FINAL REPORT

Monitoring the Microbiology of the Montecito Outflow Wastewater Plume

Heal the Ocean
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Executive Summary

Background, Goal, and Objectives

A research project was conducted by an interdisciplinary team from the University of California, Santa Barbara (UCSB), with assistance from researchers at the University of Southern California (USC) and Lawrence Berkeley National Laboratory (LBNL), between July, 2006 and December 31, 2009, involving a one-year field sampling and analysis program between November, 2007 and November, 2008. The project was funded by Heal the Ocean (HTO) with support from the California State Water Resources Control Board (SWRCB). The overall objective of the project was to examine of the fate and transport of the treated effluent plume from a wastewater treatment plant (WWTP) discharging through a diffuser at the terminus of a short, shallow outfall. The project is important because its results are applicable to the many short outfalls discharging into California's coastal waters. The approach of the project employed coordinated microbiological and chemical analysis of effluent composition with observations of effluent plume motion to quantify plume dilution, to identify plume tracers, and to describe trajectories of the plume. The WWTP discharging the effluent is operated by the Montecito Sanitary District (MSD) in Montecito, CA, which also was the site for field sampling. The specific goals of the project were to:

1. Describe the likely pathways of the WWTP effluent plume based on GPS-tracked drifter data.

2. Determine if waters located farther offshore from the WWTP outfall location move similarly.

3. Quantify culturable and DNA-based fecal indicator bacteria, amounts of DNA-based human waste markers, bacterial abundances (by extracted DNA, and also cell counts), and nutrient concentrations at the sampling locations.

4. Describe the differences and similarities in the microbial communities at the diffuser versus offshore, nearshore, and shoreline sampling locations.

5. Identify microbial taxa that appear to be tracers of the plume, and describe their abundances along the plume path.

6. Determine if and where treated effluent is most likely to enter the surf zone.

7. Estimate the plume concentration if and when it enters the surf zone.

Project Approach

The project approaches included mathematical modeling, field observations, laboratory sample analysis, and data analysis of physical oceanographic, chemical, and microbiological water characteristics. Field data were collected in four ways: 1) from moored instrumentation installed for this project near the outfall diffuser, 2) from global positioning system (GPS)-tracked drifters released from a small research vessel, 3) from vertical profiles of water properties, and 4) from boat-side sampling of ocean waters for microbiological and chemical constituents. Temperature and current profiles were measured at the mooring continuously for a year to provide data for modeling the plume vertical rise and to monitor ocean water masses and waves. Other field measurements were collected one day each week for a year. The GPS-tracked drifters recorded how ocean currents moved effluent and surface waters away from each of three release points: 1000 meters (m) offshore of the diffuser, 500 m offshore of the diffuser, and above the diffuser.

An instrument called a CTD was used to measure vertical profiles of temperature and salinity. Water samples were collected 1000 m offshore (Offshore1000) of the diffuser, above the diffuser (Diffuser), and at up to three locations (Lagrangian a, b, c) along the paths of drifters. On the same days as the offshore sampling, effluent water samples (Effluent) were collected from the WWTP discharge upstream of the outfall, and water samples were acquired from the shoreline (Shoreline) in ankle-deep water at beach locations aligned with the drifters' ending positions.

Current and temperature data collected at the mooring were inputs to a model predicting plume rise over the diffuser. CTD data from the vertical profiles allowed estimation of salinity differences and dilution as the fresher effluent plume mixed into saltier ocean waters. Drifter data tracked the plume motion over the sea surface. Water samples were analyzed for culturable fecal indicator bacteria (FIB), DNA-based markers of *Enterococcus* and of human waste-associated *Bacteroides*, enterovirus, ammonia, nitrate+nitrite, phosphate, total bacterial counts, and microbial community profile and composition using terminal restriction fragment length polymorphism (TRFLP) and PhyloChip analyses, respectively.

Quarterly stakeholder meetings were conducted throughout the project to convey progress and results to date. Attendees routinely included representatives of UCSB, HTO, MSD, and the SWRCB.

Summary of Results

Plume modeling indicated that the buoyant effluent plume always reached the sea surface within about a minute after discharge from the diffuser. Drifters deployed over the diffuser moved mainly alongshore and eastward or westward away from the diffuser. Drifters also moved in the onshore direction on 49 of the 50 sampling days. Drifter movements were consistent with known patterns of the local coastal circulation. Drifters released at the diffuser reached the surf zone over a region extending from about 0.6 km east, to about 1.6 km west, of the diffuser. Roughly half of all diffuser drifters reached the surf zone waters west of the diffuser, and the other half east of the diffuser.

Drifters deployed offshore of the diffuser moved both eastward and westward with generally higher speeds. Drifters offshore of the diffuser were less likely to reach the surf zone during the three to five hour sampling times of this study. About half of all drifters released at the diffuser location reached the surf zone while only 15% released 500 m offshore of the diffuser and 3% released 1000 m offshore of the diffuser did so.

The effluent plume was almost always detectable in surface salinity. Dilution estimates over the diffuser and following drifter motions were always greater than 100, and typically greater than 400, consistent with design criteria of WWTP effluent outfall diffusers.

Phosphate and nitrate+nitrite concentrations in effluent samples were greater than in the ocean, but ammonia concentrations were similar to the ocean. Dilution estimates based upon phosphate and nitrate+nitrite concentrations at the diffuser were similar in magnitude to estimates from salinity.

Concentrations of culturable fecal indicator bacteria (total coliforms, *E. coli* and *Enterococcus*) were generally low across all samples and, while higher at the shoreline than in effluent samples,

did not exceed State of California beach water quality criteria. Total bacteria were also typically very low in the effluent, but were higher in the nearshore region relative to offshore. DNA-based measures of *Enterococcus* and of human waste-associated *Bacteroides* were generally low and undetectable for many samples. Enteroviruses were not quantifiable in any of the samples.

Terminal restriction fragment length polymorphism (TRFLP) analyses, performed for 26 sampling events, allows for "profiling" bacterial communities using total DNA extracted from water samples. A high density phylogenetic microarray (PhyloChip), developed by LBNL, was also used to profile bacterial communities and to also potentially identify which taxa, from over 8000 microbial taxa detected by PhyloChip, were present in the samples. Due to its high cost, PhyloChip analysis was performed for a subset of 8 sampling events that were also analyzed by TRFLP.

By TRFLP and PhyloChip analyses, microbial communities in effluent samples, regardless of sample DNA content, varied but overall were distinct from all other samples. By PhyloChip analysis, several taxonomic groups, or "families" of bacteria were identified as contributing to the distinctiveness of the effluent microbial community relative to offshore. One family was shared with the nearshore region, including the shoreline. There were also many distinct taxa in the shoreline samples, meaning that the shoreline harbored bacteria that were not detected in either the effluent or the ocean.

Conclusions and Future Recommendations

A highly interdisciplinary research program was successfully performed to characterize the fate and transport of a WWTP effluent plume offshore of Montecito, CA. Effluent was extensively diluted following discharge, consistent with modeled diffuser performance and design. The plume typically moved towards shore. However, no beach water quality criteria were exceeded. The effluent fertilized the nearshore environment with phosphate and nitrate+nitrite which may have been responsible for increased overall bacterial biomass in the nearshore waters. While the shoreline microbial community shared some microbial taxa with the effluent and ocean that were not found further offshore, the exact origins of these taxa at the shoreline remain unknown. Further, the shoreline appeared to have a number of taxa that were unique to that environment, suggesting other, possibly land-based, sources. Additional data and sample analysis could provide more insight.

Glossary

ADCP: acoustic Doppler current profiler is an instrument used for measuring ocean currents vertically in the water column. The ADCP deployed in this study also measured ocean waves.

advection: horizontal movement of ocean waters due to currents

CTD: conductivity-temperature-depth instrument used for measure seawater properties such as temperature and salinity.

Diffuser: sampling station above the end of the MSD diffuser.

Diffuser and Lagrangian signature OTUs: signature OTUs (determined by PhyloChip. analysis), detected at the Effluent, Diffuser and all 3 Lagrangian locations.

Distinct OTUs: OTUs that cause the separation between groups of samples. Distinct OTUs were determined among samples for Effluent vs. Offshore1000, Offshore1000 vs. Effluent, Diffuser vs. Offshore1000, Shoreline vs. Lagrangian, and Lagrangian vs. Shoreline.

Diffuser signature OTUs: signature OTUs (determined by PhyloChip analysis), detected at the Effluent and Diffuser locations.

EC: *Escherchia coli*, one type of fecal indicator bacteria quantified in this study, using the IDEXX method.

Effluent: dechlorinated effluent sample from the MSD WWTP.

ENT: *Enterococcus* spp. or enterococci, one type of fecal indicator bacteria quantified in this study, by the IDEXX method and qPCR.

FI: fluorescence intensity, which is the quantity of signal associated with each OTU detected by the PhyloChip

FIB: Fecal Indicator Bacteria. Three types of fecal indicator bacteria are quantified in this study: total coliform, *E. coli* and enterococci.

HTO: Heal the Ocean.

Hz: Hertz or samples per second.

IDEXX: A private company that markets and sells reagents and testing platforms for fecal indicator bacteria based on a proprietary define substrate technology, and as approved for use in water quality monitoring by the U.S. EPA.

Lagrangian samples: samples taken along the path of the surface drifters. These are also designated as sampling locations 4a, b, and c. Lagrangian refers to an ocean sampling technique following a water parcel of drifter.

Lagrangian1, Lagrangian2, Lagrangian3: sampling stations along drifter trajectories

LBNL: Lawrence Berkeley National Laboratory

MDS: non-metric multidimensional scaling. A non-parametric statistical method to explore similarities between samples based on multivariate datasets.

MPN: most probable number, commonly expressed as MPN per 100 mL for FIB, which is a unit

MSD: Montecito Sanitary District.

Nearshore samples: Lagrangian and Diffuser samples.

Offshore500: sampling station 500 m directly seaward of the Diffuser station.

Offshore1000: sampling station 1000 m directly seaward of the Diffuser station.

OTU (PhyloChip): Operational Taxonomic Unit. The highest level of resolution analyzed by PhyloChip, phylogenetically. OTUs can be aggregated into higher phylogenetic levels, such as families or classes.

PCR: Polymerase Chain Reaction, a molecular microbial technique to amplify DNA.

Pf cutoff: A cutoff below which all fluorescence intensities were set to zero, used to avoid detection of multiple sequences with one OTU. Pf cutoff values of 0.7, 0.8, 0.9, 0.95 and 1 were assessed.

Positive fraction (Pf): the fraction of the probes that hybridize to the sample DNA which indicates the detection of each OTU on the PhyloChip. Positive fraction ranges between 0 and 1

Principal axis currents: Currents rotated from the geographical reference frame (i.e. east-west and north-south components) into a reference frame such that the components are not correlated. For these data the principal axis components are oriented alongshore and cross-shore and are similar to the east-west and north-south components.

qPCR: quantitative PCR, a molecular microbial technique to quantify DNA fragments.

Sampling event: the date on which samples were taken at each location.

Shoreline: sampling stations in the surf zone accessed via the shore. The beach location depended on drifter movement such that Shoreline samples were obtained directly onshore of locations where drifters were retrieved.

Signature OTUs: OTUs detected in the Effluent, Diffuser samples but not in the Offshore1000 sample. Signature OTUs were identified for each sampling event.

Stable OTUs: the 10% of OTUs for a location with the lowest variance of fluorescence intensity

SZE: Drifter Surf Zone Entry.

Surf zone: Ocean region near shore where waves are breaking.

Stratification: The strength of vertical changes in density down through the water column. When density changes greatly with depth the water column is said to be highly stratified.

SWRCB: California State Water Resources Control Board, a governmental regulatory agency

TC: Total Coliforms, one type of fecal indicator bacteria quantified in this study, by the IDEXX method.

TRFLP: Terminal Restriction Fragment Length Polymorphism, a molecular microbial technique for investigating microbial community composition and diversity.

Variable OTUs: the 10% of OTUs for a location with the highest variance of fluorescence intensity.

WWTP: Wastewater Treatment Plant.

Table of Contents

1. Introductions and Overview

This report describes the experimental design, data obtained during the study, explanations and interpretation of those data, and conclusions. The study was conducted by the faculty and staff of the University of California Santa Barbara (UCSB) pursuant to grant agreement number SB080078 between Heal the Ocean and UCSB.

1.1. Problem Statement and Existing Conditions

Roughly 37 sanitary districts discharge treated wastewater (hereafter "effluent") into the Pacific Ocean off the California coast. Discharge rates vary from 100's of millions of gallon per day for the largest sanitary districts, to much less than a million gallons per day for the smallest. To investigate the role of effluent on beach water quality, "fate and transport" of the effluent and its "water quality" are often the focus of scientific studies. Historically, these studies have focused on large discharges such as the Los Angeles City, Los Angeles County, and Orange County districts that discharge 100's of millions of gallons of treated effluent per day. Large sanitary districts typically discharge effluent many kilometers off the coast into waters 10's of meters (m) deep.

Smaller low-volume sanitary districts discharging less than one million gallons per day comprise roughly 50% of the 37 California discharges. Smaller discharge volumes allow for shorter and shallower diffusers. Despite close proximity to the shoreline at discharge, focused studies of effluent from short, shallow, low-volume diffusers have received little scientific attention. Limited observational methods that properly resolve small scales very near the coast, and presumed weak signals associated with low-volume discharges, may be part of the reason.

1.2. Geographical Setting

The focus of this study is the ocean outfall and diffuser operated by the Montecito Sanitary District (hereafter MSD, http://montsan.org/). The MSD diffuser was selected for the study because of its effluent discharge characteristics, its sampling accessibility, and its proximity to Hammonds Beach (listed by the EPA as a 303(d) impaired water body). MSD discharges ~0.72 million gallons of treated effluent per day. The highly treated effluent enters the ocean through 10 diffuser ports located along a 100 foot (ft) section of pipeline located roughly 485 m off the Montecito coastline in a water depth near 11 m (Figure 1.2-1; exact distance and depth are a function of tide height). The study area encompasses the diffuser and surrounding ocean waters from the shoreline to about 1 kilometers (km) offshore and about 2 km on either side of the diffuser. A logistical advantage of the study site is that is readily and safely accessible by small boat during all but the most extreme weather conditions.

Figure 1.2-1.. Map of the study region located just east of the Santa Barbara, CA. Red dots show sampling locations 0.5 and 1 km offshore of the outfall diffuser which is indicated with a red x. Inset shows location of Santa Barbara along the California coast.

The study area lies east of Point Conception on the mainland coast of the Santa Barbara Channel as shown in Figure 1.2-1. The diffuser is located roughly 4.5 km east of the Santa Barbara Harbor along a south facing coastline. The Northern Channel Islands lie about 40 km offshore to the south. The study site is in the lee of Point Conception which limits direct effects of prevailing northwest winds and large swells from the North Pacific. The Northern Channel Islands block southern swell from the south Pacific.

A number of discharge sources are located along the coast near the study site. These sources include: Santa Barbara's El Estero waste water treatment plant diffuser, Mission Creek, several smaller urban and semi-urban creeks, the Santa Barbara harbor, and a public anchorage with no holding tank regulations. All of these sources may discharge materials that can ultimately influence the water quality in the study region.

Combined use of high resolution water-following drifters and coordinated water sampling following motion provide the opportunity to study microbial communities as they evolve in space and time. This comprehensive interdisciplinary sampling scheme is believed to be the first of its kind thus yielding the unique data set reported on here.

2. Project Summary

2.1. Project Objectives

The overall objective of this project is to examine of the fate-and-transport of discharge from a low-volume, short, shallow diffuser, with a coordinated microbiological analysis of effluent composition following plume motion and dilution. This novel interdisciplinary experimental design, made possible by recent advances in instrumentation (Ohlmann et al. 2005, Brodie et al., 2007) enables an extended suite of microbiological constituents to be monitored following water parcels tagged with drifting buoys. Data provide descriptive and statistical summaries of the microbiological composition, advection, and relative dispersion of tagged surface water parcels as they move horizontally from the effluent discharge location.

Project goals have been modified from those in the Quality Assurance Project Plan (QAPP) of January 2008. Modifications were discussed and agreed to at the June 2008 stakeholders meeting.

Goals of this project are to:

1. describe the likely pathways of the wastewater plume based on drifter data. Plume modeling results indicate the plume always reaches the surface so the drifter trajectories should be reasonable indicators of plume movements;

2. determine if waters located farther offshore from the outfall location move similarly;

3. describe culturable and PCR-based fecal indicator bacteria abundances amounts of human waste markers, bacterial abundances (extracted DNA, and also flow cytometry), and nutrient concentrations at the sampling locations;

4. describe the differences and similarities in the microbial communities at the diffuser versus the offshore 1000 site;

5. determine where in the datasets (in space, and in time) taxa that might be plume-specific occur in the drifter trajectories, and summarize the abundances of these taxa;

6. indicate if and where treated effluent is most likely to enter the surf zone;

7. estimate the plume concentration if and when it enters the surf zone.

2.2. Project History

Ocean circulation in the Southern California Bight and the Santa Barbara Channel has been examined in a number of studies using observations from a variety of instruments including moored current meters, drifting buoys, high frequency radar for surface currents, sea-level gauges, and moored and profiling conductivity-temperature-depth instruments (CTDs). Previous studies of the regional circulation including those of Harms and Winant, (1998), Dever (2004), and Winant et al. (2003) conducted farther offshore do not describe well the dynamics affecting flow very near the coast. Regional circulation has also been investigated with numerical ocean circulation models (e.g. Dong et al., 2009). However, no existing studies or models resolve the small time and space scales of motion that advect and disperse shallow outfall plumes such as discharged from the MSD diffuser. The orientation and somewhat enclosed nature of the Santa

Barbara coastline along with associated forcing mechanisms produces complex circulation pattern such as eddies and fronts.

This project was developed after Heal the Ocean (HTO) proposed to the State of California Clean Beaches Initiative program (Proposition 50) to conduct a study aimed at understanding the influence on beach and coastal water quality of treated effluent discharged from wastewater treatment plants (hereafter WWTPs) with short, shallow outfalls.

2.3. Baseline Water Quality

Data characterizing fecal indicator bacterial (FIB) concentrations in surf zone ocean waters in the vicinity of Hammonds Beach and Butterfly Beach are available through Santa Barbara County Environmental Health Services (SBCEHS). Sampling locations on these beaches are shown in Figure 2.3-1. These data are available as a result of once per week monitoring as required through the statutes in California State Assembly Bill No. 411 of 1999 which added bacteriological ocean water quality standards and monitoring requirements to the California Health and Safety Code. A summary of the pre-study FIB exceedances collected by SBCEHS is provided in Tables 2.3-1 through 2.3-4 (exceedances are highlighted in red).

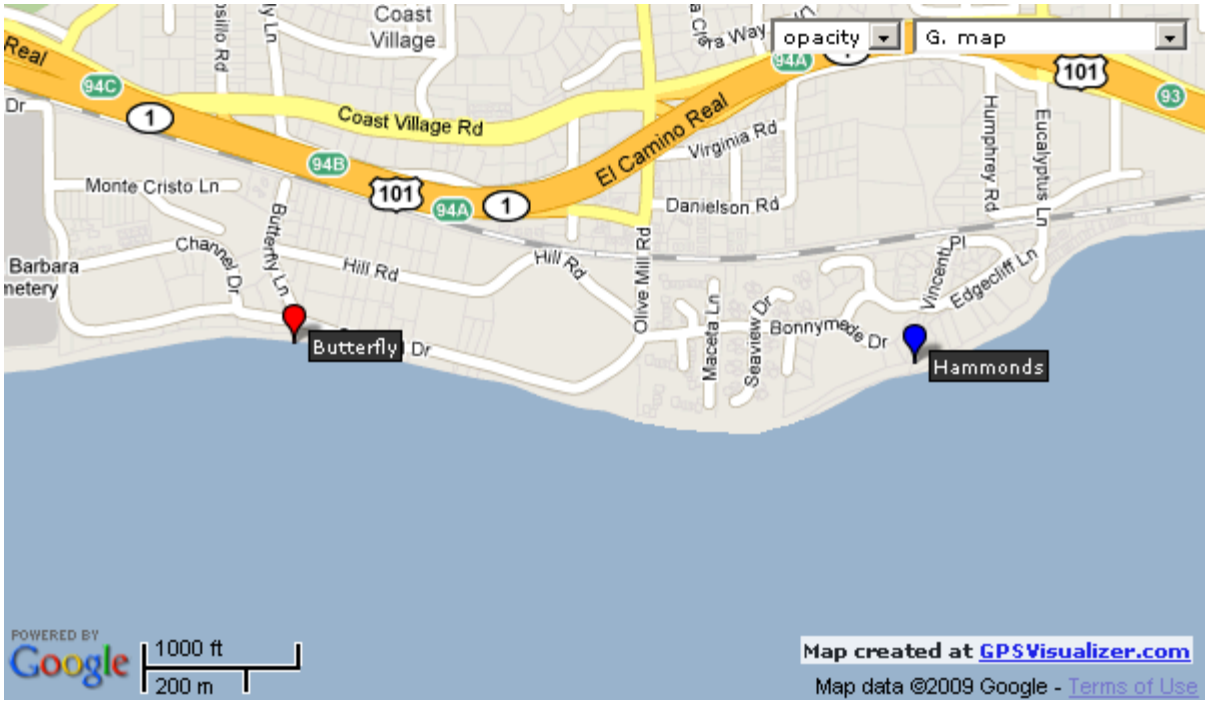


Figure 2.3-1. Santa Barbara County Environmental Health Services sampling locations at Butterfly Beach (red balloon) and Hammonds Beach (blue balloon).

Table 2.3-1. Summary of Pre-study FIB Concentrations (MPN/100 mL) and exceedances (red highlights) of AB411 at the Hammonds Beach sampling site (34° 25’ 00.9’’ North, 119° 38’ 03.3’’ West; location shown in Figure 2.3-1).

Date	Total Coliform (TC)	TC Log Mean	Fecal Coliform (FC)	EC Log Mean	Enterococcus (Ent.)	Ent. Log Mean	Ratio of fecal/total
3/17/2003	12,997	81	228	12	318	12	0.02
5/5/2003	19,863	316	670	17	216	16	0.03
7/7/2003	20	16	3	4	663	15	0.15
10/13/2003	3448	54	3448	32	402	25	1
10/27/2003	175	75	63	36	85	36	0.36
12/22/2003	243	40	74	14	645	25	0.3
3/24/2004	41	145	10	13	1565	26	0.24
7/21/2004	31	28	74	21	109	15	2.39
1/3/2005	7701	92	52	15	187	20	0.01
1/5/2005	17329	263	10	15	74	23	0
1/10/2005	24192	963	373	27	602	45	0.02
1/12/2005	15531	2920	107	40	98	66	0.01
1/18/2005	24192	8861	41	51	31	80	0
1/24/2005	24192	17563	10	45	52	105	0
1/26/2005	934	12356	10	34	10	64	0.01
1/31/2005	435	6686	20	39	10	46	0.05
2/7/2005	1281	4097	10	21	41	29	0.01
2/14/2005	670	2426	10	14	20	23	0.01
2/22/2005	24192	2426	588	22	728	38	0.02
2/24/2005	1789	1572	10	22	10	29	0.01
2/28/2005	148	1156	10	22	10	29	0.07
3/7/2005	226	1037	10	20	10	29	0.04
3/14/2005	1664	1083	10	20	20	26	0.01
4/4/2005	1281	466	185	18	31	18	0.14
7/25/2005	1259	163	288	20	72	17	0.23
3/6/2006	4884	72	20	15	328	30	0
4/3/2006	24192	290	1019	31	2359	50	0.04
4/5/2006	17329	358	63	37	292	49	0
4/10/2006	74	282	10	37	10	49	0.14
4/17/2006	546	454	51	43	52	57	0.09
4/24/2006	10	454	10	43	10	57	1
5/1/2006	354	626	10	39	10	57	0.03
5/22/2006	24192	375	882	36	3255	43	0.04
5/24/2006	1086	420	52	37	41	42	0.05
5/29/2006	52	553	10	37	30	50	0.19

6/5/2006	226	514	10	37	10	50	0.04
6/13/2006	1374	670	10	28	20	50	0.01
6/19/2006	638	804	10	28	10	45	0.02
11/6/2006	98	64	86	23	259	26	0.88
1/2/2007	110	169	41	31	121	26	0.37
6/4/2007	839	32	663	20	216	17	0.79

Table 2.3-2. Percent Exceedances at Hammonds Beach from January 1, 2003 to December 31, 2007

Year	Number of samples triggering warning status	Number of total samples	Percent exceedances
2003	6	56	11%
2004	2	54	4%
2005	17	58	29%
2006	14	56	25%
2007	2	54	4%

Table 2.3-3 - Summary of Pre-study FIB Concentrations (MPN/100 mL) and exceedances (red highlights) of AB411 at the Butterfly Beach sampling site (34° 25’ 02.2’’ North, 119° 38’ 51.2’’ West; location shown in Figure 2.3-1).

Date	Total Coliform(TC)	TC Log Mean	Fecal Coliform (FC)	EC Log Mean	Enterococcus (Ent.)	Ent. Log Mean	Ratio of fecal/total
4/7/2003	10	108	10	19	130	13	1
9/2/2003	10	30	3	5	120	10	0.3
10/27/2003	1439	48	801	28	933	27	0.56
11/24/2003	10	119	10	38	246	41	1
12/22/2003	134	36	63	14	298	34	0.47
4/26/2004	10	13	10	10	269	17	1
10/18/2004	19863	43	158	16	275	17	0.01
10/20/2004	3873	116	86	23	211	29	0.02
12/27/2004	2014	58	63	17	676	20	0.03
12/29/2004	3654	117	122	23	473	38	0.03
1/3/2005	1935	282	20	26	20	43	0.01
1/10/2005	24192	1033	2187	64	933	92	0.09
1/12/2005	14136	2418	62	86	31	111	0
1/18/2005	1396	4352	20	86	10	111	0.01

1/24/2005	374	3288	10	64	10	55	0.03
1/31/2005	529	2382	10	42	10	29	0.02
2/7/2005	364	1803	63	51	10	26	0.17
2/22/2005	12997	660	10	15	10	10	0
4/11/2005	63	143	209	37	199	16	3.32
10/17/2005	2987	52	52	18	160	22	0.02
1/3/2006	11199	83	146	40	86	14	0.01
3/6/2006	24192	109	425	25	805	21	0.02
4/3/2006	24192	325	98	27	110	37	0
4/5/2006	4352	244	132	22	554	35	0.03
4/10/2006	107	228	30	27	41	44	0.28
4/17/2006	388	373	10	27	62	60	0.03
4/24/2006	20	373	10	27	10	60	0.5
5/1/2006	31	373	10	27	10	50	0.32
9/25/2006	909	131	909	21	110	17	1
10/9/2006	432	174	122	32	201	28	0.28
12/27/2006	5794	305	109	27	183	20	0.02
4/9/2007	10	31	10	10	106	17	1

Table 2.3-4. Percent Exceedances at Butterfly Beach from January 1, 2003 to December 31, 2007

Year	Number of samples triggering warning status	Total number of samples	Percent exceedances
2003	5	55	9%
2004	5	56	9%
2005	10	55	18%
2006	11	57	19%
2007	1	53	2%

2.4. Potential Source Categories

There is the potential for terrestrial overland sources (including creeks), ocean sources (including up or down-coast from the study area), beach sands, and local WWTPs to influence coastal water quality. Some known sources that can influence water quality in the study region are explicitly indicated in Section 1.2. This study focuses solely on changes in water quality within tagged water parcels that move from three specific locations in the coastal ocean (locations indicated in Section 5.5).

2.5. Funding Program

The projected funding for the project equaled the actual cost of the project; all state funds for the project were expended by the project's end.

The project benefited from substantial leveraging provided by other funding sources. Much of the oceanographic instrumentation was borrowed from other projects which produced significant savings. Drifters used in the project with a replacement value of roughly $25k were purchased with funds provided by the U.S. Minerals Management Service (MMS) and Office of Naval research. Thermistors used on the mooring near the diffuser were also obtained with funding from the MMS. The profiling conductivity, temperature, depth (CTD) instrument was borrowed from UCSB research projects funded by private foundations and by the National Science Foundation. The only oceanographic instrument purchased for this project was the acoustic Doppler current profiler (ADCP) used for measuring ocean currents near the diffuser. Field gear in microbiological sampling was provided from sources prior to this project including State- and Measure B-funded projects with the City of Santa Barbara Creeks Division. Two additional instruments were significant to this project and provided by other sources: a BioRad qPCR machine funded through a contract with the City of Santa Barbara who received funding for the purchase from the State of California Clean Beaches Initiative, and a Synergy2 Microplate reader used for DNA quantification and other fluorometric analyses that was purchased using funds from private industry who was funded by the U.S. Department of Energy for research unrelated to this project. This project funded a significant enhancement of the microplate reader: a Time Resolved Fluorescence (TRF) detector; this project also funded the purchase of a new rotor for a high speed centrifuge used routinely in extraction and purification of samples for DNA analysis. Flow cytometry equipment was in the lab of Professor Craig Carlson at UCSB and was made available to this project for a minimal cost. Analytical instrumentation for nutrient quantification was in the Marine Science Analytical Laboratory at UCSB, whose services were made available to this project at standard on-campus recharge rates. Other facilities, including biological control cabinets, autoclaves, constant temperature rooms, freezers, and IDEXX Quantitray plate sealer, are owned by the Bren School at UCSB but made available to this project at no additional charge. Small instrumentation in the Holden Lab, purchased through grants from the National Science Foundation, the U.S Environmental Protection Agency, the U.S. DOE, and the City of Santa Barbara Creeks Division through State of California funding, was used routinely for this project at no addition cost. This equipment includes: electrophoresis gel boxes and power supply, micropipettes, and vortexers, among other items. These equipment and instruments may be available for future interdisciplinary ocean studies related to coastal ocean pollution and human health.

3. Project Activities, Tasks and Schedule of Completion

Quarterly Reporting Dates

1/16/2007
5/28/2008
7/30/2008
10/16/2008
1/19/2009
4/17/2009

9/17/2009

Project Completion Date

December 31, 2009

4. Project Implementation

The data collection phase of this project took place for an entire calendar year extending from mid-November 2007 through mid-November 2008. Sampling took place during one day each week as indicated in Table 4-1. Sampling was planned for each Monday during the year period. Sampling was occasionally rescheduled for later in the week for a variety of reasons that included University closures, boat/captain availability, and unsuitable weather. Sampling was not rescheduled during the Christmas holiday and during the first week of September. Thus data were collected on a total of 50 sampling days. Time series data were collected from November 18, 2007 through November 17, 2008. Mooring instruments were recovered quarterly to obtain data, and redeployed.

Table 4-1. Sampling dates.

Sampling event #	Date	Sampling event #	Date
1	11/26/07	26	5/28/08
2	12/3/07	27	6/2/08
3	12/10/07	28	6/9/08
4	12/17/07	29	6/16/08
5	1/2/08	30	6/23/08
6	1/8/08	31	6/30/08
7	1/14/08	32	7/7/08
8	1/22/08	33	7/14/08
9	1/28/08	34	7/21/08
10	2/5/08	35	7/28/08
11	2/11/08	36	8/4/08
12	2/20/08	37	8/11/08
13	2/27/08	38	8/18/08
14	3/3/08	39	8/25/08
15	3/10/08	40	9/8/08
16	3/18/08	41	9/15/08
17	3/24/08	42	9/22/08
18	3/31/08	43	9/29/08
19	4/7/08	44	10/6/08
20	4/14/08	45	10/13/08
21	4/21/08	46	10/20/08
22	4/28/08	47	10/27/08
23	5/5/08	48	11/3/08
24	5/12/08	49	11/10/08
25	5/19/08	50	11/17/08

5. Experimental Design and Data Collection

5.1. Sample Collection Strategy and Schedule

The interdisciplinary sampling plan includes year long time series of moored current and temperature observations, and one day each week of sampling surface currents from sets of drifters, CTD profiles, and water sampling for human-specific waste markers, measurement of fecal indicator bacteria abundances, and microbial community composition. Effluent flow and temperature data were provided by the MSD.

The main components of the observational plan employed for the project are illustrated in Figure 5.1-1. The oceanographic mooring, located just offshore of the diffuser end, was outfitted with an acoustic Doppler current profiler (ADCP) and a set of thermistors for measurements of ocean current and temperature profiles, respectively. These measurements, along with effluent flow rate and temperature data are necessary inputs to the plume model used to determine if and when discharged effluent reaches the sea surface.

During each sampling day sets of surface drifters were deployed at the diffuser (This location is hereafter denoted as Diffuser), and 500 m (hereafter Offshore500) and 1000 m (hereafter Offshore1000) seaward of the diffuser. Drifters deployed at the diffuser were used to determine how surface waters move from the diffuser location. Drifters deployed further offshore were used to determine if these waters move differently from those at the diffuser and from each other. CTD profiles were taken at the offshore end of the diffuser and farther offshore to identify the effluent plume signature in salinity, and to compare density stratification at the locations. Water samples were also taken at the locations to compare water quality very near the diffuser to water quality of the background ocean waters. CTD profiles and water samples were collected following the motion of drifters deployed at the diffuser to examine changes in salinity and water quality following motion from the deployment location. The individual components are described in greater detail below.

5.2. Oceanographic Mooring

Ocean current and temperature measurements from a mooring near the outfall provide critical environmental inputs for a near-field plume model designed to predict the vertical position of plume waters soon after discharge into the ocean. Model results indicate whether or not plume waters reach the ocean surface and provide estimates of other important plume parameters such as initial dilution. Washburn et al. (1999) used similar combined observational and modeling approaches to examine dispersion from a shallow water outfall off Carpinteria, CA.

A bottom mounted upward looking ADCP (600 kHz model, manufactured by RD Instruments, San Diego, CA) was deployed at approximately 10 m depth with the transducer head 0.4 m off the bottom. The ADCP measured current profiles and surface waves throughout the year of

shipboard sampling. Current vectors from the ADCP were averaged within 0.35m bins every 4 minutes. The instrument sampled 90 pings per ensemble at an interval of 1.05 seconds; uncertainty in the current measurements as quantified by their standard deviation was about 2 cm s^{-1}. Surface waves were measured every 2 hours with bursts of 2400 samples at a rate of 2 Hz.

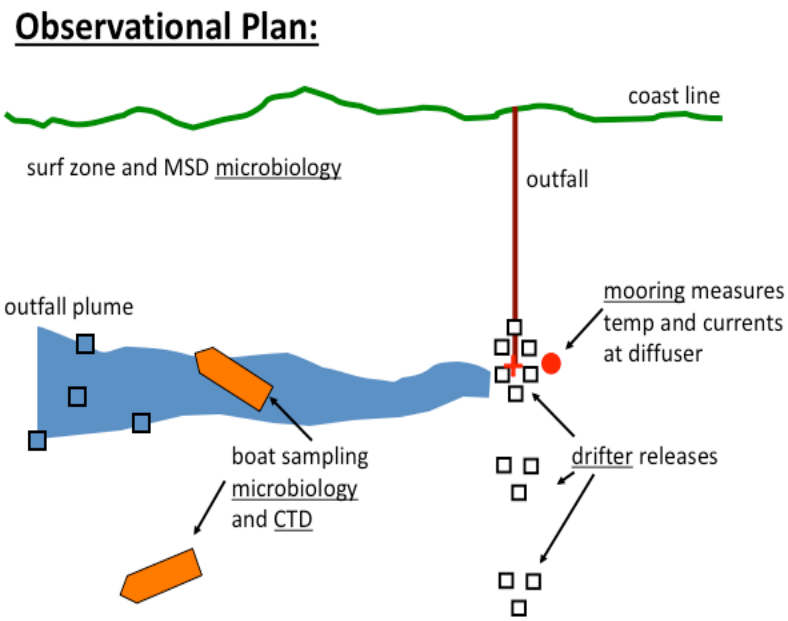


Figure 5.1-1. Schematic diagram of field sampling plan.

Three thermistors (model SBE39 manufactured by Seabird Electronics, Bellevue, WA) were attached at the top, middle, and bottom of the mooring line over the 10 m water depth. The thermistors sampled every 2 minutes with an accuracy of about 0.01 °C and a resolution of about 0.001 °C.

Thermistors from the mooring and the ADCP were retrieved quarterly to download data, clean sensors of bio-fouling, and replace batteries. Current data from the ADCP were further processed by removing values in bins near the surface contaminated by surface reflection. Magnetic north and east velocity components were rotated to true north and true east directions and then low-pass filtered with a cut off period of 1 hour to suppress noise and residual wave effects. Currents were further rotated to principal axis directions to separate alongshore and cross-shore flows. Surface wave time series data were calculated using RDI WavesMon software. The current, wave, and moored temperature data were combined by interpolating onto a common time base with a 20 minute interval.

5.3. Drifters

Drifters were deployed to track near surface water parcels at depths of about 0.5 - 1.5 m. The depth range is determined by the position of a kite-like drag structure suspended below the surface called a drogue. Multiple drifter releases from a single location provide a statistical

description of trajectories followed by water parcels. Relative motion of drifter pairs within a cluster of drifters is a measure of dispersion, key information for quantifying dilution processes. As part of the weekly sampling, a cluster of 6 drifters was deployed at the Diffuser sampling site, a cluster of 3 drifters was deployed 500 m offshore at the Offshore500 site, and a cluster of 3 drifters was deployed 1000 m offshore of the diffuser at the Offshore1000 site.

Microstar drifters (manufactured by Pacific Gyre Corporation of Carlsbad, CA), were used in the study (Figure 5.3-1). These are small drifters that record their position with GPS and transmit the position data to a host computer using a narrow band, data-only, terrestrial cellular communications system with coverage in most U.S. metropolitan regions including southern California (Ohlmann et al., 2005; Ohlmann et al., 2007). Data transmission is near real-time allowing drifter positions to be monitored from any computer with internet access. Position data is accurate to within ~5 m. The sampling frequency provides a high signal-to-noise ratio even in low velocity regimes such as the study area. The spatial accuracy and near real-time transmission enable drifters to be recovered and redeployed during the course of an experiment. The Microstar uses a collapsible scaled-down tri-star type drogue with a drag-area-ratio greater than 41. Slip is ~0.1% of the wind speed (~1 cm/s in 10 m/s of wind) which is typical of modern-day drifters. The Microstar is appropriate for the proposed work for a number of reasons. First, it has extremely high spatial and temporal resolution, required to resolve the small scales of motion that characterize coastal flows. Second, it is extremely economical. The drifters are recoverable (rather than expendable). Finally, drifter slip or leeway is minimal, and known.

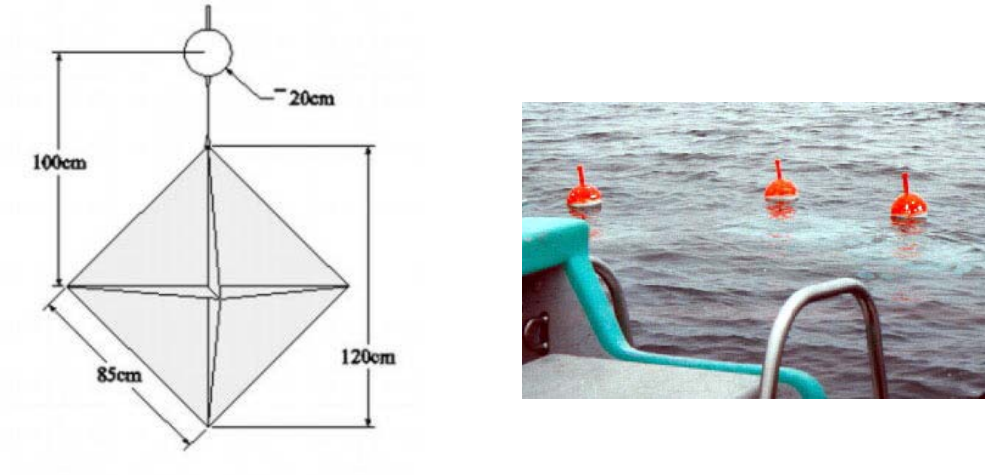


Figure 5.3-1. Schematic diagram of drifters (left) and a typical drifter deployment (right).

5.4. Profiles of Water Properties

Vertical profiles of temperature, conductivity, and of the water column were collected using a Sea-Bird SBE19-plus CTD sampling at 2 Hz. Following a 1 minute surface “soak”, the instrument was slowly lowered to the bottom and then raised to generate both downcast and upcast profiles. Temperature, conductivity, and pressure measured during profiles and water properties such as salinity and density were derived using the recommended sequence of SeaSsoft software modules and settings. Time offsets due to differences in sensor responses and the physical plumbing of the instrument were realigned during processing. Downcast and upcast profile data were separately averaged over 1 m depth bins. Only downcast data are used in the

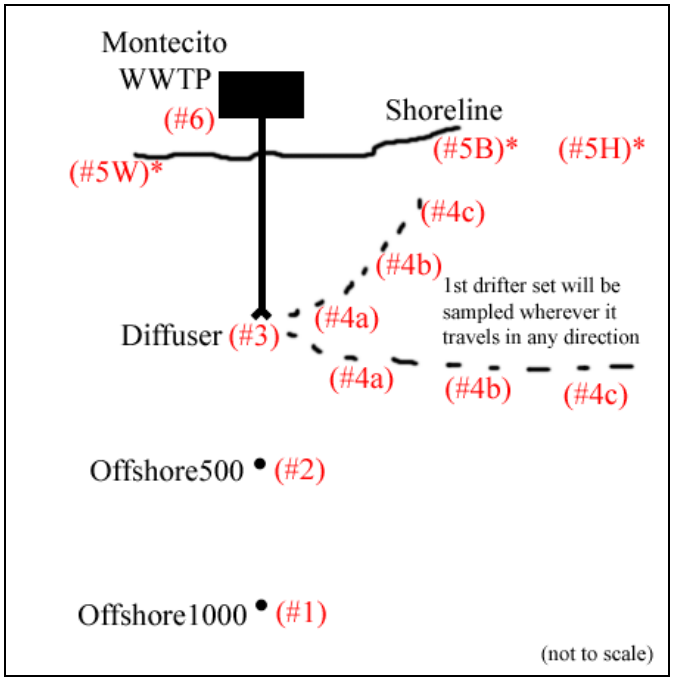
analysis. Water samples were collected immediately after CTD profiles by capturing water in 2 liter (L) Go-Flow bottles. Sample depths were nominally at 1 mdepth to correspond to the upper CTD measurements.

5.5. Weekly Small Boat Sampling and Water Collection

Ocean water sampling (via boat and shore sampling) was performed according to the Standard Operating Procedure for Microbiological Seawater Sampling that was included in the project QAPP (See Appendix Section 5). Water samples taken by boat were collected near the surface (depths of 0.5 - 1.5 m) at the Offshore1000 station, at the Diffuser station, and along the drifter trajectories at the Lagrangian1, Lagrangian2 and Lagrangian3 stations (Figure 5.5-1).

Effluent samples were collected by Montecito Sanitary District (MSD) operators, with assistance from UCSB researchers. The operators used a sterile 2L bottle attached to a pole to dip into the effluent channel in the same location where their water quality samples are taken. The effluent was then poured through sterile Miracloth into 3 x 2L bottles that were sterilized (with 200 mg of sodium thiosulfate). Each aliquot of effluent was split as equally as possible into the three sample bottles. The sterile bottle and pole was dipped into the effluent channel multiple times, until all three sample bottles were full. All samples were kept on ice after collection.

Figure 5.5-1. Station Definitions (numbered from furthest offshore):



Sampling locations:

1) Offshore1000 – location 1000 m directly seaward of the Diffuser station (#1 in Figure 5.5-1).

2) Offshore500 – location 500 m directly seaward of the Diffuser station (#2 in Figure 5.5-1).No microbiological samples were taken at Offshore500.

3) Diffuser – location at the end of the diffuser (#3 in Figure 5.5-1).

4) Lagrangian1-3 – locations along the drifter trajectories (#'s 4a, 4b, and 4c in Figure 5.5-1. CTD profiles and microbiological samples were collected at the "center" drifter from the first set of drifters deployed. Samples were obtained 3 times after drifters were deployed from the Diffuser station. These locations were variable and depended on the flow and drifter movements. The "center" drifter was subjectively determined visually while sampling. When drifters did not move toward the shore, sampling was 1 hour after drifter deployment, and then every 2 hours. When drifters were moving toward shore, sampling occurred at intervals so that 3 samples were taken at roughly equal intervals along the path from the diffuser to the surf zone. The 3 sampling times were labeled as 4a, 4b and 4c as indicated in Figure 5.5-1.

5) Shoreline– locations in ankle-to-knee deep water was sampled from shore at locations depending upon drifter movement (# 5 in Figure 5.5-1. When possible, shoreline samples were collected at the time and location where drifters reached the surf zone. Using GPS coordinates, the location of the shoreline sample was matched with the longitude of the last Lagrangian water sample. When the drifters reached the surf zone of an inaccessible location, the sample was taken at the closest safe and accessible location.

6) Effluent – dechlorinated effluent at the MSD wastewater treatment plant (#6 in Figure 5.5-1)

5.6. Water Sample Processing and Analysis

5.6.1. Water Sample Processing

Figure 5.6-1 contains a flow chart depicting the sample processing steps once samples have been brought to the laboratory. Water sample processing is also summarized in the Standard Operating Procedure for Microbiological Seawater Sampling that was included in the project QAPP (See Appendix Section 5).

Upon return to the lab, each 4L bottle from the ocean sites was split into 2 x 2L bottles through sterile Miracloth to remove any sand or debris. The split samples were stored on ice in coolers until needed. No splitting was necessary of the effluent samples since they were collected in multiple 2L bottles.

For human enterovirus analysis, 1 x 2L bottle was filtered through Millipore Sterifil filter housings that have been sterilized with Whatman GF/F filters already in place. Actual volume filtered was recorded, and the filters were removed and placed into sterile 2 oz. Whirl-Pak bags using sterile forceps. Filters were stored horizontally at -80ºC until shipment on dry ice to the laboratory of Jed Fuhrman, USC Department of Biological Sciences for analysis.

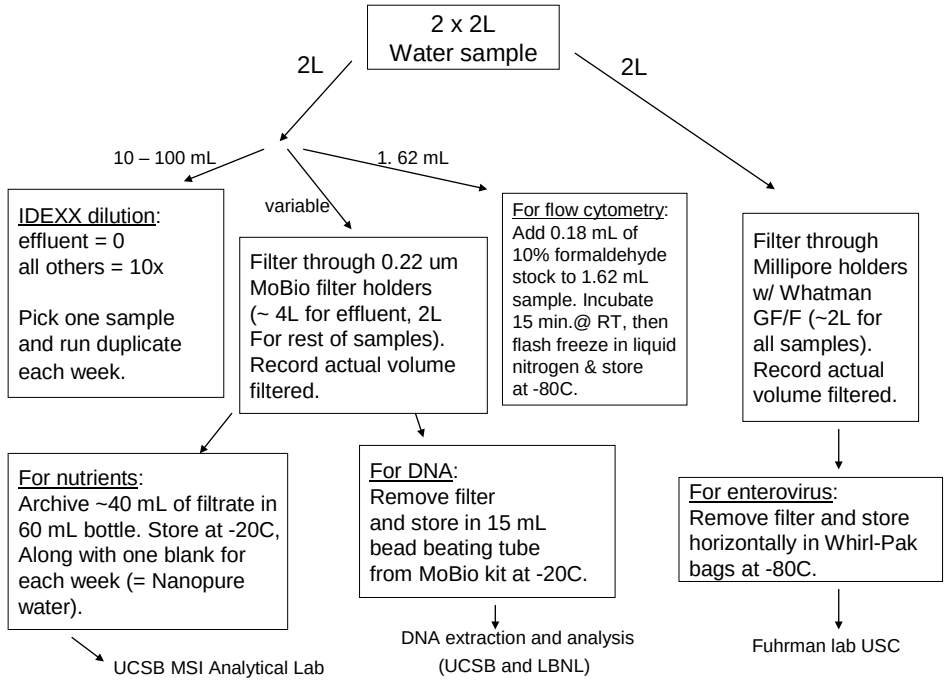


Figure 5-6-1. Flowchart depicting steps of laboratory processing of samples. Ocean samples were collected in 4L bottles and split in the lab into 2 x 2L samples. Effluent samples were compositely collected into individual 2L bottles. For both sample matrices, one 2L bottle was filtered through GF/F filter paper to archive for enterovirus analysis. The other 2L (4L for effluent) was split for multiple analyses: a small portion for cell counts via flow-cytometry (starting with the 6/16/08 sampling event), a portion for fecal indicator bacteria analysis via IDEXX, and the remainder through a 0.22 um filter for DNA extraction and downstream analyses. A portion of the filtrate was archived for nutrient analysis.

The remaining 1 x 2L bottle (2 x 2L bottles for effluent samples), were processed for DNA-based analyses. A small portion (1.62 mL) of each sample was removed for cell counts via flow cytometry (starting with the 6/16/08 sampling event). Formaldehyde (10%, 0.18 mL) was added to each aliquot, followed by incubation at room temperature for 15 minutes. Cell count samples were then flash frozen in liquid nitrogen and stored at -80ºC until analysis (Marie et al., 1999).

For fecal indicator bacteria (FIB) via IDEXX, 2 x 100 mL IDEXX bottles were prepared. For each sampling event, one sample was run in duplicate. Ocean samples were diluted with 10 mL sample and 90 mL of sterile Nanopure water. Effluent samples were not diluted. As specified by the manufacturer, Colilert and Enterolert reagents were added to the appropriate bottles and inverted to mix. Once dissolved, the IDEXX bottles were poured into IDEXX Quanti-Tray 2000 packs and sealed with a Quanti-Tray sealer. Enterolert trays were incubated for 24 hours at 41ºC, and Colilert trays for 24 hours at 35ºC.

The remainder of the sample was vacuum filtered through a 0.22 um filter included in the DNA extraction kit (UltraClean DNA Water Isolation Kit, MoBio Laboratories). Filtration was carried out until the entire sample was filtered or reached the point of refusal. Actual volume filtered was recorded, and the filters were removed using sterile forceps and stored in 15 mL conical tubes at -20ºC until extraction. A portion of the filtrates were archived for nutrients analysis in 60

mL bottles and stored at -20ºC until analysis. A storage blank consisting of Nanopure DI water was also stored with each sampling event.

Activated sludge was sampled from the MSD WWTP on 8/13/07 for use as a standard for TRFLP bacterial community analysis. An MSD operator obtained the sample by dipping a sampling bottle and pole into the activated sludge flowing from the aeration basins to the secondary clarifiers. The sample was transferred to a sterile 1 liter sample bottle and stored on ice until return to the laboratory, where the sample was filtered and processed as above for DNA extraction.

5.6.2. Selection of Dates for DNA-based Analyses

Water samples were collected and analyzed for FIB via IDEXX for 50 sampling events during this project. The budget allowed for DNA-based analysis on 26 of the 50 sampling events. Data analysis was ongoing throughout the sampling period, so selections were made in batches. The first 4 sampling events were selected for analysis at the beginning of the project, to ensure that all of our analyses would work with these sample matrices.

After the first few sampling events, selection criteria and a selection process was created. DNA was extracted from all Effluent samples during this project (n = 50). The Effluent typically had very low DNA yields, so sampling events that had enough DNA to perform the downstream analyses were prioritized for selection. At least one sampling event per month was selected, and the AB411 period was emphasized (April – October). Sampling events were also chosen so that a representation of all weather conditions and drifter path directions were captured (Table 5-1).

Based on the selected 26 dates for DNA-based analyses, sampling events were further selected for PhyloChip (8 dates) and human enterovirus (13 dates) analyses using the same criteria (Table 5-1).

Table 5-1. Summary of the 26 selected sampling events for DNA-based analyses, the 8 for PhyloChip and the 13 for human enterovirus analyses.

Date	IDEXX	DNA-based Analyses	PhyloChip	Human enteroviruses
11/26/2007	✓	✓	✓	✓
12/3/2007	✓	✓		✓
12/10/2007	✓	✓		
12/17/2007	✓	✓		
1/2/2008	✓	✓		
1/8/2008	✓	✓		✓
1/14/2008	✓	✓		✓
1/28/2008	✓	✓	✓	✓
2/5/2008	✓	✓		
2/20/2008	✓	✓		
3/24/2008	✓	✓		
4/14/2008	✓	✓		
5/5/2008	✓	✓	✓	✓
5/19/2008	✓	✓	✓	✓
5/28/2008	✓	✓	✓	✓
6/2/2008	✓	✓		
6/9/2008	✓	✓	✓	✓
6/16/2008	✓	✓		
7/7/2008	✓	✓	✓	✓
7/14/2008	✓	✓		
8/25/2008	✓	✓		✓
9/22/2008	✓	✓		
9/29/2008	✓	✓		✓
10/6/2008	✓	✓		
10/13/2008	✓	✓	✓	✓
11/17/2008	✓	✓		

5.6.3. Nutrients Analysis

Samples selected for analysis were hand delivered to the MSI Analytical Lab at UCSB. Nutrients (phosphate, nitrite+nitrate, ammonia) analysis was performed by their staff. Results were provided in an Excel worksheet. For each sampling event and each analyte, the value (if any) of the storage blank from that week was subtracted from the sample values.

5.6.4. FIB via IDEXX

After the 24 hour incubation, the Quanti-Trays were read according to the manufacturer's instructions. Total coliform values were obtained by counting the number of yellow wells on the Colilert tray. A Comparator tray from IDEXX was used to determine the minimum positive value. *E. coli* values were obtained by counting the number of yellow wells that fluoresced under a UV light. The Comparator tray was again used to determine the minimum positive value. *Enterococcus* spp. concentrations were obtained from counting the number of wells that fluoresced on the Enterolert trays. The MPN Generator computer program from IDEXX was used to calculate the most probable number (MPN) and 95% confidence limits (CL) for each sample. Sample duplicates were analyzed separately. Quality control conditions were met when the duplicates had MPN values within the 95% CL of each other.

5.6.5. DNA Extraction, Purification and Quantification

The UltraClean DNA Water Isolation Kit (MoBio Laboratories) was used to extract DNA from the archived filters for the selected sampling events. DNA was extracted according to the manufacturer's protocols, followed by ethanol precipitation. Total DNA was quantified using the Quant-iT^{TM} dsDNA BR Assay Kit (Invitrogen) and related to the volume of water filtered for reporting DNA concentration.

5.6.6. Cell counts via flow cytometry

Before analysis, archived samples were thawed and diluted 5-fold with 0.2 µm filtered ocean water (ocean samples) or 0.2 µm filtered Nanopure water (effluent samples), and stained with SYBR Green I (Molecular Probes) at a final concentration of 1:10,000 (vol:vol) for at least 30 min in the dark, and analyzed within 60 min of staining.

Cell abundances were enumerated using an LSR II flow cytometer (BD Biosciences) equipped with a 488 nm excitation laser and standard filter set (Ewart et al., 2008). The LSR II was calibrated using 3 µm Rainbow beads (Spherotech Inc.). Flow rate was calibrated by measuring change in weight of 1 ml samples of deionized water before and after 5-10 min flow runs. Data were acquired in log mode for at least 90 seconds and until 20,000 events were recorded, with the minimum green fluorescence (channel 200) set as the threshold. Gating analysis was performed using FACS Diva software (BD Biosciences). Cell abundance in cells ml^{-1} was calculated from sample flow rates and number of events recorded as described in Campbell (2001).

5.6.7. *Enterococcus* spp. qPCR

The TaqMan qPCR assay for salmon testes DNA is performed prior to the *Enterococcus* spp. assay in order to determine the lowest template dilution without inhibition. The salmon testes DNA qPCR is based on published protocols (Haugland et al. 2005; Morrison et al. 2008), and uses the same primer (300 nM) and probe (100 nM) concentrations. The qPCR master mix is spiked with salmon testes DNA, to a final concentration of 0.25 ng/reaction. Four no-sample DNA reactions (= no inhibition control) are run on each qPCR plate, in which only salmon testes DNA, PCR reagents and PCR-grade water are added. In addition, a 3-log salmon testes DNA standard curve is run to determine amplification efficiency. Diluted template DNA (2.5 µl) is added to all remaining reactions (in duplicate). Using the no -inhibition controls, the average + 3 × standard deviation cycle threshold value (Ct_{ni}) is calculated. This value is used as the upper Ct value for no inhibition. All reactions with sample DNA that produce an average Ct > Ct_{ni} are considered to be inhibited. The salmon testes assay is run first, using 1:10 diluted DNA template, to determine the occurrence of reaction inhibition. If inhibition occurs, twofold dilutions are analyzed until no inhibition occurs. The lowest template dilution without inhibition is used for *Enterococcus* spp. qPCR.

The *Enterococcus* spp. qPCR assay is based on the protocol of Haugland et al. (Haugland et al. 2005). The primer and probe concentrations used are 900 nM (forward primer), 300 nM (reverse primer) and 100 nM (probe). Baseline thresholds were set at 200 for data analyses. *Enterococcus* spp. concentrations are expressed as cell equivalents (c.eq.) per 100 ml, by assuming an *rrn* operon copy number of 6 for *Enterococcus* spp.

5.6.8. Human-specific *Bacteroides* qPCR

Human-specific *Bacteroides* qPCR was performed using a published method (Seurinck et al. 2005) for the human-specific HF 183 *Bacteroides* 16S rRNA genetic marker with SYBR® Green I detection as described before (Sercu et al. 2009). Primer design and reaction conditions were identical to the published method except for the instrument used (Bio-Rad iCycler iQ®, Hercules, CA) and the addition of fluorescein (Eurogentec, Belgium) which was necessary to enable dynamic well factor collection and data optimization on the iQ system. Each plate contained, in triplicate, a tenfold dilution of the sewage *Bacteroides* standard, ranging from 3.8 x 10^7 to 3.8 x 10^1 human-specific *Bacteroides* markers per microliter of DNA extract, a no-template control, and the samples to be analyzed. To allow comparisons, each plate was standardized by adjusting the baseline threshold position until the Ct values for the standard dilutions were less than 3% from run to run. The resulting sample Ct values were then used to calculate the number of human-specific *Bacteroides* markers per liter of sample filtered, and the triplicate values for each sample were averaged. Any replicates that did not amplify, or amplified after the lowest sewage standard, were treated as a zero value in the calculations. Samples were run at 10 ng per reaction. If no markers were detected, the sample was run again at either 5 ng or 1 ng per reaction to determine if inhibition was prohibiting amplification of the target. For samples run at multiple dilutions, the lowest dilution without inhibition was used in calculations. To ensure correct target amplification, a melt curve was run and verified for each sample.

5.6.9. 16S PCR-TRFLP

Genes encoding 16S rRNA were PCR amplified from purified DNA samples using universal primers 8F hex (fluorescently labeled) and 1389R as described before (LaMontagne and Holden 2003). PCR products were purified with the QIAquick PCR purification kit (QIAGEN, Valencia, CA), and ca. 300 ng of purified DNA was digested with *Hha*-I (New England BioLabs, Ipswich, MA). After inactivation of the restriction enzyme by heating (65°C, 20 min), the lengths of fluorescently labeled fragments were determined with an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at the Genomics Technology Support Facility (Michigan State University).

The individual peak heights of the terminal restriction fragments (TRFs) were normalized to the percentage of total height for that sample, and peaks with a relative height of less than 1% were discarded. The TRFs were aligned using the crosstab macro written by Dr. C. Walsh (http://www.wsc.monash.edu.au/~cwalsh/treeflap.xls). The aligned data was imported into the software Primer (version 6, Primer-E Ltd, U.K.), and a Bray-Curtis similarity matrix was calculated both before and after transforming the peaks to presence/absence. Non-metric Multi-Dimensional Scaling (MDS), with 100 random restarts, was used to ordinate the similarity data (Dunbar et al. 2001). SIMPER analysis was used to calculate the %similarity within sample site groups and the %dissimilarity between sample site groups. Species richness, Shannon diversity index, and Pielou's evenness were also calculated.

5.6.10. PhyloChip

Samples selected for PhyloChip analysis had their DNA concentrations adjusted to ~ 50 ng in 5 uL. Samples were shipped to LBNL on dry ice via overnight shipment. Nucleic acid preparation, scanning and probe setting, and data normalization were carried out at LBNL.

5.6.10.1. Nucleic Acid Preparation

The 16S rDNA was amplified from the gDNA using non-degenerate Bacterial primers 27F.jgi and 1492.jgi (GGT TAC CTT GTT ACG ACT T) using 3 annealing temperatures. Polymerase chain reaction (PCR) was carried out using the *TaKaRa Ex Taq* system (Takara Bio Inc, Japan). Each PCR reaction mix contained 1X *Ex Taq* buffer, 200 uM total final concentration of *TaKaRa* dNTP mixture, 0.02U/µL *TaKaRa Ex Taq* polymerase, 0.4mg/mL bovine serum albumin (BSA), and 300 pmol of each primer. PCR conditions were 1 cycle of 3 min at 95°C, followed by 25 cycles of 30 sec at 95°C, 25 sec at 48, 51.9 and 58°C (gradient block), and 2 min at 72°C, and finishing with 10 min incubation at 72°C. The gradient PCR was used to optimize the microbial diversity detection. This part of the technique was designed by the Joint Genome Institute (Walnut Creek, CA) to maximize microbial diversity amplification from environmental samples. For each array, amplicons were concentrated to a volume less than 40 µl by isopropanol precipitation. The PCR products (500 ng) were spiked with known concentrations of amplicons derived from prokaryotic metabolic genes. This mix was fragmented to 50-200 bp using DNAse I (0.02 U/µg DNA, Invitrogen) and One-Phor All buffer per Affymetrix's protocol. The complete mixture was incubated at 25 °C for 10 min., 98°C for 10 min., and then labeled. Biotin

labeling was accomplished using the GeneChip Labeling Reagent (Affymetrix) per the manufacturer's directions. The labeled DNA was then denatured (99 °C for 5 min) and hybridized to the DNA microarray at 48 °C overnight (> 16 hr). The arrays were subsequently washed and stained. Reagents, conditions, and equipment are detailed elsewhere (Masuda and Church 2002).

5.6.10.2. Scanning and Probe Set Scoring

Arrays were scanned using a GeneArray Scanner (Affymetrix, Santa Clara, CA, USA). The scan was recorded as a pixel image and analyzed using standard Affymetrix software (Microarray Analysis Suite, version 5.1) that reduced the data to an individual signal value for each probe. Background probes were identified as those producing intensities in the lowest 2% of all intensities. The average intensity of the background probes was subtracted from the fluorescence intensity of all probes. The noise value (*N*) was the variation in pixel intensity signals observed by the scanner as it read the array surface. The standard deviation of the pixel intensities within each of the identified background cells was divided by the square root of the number of pixels comprising that cell. The average of the resulting quotients was used for *N* in the calculations described below.

Probe pairs scored as positive were those that met two criteria: i) the intensity of fluorescence from the perfectly matched probe (PM) was greater than 1.3 times the intensity from the mismatched control (MM), and ii) the difference in intensity, PM minus MM, was at least 130 times greater than the squared noise value (>130 N^2). The positive fraction (pf) was calculated for each probe set as the number of positive probe pairs divided by the total number of probe pairs in a probe set. A subfamily was considered present when at least one of its subordinate OTUs had a pf > 0.90 in all three replicates.

The CEL files obtained from the Affymetrix software that produces information about the fluorescence intensity of each probe (perfect match, mismatch, and control probes) were analyzed using the CELanalysis software designed by Todd DeSantis (LBNL, Berkeley, USA). To be present, the OTU had to have at least 90% of the probe pairs in the set be positive. This is the pf cutoff of 0.9. For the remaining OTUs, any OTU with a fluorescence intensity at least 25% greater than the average of the two blanks' fluorescence intensity was also considered present in any given sample.

5.6.10.3. PhyloChip Data Normalization and Preprocessing

PhyloChip data normalization was performed using R (R Core Development Team, 2008). To correct for variation associated with quantification of amplicon target (quantification variation), and downstream variation associated with target fragmentation, labeling, hybridization, washing, staining and scanning (microarray technical variation), a two-step normalization procedure was developed. First, for each PhyloChip experiment, a scaling factor best explaining the intensities of the spiked control probes under a multiplicative error model was estimated using a maximum-likelihood procedure (Goslee and Urban, 2007). The intensities in each experiment are each multiplied by the corresponding optimal scaling factor. Second, the intensities for each

experiment are corrected for the variation in total array intensity by dividing the intensities by its corresponding total array intensity for Bacteria.

After normalization, the PhyloChip data consist of fluorescence intensity (FI) and pf for each sample/Operational Taxonomic Unit (OTU) combination. The FI is proportional to the number of gene copies present in the PCR amplified sample DNA.

Data were processed by only including FI values for each sample/OTU combination for which the pf value was equal to or greater than the pf cutoff value. FI values for which the corresponding pf value was below the pf cutoff were assigned a zero value. Pf cutoff values of 0.7, 0.8, 0.9, 0.95 and 1.0 were tested. All data were manipulated in Microsoft Excel.

5.6.10.4. Descriptive microbial community analysis

Overall spatial and temporal trends in microbial community composition by PhyloChip were investigated by non-metric multidimensional scaling (MDS), by identifying distinct OTUs, indicative of certain groups of samples, and finally by identifying stable and variable OTUs for each location.

MDS analysis was performed using PRIMER v6 software (PRIMER-E Ltd, www.primer-e.com), based on normalized and preprocessed fluorescence intensities (for $pf \geq 0.9$). Resemblances were calculated using the Bray-Curtis similarity coefficient and MDS was subsequently performed using 100 restarts, Kruskal fit scheme 1 and a minimal stress of 0.01. In addition, hierarchical cluster analysis was performed based on Bray-Curtis similarities (Clarke and Warwick, 2001), also using PRIMER v6. Significant results were analyzed using the similarity profile routine (SIMPROF) (Clarke et al., 2008), which tests for random clustering (Bradford et al., 2009). The SIMPROF test works by ordering similarities from a group of a priori unstructured samples from smallest to largest, and plotting similarities against their rank. The observed profile is compared with that expected under the null hypothesis of no meaningful structure within that group, using permutation. Repeated application of this test generates a stopping rule for a posteriori division of the samples into ever smaller subgroups, as in hierarchical cluster analysis (Clarke et al., 2008).

In order to identify distinct OTUs significantly contributing to the separation of the communities between groups of samples, a list of OTUs was generated using the SIMPER function of the PRIMER v6 software with pf cutoff of 0.9. The following groups of samples were compared: site 6 vs. site 1, site 1 vs. site 6, site 3 vs. site 1, sites 4a-c vs. site 5, and site 5 vs. site 4a-c. The SIMPER output is a list of OTUs contributing to the dissimilarity of the two groups specified, in decreasing order of importance (Clarke and Warwick, 2001). OTUs were sorted by dissimilarity/standard deviation (Diss/SD) in descending order. OTUs with ‘undefined’ Diss/SD were deleted. OTUs with Diss/SD of greater than 1 were selected. Fold differences of average relative abundance between the compared groups were used as a cutoff for the selection of representative OTUs for each group.

Variable and stable OTUs were obtained by selecting for OTUs where at least 1 sample had a pf ≥ 0.9, and sorting by variance of FI from high to low. The top decile OTUs are designated variable OTUs and the bottom decile OTUs are stable OTUs.

5.6.11. Enterovirus

Samples selected for analysis were sent to the Laboratory of Jed Fuhrman, USC Department of Biological Sciences on dry ice via overnight shipment. Analysis was performed there, and data returned in the form of mean number of gene copies or enteroviruses ± standard error of the mean, per ml of sample water.

5.6.12. Statistics

Summary statistics (average, standard deviation, standard error, minimum value, maximum value) were created for all microbiological and chemical results. The summary statistics were calculated for data type overall, by site and by individual sample date. For all data sets, any values that were below detection limit for that assay ('ND' or '<') were treated as zeros in all calculations. For IDEXX sample duplicates, the MPN values were averaged. If one of the sample duplicates had a '<' value, it was discarded and the other duplicate MPN value was used in calculations. The 95% confidence limits for the IDEXX data was not used in the statistical calculations. For statistical summaries where univariate data did not appear to vary along the Lagrangian trajectory, all Lagrangian samples (Lagrangian1, 2, 3) were lumped together into one large group.

The microbiological and chemical results were also analyzed for significant site differences via One-Way ANOVA in SPSS version 12 (SPSS Inc., Chicago, IL). Due to the unequal variances of the means across the sites, the Dunnett's T3 pairwise comparison test was used in lieu of other analysis of variance post hoc tests which assume equal variances (i.e. Tukey's HSD). Evaluations of the relationships between physical oceanographic, microbiological, and chemical results were performed via regression analysis in Microsoft Excel 2007.

5.6.13. Possible Plume Tracers

5.6.13.1. Univariate Data

The univariate data sets were evaluated as possible plume tracers by examining each sample date independently. A possible plume tracer was defined as the situation where the analyte was present in the Effluent and Diffuser samples (regardless of concentration), and absent in the Offshore1000 sample for that date.

5.6.13.2. PCR-TRFLP

For the PCR-TRFLP data set, a more overall approach was used first. All 26 Effluent samples were examined for any peaks or OTUs (operational taxonomic unit) shared amongst all samples. OTUs present in the majority of the Effluent samples were then examined and their presence was

searched for in any the ocean samples. SIMPER analysis in Primer-E was also used to identify OTUs responsible for the separation of the sample site groups, and for OTUs in common between the Effluent and Diffuser, and absent in the Offshore1000 samples. Sampling events were then looked at individually, using the possible plume tracer definition as defined above.

5.6.13.3 PhyloChip Identification of signature OTUs

An exhaustive list of signature OTUs was created, based on the datasets described in Section 5.6.10.3 for each of the 5 pf cutoff values. The criteria for each OTU to be identified as signature OTU were:

1) The FI at the Effluent (#6) was greater than zero.
2) The FI at the Diffuser (#3) was greater than two times the FI at the Offshore1000 (#1)
3) The above criteria were met in at least one out of eight sampling events.

The above procedure was performed in Microsoft Excel, by calculating for each sampling event:

1) IF FI(#3) > 2 x FI(#1), THEN "1", ELSE "0"
2) IF FI(#6) > 0, THEN "1", ELSE "0"
3) Multiply the two cells above, resulting in "1" for a signature OTU and "0" for a non-signature OTU for each sampling event.
4) For each OTU, count the number of sampling events for which the OTU was a signature OTU.
5) Rank the OTUs according to their occurrence as signature OTU.
6) Remove all OTUs with count zero.

This procedure resulted in an exhaustive list of all signature OTUs and the number of signature OTUs detected for each sampling event, for each pf cutoff value. Additional manipulations were performed in Excel to assess FI changes for all signature OTUs for each sampling event.

Additional data analysis included, for each pf cutoff value:

1) Counts of the number of signature OTUs, for each sampling event, detected at:
    a. Diffuser
    b. Diffuser + Lagrangian1
    c. Diffuser + Lagrangian1 and 2
    d. Diffuser + Lagrangian1, 2 and 3
    e. Diffuser + Lagrangian1, 2, 3 + Shoreline
2) Counts of the number of signature OTUs, detected in at least 1 to 8 sampling events.
3) Aggregation of signature OTUs for each sample event into families/classes.
4) Counts of the number of families/classes containing signature OTUs, detected in at least 1 to 8 sampling events.

5.6.13.4. Group ratio analysis (BCEEL:R ratio)

The *Bacillaceaea* (B), *Clostridiaceae* (C), *Enterobacteriaceae* (E), *Enterococcaceae* (E), and *Lachnospiraceae* (L) to *Rhodobacteraceae* (R) ratio is calculated by counting the total OTUs present (pf = 1) in each family. The count is then divided by the total number of OTUs on the G2 PhyloChip from each of the family. The sum of percent OTUs for B, C, E, E and L is divided by

percent OTU of R to obtain the BCEEL:R ratio. The rationale for selecting the BCEEL:R ratio is presented in the results section.

6. Data Quality Assessment: Data Verification and Evaluation

Data quality was evaluated throughout the project, as data was collected. Data was reported at the quarterly stakeholder meetings throughout the project duration.

Table 6-1. Measurement Quality Objectives (as stated in the project QAPP).

Parameter	Method	Units	Detection Limit	Precision	Accuracy	Complete-ness
Flow (Eulerian)	Acoustic Doppler Current Profiler	m/s	NA	NA	0.3% of current speed (= 0.003 m/s for the highest energy flows expected)	100%
Flow (Lagrangian)	Water following buoys	lat/lon position	NA	NA	GPS: ~4 m slip: 0.01-0.02 m/s	100%
CTD	CTD profiler	Siemens/m; deg C; meters	NA	NA	0.0005 C 0.005 T 0.1% D	100%
Total Coliform Bacteria (IDEXX)	Standard Method 9223B	MPN/ 100ml	1	Duplicates within 95% confidence limits R_{log} within 3.27*mean R_{log} [6]	*See section 5.1 in QAPP	90%
***E. coli* Bacteria (IDEXX)**	Standard Method 9223B	MPN/ 100ml	1	Duplicates within 95% confidence limits. R_{log} within 3.27*mean R_{log} [6]	*See section 5.1 in QAPP	90%
Enterococci Bacteria (IDEXX)	ASTM D6503-99	MPN/ 100ml	1	Duplicates within 95% confidence limits. R_{log} within 3.27*mean R_{log} [6]	*See section 5.1 in QAPP	90%
16S PCR-TRFLP	LaMontagne and Holden 2003[1]	NA	Minimum of 10 ng/uL extracted DNA	*See section 5.4	*See section 5.1 in QAPP	90%
Human-specific *Bacteroides* qPCR	Seurinck, Defoirdt, Verstraete and Siciliano 2005[2]	Human-specific *Bacteroides* markers/L	~24 human-specific markers/uL DNA extract	Coefficient of variation for standard curve 3% or less between plates	Detection efficiency estimated at 78-91% for freshwater[4]	90%

***Enterococcus* spp. qPCR**[1]	Haugland, Siefring, Wymer, Brenner and Dufour 2005[3]	cells/L	20 cells/PCR reaction[5]	95% occurrence range of ~25-400% of the mean value[5]	Geometric mean of 1088 cells for spiked sample of 1000 cells (n = 72)[5]	90%

6.1. Oceanographic Data Quality Assurance and Quality Control

Moored temperature, current, and wave data were filtered to reduce the effects of noise and under-sampled high frequency variability. Digital filtering was performed using a 5-pole low-pass Butterworth filter designed to eliminate variability on timescales less than 1 hr. After filtering data were sub-sampled to a point every 20 minutes and all data were put on the same time base (i.e. samples on the hour, 20 min after the hour, and 40 min after the hour). Moored current data are accurate to about 1 cm s^{-1} and moored temperature data to about 0.01 ºC.

Profiling CTD data were processed using standard procedures and SeaSoft software as recommended by SeaBird Electronics (Bellevue Washington). Temperature is accurate to about 0.01 ºC, salinity to about 0.01, and pressure to 0.01 dbar (1 dbar = 1 decibar; A pressure change of 0.01 dbar is equivalent to about 1 cm of seawater).

Wave statistics of significant wave height and wave period were determined from pressure measurements made from sampling "bursts for 20 minutes every 2 hr. Bursts consisted of pressure measurements at a sampling rate of 4 samples per second. Accuracy of the wave measurements depends on wave conditions themselves, but significant wave height is accurate to about 0.1 m and wave period to about ± 2 s.

Drifter position was measured by GPS to a standard deviation of roughly 3 m.

All oceanographic data were processed using MATAB analysis software.

6.2. Microbiological Data Quality Assurance and Quality Control

In compliance with the Measurement Quality Objectives as listed in the QAPP (Table 6-1), the accuracy, comparability, completeness, precision, representativeness, detection limits and existing data were evaluated on the specified data sets (IDEXX (total coliform, *E. coli*, *Enterococcus* spp.), 16S-PCR TRFLP, human-specific *Bacteroides* qPCR, and *Enterococcus* spp. qPCR).

6.2.1. Accuracy

Accuracy and bias of IDEXX data was to be evaluated as needed and appropriate. There were no indications of accuracy or bias concerns on this project, so no additional evaluations were performed. As mentioned in the QAPP in Section 5.1, the accuracy and bias of PCR-TRFLP can not be assessed for unknown complex microbial communities. The accuracy and bias for the qPCR assays is expected to fall within the ranges reported in literature.

6.2.2. Comparability

IDEXX data obtained on this project from samples taken in ankle to knee deep water at Butterfly and Hammonds beaches were compared to the appropriate weekly IDEXX data collected by Santa Barbara County Environmental Health Services (SBCEHS), and Santa Barbara Channelkeeper (SBC) for dates not tested by SBCEHS. Only data from dates that were the same date as our IDEXX samples were used for comparison purposes. For comparison with SBCEHS or SBC data from Butterfly Beach, shoreline samples from this project tagged as W (west end of Butterfly, west of MSD outfall), B (Butterfly), and BH (between Butterfly and Hammonds) were used (Figure 6.2-1). As detailed in Section 5.5., the Shoreline samples were taken at locations depending upon drifter movement for that day. Since there was only one sampling date when the drifters from the Diffuser entered the surf zone at Hammonds Beach, there is only one shoreline sample from this project that was taken at Hammonds Beach (3/31/08). For comparison with the SBCEHS data from Hammonds Beach, two shoreline samples from this project that were tagged as BH were also included (1/8/08 and 4/28/08) (Figure 6.2-2), since they were on the very eastern edge of the point between the two beaches, and therefore the closest to Hammonds. Therefore, these two dates (1/8/08 and 4/28/08) were compared twice, once against SBCEHS/SBC Butterfly Beach results (Tables 6.2-1 through 6.2-3) and then against the SBCEHS Hammonds Beach results (Tables 6.2-4 through 6.2-6). There was no IDEXX data available from either SBCEHS or SBC from 1/2/08, 10/13/08, and 10/27/08. There were no exact date matches for 2/5/08, 2/20/08, 2/27/08, 3/18/08, 5/28/08, and 8/25/08.

Total coliform results between this project and SBCEHS/SBC were generally similar, within the 95% confidence limits, and in the same order of magnitude. There were five dates with large differences between the total coliform data sets for Butterfly Beach, 1/8/08, 4/28/08, 9/15/08, 11/3/08, and 1/10/08 (Table 6.2-1), and one for Hammonds Beach (1/8/08) (Table 6.2-4). However, since our samples were not taken at the same exact location and time as the SBCEHS/SBC samples, these differences do not necessarily indicate a problem with our results.

The *E.coli*/fecal coliform and *Enterococcus* spp. results between this project and the SBCEHS/SBC samples were nearly identical once the 95% confidence limits were taken into account (Tables 6.2-2, 6.2-3, 6.2-5, 6.2-6), with one exception (*Enteroccocus* spp. on 11/10/08; Table 6.2-3). While no AB411 single sample exceedances were detected in any of the UCSB samples during the project period, there was an exceedance event for *Enterococcus* spp. on 11/10/08 according to the SBC data (Table 6.2-3).

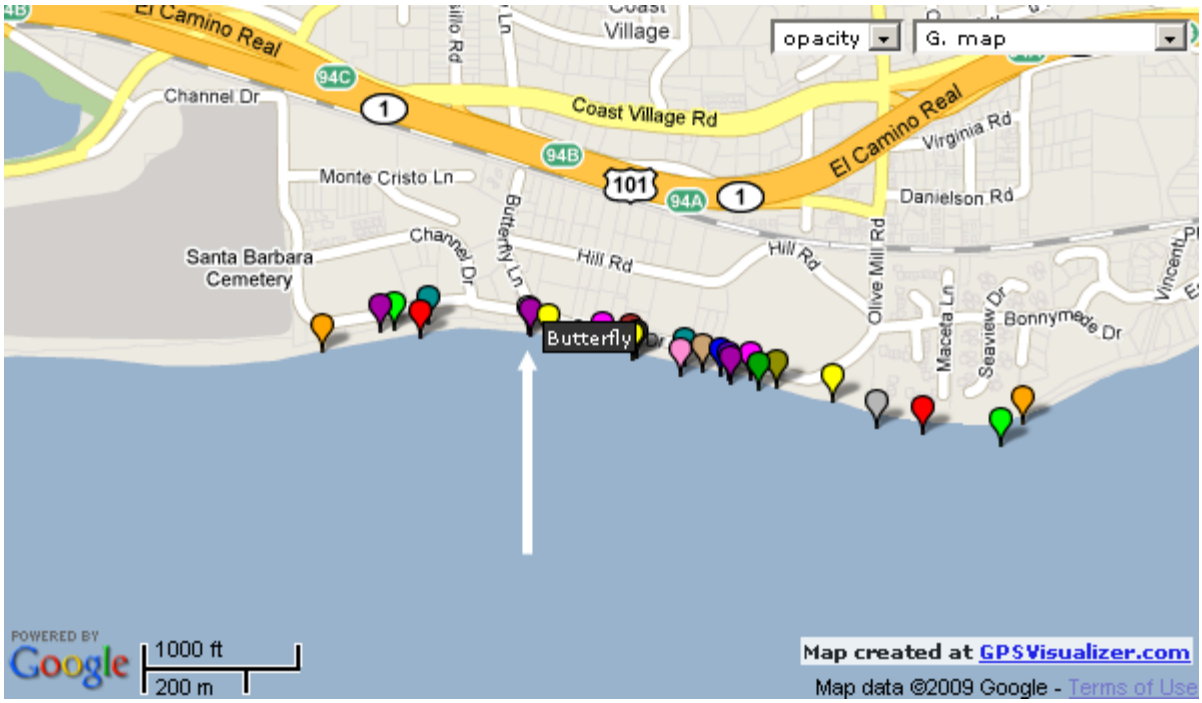


Figure 6.2-1. Santa Barbara County Environmental Health Services (SBCEHS) Butterfly Beach sampling location (white balloon just behind purple balloon, position denoted by white arrow) and the 25 shoreline samples taken in the vicinity of Butterfly for this project (colored balloons). For comparison purposes, shoreline samples tagged with W (west end of Butterfly, west of MSD outfall), B (Butterfly), and BH (between Butterfly and Hammonds) were included.

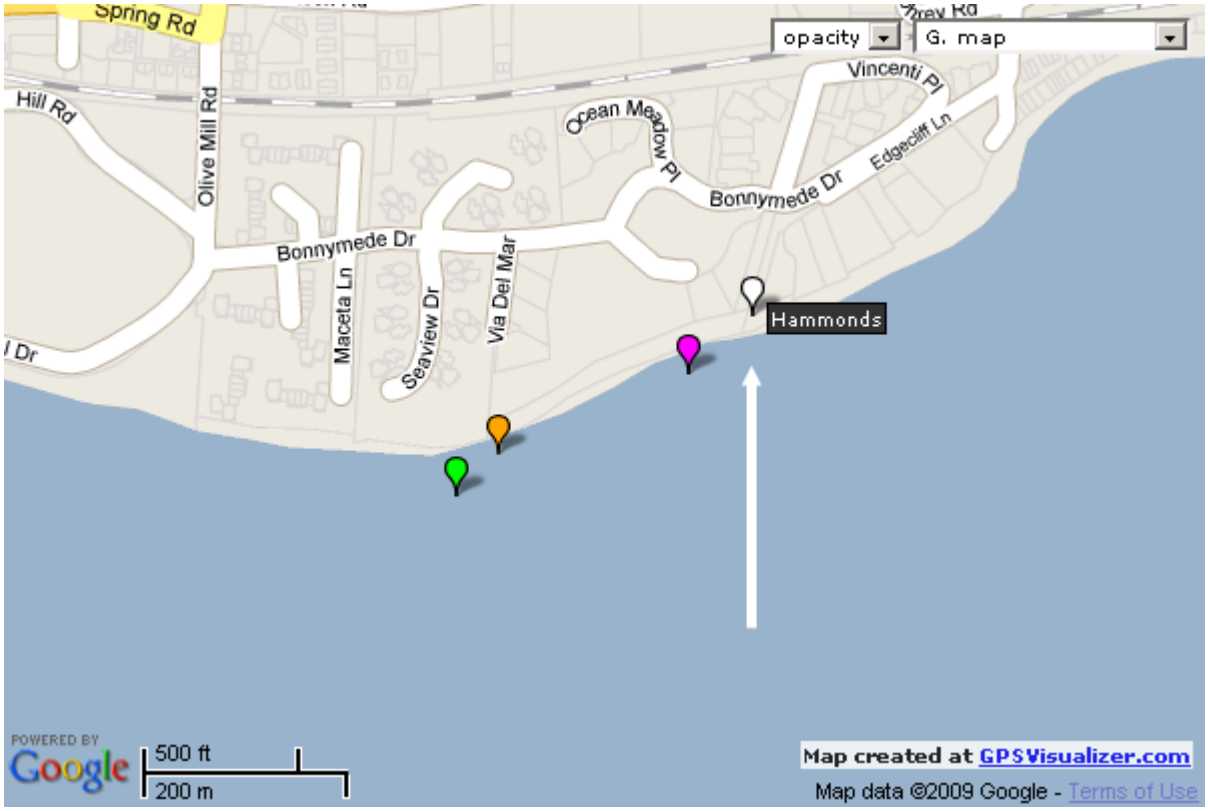


Figure 6.2-2. Santa Barbara County Environmental Health Services (SBCEHS) Hammonds Beach sampling location (white balloon) and the 3 shoreline samples taken in the vicinity of Hammonds for this project (colored balloons). Only one shoreline sample was taken at Hammonds Beach (3/31/08, purple balloon). For comparison purposes, two shoreline samples from this project that were tagged as BH (between Butterfly and Hammonds) were also included (1/8/08, green balloon; 4/28/08, orange balloon) since they were on the very eastern edge of the point between the two beaches, and therefore the closest to Hammonds.

Table 6.2-1. Total coliform via IDEXX results for Butterfly Beach from this project (UCSB) and samples from Santa Barbara County Environmental Health Services (SBCEHS) or Santa Barbara Channelkeeper (SBC)*. Values are MPN/100 mL. UCSB sample locations tagged with W (west end of Butterfly, west of MSD outfall), B (Butterfly), and BH (between Butterfly and Hammonds) were included for comparison against the Butterfly Beach SBCEHS/SBC sampling location. No samples exceeded the AB411 single sample criteria for total coliform (> 10,000 MPN/100 mL).

Date	UCSB ID	UCSB	SBCEHS/SBC
11/26/2007	H1126-5BH	63.2	134
12/3/2007	H1203-5B	20.1	< 10
12/10/2007	H1210-5W	86	96
12/17/2007	H1217-5BH	62.6	< 10
1/8/2008	H0108-5BH	488.2	95
1/14/2008	H0114-5B*	121.1	74.0
2/11/2008	H0211-5B	109.2	73
3/10/2008	H0310-5W	< 10	< 10
3/24/2008	H0324-5B	69.8	41
4/14/2008	H0414-5W	< 10	< 10
4/21/2008	H0421-5B	< 10	10
4/28/2008	H0428-5BH	104	20
5/5/2008	H0505-5B	10	31
5/12/2008	H0512-5BH	< 10	< 10
5/19/2008	H0519-5W	< 10	30
6/30/2008	H0630-5B	10	< 10
7/14/2008	H0714-5B	36.6	10
7/21/2008	H0721-5B	41.3	10
7/28/2008	H0728-5B	30.6	52
8/4/2008	H0804-5B	90.3	52
8/18/2008	H0818-5B	68.4	63
9/15/2008	H0915-5W	137.9	20
9/29/2008	H0929-5B	61.5	41
11/3/2008	H1103-5B	255.9	1331*
11/10/2008	H1110-5B	30.6	246*

Table 6.2-2. *E. coli*/fecal coliform via IDEXX results for Butterfly Beach from this project (UCSB) and samples from Santa Barbara County Environmental Health Services (SBCEHS) or Santa Barbara Channelkeeper (SBC)*. Values are MPN/100 mL. UCSB sample locations tagged with W (west end of Butterfly, west of MSD outfall), B (Butterfly), and BH (between Butterfly and Hammonds) were included for comparison against the Butterfly Beach SBCEHS/SBC sampling location. No samples exceeded the AB411 single sample criteria for *E. coli*/fecal coliform (> 400 MPN/100 mL).

Date	UCSB ID	UCSB	SBCEHS/SBC
11/26/2007	H1126-5BH	20.2	10
12/3/2007	H1203-5B	20.1	< 10
12/10/2007	H1210-5W	10	10
12/17/2007	H1217-5BH	10	< 10
1/8/2008	H0108-5BH	10	< 10
1/14/2008	H0114-5B*	< 10	10.0
2/11/2008	H0211-5B	< 10	10
3/10/2008	H0310-5W	< 10	< 10
3/24/2008	H0324-5B	< 10	10
4/14/2008	H0414-5W	< 10	< 10
4/21/2008	H0421-5B	10	< 10
4/28/2008	H0428-5BH	< 10	< 10
5/5/2008	H0505-5B	< 10	10
5/12/2008	H0512-5BH	< 10	< 10
5/19/2008	H0519-5W	< 10	< 10
6/30/2008	H0630-5B	< 10	< 10
7/14/2008	H0714-5B	< 10	< 10
7/21/2008	H0721-5B	< 10	< 10
7/28/2008	H0728-5B	10	10
8/4/2008	H0804-5B	< 10	10
8/18/2008	H0818-5B	30.6	< 10
9/15/2008	H0915-5W	< 10	20
9/29/2008	H0929-5B	< 10	20
11/3/2008	H1103-5B	< 10	41*
11/10/2008	H1110-5B	30.6	97*

Table 6.2-3. Enterococci via IDEXX results for Butterfly Beach from this project (UCSB) and samples from Santa Barbara County Environmental Health Services (SBCEHS) or Santa Barbara Channelkeeper (SBC)*. Values are MPN/100 mL. UCSB sample locations tagged with W (west end of Butterfly, west of MSD outfall), B (Butterfly), and BH (between Butterfly and Hammonds) were included for comparison against the Butterfly Beach SBCEHS/SBC sampling location. Red color indicates AB411 single sample exceedance (> 104 MPN/100 mL).

Date	UCSB ID	UCSB	SBCEHS/SBC
11/26/2007	H1126-5BH	30.6	10
12/3/2007	H1203-5B	< 10	< 10
12/10/2007	H1210-5W	< 10	20
12/17/2007	H1217-5BH	20.2	< 10
1/8/2008	H0108-5BH	20.2	< 10
1/14/2008	H0114-5B*	< 10	< 10
2/11/2008	H0211-5B	< 10	20
3/10/2008	H0310-5W	< 10	20
3/24/2008	H0324-5B	< 10	10
4/14/2008	H0414-5W	10	< 10
4/21/2008	H0421-5B	< 10	< 10
4/28/2008	H0428-5BH	20.2	< 10
5/5/2008	H0505-5B	< 10	< 10
5/12/2008	H0512-5BH	10	< 10
5/19/2008	H0519-5W	< 10	10
6/30/2008	H0630-5B	< 10	< 10
7/14/2008	H0714-5B	< 10	< 10
7/21/2008	H0721-5B	< 10	< 10
7/28/2008	H0728-5B	< 10	< 10
8/4/2008	H0804-5B	< 10	< 10
8/18/2008	H0818-5B	9.9	10
9/15/2008	H0915-5W	< 10	< 10
9/29/2008	H0929-5B	< 10	10
11/3/2008	H1103-5B	10	< 10*
11/10/2008	H1110-5B	< 10	183*

Table 6.2-4. Total coliform via IDEXX results for Hammonds Beach from this project (UCSB) and samples from Santa Barbara County Environmental Health Services (SBCEHS). Values are MPN/100 mL. UCSB sample locations tagged with H (Hammonds), and the two BH (between Butterfly and Hammonds) samples closest to Hammonds were included for comparison against the Hammonds Beach SBCEHS sampling location. No samples exceeded the AB411 single sample criteria for total coliform (> 10,000 MPN/100 mL).

Date	UCSB ID	UCSB	SBCEHS
1/8/2008	H0108-5BH	488.2	1178
3/31/2008	H0331-5H	10	< 10
4/28/2008	H0428-5BH	104	< 10

Table 6.2-5. *E. coli*/fecal coliform via IDEXX results for Hammonds Beach from this project (UCSB) and samples from Santa Barbara County Environmental Health Services (SBCEHS). Values are MPN/100 mL. UCSB sample locations tagged with H (Hammonds), and the two BH (between Butterfly and Hammonds) samples closest to Hammonds were included for comparison against the Hammonds Beach SBCEHS sampling location. No samples exceeded the AB411 single sample criteria for *E. coli*/fecal coliform (> 400 MPN/100 mL).

Date	UCSB ID	UCSB	SBCEHS
1/8/2008	H0108-5BH	10	20
3/31/2008	H0331-5H	9.9	< 10
4/28/2008	H0428-5BH	< 10	< 10

Table 6.2-6. Enterococci via IDEXX results for Hammonds Beach from this project (UCSB) and samples from Santa Barbara County Environmental Health Services (SBCEHS). Values are MPN/100 mL. UCSB sample locations tagged with H (Hammonds), and the two BH (between Butterfly and Hammonds) samples closest to Hammonds were included for comparison against the Hammonds Beach SBCEHS sampling location. No samples exceeded the AB411 single sample criteria for enterococci (> 104 MPN/100 mL).

Date	UCSB ID	UCSB	SBCEHS
1/8/2008	H0108-5BH	20.2	20
3/31/2008	H0331-5H	10	< 10
4/28/2008	H0428-5BH	20.2	< 10

Montecito Sanitary District (MSD) performs total coliform and *E. coli* via IDEXX analyses on a daily basis for treatment plant process control only. For RWQCB/NPDES reporting, MSD prepares samples for total coliform and fecal colifom via multi-tube fermentation (MTF) three times a week (Monday, Wednesday, Friday).

MTF data was not available for four of our project sampling dates (1/8/08, 1/22/08, 2/5/08, 3/18/08), as these dates were Tuesdays.

Total coliform IDEXX results between our samples and MSD's IDEXX and MTF samples for more than half of the sampling events were similar, within the 95% confidence limits, and in the same order of magnitude. There were ten dates with large differences between the data sets, 1/8/08, 1/14/08, 4/28/08, 5/12/08, 7/21/08, 8/4/08, 8/11/08, 9/29/08, 10/27/08, and 11/3/08 (Table 6.2-4). However, even though our samples were grabbed around the same time from the effluent channel, they were not split samples. MSD personnel used a grab sample approach and directly placed their sample bottles into the effluent stream. Since we needed such a large quantity of effluent to be able to extract enough DNA for our analyses, we were not able to use the same approach. MSD personnel filled our 3 x 2L bottles by dipping a sterile 2 L bottle into the effluent channel and pouring approximately 1/3 volume into each of the three sample bottles. It took approximately 5 minutes to completely fill all three sample bottles. In effect, our samples were composite samples of the effluent composition over the time duration it took to fill them, so exact results between our two data sets would not necessarily be expected.

The *E.coli*/fecal coliform IDEXX results between our samples and MSD's IDEXX and MTF were nearly identical once the 95% confidence limits were taken into account (Tables 6.2-5).

It should be noted the MTF results are what MSD must meet for its NPDES discharge permit, and that they run IDEXX for internal purposes. During the project period, there were no violations of MSD's NPDES permit requirements.

Table 6.2-4. Total coliform via IDEXX results from this project (UCSB) and via IDEXX and multi-tube fermentation from Montecito Sanitary District. Values are MPN/100 mL.

Date	UCSB IDEXX (MPN/100 mL)	MSD - IDEXX (MPN/100 mL)	MSD - Multi-tube fermentation (MTF) (MPN/100 mL)
11/26/2007	5.2	12	8
12/3/2007	1	1	2
12/10/2007	1	3.1	4
12/17/2007	2	< 1	2
1/2/2008	3.1	4.1	2
1/8/2008	54.6	1	n/a
1/14/2008	93.3	1	8
1/22/2008	1	2	n/a
1/28/2008	7.5	47.1	30
2/5/2008	1	4.1	n/a
2/11/2008	5.2	1	4
2/20/2008	5.2	13.5	8
2/27/2008	1	47.1	4
3/3/2008	13.4	40.5	2

3/10/2008	12.1	3.1	7
3/18/2008	24.7	1	n/a
3/24/2008	7.4	3.1	4
3/31/2008	12.1	17.5	50
4/7/2008	22.8	25.9	50
4/14/2008	62	12.1	4
4/21/2008	2	< 1	7
4/28/2008	325.5	17.5	11
5/5/2008	26.5	13.4	23
5/12/2008	125.7	233	8
5/19/2008	2.1	3	4
5/23/2008	< 1	3.1	2
5/28/2008	21.3	15.8	17
6/2/2008	14.5	13.4	2
6/9/2008	7.4	9.8	4
6/16/2008	3.1	24	4
6/23/2008	4.1	4.1	2
6/30/2008	3	2	< 2
7/7/2008	1	9.7	2
7/14/2008	4.1	3.1	< 2
7/21/2008	555	5.2	4
7/28/2008	11.6	4.1	< 2
8/4/2008	90.3	< 1	2
8/11/2008	18.1	98.8	50
8/18/2008	3.1	3.1	2
8/25/2008	27.2	2	2
9/8/2008	2	3.1	< 2
9/15/2008	4.1	1	4
9/22/2008	21.8	41.4	4
9/29/2008	138.9	1	8
10/6/2008	17.3	1	4
10/13/2008	4.1	68.3	2
10/20/2008	3.1	39.5	< 2
10/27/2008	387.3	4.1	< 2
11/3/2008	98.7	19.9	4
11/10/2008	12.2	28.8	< 2
11/17/2008	6.3	9.8	8

Table 6.2-5. *E. coli*/fecal coliform via IDEXX results from this project (UCSB) and via IDEXX and multi-tube fermentation from Montecito Sanitary District. Values are MPN/100 mL.

	UCSB IDEXX	**MSD - IDEXX**	**MSD - Multi-tube fermentation (MTF)**
Date	**(MPN/100 mL)**	**(MPN/100 mL)**	**FC (MPN/100 mL)**
11/26/2007	< 1.0	< 1	2
12/3/2007	< 1.0	< 1	2
12/10/2007	< 1.0	< 1	2
12/17/2007	1	< 1	2
1/2/2008	< 1.0	1	2
1/8/2008	1	< 1	n/a
1/14/2008	< 1.0	< 1	2

1/22/2008	< 1.0	< 1	n/a
1/28/2008	< 1.0	7.5	4
2/5/2008	1	< 1	n/a
2/11/2008	1	< 1	2
2/20/2008	< 1.0	1	2
2/27/2008	1	6.2	2
3/3/2008	1	4.1	< 2
3/10/2008	2	< 1	< 2
3/18/2008	< 1.0	< 1	n/a
3/24/2008	< 1.0	< 1	< 2
3/31/2008	< 1.0	< 1	4
4/7/2008	3.1	6.3	4
4/14/2008	1	3	< 2
4/21/2008	< 1.0	< 1	2
4/28/2008	1	4.1	< 2
5/5/2008	5.2	< 1	< 2
5/12/2008	< 1	< 1	2
5/19/2008	< 1	< 1	2
5/23/2008	< 1	< 1	< 2
5/28/2008	< 1	3.1	2
6/2/2008	1	2	< 2
6/9/2008	< 1.0	< 1	< 2
6/16/2008	< 1.0	1	< 2
6/23/2008	< 1	< 1	< 2
6/30/2008	< 1	< 1	< 2
7/7/2008	< 1.0	< 1	< 2
7/14/2008	1	< 1	< 2
7/21/2008	< 1	< 1	2
7/28/2008	< 1	1	< 2
8/4/2008	< 1	< 1	< 2
8/11/2008	< 1.0	1	2
8/18/2008	< 1.0	< 1	< 2
8/25/2008	< 1.0	< 1	< 2
9/8/2008	< 1.0	1	< 2
9/15/2008	1	< 1	< 2
9/22/2008	< 1.0	< 1	4
9/29/2008	1.5	< 1	< 2
10/6/2008	3.1	< 1	2
10/13/2008	< 1.0	3.1	2
10/20/2008	< 1.0	< 1	< 2
10/27/2008	< 1.0	1	< 2
11/3/2008	< 1.0	< 1	< 2
11/10/2008	< 1.0	1	< 2
11/17/2008	< 1.0	< 1	< 2

6.2.3. Completeness

Fifty-two weekly sampling events were planned for this study. Sampling was planned to occur on Mondays, and was moved to Tuesdays or Wednesdays as needed due to weather or resource issues. Due to holiday schedules and resource availability, two dates were unable to be sampled,

12/24/07 – 12/26/07 and 9/1/08 – 9/3/08. This results in 96% completeness, which is above the stated minimum expectation in Section 5.3 of the QAPP (90%).

Completeness was expected to be at least 90% for the microbiological analyses stated (IDEXX (total coliform, *E. coli*, enterococci), 16S-PCR TRFLP, human-specific *Bacteroides* qPCR, and *Enterococcus* spp. qPCR). All samples selected for DNA-based analyses were able to be successfully analyzed within the quality control guidelines for this project as stated (Table 6-1), yielding a 100% completeness rate for those samples analyzed.

6.2.4. Precision

For this project, an explicit precision study encompassing all the methods together was not performed. Instead, a more robust approach was used, assessing precision on a method by method basis to ensure measurements were within the criteria specified in Table 6-1.

For IDEXX methods, all sample duplicates were within the 95% confidence limit ranges of each other. The method precision was also assessed for each sample duplicate pair, as specified in section 9020B of Standard Methods (Table 6.2-6). To briefly summarize, for each sample matrix (ocean and treated WWTP effluent) , the first 15 positive sample duplicate pairs separately for each assay (total coliform, *E. coli*, enterococci) were first log transformed. The range (R) for each sample duplicate pair was then calculated by subtracting the log transformed data for each duplicate sample from its pair. The mean R was calculated by averaging the R for all of the first 15 positive samples for that assay and matrix combination. The precision criterion was determined by multiplying the mean R by 3.27. The R was then calculated for each sample pair throughout the project, and compared against the precision criterion for that matrix and assay combination. If a sample pair R fell within the calculated precision criterion, the level of precision was acceptable and not further action warranted. If a sample pair R fell outside of the calculated precision criterion, the level of precision was unacceptable and the following sample pairs were examined to ensure that analyst variability was not excessive.

This analysis was only able to be performed on the total coliform data on ocean samples, as the *E. coli* and enterococci results and any of the effluent duplicates did not have the 15 positive results needed to calculate the mean R.

For total coliform in ocean samples, the mean R = 0.182, and when multiplied by the 3.27 range value, the precision criterion was 0.594. Only one date had an R outside of the acceptable range (7/14/08). Since all of the sample duplicate pairs after this date had R values within the calculated precision criterion, no corrective action was required.

Table 6.2-6. Daily checks on precision of duplicate total coliform results. Precision criteria = (3.27*mean R) = 0.594. A = acceptable, U = unacceptable.

	Duplicate Analyses		Logarithims of Counts		Range of Logarithms	Acceptance of
	D_1	D_2	L_1	L_2	(R_{log})	Range
11/26/2007	30.4	30.6	1.483	1.486	0.003	A
12/3/2007	10	< 10.0	1.000	n/a	n/a	
12/17/2007	9.9	< 10.0	0.996	n/a	n/a	
1/2/2008	97.9	52.1	1.991	1.717	0.274	A
1/8/2008	30.4	40.9	1.483	1.612	0.129	A
1/14/2008	20.2	41.3	1.305	1.616	0.311	A
1/22/2008	171.2	171.2	2.234	2.234	0.000	A
1/28/2008	215.7	230.7	2.334	2.363	0.029	A
2/5/2008	40.9	40.9	1.612	1.612	0.000	A
2/11/2008	122.3	96	2.087	1.982	0.105	A
2/20/2008	354.5	383.9	2.550	2.584	0.035	A
2/27/2008	62.6	97.9	1.797	1.991	0.194	A
3/3/2008	52.1	10	1.717	1.000	0.717	A
3/10/2008	< 10.0	< 10.0	n/a	n/a	n/a	
3/18/2008	< 10.0	< 10.0	n/a	n/a	n/a	
3/24/2008	108.9	30.6	2.037	1.486	0.551	A
3/31/2008	9.9	10	0.996	1.000	0.004	A
4/7/2008	122.3	51.6	2.087	1.713	0.375	A
4/14/2008	10	10	1.000	1.000	0.000	A
4/21/2008	< 10.0	10	n/a	1.000	n/a	
4/28/2008	110	97.9	2.041	1.991	0.051	A
5/5/2008	< 10.0	10	n/a	1.000	n/a	
5/28/2008	30.6	20.2	1.486	1.305	0.180	A
6/2/2008	< 10	< 10	n/a	n/a	n/a	
6/9/2008	10	30.6	1.000	1.486	0.486	A
6/16/2008	< 10.0	10	n/a	1.000	n/a	
6/23/2008	30.6	30.6	1.486	1.486	0.000	A
6/30/2008	10	< 10.0	1.000	n/a	n/a	
7/7/2008	41.3	52.1	1.616	1.717	0.101	A
7/14/2008	10	63.2	1.000	1.801	0.801	U
8/4/2008	83.6	96.9	1.922	1.986	0.064	A
8/11/2008	74.5	41.3	1.872	1.616	0.256	A
8/18/2008	51.6	85.2	1.713	1.930	0.218	A
8/25/2008	128.4	94.2	2.109	1.974	0.135	A
9/8/2008	72.4	51.6	1.860	1.713	0.147	A
9/15/2008	41.3	30.4	1.616	1.483	0.133	A
9/22/2008	73.8	62.6	1.868	1.797	0.071	A
10/6/2008	184.9	228.1	2.267	2.358	0.091	A
10/13/2008	10	< 10	1.000	n/a	n/a	
10/20/2008	< 10	10	n/a	1.000	n/a	
10/27/2008	< 10	10	n/a	1.000	n/a	
11/3/2008	196.7	189	2.294	2.276	0.017	A
11/10/2008	61.5	30.6	1.789	1.486	0.303	A
11/17/2008	20.2	10	1.305	1.000	0.305	A

Precision for PCR-TRFLP was evaluated with the use of a reference sample (DNA extracted from an activated sludge sample from MSD) on each batch of PCR performed (10/07, 1/08, 7/08, 9/08, 12/08). The reference sample DNA was amplified fresh with each PCR performed, restricted along with the other samples in the batch, and sent to MSU for analysis. The results were then aligned with all samples on this project, and analyzed via MDS (Figure 6.2-1). The reference sample from each of the 5 batches grouped with each other on the MDS plot, indicating the nearly identical similarity in their bacterial communities.

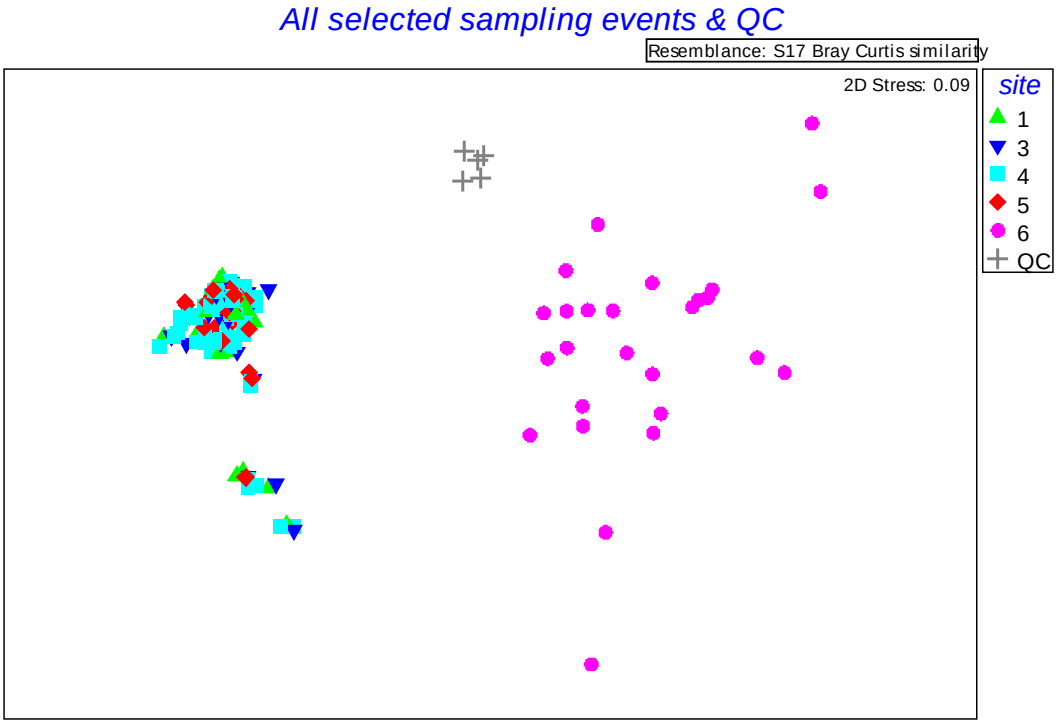


Figure 6.2.-1. MDS plot of all samples analyzed on this project, with the inclusion of the five reference samples (gray crosses on plot) that were included with every batch of samples amplified, restricted and analyzed (stress = 0.09).

As specified, human-specific *Bacteroides* qPCR (HBM qPCR) results were adjusted so that the coefficient of variation (%CV) for each of the standards was 3% or less between plates (Table 6-1).

In order to be more consistent across methods, *Enterococcus* spp. qPCR (ENT qPCR) results were not evaluated as specified Table 6-1, but instead in a method similar to the HBM qPCR results. When analyzing the ENT qPCR data, a baseline threshold of 200 was used for every plate run. To assess precision, the %CV was calculated for each standard used on every plate. The %CV for each standard was within our determined threshold of 3.5% or less for this assay.

6.2.5. Representativeness

All water samples were maintained cold (wet ice) in the field and during transport. Hold time for IDEXX analysis and sample filtration was no longer than 6 hours after sampling.

6.2.6. Method Detection Limit

No issues with method detection limit occurred, except for the PCR-TRFLP data. Table 6-1, (taken from the QAPP), stated the detection limit as a minimum of 10ng/uL of extracted DNA. The effluent samples yielded DNA values below this threshold on 22 of the 26 sampling events selected for DNA-based analyses. Attempts were made to amplify all effluent samples, including those below this threshold. All samples were able to be successfully amplified, and all electropherograms were inspected to ensure satisfactory data was generated from these low DNA yield samples. Therefore, the stated value of 10 ng/uL as a detection limit was not applicable to this study.

6.2.7. Existing Data

IDEXX data from MSD, SBEHSD, and SBC were deemed acceptable and used for quality control comparative purposes.

7. Results

7.1. Oceanographic Results

7.1.1. Drifter Trajectories

Drifter trajectories are the observed quantities from which subsequent statistical analyses of water movements are derived (Figure 7.1-1). A total of 774 drifter trajectories were obtained; these were distributed across the three stations as follows: 474 originated at the Diffuser, 151 originated at the Offshore500 site, and 149 originated at the Offshore1000 site. Drifter observations indicate alongshore movement eastward and westward away from the diffuser. These observations also indicate onshore movement away from the diffuser. Consistent offshore drifter motion was only recorded on a few days as indicated by the tracks extending offshore in Figures 7.1-1 a and b. In addition to measuring ocean currents, drifters also measure cross-shore movement resulting from waves called Stokes drift. Stokes drift moves water parcels in the

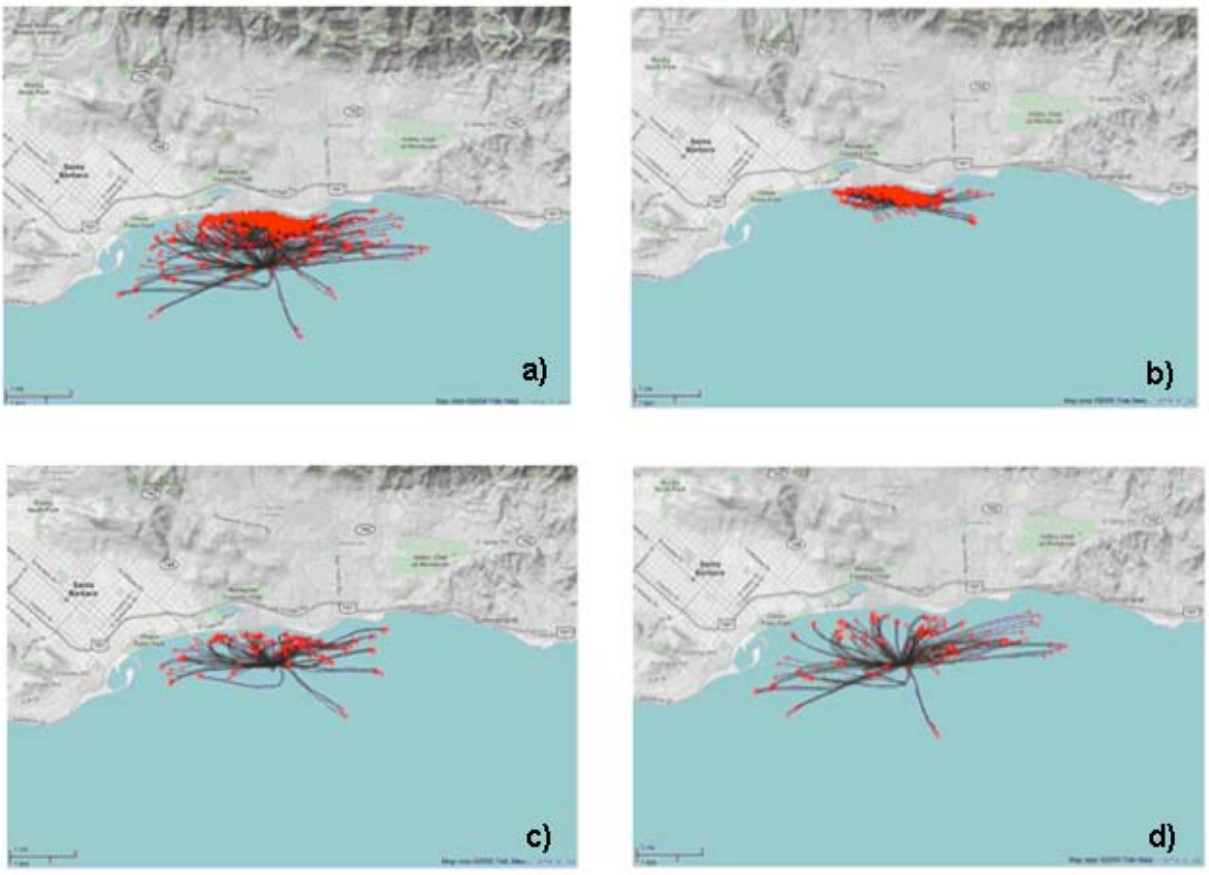


Figure 7.1-1. Trajectories of: (a) all 774 drifters; (b) drifters deployed at the diffuser; (c) drifters deployed 500 m offshore of the diffuser; and (d) drifters deployed 1000 m offshore of the diffuser. Red plus symbols indicate ending positions.

direction of wave propagation at the sea surface and offshore at depth (Hasselmann, 1973). The observed flow patterns are consistent with the regional circulation forced primarily by local winds and a larger scale pressure gradient between the Southern California Bight and the central California coast north of Pt. Conception (Harms and Winant 1998, Melton et al., 2009, Winant et al., 2003). The general onshore motion is consistent with the local sea breeze which typically blows onshore during the morning and mid-day when drifters were deployed. Some of the onshore movement near shore may also have resulted from Stokes drift.

Drifters deployed at the Offshore500 and Offshore1000 stations generally traveled greater distances than drifters deployed at the Diffuser station (Figure 7.1-1). This is partly due to the fact that drifters released near the diffuser often quickly reached a water depth of ~3 m and were recovered before entering the surf zone. Drifters released farther offshore at 500 m and 1000 m on average recorded data for longer periods. Surface currents typically exhibit increased speed with distance from shore in deeper water, consistent with other observations and theory of coastal ocean circulation.

7.1.2. Drifter Distributions

Time dependent drifter displacements are derived from individual trajectories to give statistical information about water parcel movements away from a given location. Discrete probability density functions (pdfs) are estimated by sorting locations of drifters into 100 m by 100 m spatial bins as they move over the sea surface during specified time intervals. For the graphs presented below these time intervals are 1, 2, and 3 hours. The number of drifters passing through each bin is summed and then divided by the total number of observed trajectories. The displacement pdf for drifters released at the diffuser during a 1 hr time interval after deployement indicates drifter movements are confined to a region extending 1 km east and west of the diffuser and from 0.3 km inshore of the diffuser to 0.2 km offshore of the diffuser (Figure 7.1-2). After 1 hr, the 100x100 m bin in which drifters are most likley to land (7% of the time) lies northeast of the diffuser (red square, Figure 7.1-2). Displacement pdfs after 1 hr for drifters deployed at the Offshore500 and Offshore1000 stations are similar in spatial extent to the diffuser case, but the Offshore1000 pdf shows the largest cross-shore displacements. This is consistent with the site being farthest from the shore.

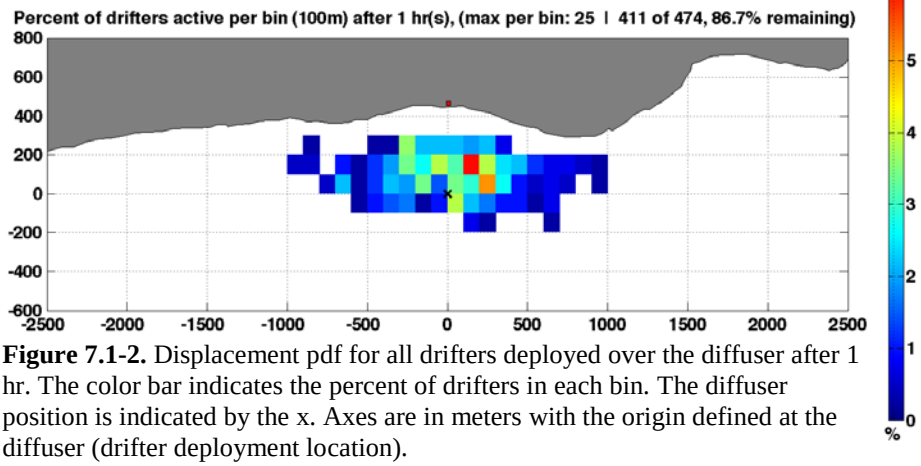


Figure 7.1-2. Displacement pdf for all drifters deployed over the diffuser after 1 hr. The color bar indicates the percent of drifters in each bin. The diffuser position is indicated by the x. Axes are in meters with the origin defined at the diffuser (drifter deployment location).

The displacement pdf for drifters released at the Diffuser staton after 2 hr shows only a slightly expanded range distribution compared with the 1 hr pattern of Figure 7.1-2. Drifters released at the diffuser moved no farther than 1.3 km alongshore (most moved less than 1 km) and up to 0.4 km cross-shore. These drifters mostly ended up in two general regions: east and slightly north of the diffuser, and northwest of the diffuser. The ending distribution to the northwest is has more drifters compared with the 1 hour distributions of Figure 7.1-2. Displacement pdfs for drifters after 2 hours from the Offshore 500 and Offshore1000 sites were greatly expanded compared with the displacements from the Diffuser. Offshore1000 drifter displacements extended up to 1.8 km in the alongshore, nearly twice the 1 hr displacements. Both the Offshore500 and Offshore1000 drifters show greater westward displacements; eastward displacements appear better correlated with onshore movement (i.e. northeastward flow) than westward displacements. This may have resulted from trends in bottom depth contours.

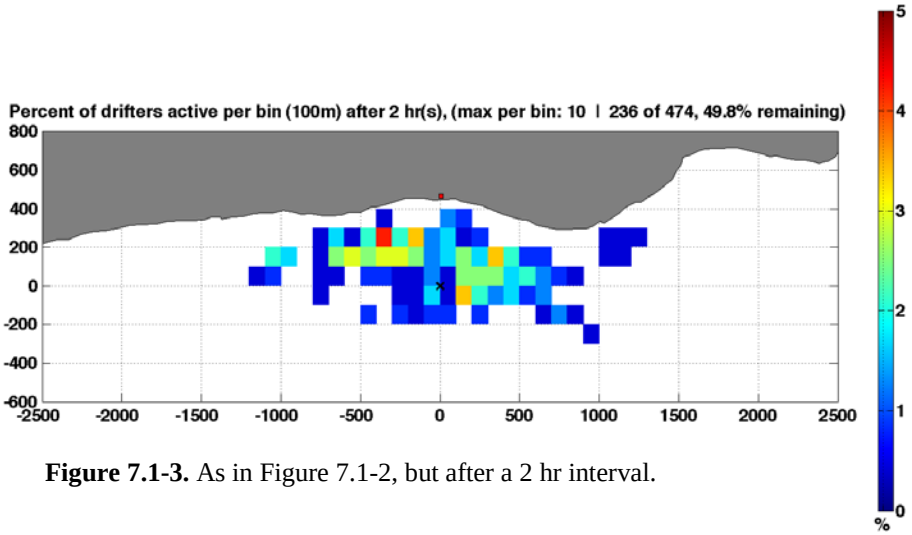


Figure 7.1-3. As in Figure 7.1-2, but after a 2 hr interval.

The displacement pdf for drifters released at the diffuser after 3 hr shows alongshore movement to 1.5 km east and west up to 0.4 km cross-shore (Figure 7.1-4). Elevated numbers of drifters occurred in three main areas after 3 hr: about 0.5 km northwest of the diffuser, 0.2- 0.3 km north (mostly directly onshore) of the diffuser, and along the very nearshore region east of the diffuser. It is interesting that nearly 10% of drifters ended up only a few hundred meters directly inshore of the diffuser after 3 hr, compared with roughly half that number for intervals of 1 and 2 hr. Additional analyses accounting for days when multiple drifters might move similarly and thus significantly elevate counts should be accounted for in subsequent analyses. Displacement pdfs for drifters traveling for 3 hr intervals from the

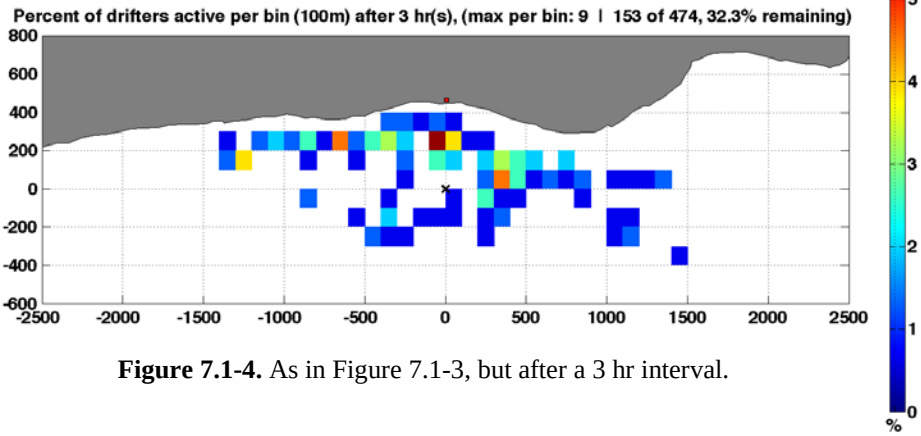


Figure 7.1-4. As in Figure 7.1-3, but after a 3 hr interval.

offshore500 and offshore1000 stations show alongshore excursions to almost 3 km, nearly twice the maximum alongshore distance traveled by drifters released at the diffuser.

The displacement pdf for diffuser drifters after 4 hr (Figure not shown here; See Appendix) indicates nearly 25% of the drifters ended in a region ~0.5 km to the northwest of the diffuser. However, only 62 drifters released at the diffuser (~13% of all drifters released at the diffuser) were still recording positions 4 hr after deployment. The others were picked up (typically over the 3 m isobath) so they did not move into the surf zone. Because of this, statistics derived from these drifters are based on relatively small sample sizes; the number of independent days represented by each bin needs further analysis. About 50% of drifters deployed at the Offshore500 site and 70% deployed at the Offshore1000 site continued recording positions after 4 hr. The discrepancy indicates that drifters deployed nearer shore were more likely to reach shallow water after 4 hr than drifters deployed farther offshore. Statistics based on drifter moving onshore to the 3 m isobath, categorized as surf zone entries, are discussed in the next section.

7.1.3. Surf Zone Entries

Drifters were always retrieved prior to moving shoreward of breaking waves. During small wave periods drifters were retrieved when they encountered water depths of ~3 m as measured by a depth sounder mounted on the research vessel. During a few large winter wave events, drifters were retrieved in even deeper waters so that the research vessel and drifters stayed offshore of the region of breaking waves. Thresholds were selected for adherence to safe boating procedures that include avoiding “swimming zones” designated in summer by Santa Barbara County lifeguards. The best estimate of “drifter surf zone entry” (hereafter SZE) for this project is thus a depth threshold that identifies drifters satisfying these criteria.

Water depths were obtained from the 3 Arc-Second Coastal Relief Model developed by the NOAA National Geophysical Data Center (Divins and Metzger, 2008). Depths from the bathymetry model were interpolated onto each drifter position for analysis. This model has a spatial resolution of roughly 75 m in the study area (~34 degrees north). Water depths from the model are referenced to mean low water and therefore account for the mixed semi-diurnal tide characteristic of the study site. Despite having the highest resolution regional bathymetry available, the model values are not the true bathymetry for three primary reasons. First, the model does not account for bathymetry variations on scales smaller than 75 m due to finite spatial resolution. Second, the values are not based on observations collected during the drifter sampling and therefore do not account for changes in the coastline and seabed. Third, the bathymetry model does not account for sea level variations caused by non-tidal processes such as upwelling, downwelling, and storm surges.

The 0.15 m contour from the NOAA bathymetry model was selected as the depth threshold for a SZE. This value is shallower than the ~3 meter depth criterion used in the field for retrieval, but it captures the set of drifters that were retrieved at the ~3 m isobath. It may also capture some drifters that did not cross the ~3 m isobath as measured by depth sounder on the research vessel. While the threshold represents a best estimate for SZE criteria, it is likely an upper bound. Shallower depth thresholds resulted in only slightly fewer SZEs. The circulation within the surf zone is very different than just beyond the surf zone, and the understanding of water exchange between the surf zone and offshore is a focus of ongoing oceanographic research. The fate of a tagged water parcel after the final drifter position is not definitively known, but surf zone entry seems likely since retrievals were typically just outside the surfzone. The Shoreline sampling

station corresponding to a particular trajectory is an estimated position based on extrapolating the last known drifter position directly shoreward to the coastline.

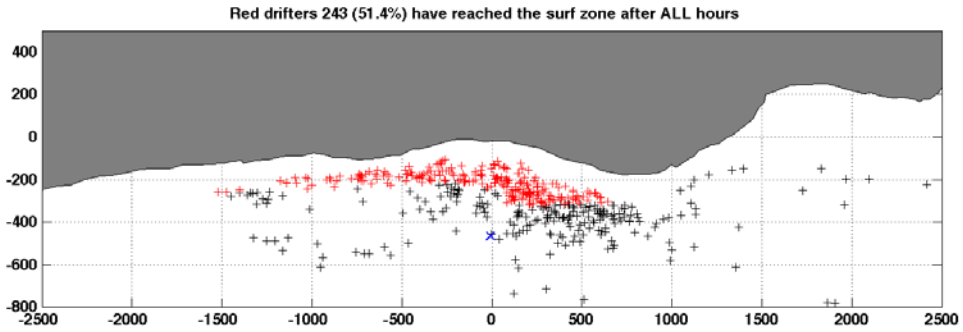


Figure 7.1-5. Ending positions of all drifters deployed at the Diffuser station. Red x's give ending positions that are inshore of the 0.15 m isobath as defined in the NOAA coastal relief model and are considered SZEs. Axes are in meters with the origin arbitrarily defined as the shoreline position of the diffuser pipe.

Drifter SZEs were determined by selecting all drifters for which the interpolated water depth (from NOAA model) at that drifter's last position was ≤ 0.15 m. Roughly 50% of all drifters deployed at the diffuser site are characterized as SZEs (Figure 7.1-5). Drifter SZEs were observed along the coast throughout a region extending from ~1500 meters westward, to ~700 meters eastward, of the diffuser location. The alongshore distribution of all SZEs was determined by binning alongshore SZE ending positions.

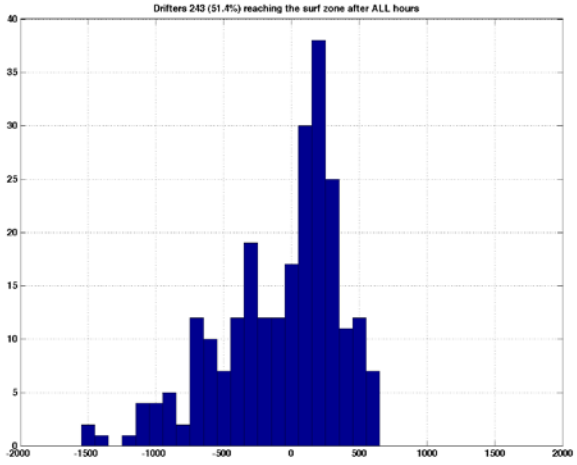


Figure 7.1-6. Probability density function of along-shore location of SZEs for all drifters deployed at the diffuser. Bins are100 m in alongshore length.

The pdf of alongshore location (Figure 7.1-6) shows roughly half of all diffuser drifter SZEs occurred west of the diffuser, and the other half to the east. West of the diffuser the SZEs occur out to ~1500 from the diffuser location and the distribution generally decays linearly with distance. Eastward of the diffuser, SZEs extend out only to ~700 meters. Roughly 40% of all drifters released at the diffuser were identified as SZEs in a region extending 400 m east of the diffuser.

Roughly 15% and 3% of drifters deployed at the offshore500 and offshore1000 stations, respectively, are identified as SZEs, considerably less than deployments at the Diffuser station.

Offshore500 SZEs are distributed similarly to the Diffuser case, with occurrences split almost evenly west and east of the diffuser with eastward occurrences spread over nearly twice the alongshore distance (Figure 7.1-7).

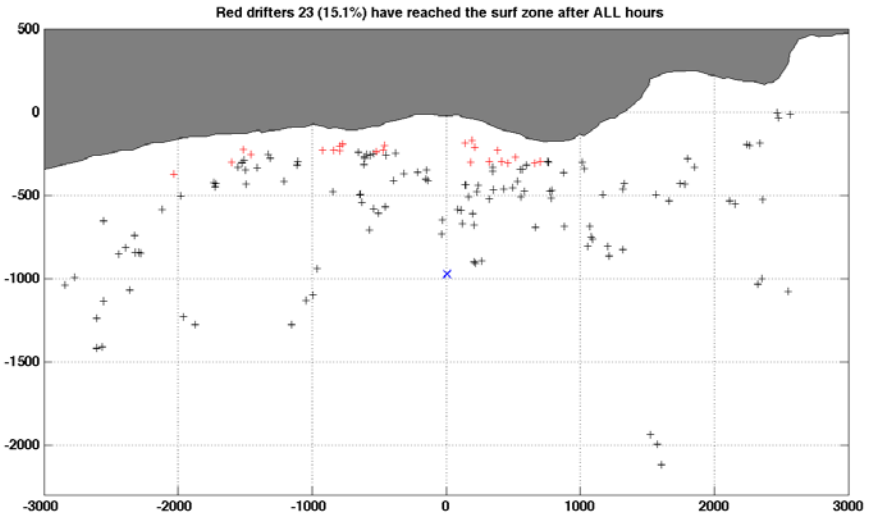


Figure 7.1-7. As in Figure 7.1-5, but for all drifters deployed at the offshore500 location.

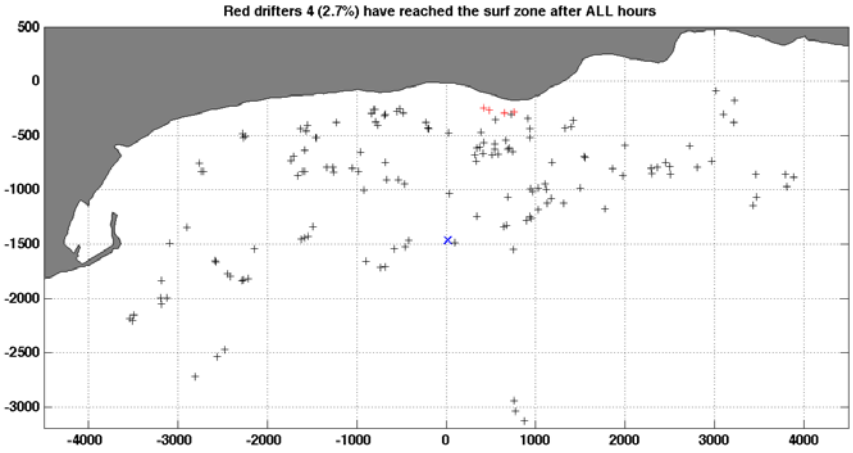


Figure 7.1-8. As in Figure 7.1-5, but for all drifters deployed at the offshore1000 location.

Offshore1000 drifter SZEs occurred near a region located ~500m eastward of the diffuser for drifters deployed only on two days (Figure 7.1-8). All Offshore500 and Offshore1000 SZEs occurred at least 2 hours after deployment. In comparison, roughly 22% of drifters released at the diffuser are flagged as SZEs within 1 hr of release.

7.1.4. Mooring Time Series

Moored currents, temperatures, and wave parameters (Section 5.2) were successfully collected for the 1 year duration of the field sampling as shown in Figure 7.1-9. To display the entire records, time series of Figures 7.1-9 a, b, and c have been low-pass filtered to remove variability with periods less than 36 hr. Figure 7.1-9a shows maximum principal axis currents (PAx U) and these are hereafter referred to as alongshore currents. Figure Figures 7.1.-9 b shows minimum principal axis currents (PAx V) hereafter referred to as cross-shore currents. Alongshore currents are typically much faster than cross-shore currents.

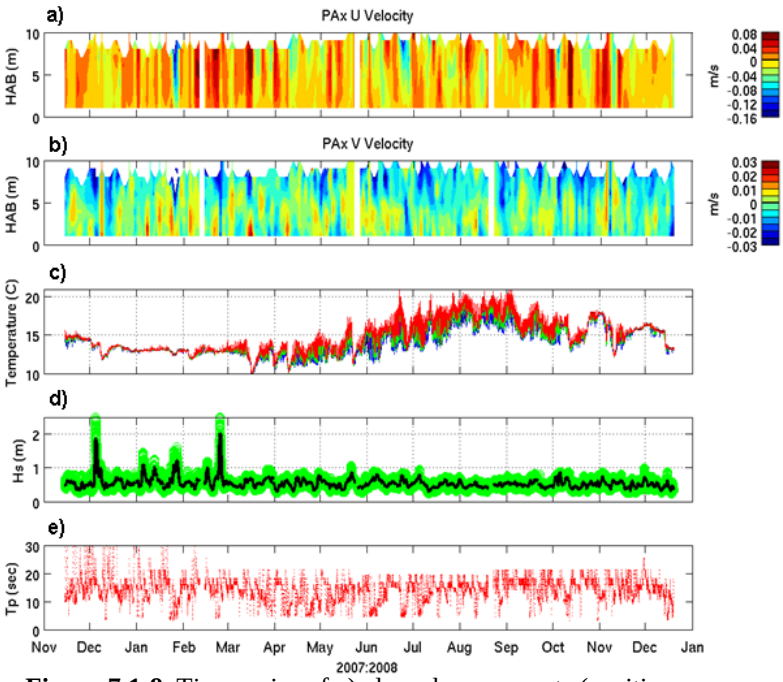


Figure 7.1-9. Time series of a) alongshore currents (positive eastward); b) cross-shore currents (positive northward); c) temperature near the surface (red), at mid-depth (green); and near the sea floor (blue); d) daily (black) and 2-hourly (green) significant wave height; e) wave period. The vertical axis is height above bottom (HAB).

Throughout the year alongshore currents alternated between several-day long periods of alternating eastward (orange to red shades, Figure 7.1.-9a) and westward flow (yellow to blue shades). Maximum eastward current speeds from the low-pass filtered time series were 0.08 m s^{-1}. Maximum westward current speeds were higher with a few approaching 0.16 m s^{-1}. The vertical orange and yellow stripe patterns in Figure 7.1-9a) indicate that alongshore currents were nearly constant with depth over much of the record. In contrast, cross-shore flow (Figure 7.1-9b) typically exhibited vertical shear with offshore flow near the surface (light to dark blue shades) and onshore flow at depth (green to red shades).

Temperatures displayed a strong seasonal cycle with well mixed cooler water during late November through early March and warmer, stratified water from March through October (Figure 7.1-9c). Surface cooling and winter storms cause the vertical mixing during winter and strong solar heating produces the temperature stratification in summer.

Waves were small during the field sampling. Apart from a few wave events in December 2007 and January and February 2008, significant wave heights (H_s) were less than 1 m; two events had periods with H_s larger at than 2 m as shown by the green peaks in December and February. (Significant wave height is a standard wave statistic and is the average height of the upper 1/3 of all waves observed.) During much of the year wave periods T_p were mostly between 5-20 s, indicating the effects of local wind waves and swell arriving from the North Pacific (Figure 7.1-9e). Some of the variability in T_p may have resulted from poorly resolved wave spectra used for estimating T_p.

7.1.5. Plume Modeling

A plume model was used to predict if and when the buoyant effluent plume typically reaches the sea surface or whether it is trapped below the surface. Knowledge of vertical plume location is necessary to verify that water parcels tagged by surface drifters represent movements of the effluent at the sea surface. Three models commonly used by scientists studying buoyant plumes were employed in the study: NRFIELD, NRFIELD2 and UM3. NRFIELD and UM3 are from the U.S. EPA's VISUAL PLUMES plume model package (Frick et al. 2003, Frick 2004). NRFIELD2 is an updated version of the NRFIELD model that accepts time-series inputs such as were measured in this study; UM3 also can incorporate time-series data. The models were obtained directly from one of the model developers, Dr. Phillip Roberts of Georgia Tech; Dr. Roberts is a leading world expert on outfall plumes. All models are appropriate for situations involving a multiport diffuser oriented normal to the general direction of ambient currents which is the case for the MSD outfall.

NRFIELD2 is an empirical model based on laboratory experiments and dimensional analysis which predicts plume dilution, rise height, and spatial extent after initial near-field mixing (Roberts et al. 1989a-c). Model inputs include the diffuser characteristics, effluent flow rate and density, background ocean stratification and ocean current. UM3 is based on physical theory of forced entrainment due to inertia, buoyancy, and velocity shear. Results presented here are from UM3, which was used primarily for its ability to handle time-series input with occasional missing data. Outputs from all models yielded similar results.

The following diffuser characteristics were used as model inputs: port diameter of 4"; port elevation of 0" (discharged at bottom); port angles of 0 degrees (horizontal discharge perpendicular to diffuser pipe); 10 ports with uniform spacing of 9 ft; port depth of 33 ft. While the MSD diffuser has discharge ports on both sides of the diffuser pipe, UM3 assumes effluent discharge on a single side only. The direction of effluent discharge is not expected to have a significant influence on the plumes ability to surface, the primary parameter of interest to this study. Effluent density at discharge is computed using daily effluent temperature data provided by MSD, and assuming zero salinity. Temperature variations (~65 to 75 C) are primarily seasonal with little variation over a single day. While the effluent salinity is not exactly zero, it is much smaller than the ocean salinity so that the assumption of zero should have negligible effect on the modeled plume evolution.

Ambient or background ocean conditions influencing plume evolution are input to the plume model hourly. Velocity profiles in 0.35 meter bins are obtained from ADCP time series data collected at the mooring near the diffuser. Stratification is determined from temperature time series located 1m, 4.5m and 8 m above the bottom and a constant salinity value of 33.2. Moored time series of salinity over the entire year sampling period are not available. Temperature – salinity (T-S) analyses from weekly CTD profiles show that stratification variations are primarily seasonal, and that vertical variations in temperature dominate the stratification.

The following parameters are output by the plume model: effluent density, effluent velocity at discharge, Froude number, time from discharge to surfacing, plume diameter at time of surfacing, depth of plume center at time of surfacing, distance from diffuser at time of surfacing, and plume dilution at time of surfacing. Dilution is defined as the volume of a seawater sample divided by the volume of effluent in that sample. The parameters of interest to this study are time

to surfacing and distance from diffuser at the time of surfacing. UM3 results indicate the effluent plume always reaches the ocean surface, and it does so within 70 seconds of discharge from the diffuser (Figure 7.1-10).

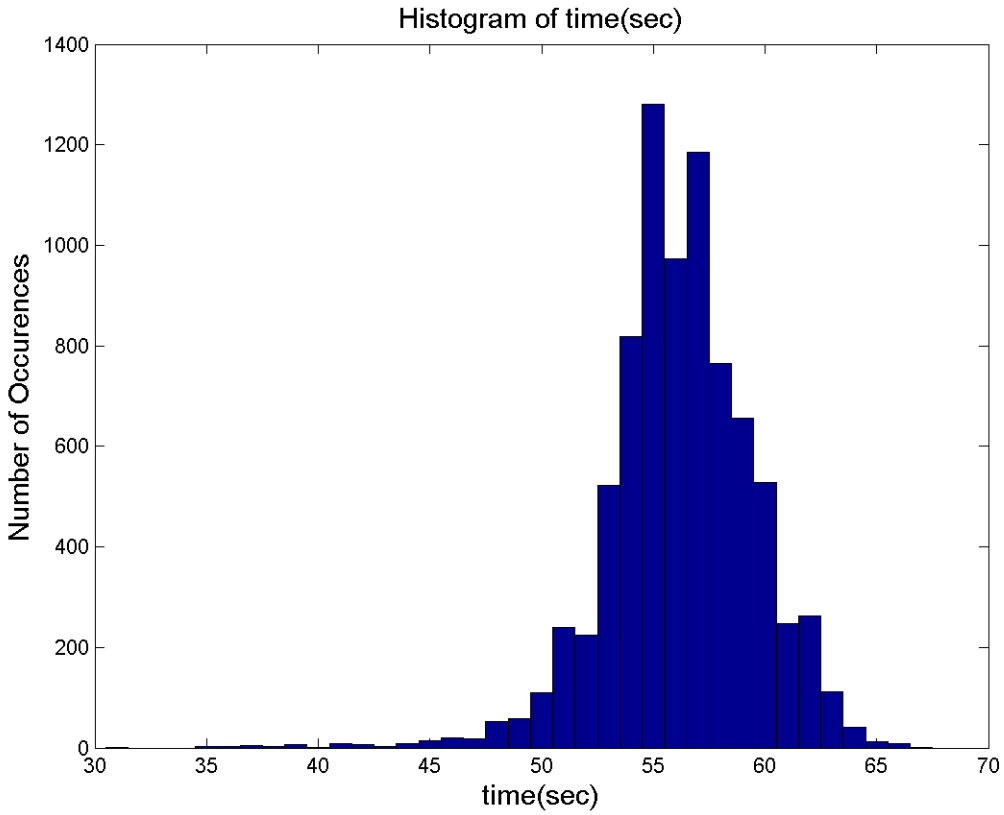


Figure 7.1-10. Distribution of time for discharged effluent to reach the ocean surface. Each occurrence represents an hourly value from the plume model run for a year.

At the time of surfacing, the model predicts that the plume center is located between ~15 and 125 feet from the diffuser in the alongshore direction, and between ~ ± 20 feet from the center of diffuser ports in the cross shore direction (Figure 7.1-11). This is consistent with CTD measurements at the Diffuser station that frequently found lower salinities indicating the presence of relatively fresh effluent waters.

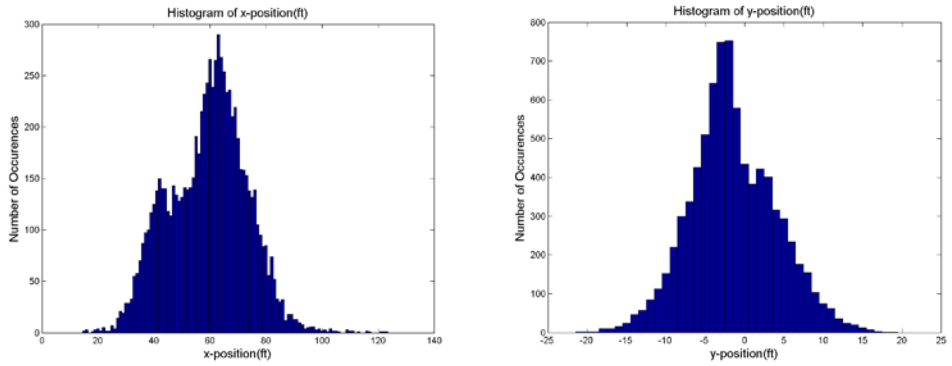


Figure 7.1-11. Distribution of alongshore (x, left-hand panel) and cross-shore (y, right-hand) locations of plume center at the time part of the plume reaches the ocean surface. Each occurrence represents an hourly value from the plume model run for a year.

This means that drifters deployed within ~10 meters of the diffuser should typically be within plume waters. Even if not deployed exactly within the surfaced effluent plume, drifter tracks that originate at the Diffuser station should be reasonable indicators of effluent plume water trajectories. The plume always surfaces within 10's of m of the Diffuser station.

7.1.6. Salinity and Dilution

Because the effluent plume is mainly fresh water, its density is less than the saltier ocean waters and it is driven upward to the surface by buoyancy forces. As the plume rises, its salinity steadily increases as it mixes with ambient ocean waters. Water parcels consisting of mixtures of effluent and ocean waters may be identified by their lower salinity compared with background ocean waters. The salinity difference between the background ocean salinity S_b and the measured salinity of a mixture of effluent and ocean water S_m is related to the dilution D according to the equation $D = (S_e-S_b)/(S_m-S_b) = S_b/\Delta S$ where S_e is the salinity of the effluent (assumed to be 0) and $\Delta S = S_b-S_m$. For this study salinity at the Offshore1000 site was assumed to represent S_b.

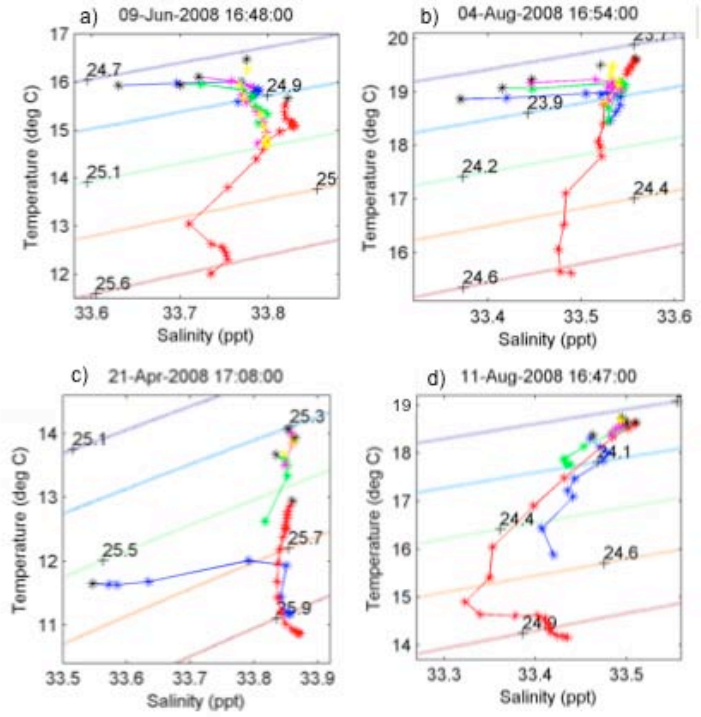


Figure 7.1-12. Temperature-Salinity diagrams from 2008 on **a)** 9 June, **b)** 4 August, **c)** 21 April, and **d)** 11 August. Asterisks are separated vertically by 1 m. Black asterisks indicate surface waters. Colors give stations: diffuser (blue), L1 (green), L2 (magenta), L3 (yellow), offshore1000 (red). Diagonal lines show constant seawater density. For example, the 25.5 line means a density of 1025.5 kg m^{-3}.

Lower salinity water due to mixing with effluent is evident in the temperature-salinity (T-S) diagrams of Figure 7.1-12. In Figure 7.1.-12a, surface waters were fresher at the diffuser station, station Lagrangian1, station Lagrangian2, and station Lagrangian3 than at the Offshore1000 station. A steady progression from fresher to saltier surface waters at the diffuser and Lagrangian1, Lagrangian2, and Lagrangian3 is evident in Figure 7.1-12b. This is consistent with increasing dilution as water parcels drifted away from the diffuser. The lowest dilution of D = 108 for the entire sampling period was recorded at the diffuser on 4/21/08. A near-field dilution of 100 is a typical design criterion for ocean outfalls (Fischer et al., 1979). In the T-S diagram of Figure 7.1.7.1c the low dilution is evident from the decrease in salinity of $\Delta S = 0.31$ between the surface and deeper waters. No surface signature in salinity was detected at Lagrangian1, Lagrangian2, or Lagrangian3, or the Offshore1000 station. Figure 7.1.7.1d from 8/11/08 is an example when no effluent was detected at any station. T-S points from the Diffuser and Lagrangian1, Lagrangian2, and Lagrangian3 stations all scatter about the Offshore1000 points and there is no clear salinity decrease as in the other figure panels.

Surface salinities at the Diffuser station were less than surface salinities at the Offshore1000 station for 41 of the 50 weeks of sampling events; for one event the salinity was higher at the diffuser and for eight weeks salinity measurements were not available at the diffuser due to instrument problems. Estimating dilution is limited by natural variability in S_m and S_b because, as ΔS becomes smaller, it becomes progressively more difficult to separate salinity decreases due

to effluent dilution from natural salinity variability. The scatter around the Offshore1000 line in Figure 7.1-12d is a consequence of this natural variability. Even in the absence of effluent, values of D could be erroneously inferred when S_m is less than S_b due to background variations.

To reduce the likelihood of estimating erroneous values, two approaches were used to estimate typical dilutions at the four sampling stations (Diffuser, Lagrangian1, Lagrangian2, and Lagrangian3). In the first approach, values of D were computed at the four stations for each sampling event only for $\Delta S \geq 0.05$. This limits the maximum detectable dilution to about 600, but reduces errors in ΔS resulting from natural variability in S_m and S_b. In the second approach, values of D were computed for $\Delta S \geq 0$ which allows higher values of D, although some of the highest may result from natural salinity variability and therefore be erroneous. Median values D_L from the first approach are interpreted as lower bounds on typical dilutions at the four stations and median values from the second approach D_U are interpreted as upper bounds. Median rather than average values of D are used since average values are more affected by outliers.

Table 7.1-1 Summary of dilution estimates

station	D_L	N_L	% $\Delta S \geq 0.05$	D_U	N_U	% $\Delta S \geq 0$
diffuser	258	27	64	447	41	98
L1	452	16	36	886	42	93
L2	415	16	34	862	41	87
L3	508	9	21	929	43	79

As shown in Table 7.1-1, median dilutions at the diffuser range from about 250-450; at Lagrangian1 and Lagrangian2 median dilutions are similar and range from about 400 to almost 900, and at Lagrangian3 they are larger and range from 500 to over 900. The numbers of dilution estimates of D_L and D_U are given in columns 3 and 6, respectively. The percentages of ΔS values exceeding 0.05 (column 4) and exceeding 0 (column 7) steadily decrease with distance from the diffuser.

7.2. Microbiological and Chemical Results

7.2.1. Site Conditions/Shoreline Sampling

As stated earlier in the report, the overall sampling program was designed for 52 weeks. Sampling actually occurred one day per week for 50 weeks (Appendix Section 7.2, Table 1); two weeks were missed due to the Christmas holiday and boat plus captain availability, and an inability to reschedule sampling to another weekday. Water sampling was performed by casting from the boat for five sites (Offshore1000, Diffuser, Lagrangian1, 2 and 3 sites), and manually in ankle deep water in the surfzone for the Shoreline samples. Effluent was gathered manually at the MSD WWTP. The sampling locations associated with the Shoreline and five ocean sites were recorded and plotted (Appendix Section 7.2, Table 1 and Figure 1-A through XX).

All 50 Shoreline samples were acquired. As stated in Section 5.5, Shoreline sampling occurred at the same longitude as the sampling location of the last Lagrangian water sample (Appendix Section 7.2, Table 2). As such, forty-five of the Shoreline samples were aligned with the sampling longitude of either the 3rd or 2nd (when there was not a 3rd) Lagrangian water sample. Of the five dates when the Shoreline sample was not acquired at the last Lagrangian sampling longitude, two were when there were either poor weather or other environmental conditions. For example, on 1/14/08, the Shoreline sample was acquired near, but not at, the target site due to high tide conditions and the need to maintain safe access. Also, on 1/22/08, only the 1st Lagrangian water sample was acquired due to high wind and rain which prevented a 2nd and 3rd Lagrangian sampling. In that case, the Shoreline sample was acquired at a location between the retrieval longitudes of the remaining Diffuser drifters. On the remaining three dates, the drifters moved rapidly, and staff reached the shore location well after the last Lagrangian drifter sampling time. Specifically, on 11/26/07, 3/31/08 and 7/21/08, there was a 15, 41 and 39 minute delay, respectively, between the 3rd Lagrangian sampling and the shoreline sample acquisition. On 11/26/07 and 3/31/08, the current position upon arrival of UCSB staff at the beach of the drifter used for the 3rd Lagrangian water sample was used to line up the Shoreline water sample location. Unfortunately, this was not possible on 7/21/08, as the drifter used for the last Lagrangian sample had to be recovered before it entered the demarked swim area; drifter retrieval took precedence over synchronized sampling. In this case, the Shoreline sample was lined up with the position the last Lagrangian water sample was taken, before it entered the swim area.

The 50 sampled Shoreline locations varied widely, without any apparent pattern over the course of the year (Table 7.2-1, Figure 7.2-1, Appendix Section 7.2, Figure 1-A through 1-XX). Most (18) Shoreline sampling locations, and thus most Lagrangian3 drifter-to-shore trajectories, were at Butterfly Beach; the rest of the locations were at the west end of Butterfly beach (9), between East and Butterfly Beaches (8), between Hammonds and Butterfly Beaches (7), at East Beach (5), at Miramar Beach (2) and at Hammonds Beach (1). Beach designations were operationally defined.

Table 7.2-1. Shoreline sample beach locations and conditions. GPS coordinates are in Appendix Section 7.2, Table 2.

Week	Date	ID	Location	Weather
1	11/26/2007	H1126-5BH	between Butterfly & Hammonds beaches	clear, sunny, windy
2	12/3/2007	H1203-5B	Butterfly Beach	clear, sunny
3	12/10/2007	H1210-5W	West end of Butterfly Beach - (west of outfall)	clear, sunny
4	12/17/2007	H1217-5BH	between Butterfly & Hammonds beaches	overcast
5	1/2/2008	H0102-5B	Butterfly Beach	clear, sunny
6	1/8/2008	H0108-5BH	between Butterfly & Hammonds beaches	cold, cloudy
7	1/14/2008	H0114-5B*	Butterfly Beach	clear, sunny
8	1/22/2008	H0122-5E*	East Beach	rainy, windy
9	1/28/2008	H0128-5Mof	Miramar Beach	clear, sunny, windy
10	2/5/2008	H0205-5B	Butterfly Beach	clear, sunny
11	2/11/2008	H0211-5B	Butterfly Beach	clear, sunny, very warm
12	2/20/2008	H0220-5BH	between Butterfly & Hammonds beaches	very windy, cold, cloudy
13	2/27/2008	H0227-5BH	between Butterfly & Hammonds beaches	clear, sunny, warm
14	3/3/2008	H0303-5M	Miramar Beach	sunny, clear, windy
15	3/10/2008	H0310-5W	West end of Butterfly Beach - (west of outfall)	sunny, hot, calm
16	3/18/2008	H0318-5Wof	West end of Butterfly Beach - (west of outfall)	sunny, warm, clear
17	3/24/2008	H0324-5B	Butterfly Beach	sunny, warm, clear
18	3/31/2008	H0331-5H	Hammonds Beach	cold, windy, overcast
19	4/7/2008	H0407-5EW	between East Beach & west end of Butterfly - (under cemetary)	clear, sunny, warm
20	4/14/2008	H0414-5W	West end of Butterfly Beach - (west of outfall)	clear, sunny, warm
21	4/21/2008	H0421-5B	Butterfly Beach	clear, sunny, warm
22	4/28/2008	H0428-5BH	between Butterfly & Hammonds beaches	sunny, warm, haze/fog moving in
23	5/5/2008	H0505-5B	Butterfly Beach	cool, overcast
24	5/12/2008	H0512-5BH	between Butterfly & Hammonds beaches	cool, overcast
25	5/19/2008	H0519-5W	West end of Butterfly Beach - (west of outfall)	clear, windy, warm
26	5/28/2008	H0528-5B	Butterfly Beach	clear, windy, warm
27	6/2/2008	H0602-5E	East Beach	warm, sunny
28	6/9/2008	H0609-5EW	between East Beach & west end of Butterfly - (under cemetary)	overcast
29	6/16/2008	H0616-5EW	between East Beach & west end of Butterfly - (under cemetary)	sunny, warm, clear
30	6/23/2008	H0623-5E	East Beach	windy, sunny, clear
31	6/30/2008	H0630-5B	Butterfly Beach	sunny, warm, clear
32	7/7/2008	H0707-5E	East Beach	sunny, warm, clear
33	7/14/2008	H0714-5B	Butterfly Beach	sunny, warm, clear
34	7/21/2008	H0721-5Bt	Butterfly Beach	sunny, warm, clear
35	7/28/2008	H0728-5B	Butterfly Beach	overcast
36	8/4/2008	H0804-5B	Butterfly Beach	partly sunny, windy
37	8/11/2008	H0811-5EW	between East Beach & west end of Butterfly - (under cemetary)	sunny, warm, clear
38	8/18/2008	H0818-5B	Butterfly Beach	sunny, warm, clear
39	8/25/2008	H0825-5W	West end of Butterfly Beach - (west of outfall)	sunny, warm, clear
40	9/8/2008	H0908-5EW	between East Beach & west end of Butterfly - (under cemetary)	sunny, warm, clear
41	9/15/2008	H0915-5W	West end of Butterfly Beach - (west of outfall)	sunny, warm, clear
42	9/22/2008	H0922-5EW	between East Beach & west end of Butterfly - (under cemetary)	sunny, warm, clear
43	9/29/2008	H0929-5B	Butterfly Beach	sunny, warm, clear
44	10/6/2008	H1006-5E	East Beach	sunny, warm, clear
45	10/13/2008	H1013-5W	West end of Butterfly Beach - (west of outfall)	sunny, warm, clear
46	10/20/2008	H1020-5EW	between East Beach & west end of Butterfly - (under cemetary)	sunny, warm, clear
47	10/27/2008	H1027-5W	West end of Butterfly Beach - (west of outfall)	sunny, warm, clear
48	11/3/2008	H1103-5B	Butterfly Beach	sunny, very windy
49	11/10/2008	H1110-5B	Butterfly Beach	sunny, warm, clear
50	11/17/2008	H1117-5EW	between East Beach & west end of Butterfly - (under cemetary)	hot, clear, sunny, calm

* = could not match position with last Lagrangian sample
of = all drifters headed offshore instead of towards shore
t = time delay and drifter from last Lagrangian recovered before sampling

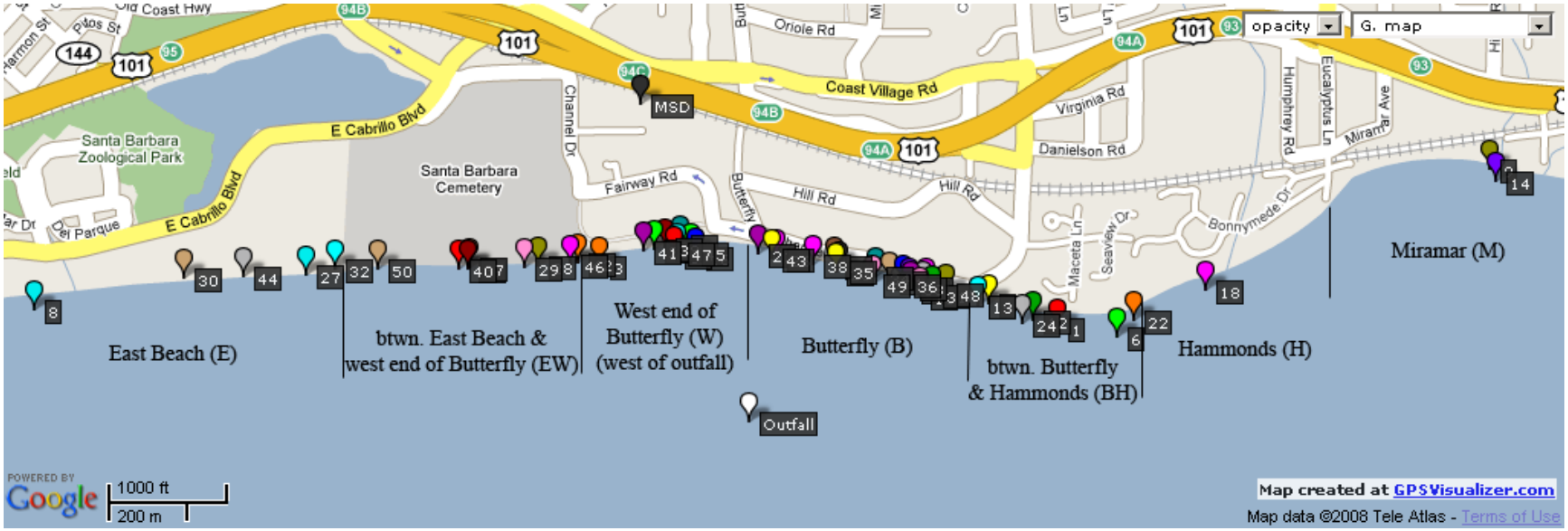


Figure 7.2-1. Locations of all 50 Shoreline samples taken during this project. Numbers represent sampling event (= week number). The end of Montecito Sanitary District's Outfall is marked with a white balloon (= Diffuser sampling location). Beach designations were operationally defined. GPS coordinates are in Appendix Section 7.2, Table 2.

7.2.2. Nutrients

As stated in Section 5.6.2, 26 of the 52 sampling events were budgeted for analysis of DNA and also of dissolved nutrients. Most water samples from all 26 events were analyzed for dissolved nitrate/nitrite, ammonia, and phosphate. Concentrations were reported in the units "μM" which, for these nutrients, is equivalent to reporting "as μM N" or as "μM P", respectively. Conversion to mg/L-N or mg/L-P, as appropriate, was performed for plotting purposes by multiplying the micromolar concentration by the molecular weight of either N or P, respectively, and dividing by 1000.

Out of the total possible 179 samples (23 events for 7 sites, and 3 events for 6 sites), 177 were analyzed for dissolved nutrients; the two samples not archived for analysis were the Effluent sample from 12/3/07 and the Shoreline sample from 1/2/08 (Appendix Section 7.2, Table 3).

Dissolved phosphate-P was quantifiable in all available samples (177, as above), and the concentrations appeared highest, by at least one order of magnitude, in the Effluent sample within each sampling set (Figure 7.2-2; Appendix Section 7.2, Table 3). When averaged across all sampling sets, the mean dissolved phosphate-P concentration was statistically significantly higher for the Effluent samples as compared to samples from the other sites, and the sample with the absolute highest dissolved phosphate-P concentration was an Effluent sample (Table 7.2-2; Appendix Section 7.2, Table 3). Across all 26 sampling events, the Effluent sample dissolved phosphate-P concentration varied by approximately 5% as determined by dividing the standard error of the mean into the mean concentration (Table 7.2-2). Typical effluent total phosphorous concentrations in secondary WWTPs range between 4 and 10 mg/L (Carey and Migliaccio, 2009), and thus P concentrations in the MSD Effluent appear to be lower than in typical secondary WWTP effluents.

Dissolved nitrate/nitrite-N was quantifiable in 137 samples (Figure 7.2-2; Appendix Section 7.2, Table 3). For 12 of the 26 weekly sampling sets, dissolved nitrate/nitrite-N was quantifiable in all samples. Of the 42 samples for which dissolved nitrate/nitrate-N was undetectable, 13 were from the Offshore1000 site, 2 were from the Diffuser site, 20 were from the Lagrangian sites and 7 were from the Shoreline sites. Thus, besides the Effluent for which dissolved nitrate/nitrate-N was detectable in all available samples, dissolved nitrate/nitrite-N was most frequently detected at the Diffuser. Notably, dissolved nitrate/nitrate-N was detectable in all samples available during the period 12/3/07 to 3/24/08 (Appendix Section 7.2, Table 3). Also, on two dates (7/7/08 and 10/6/08), dissolved nitrate/nitrite-N was only quantifiable in the Effluent sample, and on two other dates (5/19/08 and 6/16/08), dissolved nitrate/nitrate-N was only quantifiable in the Effluent and Diffuser samples (Appendix Section 7.2, Table 3).

The concentrations of dissolved nitrate/nitrite-N appeared highest in the Effluent by up to several orders of magnitude (Figure 7.2-3; Appendix Section 7.2, Table 3), and the mean dissolved nitrate/nitrite-N concentration was significantly higher for the Effluent samples when compared to the other sampling sites (Table 7.2-3). Across the analyzed 26 sampling events, the Effluent sample dissolved nitrate/nitrite concentration-N varied by approximately 12% as determined by dividing the standard error of the mean into the mean concentration. Also, the highest single sample dissolved nitrate/nitrite-N concentration was for an Effluent sample (Table 7.2-3).

Typical effluent nitrate concentrations in secondary WWTPs range between 10 and 30 mg/L (Carey and Migliaccio, 2009), and thus nitrate+nitrite N concentrations in the MSD Effluent appear to be mostly lower than in typical secondary WWTP effluents.

Dissolved ammonia-N was quantifiable in all available samples (177, as above). Ammonia-N concentrations appeared highest in the Effluent, by at least one order of magnitude, for only 3 of the 26 sampling dates (11/16/07, 5/19/08 and 7/7/08); for the rest, i.e. majority of the sampling dates, ammonia-N concentrations in the Effluent were more comparable to all other sites (Figure 7.2-4; Appendix Section 7.2, Table 3) and, overall, there were no significant differences across sampling sites in ammonia-N concentrations (Table 7.2-4). Typical effluent ammonia-N concentrations in secondary WWTPs range between 1 and 10 mg/L (Carey and Migliaccio, 2009), and thus ammonia-N concentrations in the MSD Effluent appear to be mostly lower than typical secondary WWTPs (Figure 7.2-4; Appendix Section 7.2, Table 3). The three "spikes" in ammonia-N in the MSD WWTP effluent, occurring on 11/26/07, 5/19/08 and 7/7/08, contained 3.7, 3.6 and 6.9 mg/L ammonia-N, respectively, which is still lower than typical secondary WWTP effluents (Carey and Migliaccio, 2009) even though concentrations on these dates were between 1 and 2 orders of magnitude higher than Effluent sample concentrations for other dates (Appendix Section 7.2, Table 3).

The dilution of effluent into the ocean at the Diffuser site was estimated by dividing the Effluent sample concentration for each nutrient by the Diffuser sample concentration. Dilution factors for the three nutrient characteristics ranged between 1 and 2 orders of magnitude when comparing the lowest and highest ratios within each nutrient type. On average, nitrate/nitrite-N was diluted to the greatest extent, followed by phosphate-P and ammonia-N (Table 7.2-5).

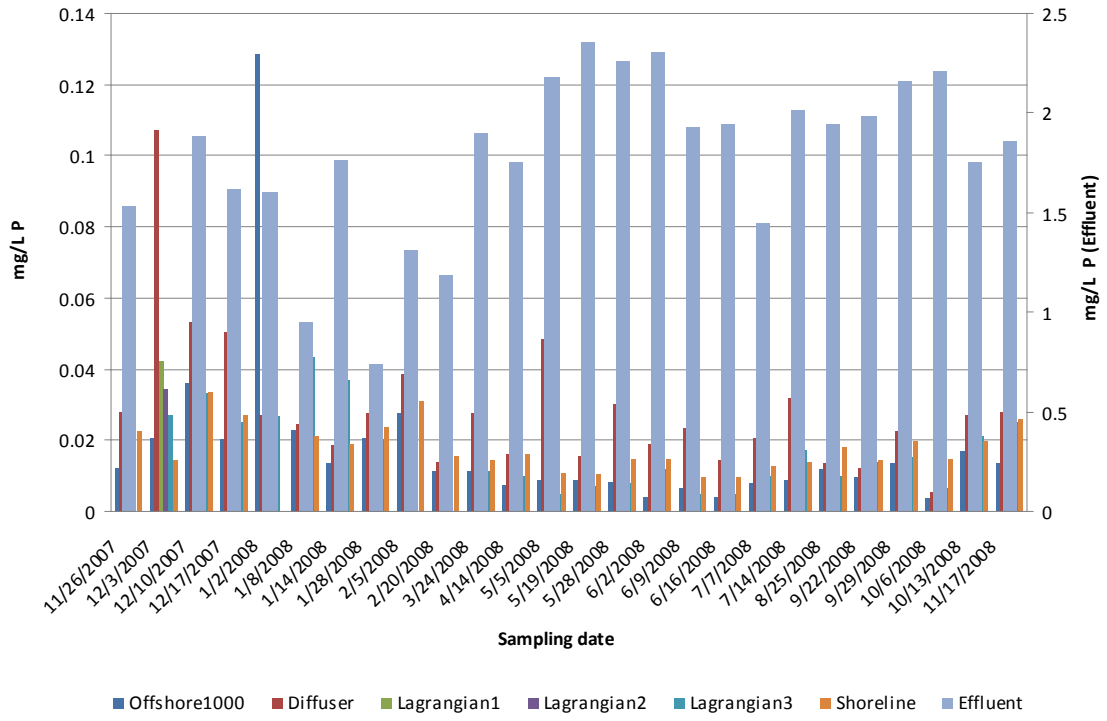


Figure 7.2-2. Phosphate-P concentrations by date and location.

Table 7.2-2. Summary statistics overall and by site for dissolved phosphate-P. Values are expressed as µM. SD = standard deviation, SE = standard error, Min = minimum value, Max = maximum value. Superscripts indicate sites with significant difference. When analyzed by site, the Effluent samples were significantly different from all of the other sites (One-Way ANOVA with Dunnett's T3, $\alpha = 0.05$).

	Overall	Offshore1000[a]	Diffuser[b]	Lagrangians[c]	Shoreline[d]	Effluent[a,b,c,d]
Average	8.72	0.58	0.93	0.63	0.58	57.69
SD	20.52	0.77	0.64	0.40	0.21	13.33
SE	1.54	0.15	0.13	0.05	0.04	2.67
Min	0.13	0.13	0.18	0.16	0.31	24.02
Max	76.09	4.16	3.47	2.35	1.09	76.09
# samples	177	26	26	75	25	25

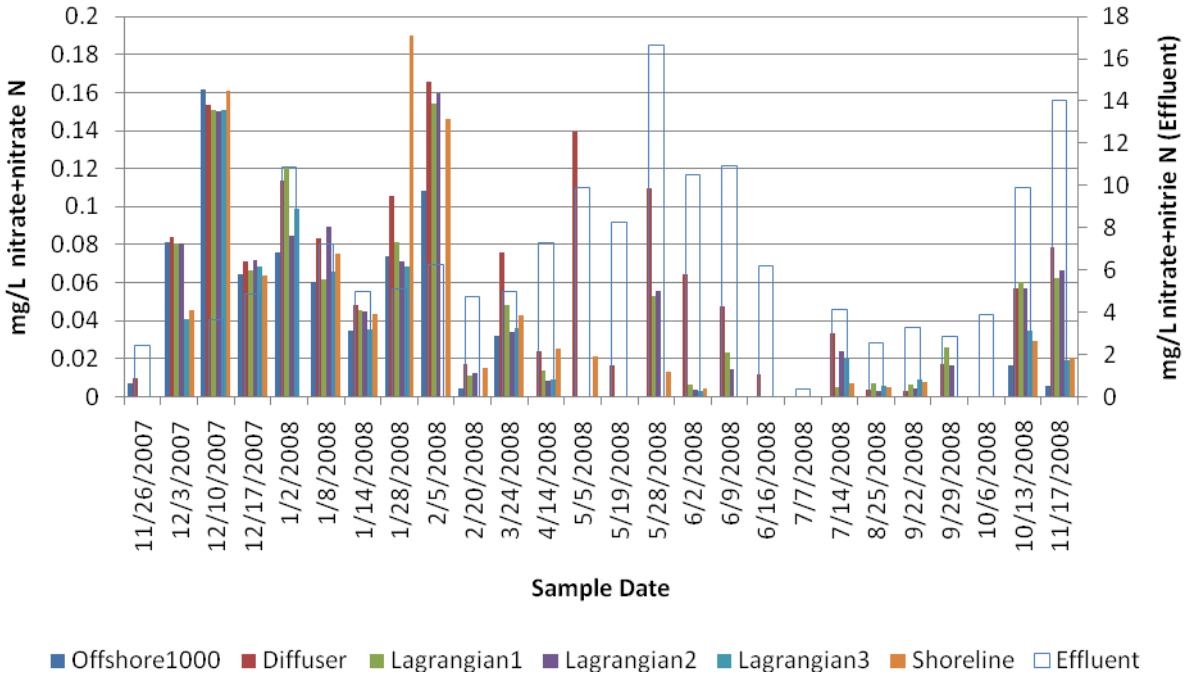


Figure 7.2-3. Nitrate+nitrite-N concentrations by date and location.

Table 7.2-3. Summary statistics overall and by site for dissolved nitrite/nitrate-N. Values are expressed as μM. SD = standard deviation, SE = standard error, Min = minimum value, Max = maximum value. Superscripts indicate sites with significant difference. When analyzed by site, the Effluent samples were significantly different from all of the other sites (One-Way ANOVA with Dunnett's T3, $\alpha = 0.05$).

	Overall	Offshore1000[a]	Diffuser[b]	Lagrangians[c]	Shoreline[d]	Effluent[a,b,c,d]
Average	69.30	1.99	4.21	2.67	2.62	473.54
SD	194.16	3.05	3.55	3.13	3.81	279.57
SE	14.59	0.60	0.70	0.36	0.76	55.91
Min	0.00	0.00	0.00	0.00	0.00	25.75
Max	1186.80	11.53	11.82	11.40	13.59	1186.80
# samples	177	26	26	75	25	25

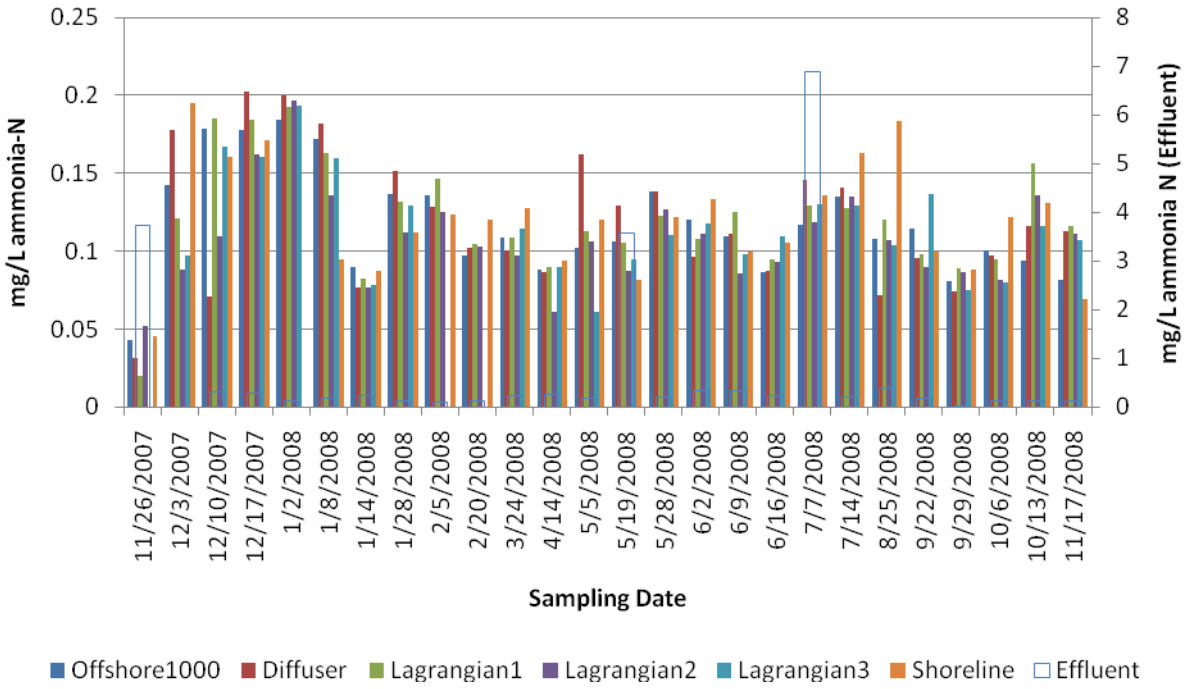


Figure 7.2-4. Ammonia-N concentrations by date and location.

Table 7.2-4. Summary statistics overall and by site for dissolved ammonia-N. Values are expressed as μM. SD = standard deviation, SE = standard error, Min = minimum value, Max = maximum value. When analyzed by site, no significant differences were detected (One-Way ANOVA with Dunnett's T3, $\alpha = 0.05$).

	Overall	Offshore1000	Diffuser	Lagrangians	Shoreline	Effluent
Average	14.63	8.37	8.48	8.18	8.53	53.02
SD	45.07	2.45	3.07	2.38	2.55	114.32
SE	3.39	0.48	0.60	0.28	0.51	22.86
Min	1.43	3.09	2.24	1.43	3.24	2.15
Max	492.53	13.16	14.46	14.04	13.95	492.53
# samples	177	26	26	75	25	25

Table 7.2-5. Ratio (dimensionless) of Effluent to Diffuser concentrations for nutrients, calculated by dividing the concentrations in the Effluent samples by the concentrations in the Diffuser samples. "NaN" = "not a number" because the Diffuser concentration = 0.

Date	Phosphate	Nitrite+Nitrate	Ammonia
11/26/2007	54	259	119
12/3/2007	Effluent sample not archived		
12/10/2007	35	24	4
12/17/2007	32	68	1
1/2/2008	59	96	1
1/8/2008	39	87	1
1/14/2008	94	103	3
1/28/2008	27	48	1
2/5/2008	34	38	1
2/20/2008	85	276	1
3/24/2008	69	66	2
4/14/2008	108	302	3
5/5/2008	45	71	1
5/19/2008	150	510	28
5/28/2008	74	151	1
6/2/2008	120	164	3
6/9/2008	83	229	3
6/16/2008	136	523	3
7/7/2008	70	NaN	47
7/14/2008	63	124	2
8/25/2008	142	651	5
9/22/2008	160	1035	2
9/29/2008	96	162	0
10/6/2008	400	NaN	1
10/13/2008	64	174	1
11/17/2008	66	178	1
Min	27	24	0

Avg	92	232	9
Max	400	1035	119

7.2.3. Fecal Indicator Bacteria (FIB) – IDEXX

As per Section 5.6, FIB were quantified using IDEXX reagents in a Quantitray 2000 MPN format. IDEXX-based analyses of total coliform (TC), *Escherichia coli* (EC) and enterococci (ENT) were performed for all 50 sampling events (Appendix Section 7.2, Table 7). When compared to CA AB411 ocean water quality criteria (http://www.cdph.ca.gov/HealthInfo/environhealth/water/Documents/Beaches/AppendixA.pdf), no sample analyzed during this study exceeded any of the single sample criteria defined by the State of California. No samples were lost; further, when FIB were determined to be below detection limits, most samples were reported as <10, which indicates that the selected dilution yielded a detection limit well within the single sample standards defined in AB411.

TC were quantifiable in all 50 Effluent samples, and in 44 Shoreline, 36 Diffuser, 40 Lagrangian (1 or more), and 20 Offshore1000 samples (Appendix Section 7.2, Table 7). For 10 samples, TC were not quantifiable in any Lagrangian samples. For one date (3/18/08), TC was only quantifiable in the Effluent sample. For five dates (2/5/08, 4/7/08, 4/14/08, 4/21/08, and 6/16/08), TC were quantifiable in only the Effluent and Shoreline samples. For one date (6/2/08), TC were quantifiable in only the Effluent and Diffuser samples (Appendix Section 7.2, Table 7).

As above, all FIB values were below the AB411 water quality criteria concentration. Still, it is useful to review the patterns of quantification during this study, as quantification frequency provides another insight into the prevalence and consistency of FIB across sites. EC were quantifiable in some samples for most events, but were not detected in any samples for 11 of the 50 events (Appendix Section 7.2, Table 7). Ten of the 11 events where EC were not detected in any sample were also those for which no ENT were detected in any sample (below). Nineteen (19) of the Shoreline samples had quantifiable EC, and for 11 of those events, EC was present in only the Shoreline samples. For 16 events, EC was quantifiable in the Effluent; of those, 11 were for events where only the Effluent (i.e. no other samples) had quantifiable EC. Of the 5 events where EC were quantifiable in an Effluent sample and other samples, 3 events were where the Shoreline sample had quantifiable EC. For the other 2 events where EC were quantifiable in the Effluent and another sample, one other sample was the Diffuser and the other was a Lagrangian. On three occasions (5/19/08, 6/30/08 and 8/4/08), EC were only quantifiable in the Diffuser samples.

Enterococci concentrations were below detection limits across all samples for 26 of the 50 sampling events (Appendix Section 7.2, Table 7). The highest frequency of enterococci detections was for Shoreline samples: enterococci were quantified in only Shoreline samples for 13 sampling events. For 5 sampling events, enterococci were quantified in only the Effluent samples. For two sampling events, enterococci were quantified in only the Shoreline and Effluent samples, or in only one or more Lagrangian samples. For one sampling event each, enterococci were quantified in either only the Offshore1000 sample, or in a majority (6 of 7) of

samples. The latter event (1/28/08) was the only one for which enterococci were quantified in a Diffuser sample (Appendix Section 7.2, Table 7).

Table 7.2-6. Summary statistics overall and by site for total coliform via IDEXX. Values are expressed as most probable number (MPN). SD = standard deviation, SE = standard error, Min = minimum value, Max = maximum value. Superscripts indicate sites with significant difference. When analyzed by site, the Shoreline samples were significantly different from the Offshore1000 samples (One-Way ANOVA with Dunnett's T3, $\alpha = 0.05$). However, it should be noted that statistics were performed using the MPN values and the 95% confidence limits were not taken into account.

	Overall	Offshore1000[a]	Diffuser	Lagrangians	Shoreline[a]	Effluent
Average	42.1	18.0	37.8	38.7	78.3	44.0
SD	101.3	55.1	60.0	116.3	115.0	104.3
SE	5.5	7.8	8.3	9.8	16.3	14.8
Min	0	0	0	0	0	1
Max	1161.9	378.6	354.5	1161.9	573.1	555
# samples	344	50	52	142	50	50

When comparing across sites for all sampling events, the average Shoreline sample TC concentration was statistically highest while the average Offshore1000 was statistically the lowest concentration (Table 7.2-6). The average concentrations of TC across the Diffuser, Lagrangian(s) and Effluent sites were not significantly different (Table 7.2-6). The average Shoreline sample EC concentration, across all sampling events, was statistically higher than the average EC concentrations for all other sites which were not statistically different from one another (Table 7.2-7). The average Shoreline sample ENT concentration, across all sampling events, appeared statistically higher than the average ENT concentrations for all other sites, yet there was more spread in the ENT data and thus average concentrations across sites overlapped more so than for either EC or TC concentrations (Table 7.2-8). However, taking into account the 95% confidence intervals for all FIB data, differences between sites based on any of the three FIB categories appeared to be insignificant (Appendix Section 7.2, Table 7).

Table 7.2-7. Summary statistics overall and by site for *E. coli* via IDEXX. Values are expressed as most probable number (MPN). SD = standard deviation, SE = standard error, Min = minimum value, Max = maximum value. Superscripts indicate sites with significant difference. When analyzed by site, the Shoreline samples were significantly different from the samples from all the other sites (One-Way ANOVA with Dunnett's T3, $\alpha = 0.05$). However, it should be noted that statistics were performed using the MPN values and the 95% confidence limits were not taken into account.

	Overall	Offshore1000[a]	Diffuser[b]	Lagrangians[c]	Shoreline[a,b,c,d]	Effluent[d]
Average	1.9	0.4	1.6	0.7	8.4	0.5
SD	6.8	2.0	3.9	2.6	15.3	1.0
SE	0.4	0.3	0.5	0.2	2.2	0.1
Min	0	0	0	0	0	0
Max	80.8	10	15.1	10	80.8	5.2
# samples	344	50	52	142	50	50

Table 7.2-8. Summary statistics overall and by site for enterococci via IDEXX. Values are expressed as most probable number (MPN). SD = standard deviation, SE = standard error, Min = minimum value, Max = maximum value. Superscripts indicate sites with significant difference. When analyzed by site, the Shoreline samples were significantly different from the Offshore1000, Lagrangian and Effluent samples (One-Way ANOVA with Dunnett's T3, $\alpha = 0.05$). However, it should be noted that statistics were performed using the MPN values and the 95% confidence limits were not taken into account.

	Overall	Offshore1000[a]	Diffuser	Lagrangians[b]	Shoreline[a,b,c]	Effluent[c]
Average	1.1	0.6	0.8	0.4	4.8	0.4
SD	4.5	3.2	5.7	2.2	8.3	1.2
SE	0.2	0.4	0.8	0.2	1.2	0.2
Min	0	0	0	0	0	0
Max	41.3	20.2	41.3	20.2	30.8	6.3
# samples	344	50	52	142	50	50

7.2.4. DNA Yield

Total DNA was extracted from all 50 Effluent samples for the purposes of quantifying Effluent sample DNA concentrations and, where Effluent DNA concentrations were sufficient and other criteria for further analysis applied (see Section 5.6), for analyzing DNA for DNA-based analyses (i.e. qPCR HBM, qPCR ENT, TRFLP, and PhyloChip). For the other sites (Offshore1000, Diffuser, Lagrangians, and Shoreline), 26 sampling events were selected for DNA-based analyses and had their samples extracted (see Section 5.6.2 for selection criteria and dates selected).

Out of all 50 events for Effluent and 26 events for the Offshore1000, Diffuser, Lagrangians and Shoreline samples, extracted DNA was quantifiable in all samples (Appendix Section 7.2, Table 11). For 36 of the 50 events, the DNA concentration in the extracted Effluent sample was below 100 ng/L (Appendix Section 7.2, Table 11) and was significantly lower than for other sites when averaged across all events (Table 7.2-9). For three events (11/26/07, 5/19/08, 7/7/08), Effluent sample DNA concentrations were at similar magnitudes to other samples. Across all events, DNA concentrations appeared lower in the Offshore1000 samples as compared to other ocean samples (Table 7.2-9). Also, DNA concentrations in Diffuser, Lagrangian and Shoreline samples appeared similar when compared across all events (Table 7.2-9).

Table 7.2-9. Summary statistics overall and by site for DNA yield. Values are expressed as ng/L. SD = standard deviation, SE = standard error, Min = minimum value, Max = maximum value. Superscripts indicate sites with significant difference. When analyzed by site, the Effluent samples were significantly different from the Diffuser, Lagrangian and Shoreline samples (One-Way ANOVA with Dunnett's T3, $\alpha = 0.05$).

	Overall	Offshore1000	Diffuser[a]	Lagrangians[b]	Shoreline[c]	Effluent[a,b,c]
Average	2303.1	1763.9	3136.5	2780.2	3871.6	618.9
SD	3019.6	1571.7	3798.7	2592.3	3850.3	2486.5
SE	211.9	308.2	745.0	299.3	755.1	351.6
Min	7.6	86.2	426.7	353.3	481.6	7.6
Max	17865.9	7123.9	17865.9	14111.1	15444.4	16315.8
# samples	203	26	26	75	26	50

7.2.5. Cell Counts via Flow Cytometry

Beginning on 6/16/08, subsamples of each water sample were archived (as per Section 5.6) for counting total cells by flow cytometry. Samples from only 9 events were subjected to flow cytometry as the decision was made to quantify cells only for samples that were to be processed for DNA-based analyses. Because of the robust sample fixation and storage protocol followed, samples archived for possible flow cytometric analysis could be selected for that analysis at the same time, and using similar criteria, as for DNA analysis.

Flow cytometry was used to quantify cells in samples for 9 sampling events (Appendix Section 7.2, Table 13). All samples in those 9 events were analyzed, with the exception of the Effluent samples. Due to large (> 2 μm) particles in the Effluent samples that caused shadowing which prevented quantifying bacterial cells, flow cytometry was not valid for quantifying bacteria in those samples (Table 7.2-10; Appendix Section 7.2, Table 13). For all other samples, cell concentrations ranged between ca. 0.3E9 and 4.7E9 cells /liter, and the means for each site, across all events were not significantly different from one another using One-Way ANOVA (Table 7.2-10). However, paired t-tests indicated significantly different and higher cell concentrations at the Diffuser, all 3 Lagrangians and the Shoreline samples compared to the Offshore1000 samples ($p < 0.05$).

Table 7.2-10. Summary statistics overall and by site for cell counts via flow cytometry. Values are expressed as cells/L. SD = standard deviation, SE = standard error, Min = minimum value, Max = maximum value. Effluent samples were unable to be quantified using this method.

	Overall	Offshore1000	Diffuser	Lagrangians	Shoreline	Effluent
Average	2.2E+09	1.6E+09	2.1E+09	2.4E+09	2.2E+09	N/A
SD	1.0E+09	9.5E+08	1.1E+09	1.0E+09	9.6E+08	N/A
SE	1.4E+08	3.2E+08	3.7E+08	2.0E+08	3.2E+08	N/A
Min	2.9E+08	2.9E+08	7.0E+08	8.6E+08	7.1E+08	N/A
Max	4.7E+09	3.1E+09	3.8E+09	4.7E+09	3.1E+09	N/A
# samples	54	9	9	27	9	9

7.2.6 *Enterococcus* spp. qPCR

Total *Enterococcus* spp. concentrations were analyzed, in addition to IDEXX analysis of culture-dependent bacteria, by culture-independent quantitative polymerase chain reaction (qPCR) as per Section 5.6. *Enterococcus* spp. concentrations by this method are abbreviated here as qPCR ENT. qPCR ENT was analyzed for all 26 sampling events for which DNA was extracted. All samples were analyzed in triplicate, and the mean reported as "markers per liter" to indicate the number of DNA markers of *Enterococcus* spp. per liter (Appendix Section 7.2, Table 15). The number of *Enterococcus* spp. cells was estimated by assuming 6 copies of the DNA marker per cell (Appendix Section 7.2, Table 15), as per Section 5.6.7.

Across all sampling events, qPCR ENT was quantifiable in 19 Shoreline samples, in 18 Effluent samples, and in 12 Diffuser samples (Appendix Section 7.2, Table 15). There were 6 sampling events (12/3/07, 1/2/08, 1/28/08, 2/5/08, 2/20/08, and 3/24/08) for which qPCR ENT was quantifiable in all samples. There were four events (5/28/08, 6/9/08, 9/22/08 and 11/17/08) for which qPCR ENT was not quantifiable in any sample. qPCR ENT was quantifiable in 11 of the Offshore1000 samples. For one event (9/29/08), qPCR ENT was quantifiable in the Diffuser but not in the Effluent sample. For 3 events (6/16/08, 8/25/08 and 9/29/08), qPCR ENT was quantifiable in the Shoreline sample but not in the Effluent sample. For 12 events, qPCR ENT was quantifiable in the Shoreline and Diffuser samples; out of these, there were 7 events for which qPCR ENT was also quantifiable in all 3 Lagrangian samples, 1 event in which qPCR ENT was quantifiable in 2 Lagrangian samples, 3 events in which qPCR was quantifiable in 1 Lagrangian sample, and 1 event for which qPCR ENT was not quantifiable in any Lagrangian samples. Thus, when qPCR ENT was quantifiable in Diffuser samples, it was also quantifiable in the Shoreline samples and in most of the Lagrangian samples.

Across all sampling events, qPCR ENT in the Effluent appeared highest when compared to other sites, and qPCR ENT was similar across the other sites (Table 7.2-11). There were three events (11/26/07, 5/19/08, 7/7/08) for which qPCR ENT was much higher in the Effluent samples when compared to other Effluent samples from other events. On 11/26/07, qPCR ENT was quantifiable in the Effluent and in the Offshore1000, and appeared relatively higher in the Effluent (Table 7.2-12). On 5/19/08, qPCR ENT appeared highest in the Effluent, but was also quantifiable in the Lagrangians and Shoreline samples. On 7/7/08, qPCR ENT also appeared

highest in the Effluent, but was also quantifiable in all but the Offshore1000 sample (Table 7.2-12).

Table 7.2-11. Summary statistics overall and by site for *Enterococcus* qPCR results. Values are expressed as markers/L. SD = standard deviation, SE = standard error, Min = minimum value, Max = maximum value. Superscripts indicate sites with significant difference. Samples were analyzed in triplicate. When analyzed by site, the Shoreline samples were significantly different from the Offshore1000 and Diffuser samples (One-Way ANOVA with Dunnett's T3, $\alpha = 0.05$).

	Overall	**Offshore1000[a]**	**Diffuser[b]**	**Lagrangians**	**Shoreline[a,b]**	**Effluent**
Average	1.9E+04	1.1E+03	1.8E+03	2.9E+03	4.6E+03	1.2E+05
SD	2.0E+05	2.2E+03	2.9E+03	1.7E+04	6.5E+03	5.0E+05
SE	8.4E+03	2.4E+02	3.2E+02	1.1E+03	7.4E+02	5.7E+04
Min	0	0	0	0	0	0
Max	2.7E+06	8.9E+03	1.1E+04	1.6E+05	3.2E+04	2.7E+06
# replicates	537	78	78	225	78	78

Table 7.2-12. *Enterococcus* qPCR results for 11/26/07, 5/19/08 & 7/7/08. Values are expressed as markers/L. SE = standard error. Superscripts indicate sites with significant difference. Samples were analyzed in triplicate, and these dates were independently analyzed for significance between the sites. On 11/26/07, 5/19/08 & 7/7/08, the Effluent samples were significantly different from all of the other sites on that date. Also on 7/7/08, the Diffuser samples were significantly different from the Offshore1000 and Lagrangian sites (One-Way ANOVA with Dunnett's T3, α = 0.05).

11/26/07

	Offshore1000[a]	**Diffuser[b]**	**Lagrangians[c]**	**Shoreline[d]**	**Effluent[a,b,c,d]**
Average	6.1E+03	0.0E+00	0.0E+00	0.0E+00	1.9E+05
SE	8.7E+02	0.0E+00	0.0E+00	0.0E+00	6.6E+03

5/19/2008

	Offshore1000[e]	**Diffuser[f]**	**Lagrangians[g]**	**Shoreline[h]**	**Effluent[e,f,g,h]**
Average	0.0E+00	0.0E+00	4.9E+04	1.6E+03	1.8E+05
SE	0.0E+00	0.0E+00	2.4E+04	1.6E+03	7.7E+03

07/07/08

	Offshore1000[i,m]	**Diffuser[j,m,n]**	**Lagrangians[k,n]**	**Shoreline[l]**	**Effluent[i,j,k,l]**
Average	0.0E+00	7.4E+03	1.1E+03	2.1E+03	2.6E+06
SE	0.0E+00	6.6E+02	7.5E+02	2.1E+03	7.6E+04

7.2.7. Human-specific *Bacteroides* qPCR

As above, DNA was extracted from the Effluent for all 50 events, and was extracted for all sites/samples for 26 events. Because of the importance of the human specific *Bacteroides* marker (HBM) for specifically quantifying evidence of human sewage (Sercu et al., 2009), it was decided to attempt quantification of HBM in DNA extracted from all of the Effluent samples in addition to the DNA extracted from the selected 26 samples for complete DNA (i.e. by qPCR ENT, HBM, TRFLP and PhyloChip) analysis.

HBM was quantifiable in some samples for 26 events, but was not quantifiable in any samples for 24 events (Appendix Section 7.2, Table 17). Of the 24 events for which HBM was not quantifiable, 17 events where when only Effluent DNA was extracted and analyzed. Thus, there were 7 events targeted for full DNA analysis for which HBM markers were not quantifiable in any sample. Of the 26 events targeted for full DNA analysis, there were 13 for which HBM was quantifiable in at least 1 Lagrangian sample, 11 for which HBM was quantifiable in the Diffuser samples, 9 for which HBM was quantifiable in the Offshore1000 samples, and 8 for which HBM was quantifiable in the Shoreline samples. For two events, HBM was quantifiable at the

Shoreline but not in any Lagrangian sample: on 2/5/08, HBM was however present in the Diffuser and Offshore1000 samples but not in the Effluent sample, and on 4/14/08, HBM was present also in the Effluent but not in other samples in addition to the Shoreline (Appendix Section 7.2, Table 17).

Across all events, the Effluent samples contained relatively more HBM as compared to the other sites (Table 7.2-13). While the concentrations of HBM were generally low overall, including in most Effluent samples, there were three dates (11/26/07, 5/19/08, 7/7/08) when HBM concentrations in the Effluent were relatively high, and in fact were the highest values quantified for any sample across all events (Table 7.2-14; Appendix Section 7.2, Table 17). The concentrations were, respectively, 5.7E6 ± 1.4E5, 1.9E6 ± 9.3E4, and 3.5E6 ± 1.8E5 HBM per liter. On one occasion (see Section 5.6.1), MSD activated sludge was sampled and HBM quantified from extracted DNA; the concentration for that sample was 1.6E7±5.9E6. Thus, for the three events for which Effluent HBM concentrations were highest, these concentrations were approximately 3 to 8 times lower than the concentration measured for one sample of MSD activated sludge.

Table 7.2-13. Summary statistics overall and by site for human-specific *Bacteroides* qPCR results. Values are expressed as markers/L. SD = standard deviation, SE = standard error, Min = minimum value, Max = maximum value. Superscripts indicate sites with significant difference. Samples were analyzed in triplicate. When analyzed by site, the Effluent samples were significantly different from all of the other sites (One-Way ANOVA with Dunnett's T3, $\alpha = 0.05$).

	Overall	**Offshore1000[a]**	**Diffuser[b]**	**Lagrangians[c]**	**Shoreline[d]**	**Effluent[a,b,c,d]**
Average	6.0E+04	3.2E+03	2.4E+03	2.5E+03	5.1E+03	2.3E+05
SD	4.9E+05	9.9E+03	5.3E+03	7.5E+03	1.8E+04	9.6E+05
SE	2.0E+04	1.1E+03	6.0E+02	5.0E+02	2.0E+03	7.8E+04
Min	0	0	0	0	0	0
Max	5.9E+06	5.4E+04	3.4E+04	5.3E+04	1.3E+05	5.9E+06
# replicates	610	78	78	224	78	152

Table 7.2-14. Human-specific *Bacteroides* qPCR results for 11/26/07, 5/19/08 & 7/7/08. Values are expressed as markers/L. SE = standard error. Superscripts indicate sites with significant difference. Samples were analyzed in triplicate, and these dates were independently analyzed for significance between the sites. On all 3 dates, the Effluent samples were significantly different from all of the other sites on that date (One-Way ANOVA with Dunnett's T3, $\alpha = 0.05$).

11/26/2007

	Offshore1000[a]	Diffuser[b]	Lagrangians[c]	Shoreline[d]	Effluent[a,b,c,d]
Average	2.6E+03	6.5E+03	0.0E+00	0.0E+00	5.7E+06
SE	3.6E+02	3.3E+03	0.0E+00	0.0E+00	1.4E+05

5/19/2008

	Offshore1000[e]	Diffuser[f]	Lagrangians[g]	Shoreline[h]	Effluent[e,f,g,h]
Average	0.0E+00	7.0E+03	8.0E+03	0.0E+00	1.9E+06
SE	0.0E+00	1.5E+03	4.0E+03	0.0E+00	9.3E+04

7/7/2008

	Offshore1000[i]	Diffuser[j]	Lagrangians[k]	Shoreline[l]	Effluent[i,j,k,l]
Average	0.0E+00	5.0E+03	5.9E+03	1.5E+04	3.5E+06
SE	0.0E+00	2.5E+03	5.9E+03	1.5E+04	1.8E+05

7.2.8. Human Enterovirus

Thirteen sampling events (11/26/07, 12/3/07, 1/8/08, 1/14/08, 1/28/08, 5/5/08, 5/19/08, 5/28/08, 6/9/08, 7/7/08, 8/25/08, 9/29/08 & 10/13/08) were selected for analysis of enterovirus (performed in the laboratory of Dr. Jed Fuhrman at the University of Southern California). As per Section 5.6, the procedure at UCSB was to filter water samples per USC protocols, to freeze the filters, then ship the filters frozen to USC for analysis. A report of the results was returned to UCSB and to HTO. Those results are summarized here.

Out of the 13 events for which samples were analyzed for enterovirus, only 5 of the 90 samples contained amplifiable markers of enterovirus: the Diffuser & Effluent samples on 11/26/07, the Offshore1000 sample on 12/3/07, the Lagrangian1 sample on 1/14/08, and the Offshore1000 sample on 5/19/08 (Table 7.2-15). However, for all of these samples, only 1 of 2 analytical duplicates amplified, so the values were the same as the values of standard errors. Since the values on the dates detected were so close to the detection limit of the assay, as evidenced by the presence in only 1 of the analytical duplicates, no further analysis or quantification can be performed with these results.

Quoting the reports from the Furhman lab: “We are particularly uncertain about these instances where one replicate is positive and one negative, which we believe occurs when contamination is very low but present. It is important to note that there are currently no formal standards set to evaluate "acceptable" or "unacceptable" levels of these contaminants as measured genetically, and that highly sensitive methods like this may possibly be detecting levels that are not necessarily harmful (in the same way that classical fecal indicators may have detectable levels that are not thought to be harmful). The lack of enteroviruses is also not necessarily an indication of the lack of fecal contamination in a given sample because not all fecal material comes from people shedding enteroviruses. Thus, sewage from a large population is more likely to have enteroviruses present.”

Further, the Furhman lab report stated, when there were no positive values, that: “None of the samples were positive; however, it is possible that dilution to remove inhibition may have reduced the sensitivity of the assay.” This latter statement reflects the perspective of the analyst in a molecular ecology lab, in that it is possible that dissolved chemicals in water samples, including natural organic matter, can interfere with standard methods used in molecular biological analysis of viruses and other microbes in water. To reduce the level of interference, and thus to enhance the likelihood of detecting analytes that are present, it is standard in this type of research to test and apply a dilution step at a level that would diminish the concentration of chemical inhibitors while not relegating the analyte concentration to below detection limits.

Table 7.2-15. Enterovirus results provided by the Laboratory of Jed Fuhrman, USC Department of Biological Sciences. Values are mean number of gene copies or enteroviruses ± standard error of the mean, per ml of sample water.

Sample ID	Enterovirus/mL±SEM*
H1126-1	0.0 ± 0.0
H1126-3	0.756 ± 0.756
H1126-4a	0.0 ± 0.0
H1126-4b	0.0 ± 0.0
H1126-5BH	0.0 ± 0.0
H1126-6	0.200 ± 0.200
H1203-1	0.332 ± 0.332
H1203-3	0.0 ± 0.0
H1203-4a	0.0 ± 0.0
H1203-4b	0.0 ± 0.0
H1203-4c	0.0 ± 0.0
H1203-5B	0.0 ± 0.0
H1203-6	0.0 ± 0.0
H0108-1	0.0 ± 0.0
H0108-3	0.0 ± 0.0
H0108-4a	0.0 ± 0.0
H0108-4b	0.0 ± 0.0
H0108-4c	0.0 ± 0.0
H0108-5BH	0.0 ± 0.0
H0108-6	0.0 ± 0.0

H0114-1	0.0 ± 0.0
H0114-3	0.0 ± 0.0
H0114-4a	0.54 ± 0.54
H0114-4b	0.0 ± 0.0
H0114-4c	0.0 ± 0.0
H0114-5B	0.0 ± 0.0
H0114-6	0.0 ± 0.0
H0128-1	0.0 ± 0.0
H0128-3	0.0 ± 0.0
H0128-4a	0.0 ± 0.0
H0128-4b	0.0 ± 0.0
H0128-4c	0.0 ± 0.0
H0128-5Mof	0.0 ± 0.0
H0128-6	0.0 ± 0.0
H0519-1	0.313 ± 0.313
H0519-3	0.0 ± 0.0
H0519-4a	0.0 ± 0.0
H0519-4b	0.0 ± 0.0
H0519-4c	0.0 ± 0.0
H0519-5W	0.0 ± 0.0
H0519-6	0.0 ± 0.0
H0707-1	0.0 ± 0.0
H0707-3	0.0 ± 0.0
H0707-4a	0.0 ± 0.0
H0707-4b	0.0 ± 0.0
H0707-4c	0.0 ± 0.0
H0707-5E	0.0 ± 0.0
H0707-6	0.0 ± 0.0
H0505-1	0.0 ± 0.0
H0505-3	0.0 ± 0.0
H0505-4a	0.0 ± 0.0
H0505-4b	0.0 ± 0.0
H0505-4c	0.0 ± 0.0
H0505-5B	0.0 ± 0.0
H0505-6	0.0 ± 0.0
H0528-1	0.0 ± 0.0
H0528-3	0.0 ± 0.0
H0528-4a	0.0 ± 0.0
H0528-4b	0.0 ± 0.0
H0528-4c	0.0 ± 0.0
H0528-5B	0.0 ± 0.0
H0528-6	0.0 ± 0.0
H0609-1	0.0 ± 0.0

H0609-3	0.0 ± 0.0
H0609-4a	0.0 ± 0.0
H0609-4b	0.0 ± 0.0
H0609-4c	0.0 ± 0.0
H0609-5EW	0.0 ± 0.0
H0609-6	0.0 ± 0.0
H0825-1	0.0 ± 0.0
H0825-3	0.0 ± 0.0
H0825-4a	0.0 ± 0.0
H0825-4b	0.0 ± 0.0
H0825-4c	0.0 ± 0.0
H0825-5W	0.0 ± 0.0
H0825-6	0.0 ± 0.0
H0929-1	0.0 ± 0.0
H0929-3	0.0 ± 0.0
H0929-4a	0.0 ± 0.0
H0929-4b	0.0 ± 0.0
H0929-4c	0.0 ± 0.0
H0929-5B	0.0 ± 0.0
H0929-6	0.0 ± 0.0
H1013-1	0.0 ± 0.0
H1013-3	0.0 ± 0.0
H1013-4a	0.0 ± 0.0
H1013-4b	0.0 ± 0.0
H1013-4c	0.0 ± 0.0
H1013-5W	0.0 ± 0.0
H1013-6	0.0 ± 0.0

7.2.9. 16S-PCR TRFLP

TRFLP is a method of “profiling” bacterial communities in DNA extracted from water or other environmental samples. Here, TRFLP was used as one of two community analysis approaches, the other being PhyloChip (Section 7.2.10). The rationale for using community analysis in this study was that such methods allow for interrogating a large amount of the entire bacterial community in a water sample, regardless of bacterial culturability. Neither approach encompasses the entire diversity of any environmental sample; no method as such is currently available as microbial community diversity is still vastly understood for all environments. However, each method provides for more comprehensively examining bacterial community differences between samples, and each has the potential to reveal individual taxa or taxa groups that prevail in the Effluent and thus which could be used for tracing the effluent plume within data sets acquired for samples from the near shore environment. Both methods were used because TRFLP is relatively inexpensive and rapid to perform, but PhyloChip, while more expensive and slower, yields significantly more detailed information. The two methods, except for using the same extracted DNA, are performed completely independently of one another.

Therefore, where communities are to be compared across sites and events, similar patterns using each method could indicate a lack of significant bias in either method.

As described in Section 5.6, PCR-TRFLP (here referred to as TRFLP) was performed for 26 events for which DNA was extracted from all samples (Appendix Section 7.2, Table 19). TRFLP profiles were acquired for all samples in each of the 26 events. For the additional 24 events for which DNA was extracted from only the Effluent samples, TRFLP was not performed due to budgetary constraints.

Taxa richness, as estimated in TRFLP by the number of terminal restriction fragments (TRFs) in a profile for a sample, ranged from 6 to 23 (Table 7.2-16; Appendix Section 7.2, Table 19). Across all dates, samples from the Shoreline site had significantly lower TRF richness as compared to either the Offshore1000 or Effluent samples (Table 7.2-16). Taxa evenness, a measure of the proportional distribution of TRFLP signal across the profile TRFs, was (as per Section 5.6.9) calculated as a "Pielou's evenness" index. Across all events, TRF evenness was relatively similar across sites (Appendix Section 7.2, Table 19), except that the average evenness of the Effluent samples was relatively higher than the average evenness of the Shoreline samples (Table 7.2-17). Taxa diversity, calculated as a Shannon index, was relatively similar across all sampling sites for all events (Appendix Section 7.2, Table 19), except that, across all events, the average diversity of the bacterial communities in the Effluent and Offshore1000 samples were each significantly higher than the average diversity of the bacterial communities in the Shoreline samples (Table 7.2-18).

Table 7.2-16. Summary statistics overall and by site for taxa (TRF) richness (S) as calculated from the 16S-PCR TRFLP data. Values are expressed as number of peaks. SD = standard deviation, SE = standard error, Min = minimum value, Max = maximum value. Superscripts indicate sites with significant difference. When analyzed by site, the Shoreline samples were significantly different from the Offshore1000 and Effluent samples. (One-Way ANOVA with Dunnett's T3, $\alpha = 0.05$).

	Overall	**Offshore1000[a]**	**Diffuser**	**Lagrangians**	**Shoreline[a,b]**	**Effluent[b]**
Average	14	15	13	13	12	15
SD	3	3	3	2	2	4
SE	0	1	1	0	0	1
Min	6	10	9	10	8	6
Max	23	23	19	23	18	21
# samples	179	26	26	75	26	26

Table 7.2-17. Summary statistics overall and by site for evenness (J', Pielou's evenness) as calculated from the 16S-PCR TRFLP data. SD = standard deviation, SE = standard error, Min = minimum value, Max = maximum value. Superscripts indicate sites with significant difference. When analyzed by site, the Shoreline samples were significantly different from the Effluent samples. (One-Way ANOVA with Dunnett's T3, $\alpha = 0.05$).

	Overall	**Offshore1000**	**Diffuser**	**Lagrangians**	**Shoreline[a]**	**Effluent[a]**
Average	0.7116	0.7232	0.6984	0.7030	0.6792	0.7700
SD	0.0816	0.0664	0.0685	0.0739	0.0712	0.1089

SE	0.0061	0.0130	0.0134	0.0085	0.0140	0.0214
Min	0.4584	0.5485	0.5432	0.5451	0.5595	0.4584
Max	0.8973	0.8455	0.8197	0.8423	0.7947	0.8973
# samples	179	26	26	75	26	26

Table 7.2-18. Summary statistics overall and by site for taxa (TRF) diversity (H', Shannon diversity index, $\log_e$) as calculated from the 16S-PCR TRFLP data. SD = standard deviation, SE = standard error, Min = minimum value, Max = maximum value. Superscripts indicate sites with significant difference. When analyzed by site, the Shoreline samples were significantly different from the Offshore1000 and Effluent samples. (One-Way ANOVA with Dunnett's T3, $\alpha = 0.05$).

	Overall	**Offshore1000[a]**	**Diffuser**	**Lagrangians**	**Shoreline[a,b]**	**Effluent[b]**
Average	1.849	1.937	1.785	1.807	1.697	2.096
SD	0.315	0.243	0.241	0.245	0.230	0.503
SE	0.024	0.048	0.047	0.028	0.045	0.099
Min	1.007	1.363	1.251	1.307	1.288	1.007
Max	2.688	2.318	2.273	2.384	2.277	2.688
# samples	179	26	26	75	26	26

TRF richness, evenness and diversity can be useful indices for comparing bacterial communities in samples that vary in space or in time. However, TRFLP data are most often analyzed as multivariate data sets to compare bacterial community profiles between sites or across time. As described in the Section 5.6.9, the common multivariate technique of non-metric Multi-Dimensional Scaling (MDS) was used for comparing overall similarities and differences between bacterial communities of different sites based on their TRFLP profiles. Across all sites and events, the bacterial communities in the Effluent samples appeared distinct from all other sites (Figure 7.2-5). The bacterial communities in the Effluent samples, across all events, were quite variable (Figure 7.2-5), but three were more distinct from the rest: those from 11/26/07, 5/19/08 and 7/7/08 (Figure 7.2-6). As above, Effluent samples from those dates were also distinct in their HBM and qPCR ENT contents, with concentrations highest across all samples for those events (Appendix Section 7.2, Tables 15 & 17). These were also events where the Effluent DNA yield was higher relative to Effluent DNA from all other events (Appendix Section 7.2, Table 11). Effluent FIB concentrations were not unusual for those events (Appendix Section 7.2, Table 7). Taken together, this suggests that Effluent DNA yields could have significantly affected the culture-independent results, particularly for the qPCR-based (HBM and ENT) methods. However, the bacterial community profiles for these three "key" dates appear more similar to one another than to all other samples (Figure 7.2-5). One explanation may be that qPCR-based markers (HBM and ENT) are such a small fraction of the entire Effluent microbial community, that variations in these marker abundances would not significantly affect the TRFLP profile.

When evaluated by TRFLP, DNA extracted from the Shoreline samples did not vary by the location of the sample (Figure 7.2-7), i.e. the beach aligned with the longitude of the last Lagrangian water sample.

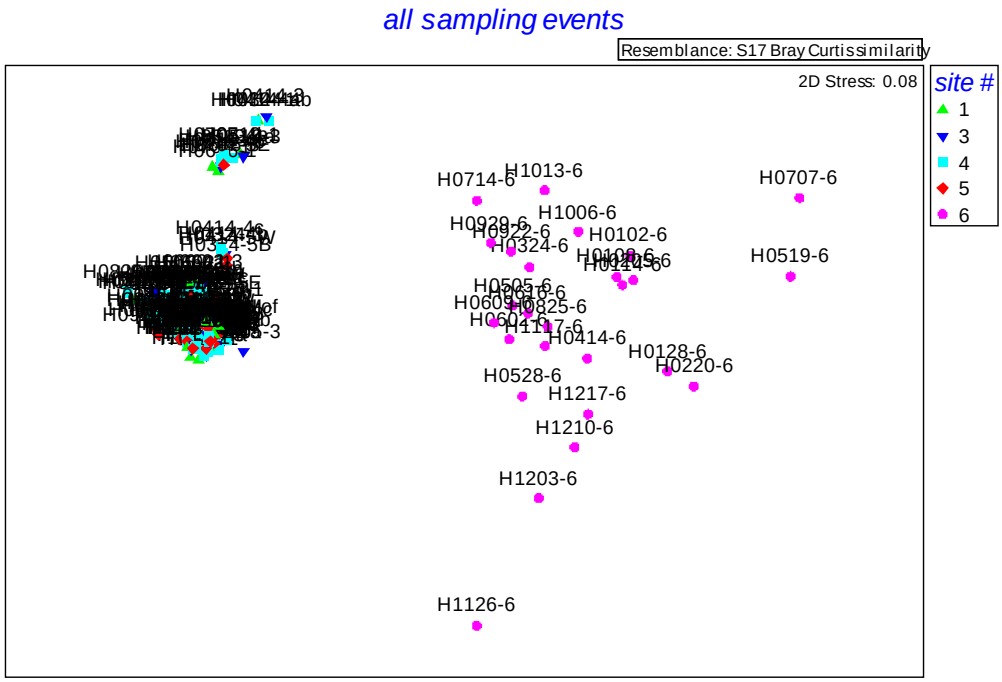


Figure 7.2-5. Non-metric Multi-Dimensional Scaling (MDS) plot of all samples grouped by site from the 26 selected sampling events for DNA-based analysis (stress = 0.08). Site numbers are 1 (Offshore1000), 3 (Diffuser), 4 (Lagrangians), 5 Shoreline, and 6 (Effluent). The Effluent samples appear distinct from the rest of the sites.

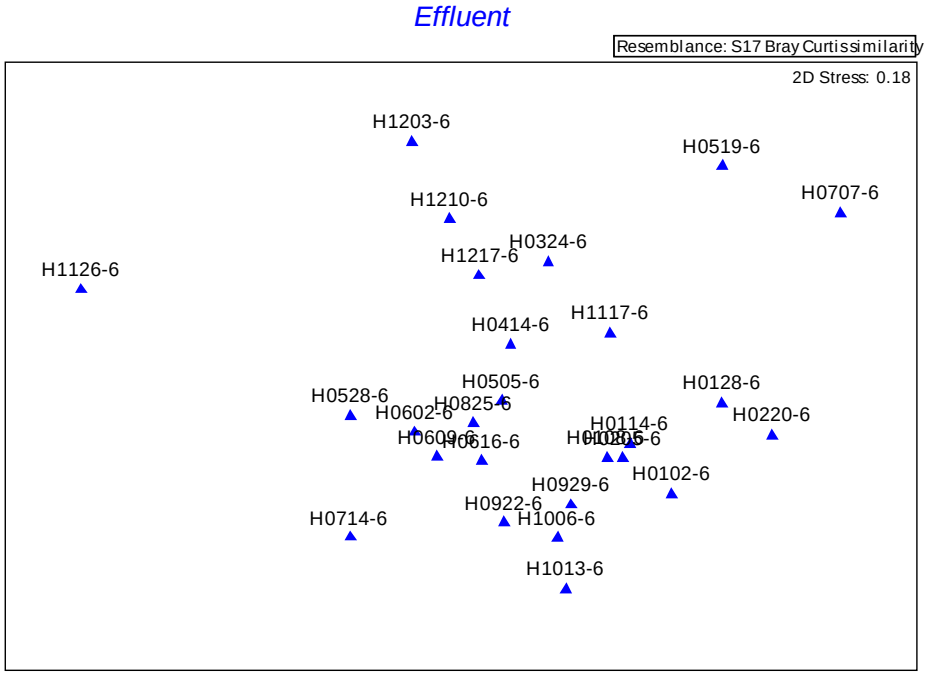


Figure 7.2-6. Non-metric Multi-Dimensional Scaling (MDS) plot of Effluent samples (stress = 0.18). Three dates (11/26/07, 5/19/08 and 7/7/08) appear distinct from the rest of the Effluent samples.

By TRFLP, the bacterial communities at the Offshore1000 and Diffuser sites were indistinguishable from one another (Figure 7.2-8), as were the Offshore1000, Lagrangians and Shoreline bacterial communities (Figure 7.2-9). The latter, in particular, would indicate that, by TRFLP, nearshore bacterial communities are not distinctive from communities offshore.

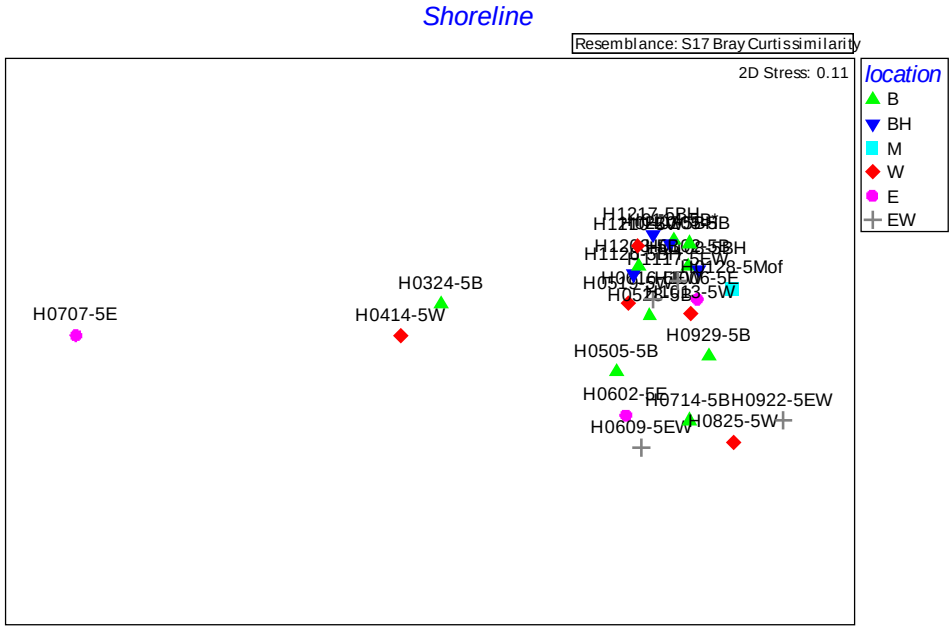


Figure 7.2-7. Non-metric Multi-Dimensional Scaling plot of Shoreline samples by beach designation (stress = 0.11). Beach designations are B (Butterfly Beach), BH (between Butterfly & Hammonds beaches), M (Miramar Beach), W (west end of Butterfly Beach, west of MSD outfall), E (East Beach), EW (between East Beach and west end of Butterfly Beach). There is no apparent grouping of the Shoreline samples by beach designation. Note that there is not a sample shown for Hammonds Beach as the sampling event for which drifters migrated towards Hammonds was not an event selected for DNA analysis.

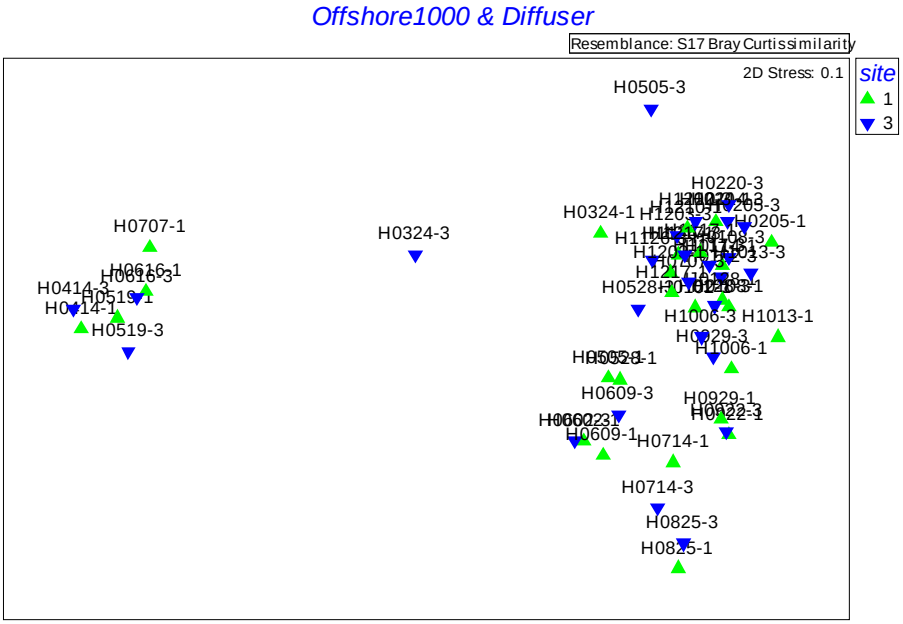


Figure 7.2-8. Non-metric Multi-Dimensional Scaling (MDS) plot of Offshore1000 and Diffuser samples (stress = 0.1). Site numbers are 1 (Offshore1000) and 3 (Diffuser). There is no apparent difference between the bacterial communities at the Offshore1000 and Diffuser site.

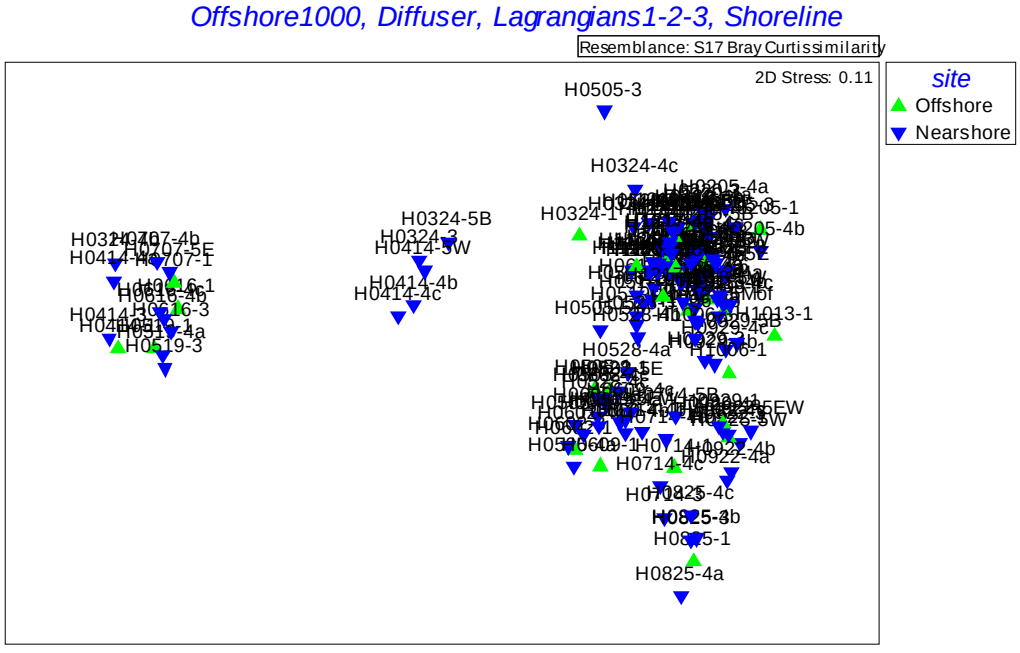


Figure 7.2-9. Non-metric Multi-Dimensional Scaling plot comparing the offshore to nearshore samples (stress = 0.11). Sites are offshore (Offshore1000) and nearshore (Diffuser, Lagrangians, Shoreline). There is no apparent difference between the offshore and nearshore bacterial communities.

Using an algorithm in the statistical software (Primer) used for MDS analysis (the SIMPER routine), it was determined that, across all sample events, TRFLP profiles for the "ocean" samples (Offshore1000, Diffuser, Lagrangians, and Shoreline) were more similar to each other (50-60% similarity) than TRFLP profiles in the Effluent samples were to one another (26% similarity; Table 7.2-19). This finding is consistent with the distribution of points representing TRFLP profiles in across event and across samples MDS plots (Figure 7.2-5) where Effluent bacterial communities appeared to be quite variable. Similarly, the multivariate statistical software allows for computing the "dissimilarity" between TRFLP profiles. By this approach, TRFLP profiles generated from DNA extracted from Effluent samples were more dissimilar to all other samples while the other samples (ocean) were less dissimilar to one another (Table 7.2-20).

Taken together, TRFLP data indicate that the bacterial communities in Effluent samples were distinct from all other samples, yet highly variable as a group.

Table 7.2-19. Average % similarity within the sample group for each site (via SIMPER analysis and MDS analysis). The ocean samples (Offshore1000, Diffuser, Lagrangians, and Shoreline) are more similar within their groups than the Effluent group.

Site	Average % Similarity within group
Offshore1000	51.15
Diffuser	52.25
Lagrangians	57.37
Shoreline	60.92
Effluent	26.15

Table 7.2-20. Average % dissimilarity between sample groups from each site (via SIMPER analysis and MDS analysis). The ocean samples (Offshore1000, Diffuser, Lagrangians, and Shoreline) when compared to each other were less dissimilar than when compared to the Effluent group.

Site comparison	Average % Dissimilarity between groups
Offshore1000 & Diffuser	47.71
Offshore1000 & Lagrangians	45.84
Offshore1000 & Shoreline	45.93
Diffuser & Lagrangians	44.54
Diffuser & Shoreline	43.42
Lagrangians & Shoreline	41.21
Offshore1000 & Effluent	97.32
Diffuser & Effluent	97.44
Lagrangians & Effluent	97.54
Shoreline & Effluent	97.52

7.2.10. PhyloChip

First, the microbial community composition by PhyloChip was compared using MDS analysis for all samples of all 8 sampling events (Fig. 7.2-10). A pf cutoff of 0.9 was used for this analysis. For this dataset (55 samples), 2627 OTUs on the PhyloChip were detected. The MDS plot shows distinct grouping of all Effluent samples, and of the Diffuser and all 3 Lagrangian samples on 01/28. Most of the other ocean samples (Diffuser, Lagrangian and Shoreline) grouped together. Note that the Offshore1000 (#1) and Shoreline (#5) samples on 01/28/08 did not group separately, but grouped with the remaining ocean samples. However, the 01/28/08 Effluent sample was distinct from the others. Finally, the Diffuser sample of 11/26/07 was also separated from the majority of the ocean samples.

Second, a more detailed analysis was performed of the microbial diversity for each group of samples: Effluent (site 6), Diffuser (site 3), Lagrangian samples (sites 4a, 4b, 4c) and Shoreline (site 5). The analysis focused on the differences between sites (by identifying distinct OTUs) and on the temporal variability of the microbial community composition (by identifying stable and variable OTUs).

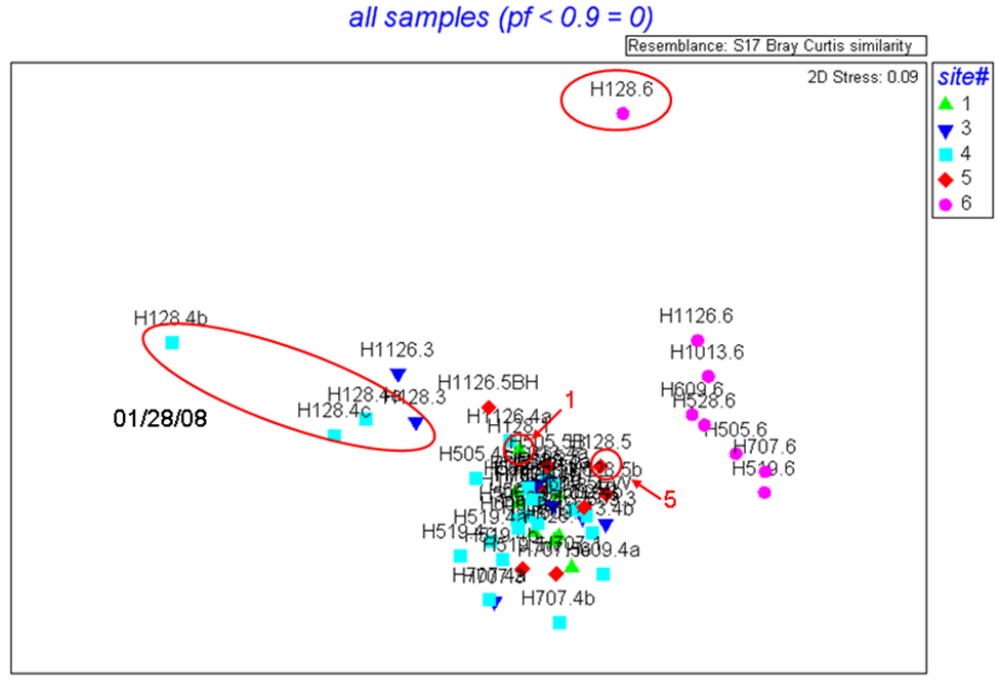


Figure 7.2.10. MDS plot showing similarities between PhyloChip-based microbial community composition of all samples of the eight sampling events selected for PhyloChip analysis. Symbols indicate the sites Offshore (1), Diffuser (3), Lagrangian a-c (4), Shoreline (5), and Effluent (6).

For the Effluent, 266 distinct OTUs were identified (indicative of the Effluent but not the Offshore1000 samples), where distinct was defined as fluorescence intensities of Effluent OTUs

being at least 5 times higher than those in the Offshore1000. Most distinct Effluent OTUs (56%) belonged to the families *Comamonadaceae, Enterobacteriaceae, Bacillaceae, Clostridiaceae* and *Lachnospiraceae* (Table 7.2-21). However, 46 other families also contained distinct OTUs. All of the 5 families containing most of the distinct Effluent OTUs are typically found in human fecal or WWTP activated sludge samples (Eckburg et al., 2005; Lu et al., 2009; Sanapareddy et al., 2009; Suau et al., 1999), and are generally not abundant or typical in ocean samples (Dang et al., 2008; Pommier et al., 2007; Venter et al., 2004).

Most of the 221 stable OTUs (52%) in the Effluent belonged to the classes *Actinobacteria*, *Gammaproteobacteria*, *Bacilli* and *Clostridia* (Fig. 7.2-11). The families including most (22%) of the stable OTUs were *Staphylococcaceae* (*Bacilli*), *Lachnospiraceae* (*Clostridia*), *Spirochaetaceaea* (*Spirochaetes*) and *Streptomycetaceae* (*Actinobacteria*). Most of the 220 variable OTUs (57%) belonged to the classes *Actinobacteria, Alphaproteobacteria, Betaproteobacteria* and *Epsilonproteobacteria* (Fig. 7.2-11). The families including most (29%) of the variable OTUs were *Helicobacteraceae* and *Campylobacteraceae* (*Epsilonproteobacteria*), *Mycobacteriaceae* (*Actinobacteria*), and *Flavobacteriaceae* (*Flavobacteria*). Therefore, the distinct OTUs were not necessarily the most stable or variable ones, except for the *Lachnospiraceae*.

A more detailed look at the variable OTUs indicated that the *Helicobacteraceae* and *Campylobacteriaceae* (containing 16% of the variable OTUs) exhibited consistently higher abundances on 11/26/07 and lower abundances on 05/05/08. The *Mycobacteriaceae* (9% of variable OTUs) showed consistently high abundances on 01/28/08 and low abundances on 10/13/08. The latter OTUs are likely responsible for the separate grouping of the 01/28/08 Effluent sample in Fig. 7.2-10. The grouping of the *Flavobacteriaceae* did not appear consistently high or low for certain sampling events.

Table 7.2-21. Number of OTUs indicative of site 6 (Effluent) and not site 1 (Offshore1000) by family, expressed as total numbers (Total) and percent of total number (% total). Unclassified indicates that the OTUs could not be classified at the family level.

Count of Family Fold Diff>=5		
Family	Total	% total
Acidithiobacillaceae	1	0.37593985
Acidobacteriaceae	1	0.37593985
Actinosynnemataceae	1	0.37593985
Aeromonadaceae	3	1.12781955
Alcaligenaceae	2	0.7518797
Alteromonadaceae	1	0.37593985
Bacillaceae	29	10.9022556
Beijerinck/Rhodoplan/Methylocyst	2	0.7518797
Burkholderiaceae	4	1.5037594
Carnobacteriaceae	1	0.37593985
Caryophanaceae	1	0.37593985
Clostridiaceae	27	10.1503759
Comamonadaceae	41	15.4135338
Crenotrichaceae	2	0.7518797
Desulfobacteraceae	1	0.37593985
Enterobacteriaceae	36	13.5338346
Enterococcaceae	7	2.63157895
Erysipelotrichaceae	3	1.12781955
Flavobacteriaceae	3	1.12781955
Gordoniaceae	1	0.37593985
Halobacillaceae	2	0.7518797
Hyphomicrobiaceae	1	0.37593985
Kineosporiaceae	1	0.37593985
Lachnospiraceae	17	6.39097744
Lactobacillaceae	1	0.37593985
Moraxellaceae	1	0.37593985
Mycobacteriaceae	1	0.37593985
Neisseriaceae	1	0.37593985
Nitrosomonadaceae	1	0.37593985
Nitrospiraceae	3	1.12781955
Nocardiaceae	2	0.7518797
Oxalobacteraceae	3	1.12781955
Paenibacillaceae	2	0.7518797
Peptococc/Acidaminococc	2	0.7518797
Peptostreptococcaceae	6	2.2556391
Polyangiaceae	1	0.37593985
Porphyromonadaceae	1	0.37593985
Prevotellaceae	1	0.37593985
Procabacteriaceae	1	0.37593985
Pseudomonadaceae	5	1.87969925
Rhodocyclaceae	6	2.2556391
Rickettsiaceae	1	0.37593985
Sphingomonadaceae	1	0.37593985
Sporolactobacillaceae	1	0.37593985
Staphylococcaceae	4	1.5037594
Streptococcaceae	4	1.5037594
Thermoactinomycetaceae	1	0.37593985
Thiotrichaceae	1	0.37593985
Unclassified	20	7.51879699
Verrucomicrobia subdivision 5	1	0.37593985
Xanthomonadaceae	6	2.2556391
Grand Total	266	

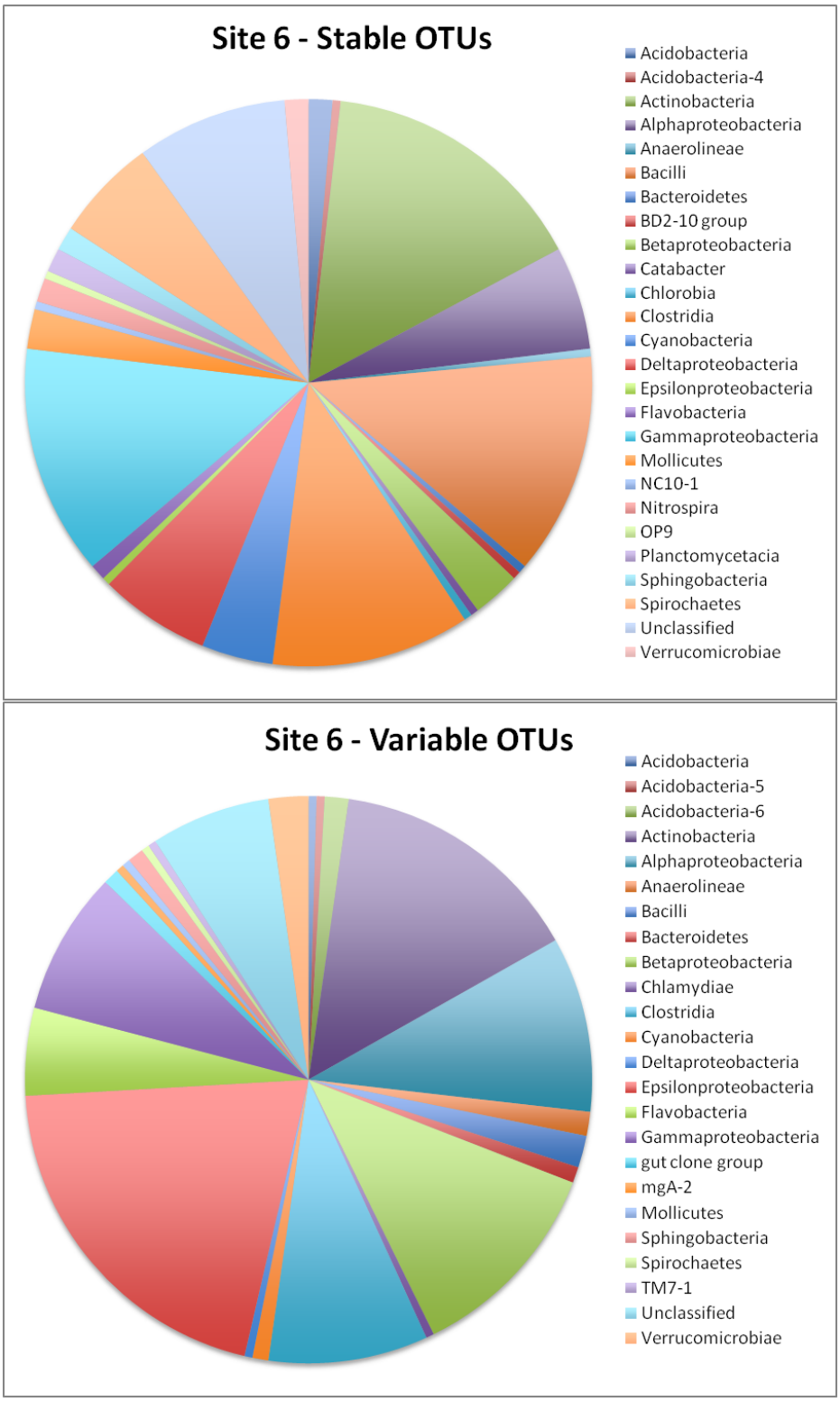


Figure 7.2-11. Stable and variable OTUs in site 6 (Effluent), aggregated into class level.

For the Offshore1000 samples, 231 distinct OTUs were identified (indicative of the Offshore1000 but not the Effluent samples) (Table 7.2-22), with fluorescence intensities in the distinct Offshore1000 OTUs at least two times higher than those in the Effluent. Those distinct Offshore1000 OTUs mostly belonged to the *Rhodobacteraceae* (23%), but also to the *Flavobacteraceae* (6%) and *Alteromonadaceae* (4%). Chloroplasts were also identified due to the similarity of their genes to bacterial genes encoding 16S rRNA. A total of 58 families were identified. The family *Rhodobacteraceae*, containing most of the distinct Offshore1000 OTUs, contains bacteria that are typically widely distributed and of high abundance in ocean environments (Dang et al., 2008; Du et al., 2006), while *Alteromonadaceae* are marine bacteria typical found in lower abundances (Allers et al., 2007).

Most of the 188 stable Offshore1000 OTUs belonged to the classes *Gammaproteobacteria* (37%), *Alphaproteobacteria* (13%) and *Betaproteobacteria* (14%) (Figure 7.2-12). The families including most (29%) of the stable OTUs were *Enterobacteriaceae* and *Pseudomonadaceae* (*Gammaproteobacteria*), *Spirochaetaceaea* (*Spirochaetes*) and *Comamonadaceae* (*Betaproteobacteria*). Most of the 187 variable OTUs (54%) belonged to the classes *Alphaproteobacteria*, *Flavobacteria* and *Cyanobacteria*. The families including most (38%) of the variable OTUs were *Rhodobacteraceae* (*Alphaproteobacteria*), *Flavobacteriaceae* (*Flavobacteria*) and Chloroplasts (*Cyanobacteria*).

The *Rhodobacteraceae* (containing 16% of the variable OTUs) showed fairly consistent higher abundances on 01/28/08 and lower abundances on 07/07/08. The Chloroplasts (7% of variable OTUs) showed fairly consistent high abundances on 10/13/08 and low abundances on 11/26/07. For *Flavobacteriaceae*, a general trend of higher abundances on 05/05/08 was observed, although less consistent.

Table 7.2-22. Number of OTUs indicative of site 1 (Offshore1000) but not site 6 (Effluent) by family, expressed as total numbers (Total) and percent of total number (% total). Unclassified indicates that the OTUs could not be classified at the family level.

Count of Family Fold Diff>=2		
Family	Total	%Total
Acidimicrobiaceae	1	0.43290043
Acidithiobacillaceae	1	0.43290043
Acidobacteriaceae	1	0.43290043
Acidothermaceae	1	0.43290043
Alcanivoraceae	1	0.43290043
Alteromonadaceae	10	4.32900433
Bacteroidaceae	1	0.43290043
Bradyrhizobiaceae	1	0.43290043
Brucellaceae	1	0.43290043
Chlorobiaceae	1	0.43290043
Chloroplasts	12	5.19480519
Chromatiaceae	1	0.43290043
Clostridiaceae	1	0.43290043
Crenotrichaceae	1	0.43290043
Cryomorphaceae	1	0.43290043
Desulfobacteraceae	2	0.86580087
Dictyoglomaceae	1	0.43290043
Ectothiorhodospiraceae	1	0.43290043
Enterobacteriaceae	2	0.86580087
Eubacteriaceae	1	0.43290043
Flavobacteriaceae	13	5.62770563
Flexibacteraceae	4	1.73160173
Geobacteraceae	1	0.43290043
Halobacteroidaceae	1	0.43290043
Halomonadaceae	3	1.2987013
Helicobacteraceae	2	0.86580087
Hydrogenothermaceae	2	0.86580087
Hyphomonadaceae	1	0.43290043
Kineosporiaceae	1	0.43290043
Methylococcaceae	1	0.43290043
Microbacteriaceae	2	0.86580087
Micrococcaceae	1	0.43290043
Mycoplasmataceae	1	0.43290043
Phyllobacteriaceae	1	0.43290043
Pirellulae	1	0.43290043
Planctomycetaceae	1	0.43290043
Polyangiaceae	1	0.43290043
Prevotellaceae	1	0.43290043
Rhodobacteraceae	53	22.9437229
Rikenellaceae	4	1.73160173
SAR11	2	0.86580087
Sphingobacteriaceae	2	0.86580087
Sphingomonadaceae	2	0.86580087
Spirochaetaceae	1	0.43290043
Unclassified	81	35.0649351
Verrucomicrobia subdivision 5	1	0.43290043
Verrucomicrobia subdivision 7	1	0.43290043
Verrucomicrobiaceae	4	1.73160173
Grand Total	231	

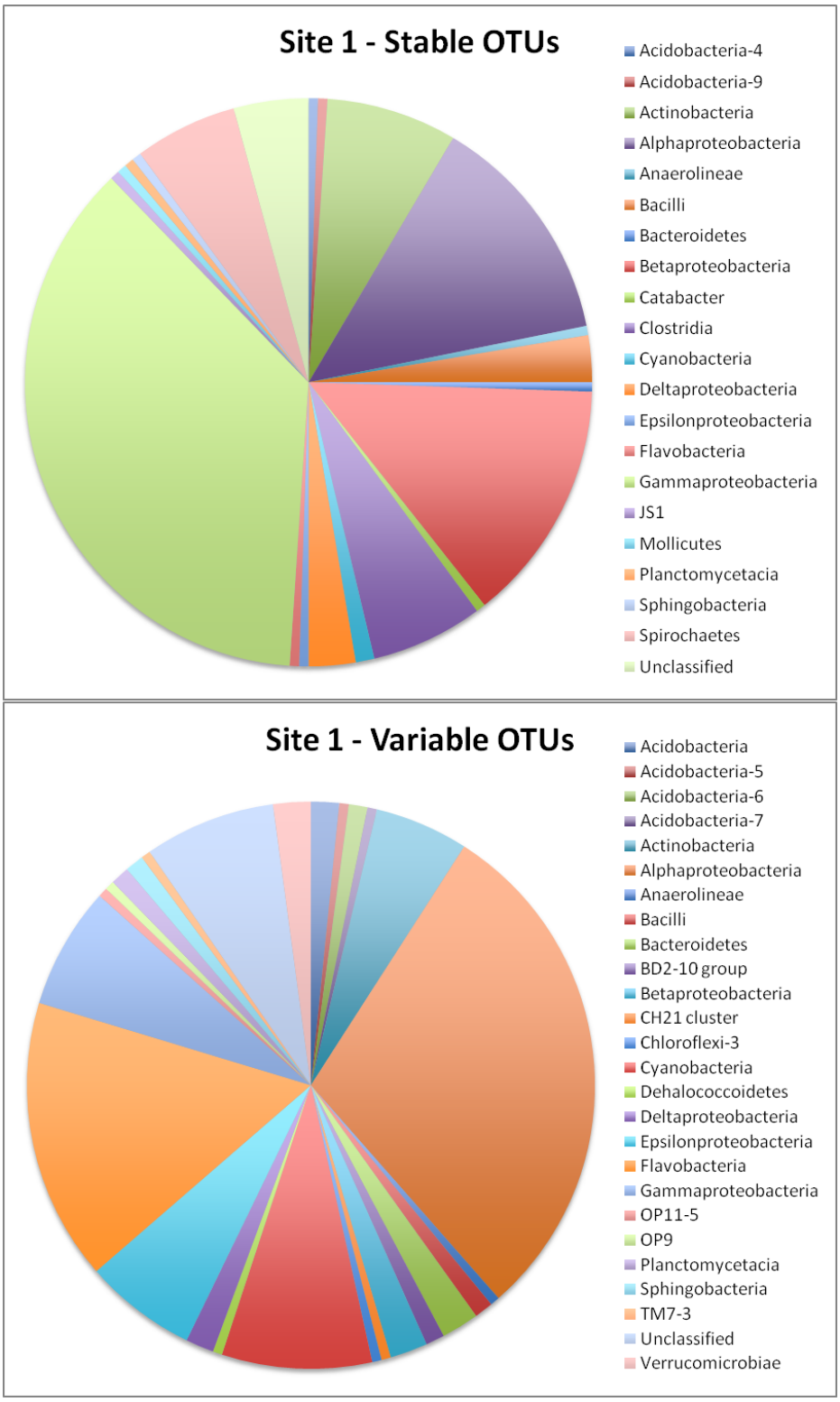


Figure 7.2-12. Stable and variable OTUs in site 1 (Offshore1000), aggregated into class level.

For the Diffuser, only 78 distinct OTUs were identified (Table 7.2-23), indicative of the Diffuser but not the Offshore1000 samples, with fluorescence intensities in the distinct Diffuser OTUs at least 1.5 times higher than those in the Offshore1000 samples. This indicates that most of the OTUs at the Diffuser also occurred in the Offshore1000 samples. Interestingly, most of the distinct OTUs (28%) belonged to the family *Comamonadaceae*, which was also identified as distinctive for the Effluent. This indicates the Effluent influences the microbial community composition at the Diffuser. There is lower diversity of OTUs in distinct Diffuser communities than in the distinct Offshore1000 and Effluent communities, most likely due to the similarity of the Diffuser communities to Offshore1000 communities as illustrated by the overall community analysis (Figure 7.2-10).

The analysis for stable and variable OTUs was performed on the Diffuser site grouped with all 3 Lagrangian samples, together defined as nearshore ocean samples. Most of the 216 stable near shore OTUs belonged to the classes *Gammaproteobacteria* (33%), *Alphaproteobacteria* (20%) and *Betaproteobacteria* (10%) (Figure 7.2-13). The families including most (23%) of the stable OTUs were *Enterobacteriaceae* and *Pseudomonadaceae* (*Gammaproteobacteria*), *Rhizobiaceae* (*Alphaproteobacteria*) and *Lachnospiraceae* (*Clostridia*). Most of the 216 variable OTUs (57%) belonged to the classes *Alphaproteobacteria*, *Epsilonproteobacteria* and *Flavobacteria*. The families including most (35%) of the variable OTUs were *Rhodobacteraceae* (*Alphaproteobacteria*), *Flavobacteriaceae* (*Flavobacteria*) and *Helicobacteraceae* (*Epsilonproteobacteria*). The family and class affiliations of the stable and variable OTUs in the near shore samples were a mix of those found in the Offshore1000 and Effluent samples.

In general, the patterns of high and low abundances for the variable families were slightly different between nearshore locations. *Rhodobacteraceae* (containing 15% of the variable OTUs) intensities were highest on 1/28/08 for the Lagrangian samples, but were highest on 11/26/07 for the Diffuser sample. The lowest *Rhodobacteraceae* abundances were generally observed on 06/09/08 and 07/07/08, except for location 4c (Lagrangian3), for which lowest abundances occurred on 10/13/08. The *Helicobacteraceae* (9% of variable OTUs) abundances followed the same patterns as the *Rhodobacteraceae*. For *Flavobacteriaceae*, the patterns were less consistent. At the Diffuser, *Flavobacteriaceae* were generally most abundant on 11/26/07. At 4a (Lagrangian1) *Flavobacteriaceae* were generally most abundant on 11/26/07 and 01/28/08. At 4b (Lagrangian2), *Flavobacteriaceae* were generally most abundant on 01/28/08 and least abundant on 07/07/08 and 10/13/08. At 4c (Lagrangian3) *Flavobacteriaceae* were generally most abundant on 05/05/08 and least abundant on 10/13/08.

The variable OTUs belonging to the families *Rhodobacteraceae*, *Helicobacteraceae* and *Flavobacteriaceae* are likely responsible for the separate grouping of the nearshore samples on 01/28/08 in Figure 7.2-10. Those families were identified before as containing distinct OTUs of the Offshore1000 samples (*Rhodobacteraceae*, *Flavobacteriaceae*), and a large part of the variable OTUs in the Offshore1000 (*Rhodobacteraceae*, *Flavobacteriaceae*) and Effluent (*Helicobacteraceae*) samples. Therefore, the separate grouping of the 01/28/08 nearshore samples appears to be caused by mostly typical Offshore1000 taxa, and some Effluent taxa.

Table 7.2-23. Number of OTUs indicative of site 3 (Diffuser) but not site 1 (Offshore1000) at the family level, expressed as total number (Total) and percent of total number (% total). Unclassified indicates that the OTUs could not be classified at the family level.

Count of Family		
Family	Total	%Total
Acidithiobacillaceae	1	1.28205128
Acidobacteriaceae	1	1.28205128
Alcaligenaceae	2	2.56410256
Alicyclobacillaceae	1	1.28205128
Alteromonadaceae	5	6.41025641
Beijerinck/Rhodoplan/Methylocyst	1	1.28205128
Bradyrhizobiaceae	3	3.84615385
Burkholderiaceae	1	1.28205128
Campylobacteraceae	1	1.28205128
Chromatiaceae	1	1.28205128
Clostridiaceae	2	2.56410256
Comamonadaceae	22	28.2051282
Corynebacteriaceae	1	1.28205128
Desulfobacteraceae	2	2.56410256
Helicobacteraceae	1	1.28205128
Hyphomicrobiaceae	3	3.84615385
Methylobacteriaceae	1	1.28205128
Mycobacteriaceae	2	2.56410256
Nitrosomonadaceae	1	1.28205128
Oxalobacteraceae	2	2.56410256
Peptostreptococcaceae	2	2.56410256
Pseudomonadaceae	3	3.84615385
Rhodobacteraceae	1	1.28205128
Rhodocyclaceae	1	1.28205128
Sphingomonadaceae	2	2.56410256
Spirochaetaceae	1	1.28205128
Unclassified	13	16.6666667
Verrucomicrobiaceae	1	1.28205128
Grand Total	78	

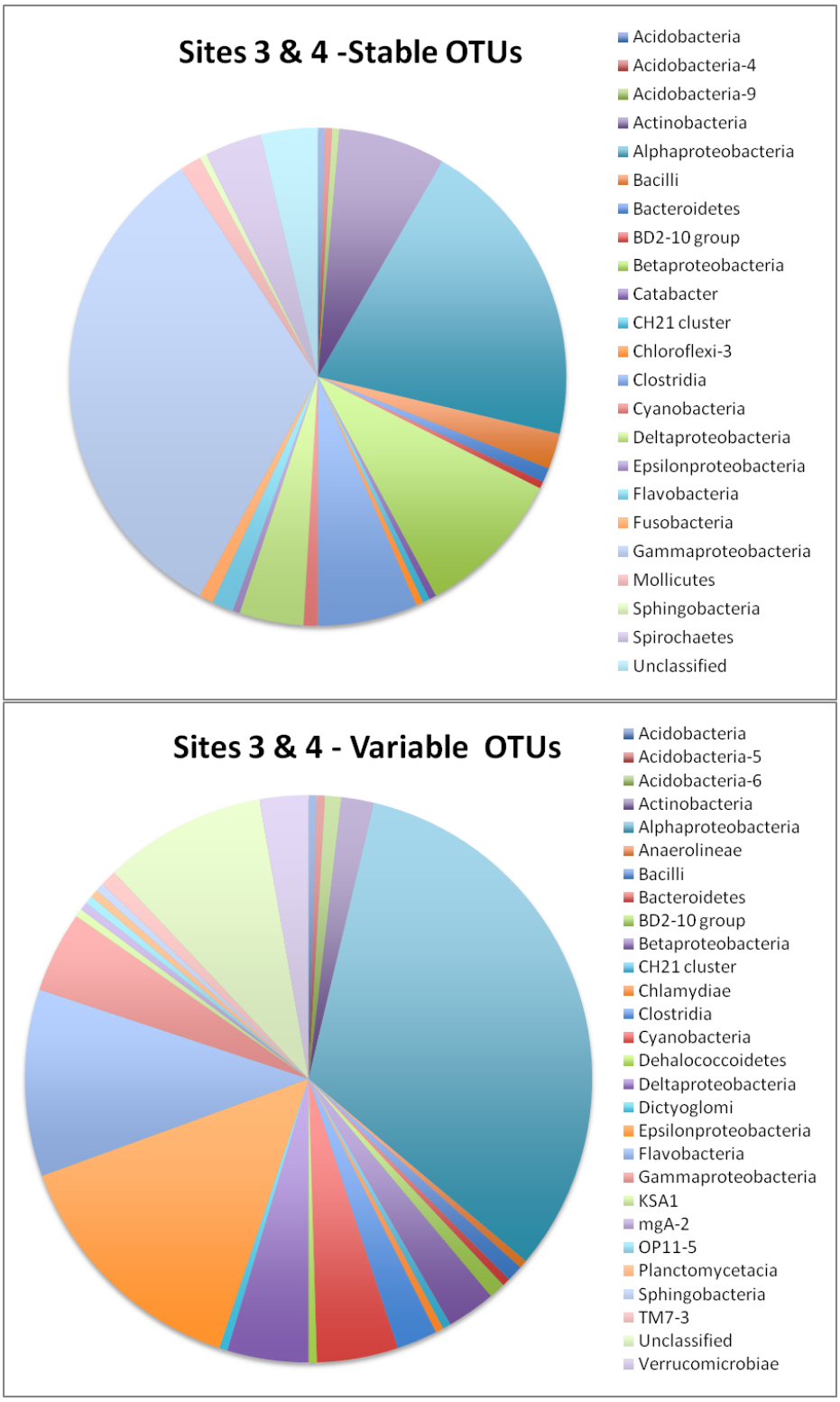


Figure 7.2-13. Stable and variable OTUs in sites 3 (Diffuser) and 4 (near shore, Lagrangians1, 2, and 3), aggregated into class level.

Finally, an assessment of the distinct Shoreline versus Lagrangian OTUs was performed. 229 distinct OTUs were mainly found in the Shoreline but not in the Lagrangian samples, with fluorescence intensities in the distinct Shoreline OTUs at least 1.5 times higher than those in the Lagrangian samples. Most OTUs belonged to the families *Clostridiaceae* (12%), *Lachnospiraceacea* (7%) and *Bradyrhizobiaceae* (5%) (Table 7.2-24). However, only 35 OTUs were found indicative of the Lagrangian samples and not of the Shoreline samples, with fluorescence intensities in the distinct Lagrangian OTUs at least 1.5 times higher than those in the Shoreline samples. The top family was *Flavobacteriaceae* (9%). *Neisseriaceae, Nocardiaceae, Rhodobacteriaceae* and *Sphingomonadaceae* contained 6% of distinct OTUs each (Table 7.2-25). The higher number of distinct OTUs found in Shoreline compared to Lagrangian samples could indicate a possible source of bacteria near the Shoreline samples, besides the near shore waters. For instance, it has been demonstrated that beach sediments are reservoirs for bacteria (Ishii et al., 2006; Hartz et al., 2008; Yamahara et al., 2009), and wave action is a potential source of resuspended sediment-associated bacteria to the water column. Bacteria in the families of *Clostridiaceae* and *Lachnospiraceae* are commonly associated with fecal bacteria (Savichtcheva and Okabe, 2006; Wery et al., 2008). However, they are also found in various anaerobic environmental samples analyzed by PhyloChip (unpublished data, LBNL). *Flavobacteriaceae* and *Rhodobacteriaceae* were families identified as distinct Offshore1000 OTUs in the study (Table 7.2-11), thus, corroborating our observations.

Most of the 206 stable Shoreline OTUs belonged to the classes *Gammaproteobacteria* (37%), *Alphaproteobacteria* (15%) and *Clostridia* (9%) (Figure 7.2-14). The families including most (23%) of the stable OTUs were *Enterobacteriaceae* and *Alteromonadaceae* (*Gammaproteobacteria*), and *Spirochaetaceaea* (*Spirochaetes*). Most of the 206 variable OTUs (60%) belonged to the classes *Alphaproteobacteria*, *Epsilonproteobacteria* and *Flavobacteria*. The families including most (39%) of the variable OTUs were *Rhodobacteraceae* (*Alphaproteobacteria*), *Flavobacteriaceae* (*Flavobacteria*) and *Helicobacteraceae* (*Epsilonproteobacteria*), the same families as for the nearshore (combined Diffuser and Lagrangian) samples.

The abundance trends for all variable families were consistent, with higher abundances on 11/26/07 and lower abundances on 07/07/08 for *Rhodobacteraceae* (containing 17% of the variable OTUs), *Helicobacteraceae* (10% of variable OTUs), and *Flavobacteriaceae* (11% of variable OTUs). *Helicobacteraceae* showed generally low abundances on 05/19/08 as well.

Table 7.2-24. Number of OTUs indicative for site 5 (Shoreline) but not Lagrangian (site 4) samples at the family level, expressed as total number (Total) and percent of total number (% total). Unclassified indicates that the OTUs could not be classified at the family level.

Count of Family Fold Diff 5/4 >1.5		
Family	Total	% Total
Acidobacteriaceae	3	1.310044
Actinomycetaceae	1	0.436681
Aeromonadaceae	9	3.930131
Alcaligenaceae	1	0.436681
Alteromonadaceae	8	3.49345
Bacillaceae	1	0.436681
Bacteroidaceae	1	0.436681
Bradyrhizobiaceae	11	4.803493
Brucellaceae	1	0.436681
Cellulomonadaceae	1	0.436681
Chromatiaceae	1	0.436681
Clostridiaceae	27	11.79039
Coriobacteriaceae	1	0.436681
Crenotrichaceae	1	0.436681
Dermabacteraceae	1	0.436681
Desulfobacteraceae	6	2.620087
Desulfobulbaceae	5	2.183406
Desulfovibrionaceae	1	0.436681
Desulfuromonaceae	1	0.436681
Ectothiorhodospiraceae	1	0.436681
Enterobacteriaceae	6	2.620087
Flavobacteriaceae	2	0.873362
Fusobacteriaceae	1	0.436681
Halobacillaceae	1	0.436681
Helicobacteraceae	3	1.310044
Lachnospiraceae	17	7.423581
Legionellaceae	1	0.436681
Microbacteriaceae	1	0.436681
Micromonosporaceae	1	0.436681
Moraxellaceae	1	0.436681
Neisseriaceae	1	0.436681
Nitrosomonadaceae	1	0.436681
Nitrospiraceae	1	0.436681
Oceanospirillaceae	2	0.873362
Paenibacillaceae	1	0.436681
Peptococc/Acidaminococc	6	2.620087
Peptostreptococcaceae	10	4.366812
Pirellulae	1	0.436681
Piscirickettsiaceae	2	0.873362
Planctomycetaceae	1	0.436681
Polyangiaceae	4	1.746725
Porphyromonadaceae	7	3.056769
Prevotellaceae	4	1.746725
Pseudomonadaceae	4	1.746725
Pseudonocardiaceae	1	0.436681
Rhodobacteraceae	5	2.183406
Rikenellaceae	1	0.436681
Spirochaetaceae	7	3.056769
Sporolactobacillaceae	1	0.436681
Streptomycetaceae	9	3.930131
Syntrophaceae	1	0.436681
Thermomonosporaceae	1	0.436681
Unclassified	30	13.10044
Verrucomicrobia subdivision 5	1	0.436681
Verrucomicrobiaceae	1	0.436681
Vibrionaceae	9	3.930131
Xanthomonadaceae	1	0.436681
Grand Total	229	

Table 7.2-25. Number of OTUs indicative of Lagrangian (site 4) and not Shoreline (site 5) samples at the family level, expressed as total number (Total) and percent of total number (% total). Unclassified indicates that the OTUs could not be classified at the family level.

Count of Family Fold Diff 4/5 >1.5	
Family	Total
Comamonadaceae	1
Desulfobacteraceae	1
Desulfovibrionaceae	1
Dietziaceae	1
Flavobacteriaceae	3
Neisseriaceae	2
Nocardiaceae	2
Oxalobacteraceae	1
Peptococc/Acidaminococc	1
Rhodobacteraceae	2
Sphingomonadaceae	2
Syntrophobacteraceae	1
Thermomonosporaceae	1
Unclassified	16
Grand Total	35

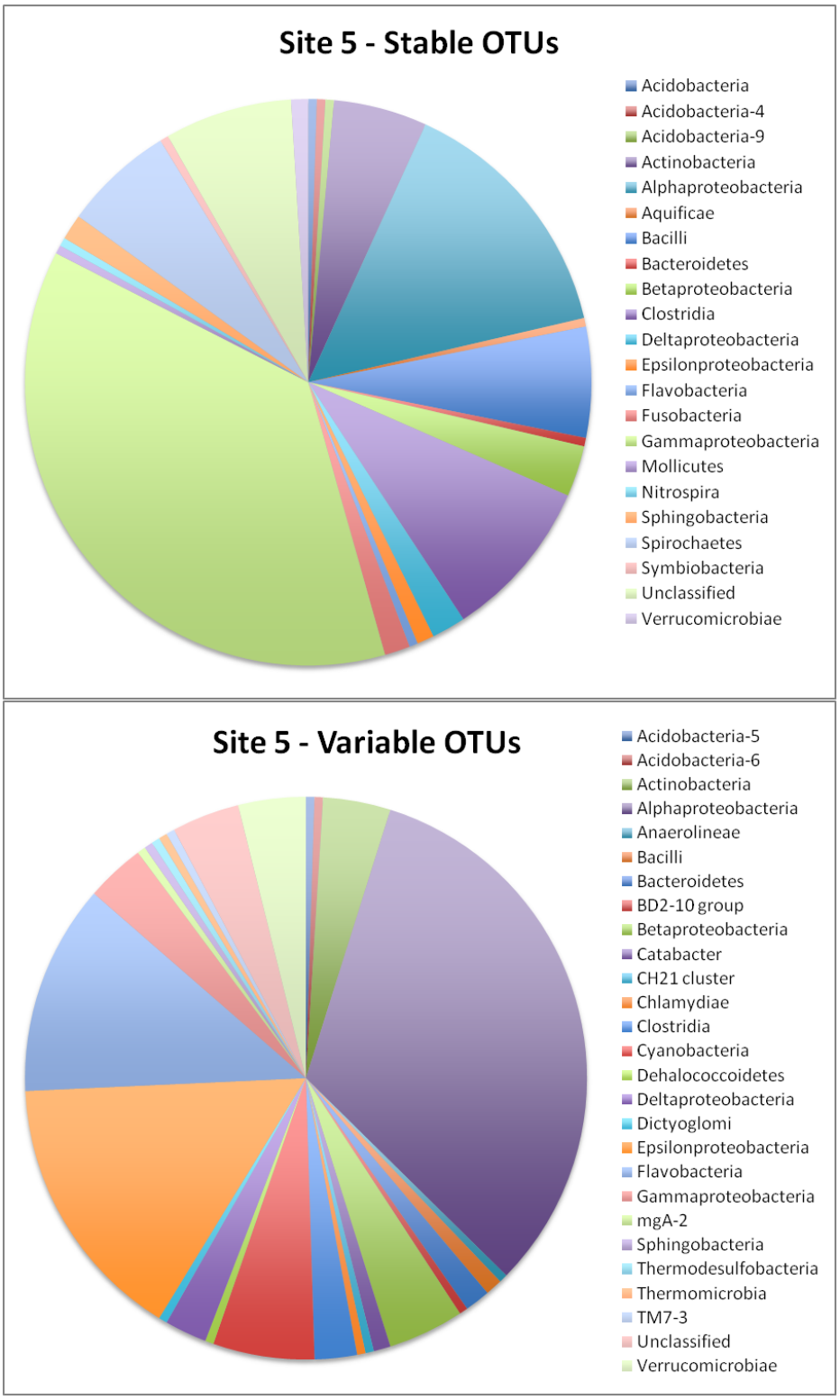


Figure 7.2-14. Stable and variable OTUs in site 5 (Shoreline), aggregated into class level.

Finally, a more detailed investigation of the variable and stable OTUs shared between locations was performed. This is necessary because shared families and classes can contain different OTUs.

In Table 7.2-26 and 7.2-27 an overview is presented of the number of OTUs shared between locations for the stable and variable OTUs, respectively. In addition, the dominant family affiliations of those shared OTUs and the number of OTUs belonging to those families are indicated.

For each location, 188 - 221 stable and variable OTUs were identified before. In general, more variable OTUs (40 – 157) were shared between locations compared to stable OTUs (27 – 87). The number of shared OTUs was always lowest between Offshore and Effluent, and slightly higher between Effluent and Diffuser/Lagrangian or Shoreline samples. The number of shared OTUs was highest between Diffuser/Lagrangian and Offshore or Shoreline samples.
Some families were shared between all locations (*Spirochaetaceae* and *Helicobacteraceae*), or all ocean locations (*Enterobacteriaceae, Flavobacteriaceae, Rhodobacteraceae, Chloroplasts*), and one was only shared between Effluent and Diffuser/Lagrangian and Shoreline samples (*Lachnospiraceae*).

In general, the shared OTUs suggest a microbiological link between Effluent and ocean (Offshore, Diffuser/Lagrangian or Shoreline) samples, although not as strong as the microbiological link between those ocean samples. The family affiliations suggest a long-term effect of the Effluent on the nearshore locations (not Offshore1000) by inoculating with *Lachnospiraceae*. This effect could only be observed through detailed PhyloChip analysis. Finally, the differences between the numbers of shared stable and variable OTUs suggest that the stable microbial populations are more distinct between locations, and that variations in microbial community composition are often shared by locations, in particular by Diffuser/Lagrangian and Shoreline locations. Because analysis of the stable OTUs suggests that *Lachnospiraceae* in the ocean are derived from the WWTP effluent, a more detailed analysis of the distribution of all OTUs in this family is presented in Section 7.2.11.3.6.

Table 7.2-26. Number of stable OTUs shared between Effluent, Diffuser/Lagrangian, Shoreline and Offshore locations. Total number of stable OTUs are indicated between parentheses for each location. Total number of stable OTUs shared between two locations are shown in bold, number of stable OTUs belonging to each family are added between parentheses.

STABLE	**Offshore (188)**	**Effluent (221)**	**Diffuser/Lagrangian (216)**
Effluent (221)	**27** *Spirochaetaceae* (7) *Lachnospiraceae* (0)		
Diffuser/ Lagrangian (216)	**87** *Spirochaetaceae* (7) *Enterobacteriaceae* (14) *Rhizobiaceae* (6)	**39** *Spirochaetaceae* (6) *Lachnospiraceae* (6)	
Shoreline (206)	**55** *Spirochaetaceae* (9) *Enterobacteriaceae* (7) *Pseudomonadaceae* (6)	**34** *Spirochaetaceae* (7) *Lachnospiraceae* (5)	**71** *Spirochaetaceae* (6) *Enterobacteriaceae* (9) *Rhizobiaceae* (7)

Table 7.2-27. Number of variable OTUs shared between Effluent, Diffuser/Lagrangian, Shoreline and Offshore locations. Total number of variable OTUs are indicated between parentheses for each location. Total number of variable OTUs shared between two locations are shown in bold, number of stable OTUs belonging to each family are added between parentheses.

VARIABLE	**Offshore (187)**	**Effluent (220)**	**Diffuser/Lagrangian (216)**
Effluent (220)	**40** *Helicobacteraceae* (8) *Flavobacteriaceae* (6)		
Diffuser/ Lagrangian (216)	**119** *Helicobacteraceae* (8) *Flavobacteriaceae* (14) *Rhodobacteraceae* (26) *Chloroplasts* (9)	**67** *Helicobacteraceae* (20) *Flavobacteriaceae* (3)	
Shoreline (206)	**109** *Helicobacteraceae* (8) *Flavobacteriaceae* (15) *Rhodobacteraceae* (27) *Chloroplasts* (8)	**62** *Helicobacteraceae* (21) *Flavobacteriaceae* (2)	**157** *Helicobacteraceae* (20) *Flavobacteriaceae* (19) *Rhodobacteraceae* (31) *Chloroplasts* (9) *Sphingomonadaceae* (8)

7.2.11. Possible Plume Tracers

7.2.11.1 Univariate Data

Similarly to the evaluation of salinity difference as a potential plume tracer (Section 7.1.6), we evaluated univariate microbiological (total coliform, *E. coli*, enterococcus, qPCR ENT, qPCR HBM, enterovirus, DNA yield, cell count, TRFLP OTU richness, TRFLP OTU diversity, TRFLP OTU evenness) and chemical (phosphate, nitrate/nitrite, ammonia) data for their potential to indicate the plume, independently of the salinity data. This effort acknowledges that the WWTP effluent can alter microbiological and chemical characteristics in the plume trajectory nonconservatively, as opposed to salinity which is a conservative tracer. For example, the continuous discharge of WWTP effluent may be altering microbiological characteristics if Effluent microbes are flourishing in the ocean, or if introduced nutrients are altering microbiological populations. While the microbial community composition overall (Sections 7.2.9 & 7.2.10) by either TRFLP or PhyloChip does not appear to be distinct in the Offshore 1000 versus nearshore (Diffuser plus Lagrangian) sites, it is still possible that either individual, perhaps rare, populations do vary across Offshore 1000 and nearshore sites. Similarly, the univariate measures may vary as well. The potential for univariate characteristics to indicate the plume is defined as the delta, i.e. where the value for a characteristic at the Diffuser site is greater than at the Offshore for a given date.

As per Figure 7.2.2, the phosphate P concentrations within the Effluent were rather constant and appeared to not vary with the concentrations of P within other samples. However, the concentrations of nitrate+nitrite did appear to vary in the ocean samples, especially between April and September 2008, in a pattern that was similar to the Effluent concentration pattern (Figure 7.2.3). Consistently, for 11 events between April and September, 2008, nitrate+nitrite concentrations met the criteria as plume tracers (Table 7.6-28). Ammonia concentrations did not meet the criteria as plume tracers for any events.

Enterococcus qPCR concentrations met the criteria a plume tracers, but for only 3 of 26 sampling events (Table 7.2-29). One of the events (7/7/08) was also one of the three events for which Effluent DNA concentrations were relatively high. However, qPCR ENT concentrations were very low overall and the patterns along the trajectory of Diffuser-Lagrangian, in two events (1/8/08 and 5/8/08), resulted in an apparent increase in concentration (Table 7.2-29). Thus, qPCR ENT could be regarded as a tracer only on 7/7/08.

HBM concentrations (Table 7.2-30) met the criteria as plume tracers for 5 events, two of which were also events in which DNA concentrations in the Effluent were relatively high (5/19/08 and 7/7/08). For both of these events, HBM met the criteria as a plume tracer. However, of the other dates, two (5/5/08 and 9/29/08) showed increasing HBM concentrations between the Effluent and the Diffuser, and one (11/17/08) indicated complete dilution of the effluent at the diffuser and beyond. Thus, HBM was a tracer only on 5/19/08 and 7/7/08.

Table 7.2-28. Possible plume tracers from nitrite+nitrate data. Eleven dates meet criteria (present in Effluent & Diffuser, absent at Offshore1000). Values are uM.

	4/14/2008	**5/5/2008**	**5/19/2008**	**5/28/2008**	**6/2/2008**	**6/9/2008**	**6/16/2008**	**7/14/2008**	**8/25/2008**	**9/22/2008**	**9/29/2008**
Effluent	521.96	708.49	590.08	1186.80	751.01	780.20	444.14	295.97	179.78	234.47	201.90
Diffuser	1.73	9.97	1.16	7.84	4.58	3.40	0.85	2.38	0.28	0.23	1.24
Lagrangian1	0.97	0	0	3.78	0.45	1.65	0	0.36	0.49	0.46	1.84
Lagrangian2	0.61	0	0	3.98	0.26	1.02	0	1.72	0.20	0.30	1.17
Lagrangian3	0.63	0	0	0	0.21	0	0	1.42	0.39	0.66	0
Shoreline	1.82	1.54	0	0.96	0.30	0	0	0.49	0.36	0.57	0
Offshore1000	0	0	0	0	0	0	0	0	0	0	0

Table 7.2-29. Possible plume tracers from *Enterococcus* qPCR data. Three dates (1/8/08, 5/8/08 & 7/7/08) meet criteria (present in Effluent & Diffuser, absent at Offshore1000). Values are average markers/L. Values in red are when only one of three analytical replicates amplified with the target (mean = standard error).

	1/8/2008	5/8/2008	7/7/2008
Effluent	4.2E+03	8.5E+02	2.6E+06
Diffuser	6.0E+02	4.0E+02	7.4E+03
Lagrangian1	9.2E+02	0	3.4E+03
Lagrangian2	1.4E+03	2.3E+03	0
Lagrangian3	3.2E+03	1.3E+03	0
Shoreline	3.0E+03	4.2E+03	2.1E+03
Offshore1000	0	0	0

Table 7.2-30. Possible plume tracers from human-specific *Bacteroides* qPCR data. Four dates (5/5/08, 5/19/08, 7/7/08, 9/29/08 & 11/17/08) meet criteria (present in Effluent & Diffuser, absent at Offshore1000). Values are average markers/L. Values in red are when only one of three analytical replicates amplified with the target (mean = standard error).

	5/5/2008	5/19/2008	7/7/2008	9/29/2008	11/17/2008
Effluent	1.2E+03	1.9E+06	3.5E+06	2.6E+02	4.4E+02
Diffuser	2.6E+03	7.0E+03	5.0E+03	1.2E+04	2.4E+02
Lagrangian1	0	5.8E+03	1.8E+04	1.1E+04	0
Lagrangian2	0	5.8E+02	0	5.0E+03	5.4E+03
Lagrangian3	0	1.8E+04	0	7.8E+03	0
Shoreline	0	0	1.5E+04	0	0
Offshore1000	0	0	0	0	0

7.2.11.2 TRFLP

An exhaustive analysis of TRFLP data was performed in order to identify potential plume tracers that met the stated criteria. Initially, tracer OTUs were sought that were in common across all events. None were identified as such, owing probably to the highly variable microbiological composition of the Effluent samples, but also due to variations in the microbial communities in the ocean across events. Since no tracer OTUs appeared in common across all events, each sampling event was then analyzed separately. For 3 events out of 26, 4 different OTUs were identified as potential tracers (Table 7.2-31). Note that, given the OTU richness reported herein (Table 7.2-16) each tracer then represents only approximately 5% of the richness for any given event, and is a small percentage of the total TRF signal for a given sample (Table 7.2-32). For one event (2/5/08), two OTUs appeared as possible tracers, but only one (OTU 570/1) appeared to decrease between the Effluent and the Diffuser (Table 7.2-31). For the other two events, two different OTUs were identified as potential tracers. While all three OTUs, across three different events, thus appeared to decrease from the Effluent into the Diffuser, the decrease in concentration was not consistent with a simple dilution. Still, given the well-recognized coarse resolution of TRFLP, which is intended mainly as a community profiling technique, it is worth

further evaluating the potential that these three OTUs may have for uniquely indicating the influence of effluent microbial communities in the near shore region.

Table 7.2-31. Possible plume tracers from 16S-PCR TRFLP data. Three dates (2/5/08, 6/2/08 & 11/17/08) have a total of 4 OTUs that meet criteria (present in Effluent & Diffuser, absent at Offshore1000). Values are in %peak height.

	2/5/2008	**2/5/2008**	**6/2/2008**	**11/17/2008**
	OTU 205/6	**OUT 570/1**	**OTU 88**	**OTU 77/8**
Effluent	1.3	4.5	4.4	4.4
Diffuser	1.7	1.2	1.3	1.1
Lagrangian1	1.5	0	1.4	1.1
Lagrangian2	1.3	0	0	1.1
Lagrangian3		0	0	0
Shoreline	0	0	0	1.1
Offshore1000	0	0	0	0

Table 7.2-32. Possible plume tracers from 16S-PCR TRFLP data. Three dates (2/5/08, 6/2/08 & 11/17/08) have a total of 4 OTUs that meet criteria (present in Effluent & Diffuser, absent at Offshore1000). Values are as fractions of raw OTU peak height to total peak height for that sample. OTU 77/8 on 11/17/08 is present in the Offshore1000 sample, but the fraction is equal to less than 1%, and is cutoff during the normalization process.

	2/5/2008	**2/5/2008**	**6/2/2008**	**11/17/2008**
	OTU 205/6	**OTU 570/1**	**OTU 88**	**OTU 77/8**
Effluent	146/12288	511/12288	281/6915	218/4998
Diffuser	93/5629	65/5629	79/6158	82/7635
Lagrangian1	69/4751	0/4751	91/6769	79/7344
Lagrangian2	79/5927	0/5927	57/6371	66/6210
Lagrangian3			85/9545	61/7077
Shoreline	0/3674	0/3674	65/11329	83/8116
Offshore1000	0/4369	0/4369	92/12381	64/10386 = < 1% cutoff

7.2.11.3. PhyloChip

7.2.11.3.1 Counts of signature OTUs

Signature OTUs are defined as OTUs indicative of the Effluent in at least one sampling event. This approach is potentially more sensitive for finding evidence of effluent plume microbes in the ocean compared to the distinct OTU approach, because it analyses the microbial communities separately for each sampling event, and not for the 8 sampling events overall. An overview of the number of signature OTUs identified for each sampling event, including the effect of pf cutoff value and inclusion of one or more subsequent Lagrangian samples, is presented in Figure 7.2-15.

The lowest numbers of signature OTUs were detected on 1/28/08 and 07/07/08, regardless of the number of Lagrangians included in the analysis. When considering signature OTUs detected in the Effluent/Diffuser only (hereafter referred to as Diffuser signature OTUs), the highest number of signature OTUs (up to 75) were detected on 05/05/08, 05/19/08 and 06/09/08. When considering signature OTUs detected in the Effluent/Diffuser and all Lagrangians (hereafter referred to as Diffuser/Lagrangian signature OTUs), the highest number of signature OTUs (up to 74) was detected on 05/28/08. Note that only 2 Lagrangian locations were sampled on 11/26/07. When including 1 or 2 Lagrangians, less than 30 signature OTUs were detected for all sampling events. The latter was surprising, as one would expect the number of signature OTUs to remain constant or decrease when including an increasing number of Lagrangian locations in the analysis, depending on the dilution of the effluent plume after it reached the ocean surface. To further validate the accuracy of signature OTUs to detect diluted WWTP effluent in the ocean, the distribution of those signature OTUs for all sampling events was compared for the scenarios of considering all or no Lagrangians (Section 7.2.11.3.2).

In order to investigate the overall patterns of OTUs that were included or excluded as signature OTUs, the number of OTUs belonging to each of the following groups was counted for each sampling event and pf cutoff (Figure 7.2-16):

- S: FI(#6) > 0 AND FI(#3) > 2 x FI(#1) (signature OTU)
- NS1: FI(#6) > 0 AND FI(#3) < 2 x FI(#1) AND FI(#3) > 0 (non-signature OTU, because the FIs at the Diffuser and Offshore1000 were too similar)
- NS3: FI(#6) > 0 AND FI(#3) = 0 (non-signature OTU, because the FI at the Diffuser was zero)
- NS6: FI(#6) = 0 (non-signature OTU, because the FI in the Effluent was zero)

The data indicate that at a low pf cutoff (0.7), the majority of the OTUs were not selected as signature OTUs because FI(#1) was too high (group NS1), but at the highest pf cutoff (1), the reason was most frequently that FI(#6) = 0 (group NS6).

Based on the OTU counts presented in Figures 7.2-15 and 7.2-16, a pf cutoff of 0.9 – 0.95 is optimal. Those cutoff values strike a balance between excluding OTUs as signature OTUs because FI(#1) is too high or because FI(#6) equals zero, and allow a maximal number of signature OTUs to be identified.

In addition, Figure 7.2-16 indicates that on 01/28/08, more OTUs were excluded as signature OTU because FI(#6) = 0, compared to the other sampling events. However, such a pattern was not observed that could explain the lower number of signature OTUs on 07/07/08. The low number of signature OTUs on 01/28/08 further indicates that the separate grouping of the 01/28/08 nearshore samples in Fig. 7.1 was not caused by a treated effluent plume effect, as was also suggested by analyzing the OTU phylogenetic affiliations.

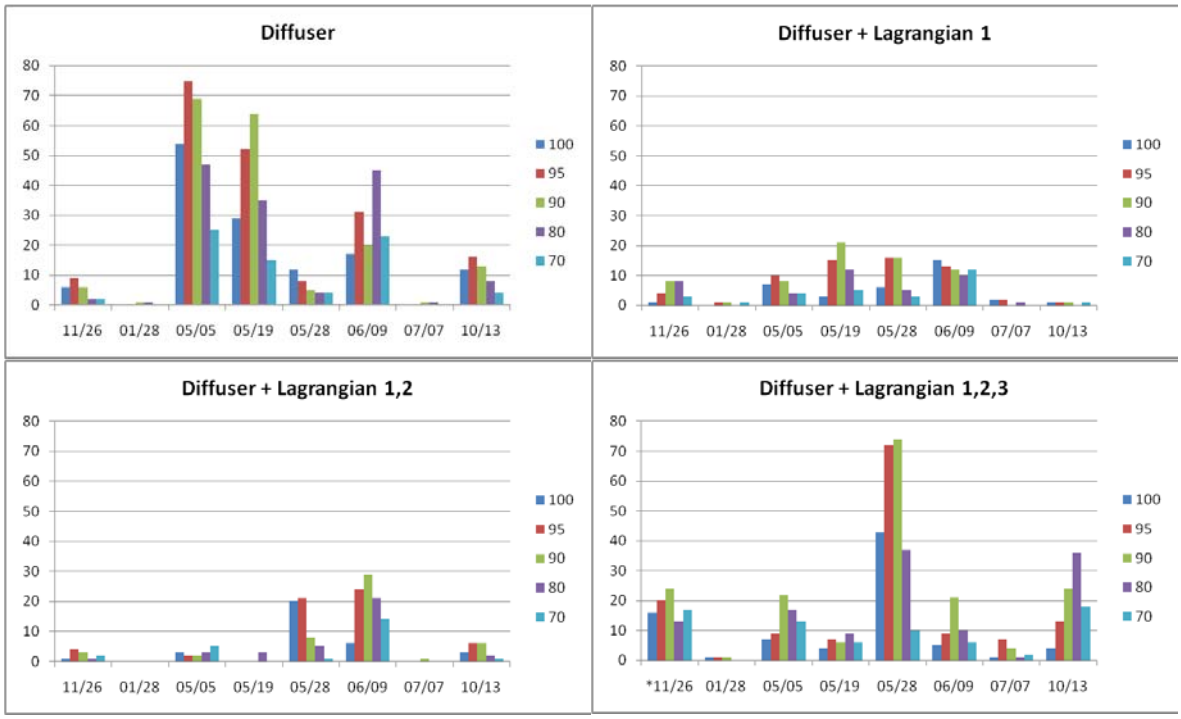


Figure 7.2-15. Number of signature OTUs detected at each sampling event, based on signature OTU detection at Diffuser, Diffuser + Lagrangian 1, Diffuser + Lagrangian1 and 2, Diffuser + Lagrangian 1,2 and 3 (irrespective of detection at Shoreline). Pf cutoff values ranged from 0.7 to 1. Note that only 2 Lagrangian samples were taken on 11/26/07, and those counts are presented in the graph showing "Diffuser + Lagrangian 1,2,3" as well (marked by *).

Figure 7.2-16. Percentage of OTUs present in each of the following groups: S (FI(#6) > 0 AND FI(#3) > 2 x FI(#1)); NS1 (FI(#6) > 0 AND FI(#3) < 2 x FI(#1) AND FI(#3) > 0); NS3 (FI(#6) > 0 AND FI(#3) = 0); NS6: (FI(#6) = 0). Pf cutoff values range between 0.7 and 1.

7.2.11.3.2 Distribution of signature OTUs across sampling events

To investigate to what extent signature OTUs are shared between sampling events, the number of signature OTUs detected in at least "x" sampling events ("x" from 1 – 8) were counted (Figure 7.2-17). Based on the results described in section 7.2.11.3.1, this analysis is only performed for pf cutoff values of 0.9 and 0.95. The counts of signature OTUs for each week are also shown in Figure 7.2-17 for comparison, in this case only for pf cutoff values of 0.9 and 0.95.

When considering Diffuser signature OTUs, about 80% of the OTUs were unique to each sampling event. Only 19-23 OTUs were shared by 2 sampling events, and 5 - 7 OTUs were shared by 3 sampling events.
When considering signature OTUs detected in the Effluent/Diffuser and one or two Lagrangians, only 4-7 OTUs were shared by 2 sampling events.
When considering Diffuser/Lagrangian signature OTUs, only 1-3 OTUs were shared by 2 sampling events.
Generally, the proportion of unique OTUs increased when including more Lagrangian samples in the analysis. When including two or more Lagrangian samples, none of the signature OTUs were shared by more than 2 sampling events.

A more detailed analysis of the distribution of signature OTUs across sampling events is presented in Figs. 7.2-18 and 7.2-19. This analysis was performed because signature OTUs identified for one sampling event also occurred during the remaining sampling events, although they were not identified as signature OTUs during the latter because the criteria (defined in 5.6.13.3) were not met. Two extreme scenarios can occur. In scenario 1, the signature OTUs identified on one sampling event are not detected for the remaining sampling events. This scenario represents the case of signature OTUs highly specific for the diluted effluent plume, but unique for each sampling event. In scenario 2, the signature OTUs identified on one sampling event are also detected at all locations during the other sampling events. This scenario represents the case of signature OTUs generally present in Effluent and all ocean samples, but still classified as signature OTUs for a given sampling event because of their absence in the Offhore1000 samples, probably due to temporal or spatial variability.

Figure 7.2-17. Counts of signature OTUs for each sampling event (left graphs). Counts of signature OTUs present in at least x sampling events (x = 1 – 8), based on signature OTU detection in at least Diffuser, Diffuser + Lagrangian 1, Diffuser + Lagrangian 1,2, and Diffuser + Lagrangian 1,2,3 samples (right graphs). Pf cutoff values are 0.9 and 0.95.

The Diffuser/Lagrangian signature OTUs defined for one sampling event were commonly detected at all locations during the other sampling events (Fig. 7.2-18). For instance, on 05/28/08, 74 Diffuser/Lagrangian signature OTUs were identified. Most of those 05/28 Diffuser/Lagrangian signature OTUs were detected during the other sampling events in the Effluent, Diffuser Lagrangian and Offshore1000 samples. Especially the frequent detection at the Offshore1000 is important, because it causes the 05/28/08 Diffuser/Lagrangian signature OTUs not to be classified as signature OTUs for the other sampling events. For instance, at least 50 of the seventy-four (74) 05/28/08 Diffuser/Lagrangian signature OTUs were detected at the Offshore1000 on all but one of the other sampling events. This pattern was observed for the Diffuser/Lagrangian signature OTUs of all sampling events, and was highly similar to scenario 2 mentioned above, suggesting that Diffuser/Lagrangian signature OTUs are not specific to the effluent plume but are commonly present in the nearshore and Offshore environment.

The Diffuser signature OTUs for each sampling event were also detected during the other sampling events, but less frequently compared to the Diffuser/Lagrangian signature OTUs, as is evident from the generally shorter bars in Fig. 7.2-19 compared to 7.2-18. More specifically, Diffuser signature OTUs of a given sampling event were less frequently detected in the Offshore1000 samples during the other sampling events. For instance, less than 30 of the 69 05/05/08 Diffuser signature OTUs were detected at the Offshore1000 locations on all but one of the other sampling events.
The distribution of the 06/09/08 Diffuser signature OTUs corresponded better to the ideal scenario 1 presented above, as most of the 20 Diffuser signature OTUs were not detected in the ocean during the other sampling events. Their common detection in the Effluent corroborates their usefulness as signature OTUs for the Effluent.

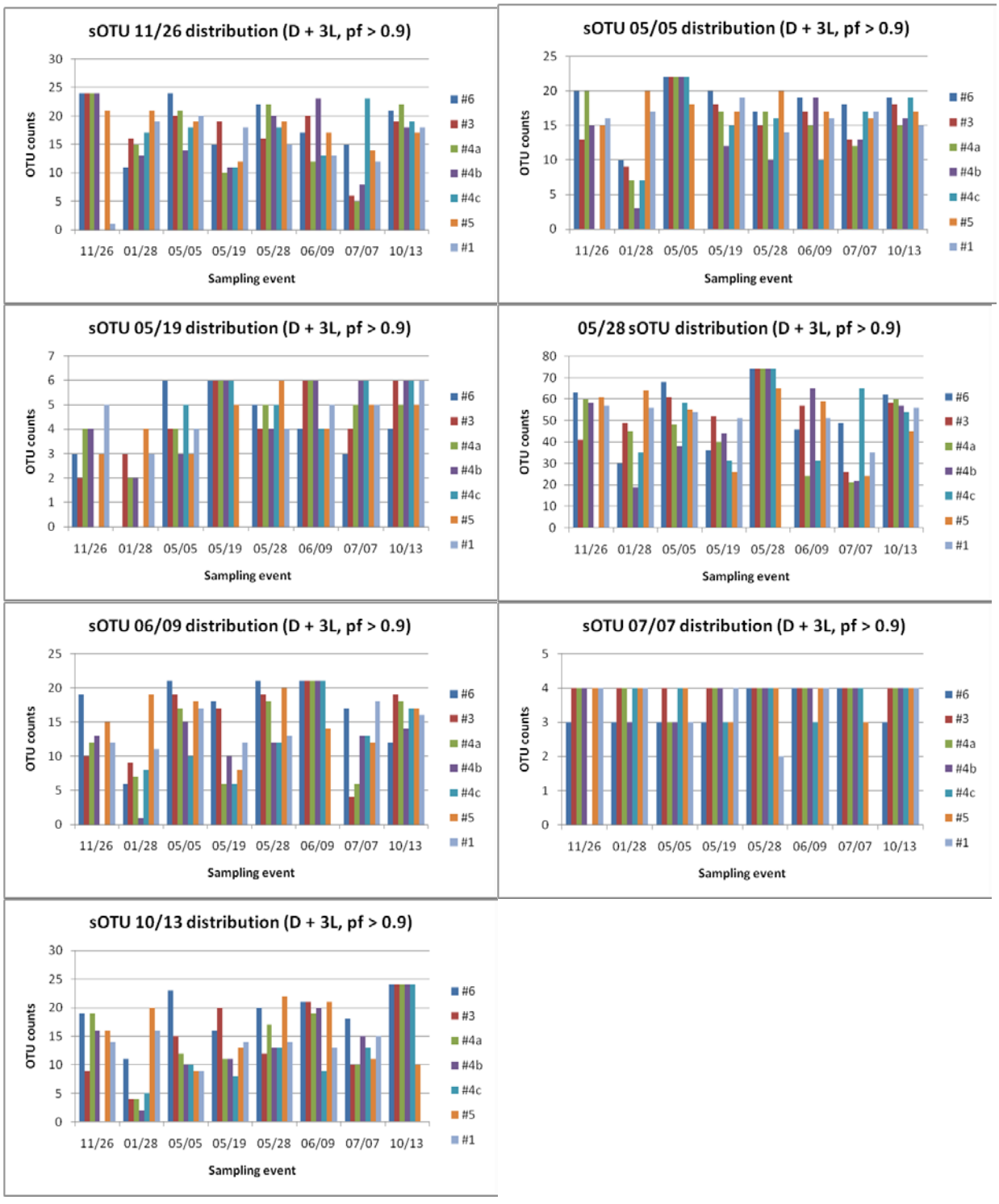


Figure 7.2-18. Distribution of Diffuser/Lagrangian signature OTUs across sampling events and locations. Each graph shows the number of Diffuser/Lagrangian signature OTUs identified for one sampling event, and the number of Diffuser/Lagrangian signature OTUs detected at all locations during the other sampling events. Pf is 0.9. Diffuser/Lagrangian signature OTUs were not presented for 01/28 because only 1 signature OTU was found.

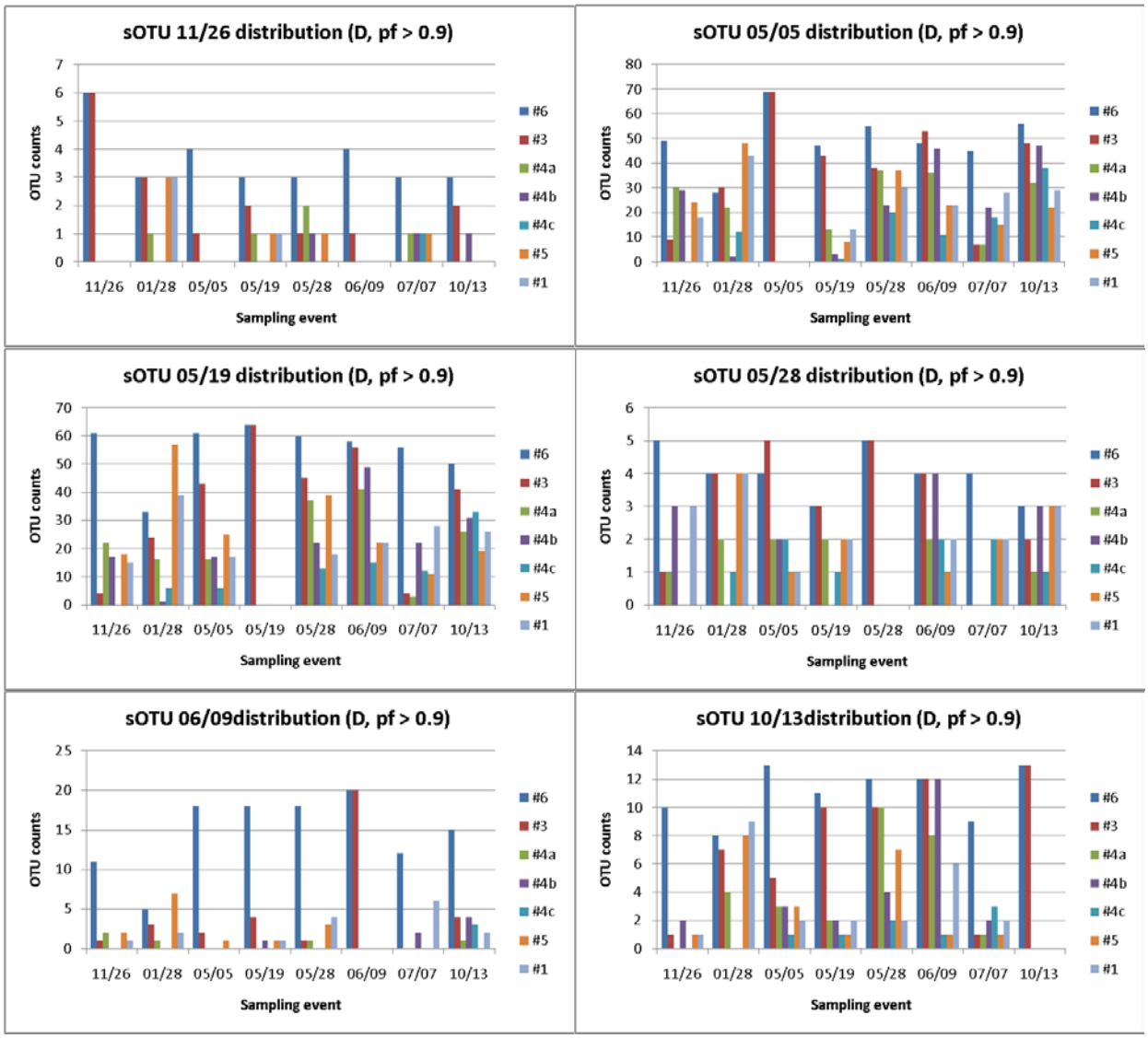


Figure 7.2-19. Distribution of Diffuser signature OTUs across sampling events and locations. Each graph shows the number of Diffuser signature OTUs identified for one sampling event, and the number of Diffuser signature OTUs detected at all locations during the other sampling events. Pf cutoff value is 0.9. Diffuser signature OTUs were not presented for 01/28 and 07/07 because only 1 signature OTU was found.

7.2.11.3.3 Phylogenetic affiliation of signature OTUs

The occurrence and distribution of signature families or classes (containing signature OTUs) across sampling events were investigated. This analysis was performed for Diffuser/Lagrangian signature OTUs (pf > 0.9 and pf > 0.95), and Diffuser signature OTUs (pf > 0.9).

Compared to the signature OTUs, the signature families/classes were more often shared across weeks, and the fraction of unique signature families/classes is lower (Figure 7.2-20). For Diffuser/Lagrangian signature OTUs, 2 – 3 families were shared on 4 sampling events (excluding "unclassified" families), and 3 classes were shared on 6 sampling events (excluding "unclassified" classes). For Diffuser signature OTUs, 3 families and classes (excluding "unclassified" taxa) were shared on 4 sampling events.

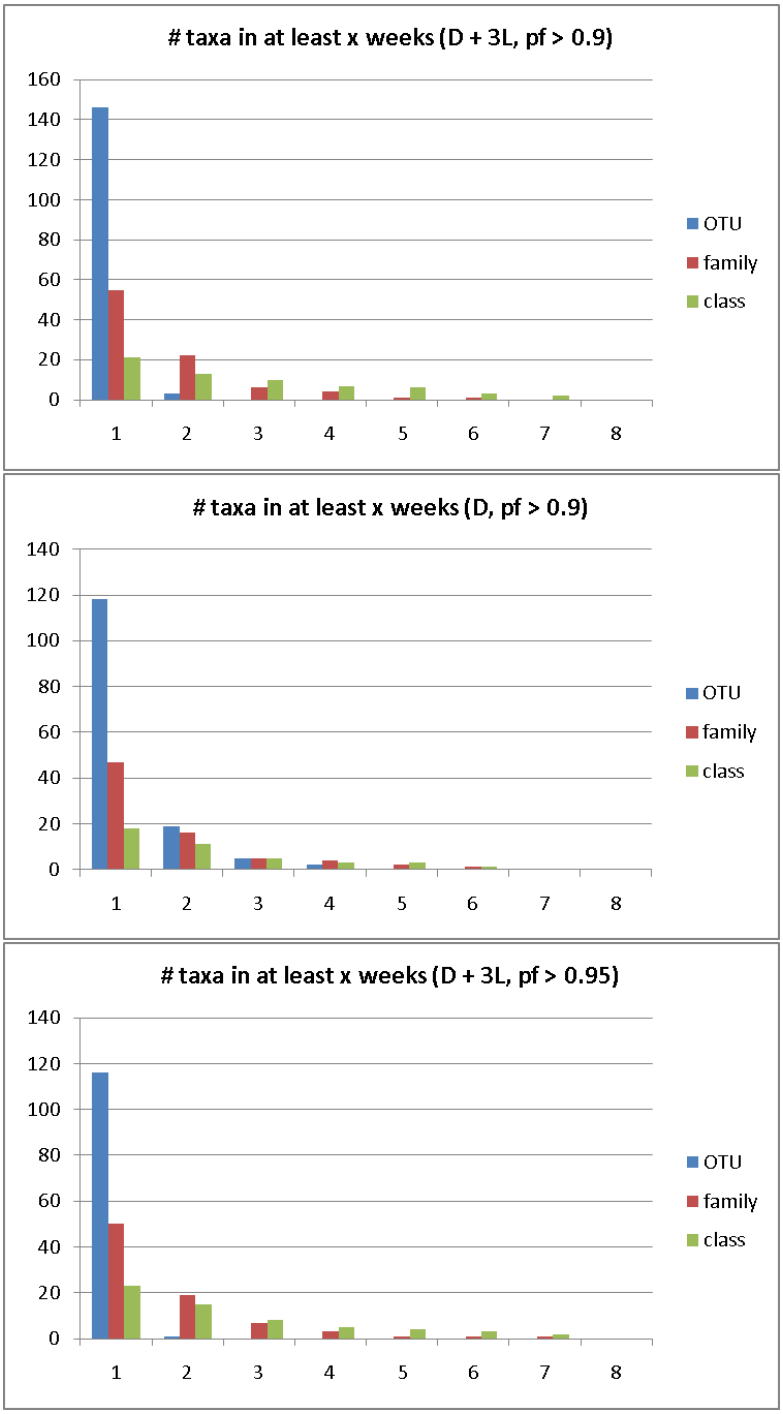


Figure 7.2-20. Counts of signature OTUs and families/classes containing signature OTUs, present in at least x sampling events (x = 1 – 8). Counts were performed based on Diffuser/Lagrangian (D + 3L) and Diffuser (D) signature OTUs. Pf cutoff values are 0.9 and 0.95.

The phylogenetic affiliations of the Diffuser/Lagrangian and Diffuser signature OTUs were presented per sampling event at the family (Figures 7.2-21) and class (Fig. 7.2-22) levels for pf >

0.9. Family and class affiliations for Diffuser/Lagrangian signature OTUs for pf > 0.95 are presented in Fig. 7.2-23.

As already presented in Fig. 7.2-20, only a few families containing signature OTUs were shared between 3 or more sampling events.
For the Diffuser/Lagrangian signature OTUs, the families consistently shared on at least 3 sampling events (for pf > 0.9) were *Alteromonadaceae*, *Clostridiaceae*, *Enterobacteriaceae*, *Lachnospiraceae* and *Rhodobacteraceae*. Those families contained 8 – 33% of the Diffuser/Lagrangian signature OTUs for all sampling events (excluding sampling events with only 1 Diffuser signature OTU detected). However, for a pf > 0.95 other families were shared on at least 3 sampling events (*Burkholderiaceae*, *Comamonadaceae*, *Clostridiaceae*, *Flavobacteriaceae*, *Pseudomonadaceae* and *Rhodobacteriaceae*), accounting for 15 – 57% of the Diffuser/Lagrangian signature OTUs. Therefore, analysis of Diffuser/Lagrangian signature OTUs appears highly dependent on pf cutoff value.
For the Diffuser signature OTUs, the families consistently shared on at least 3 sampling events (pf > 0.9) were *Burkholderiaceae*, *Comamonadaceae*, *Pseudomonadaceae* and *Rhodocyclaceae*, together accounting for 25 – 62% of the Diffuser signature OTUs (excluding sampling events with only 1 Diffuser signature OTU detected).
Clostridiaceae, *Comamonadaceae*, *Lachnospiraceae*, *Enterobaceriaceae* were identified before as OTUs distinguishing the Effluent from Offshore1000 samples (Table 7.2-21). Although distinct OTUs were identified based on common patterns across all sampling events, and signature OTUs based on individual sampling events, 4 families were identified by both analyses. Those 4 families were not identified as distinct for the Offshore1000 samples (Table 7.2-22). However, *Alteromonadaceae*, *Rhodobacteriaceae* and *Flavobacteriaceae* contained many of the distinct Offshore1000 OTUs.

The signature OTU class affiliations were more often shared across sampling events, which was expected when aggregating OTUs into higher level phylogenetic taxa. The top 4 classes containing most of the Diffuser/Lagrangian signature OTUs were *Alphaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria* and *Clostridia* (pf > 0.9) and *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Clostridia* (pf > 0.95). Those 4 families accounted for 45 – 86% (pf > 0.9) and 44 – 86% (pf > 0.95) of the Diffuser/Lagrangian signature OTUs, and were shared on at least 4 sampling events. Still, the class affiliations of the Diffuser/Lagrangian signature OTUs varied between sampling events (Fig. 7.2-22).
The top 4 classes containing most of the Diffuser signature OTUs (pf > 0.9) were *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Actinobacteria*, together accounting for 77 – 83% of the Diffuser signature OTUs. Therefore, the Diffuser signature OTUs are more consistently shared at the class level compared to the Diffuser/Lagrangian signature OTUs.
Alphaproteobacteria, *Betaproteobacteria*, *Gammaproteobacteria*, *Clostridia* and *Actinobacteria* were identified before as containing most of the stable and variable OTUs in the Effluent (Fig. 7.2-11). However, *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* were also identified as the classes containing most of the stable OTUs in the Offshore1000 samples (Fig. 7.2-12), therefore those classes cannot be considered to be specific or indicative of the Effluent.

Many of the families and especially classes containing signature OTUs are also numerically important in the Offshore1000 samples, indicating that aggregating into families or classes reduces the specificity. *Alphaproteobacteria* and *Gammaproteobacteria* are generally the classes containing most of the bacteria in the ocean (Pommier et al., 2007, Venter et al., 2004), while *Betaproteobacteria* and *Clostridia* have been shown to contain the majority of bacteria in human fecal or WWTP activated sludge samples (Eckburg et al., 2005; Sanapareddy et al., 2009; Wery et al., 2009; Suau et al., 1999). *Actinobacteria* are important activated sludge bacteria, and are often associated with bulking sludge, but are also found in the ocean (Seviour et al., 2008, Venter et al., 2004).

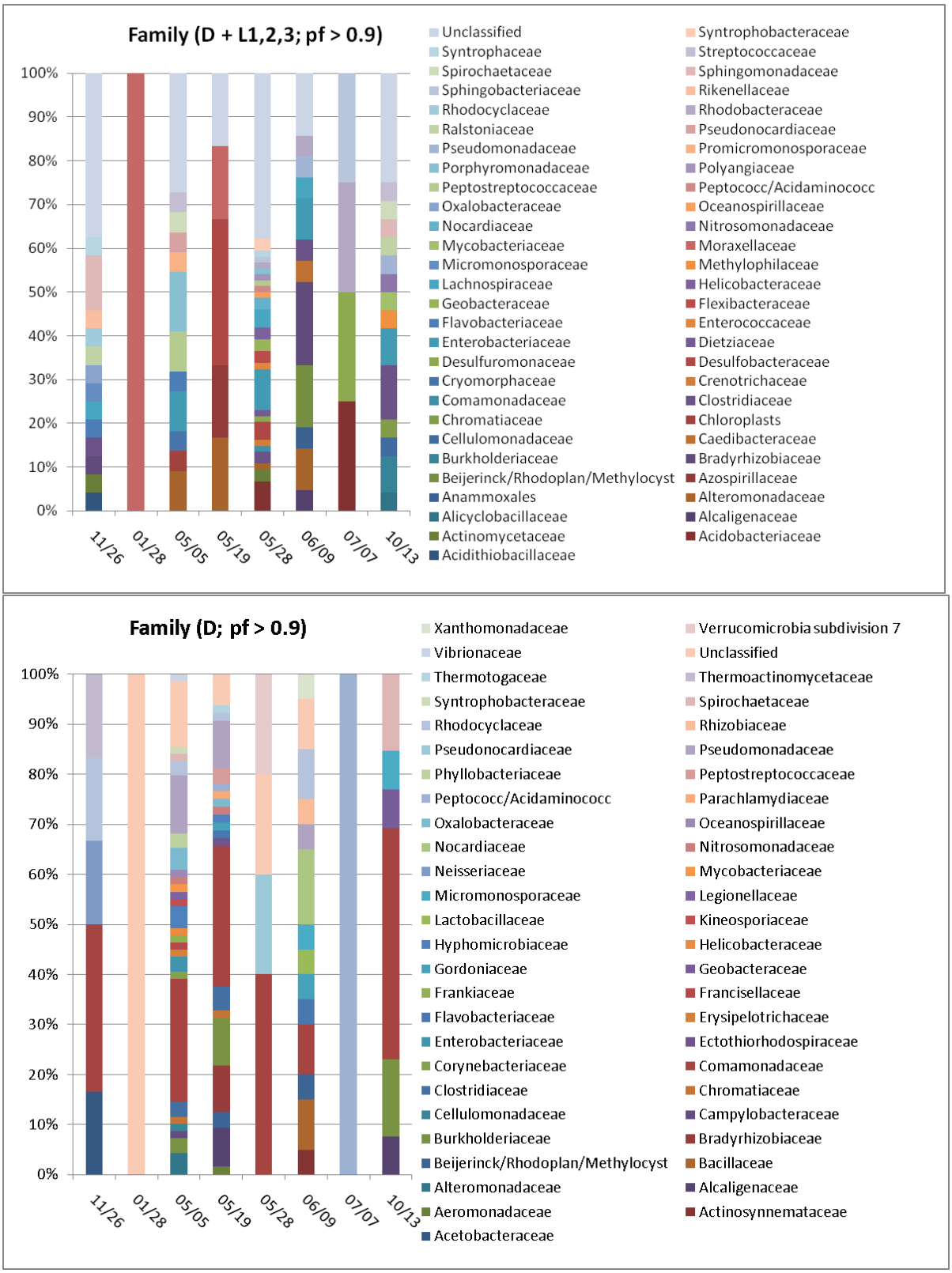


Figure 7.2-21. Phylogenetic affiliations of Diffuser/Lagrangian (D + L1,2,3) and Diffuser (D) signature OTUs for each sampling event, in % of total signature OTUs. Total number of signature OTUs are shown above each bar chart. Pf > 0.9.

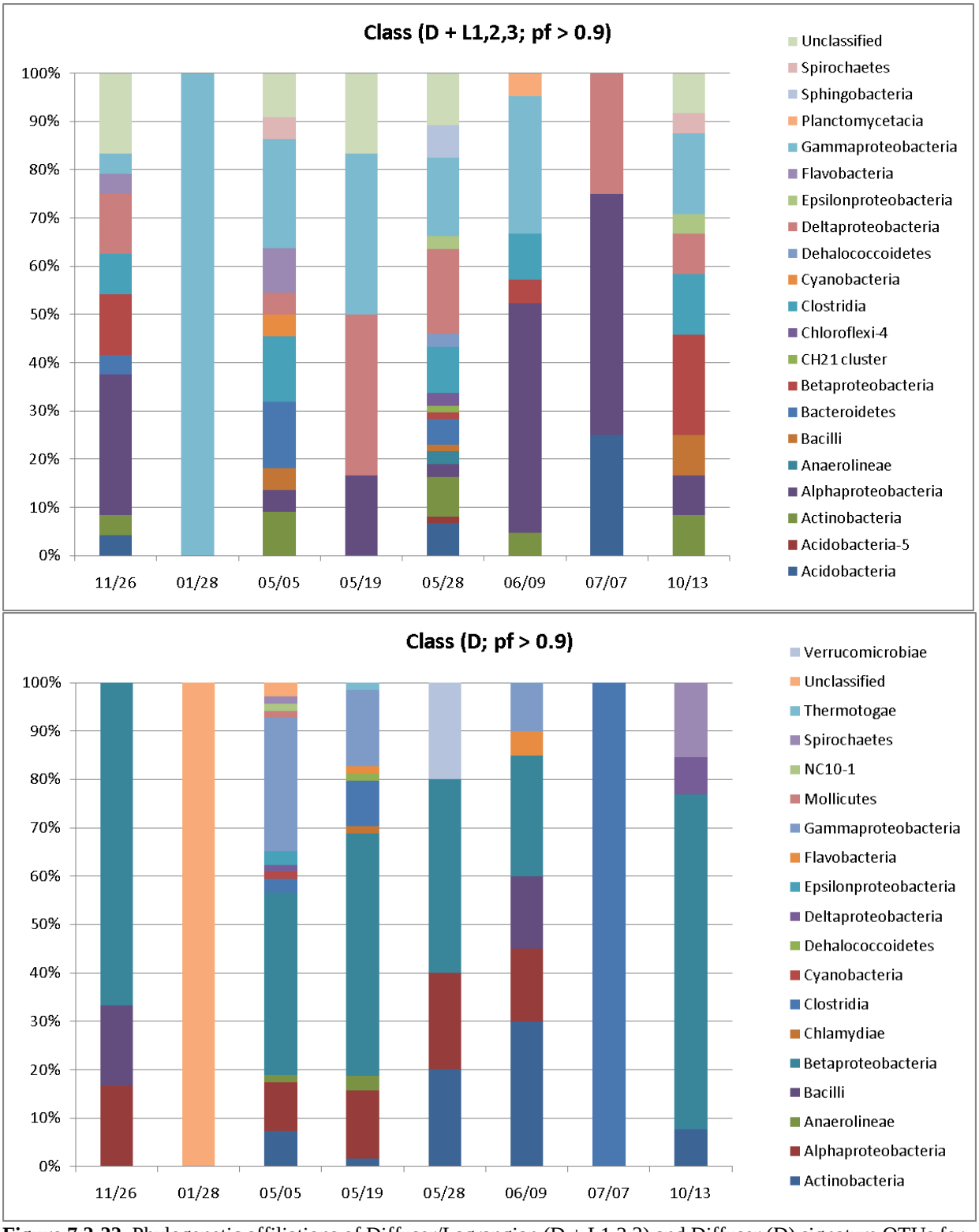


Figure 7.2-22. Phylogenetic affiliations of Diffuser/Lagrangian (D + L1,2,3) and Diffuser (D) signature OTUs for each sampling event, in % of total signature OTUs. Total number of signature OTUs are shown above each bar chart. Pf > 0.9.

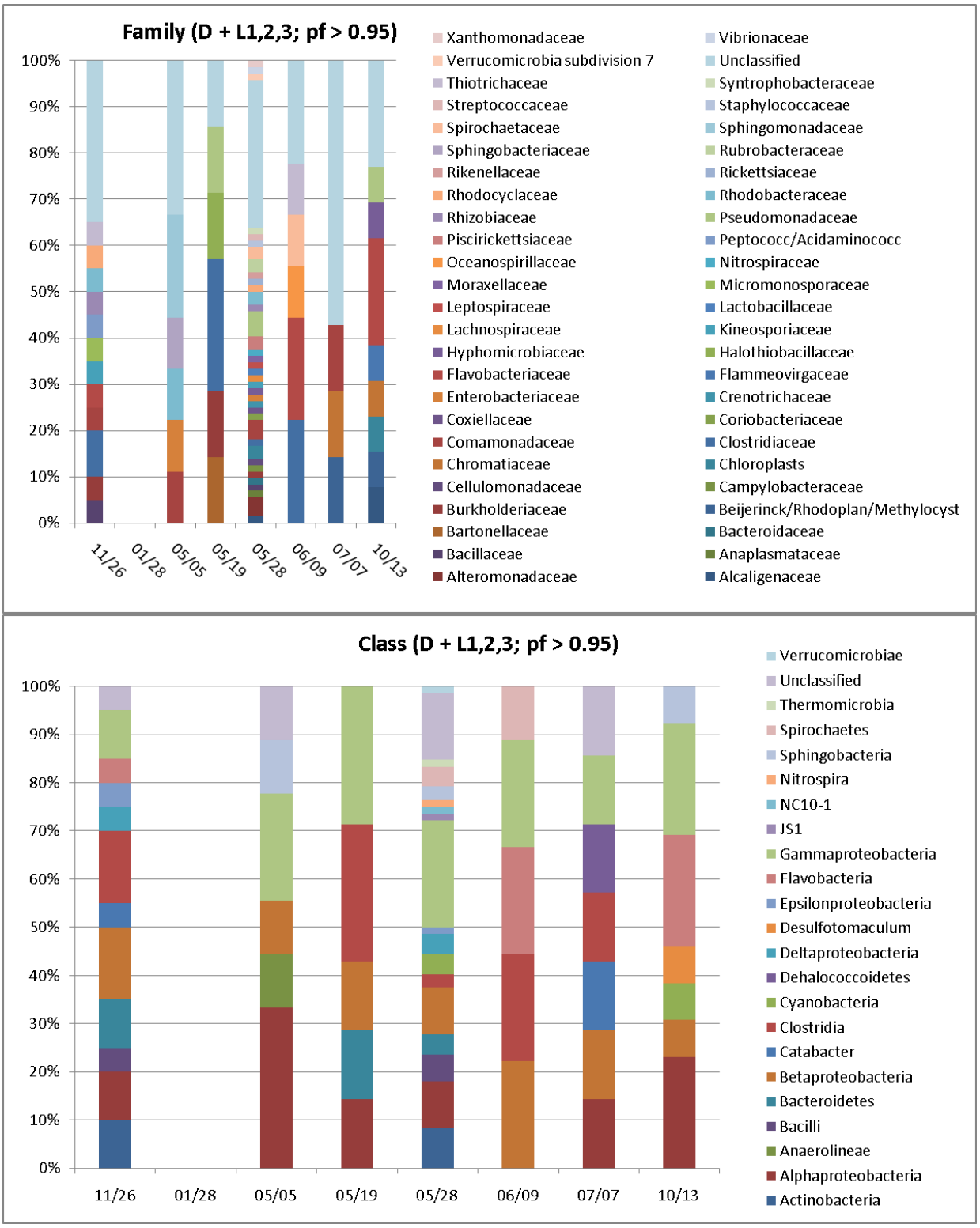


Figure 7.2-23. Phylogenetic affiliations of Diffuser/Lagrangian signature OTUs for each sampling event, in % of total signature OTUs. Total number of signature OTUs are shown above each bar chart. Pf > 0.95.

7.2.11.3.4. Quantitative analysis of Diffuser/Lagrangian signature OTUs

The normalized and averaged FI values of Diffuser/Lagrangian signature OTUs, were fairly constant between sites 3 (Diffuser) and 4c (Lagrangian3) for all sampling events, indicating minimal attenuation (Figure 7.2-24). In addition, narrow 95% confidence intervals indicated that the patterns are similar for all signature OTUs within each sampling event.

Still, for each sampling event, the normalized and averaged FI values were investigated for each family containing at least 3 Diffuser/Lagrangian signature OTUs, in order to identify the FI patterns of phylogenetically related signature OTUs (Table 7.2-33). Again, in most cases the FI values were stable between locations 3 and 4c. Only for the *Sphingomonadaceae* on 11/26/07, there was a decrease of ca. 1500 FI units between the Diffuser and Lagrangian2 (Lagrangian3 not sampled), corresponding to a 10 to 100-fold relative abundance decrease.

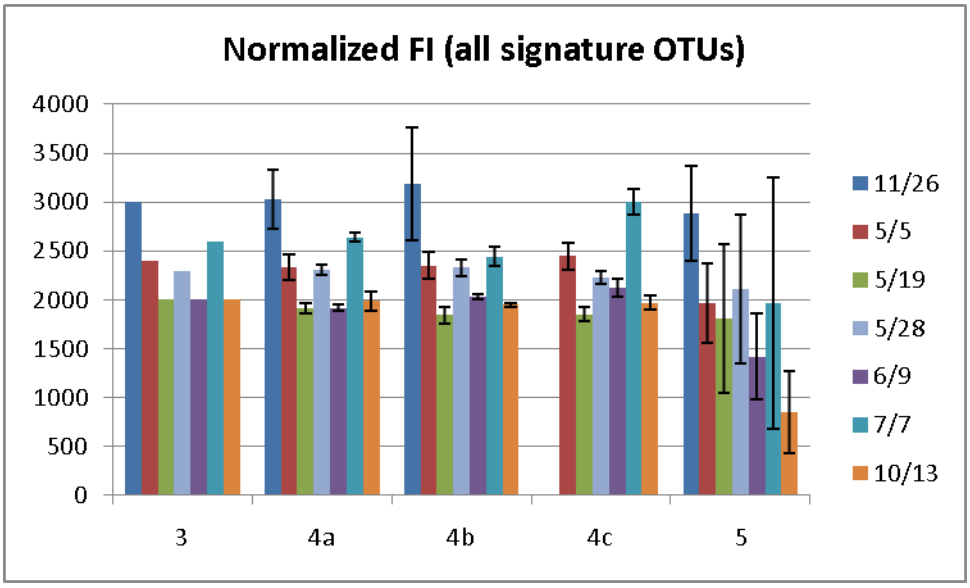


Figure 7.2-24. Normalized fluorescence intensity (FI) averaged over all Diffuser/Lagrangian signature OTUs for each sampling event (pf > 0.9). Event 1/28 was not included because only one signature OTUs was identified. Location 4c (Lagrangian3) was not sampled on 11/26/07. Error bars indicate 95% confidence intervals. FI was normalized to the average value at location 3 (Diffuser) for each sampling event.

In general, most of the Diffuser/Lagrangian signature OTUs were detected in the Diffuser and Lagrangian samples, but also in the Shoreline samples (Table 7.2-34). The fraction of signature OTUs not detected at the Shoreline was highest for sampling events 06/09/08 and 10/13/08.

The FI of Diffuser/Lagrangian signature OTUs in the Shoreline samples was usually similar to those in the last Lagrangian sample (Fig. 7.2-37). Only for sampling events 05/05/08, 06/09/08 and 10/13/08 there was a significant decrease in FI observed between locations 4c (Lagrangian3) and 5 (Shoreline; paired t-test using non-normalized FI, $p < 0.05$). Also families containing at least 3 Diffuser/Lagrangian signature OTUs were usually detected in the Shoreline and Lagrangian samples with similar FIs (Table 7.2-33). When Diffuser/Lagrangian signature OTUs

were not detected at the Shoreline, this was usually not consistent with the other family members, as evident from the relatively high confidence intervals. The *Bradyrhizobiaceae* on 06/09/08 were the exception, and were consistently not detected in the Shoreline samples.

Based on the analysis of FIs we can conclude that Diffuser/Lagrangian signature OTUs are present at all locations with similar FIs, except the Offshore1000 sample (FI equals zero). As presented in Section 7.2.11.3.2, Diffuser/Lagrangian signature OTUs for a given sampling event were also commonly detected at all locations (including Offshore1000) on the other sampling events. Therefore, the Diffuser/Lagrangian signature OTUs appear not specific to the effluent plume or even nearshore environment.

Table 7.2-33. Normalized and averaged fluorescence intensity per sampling event (FI ± 95% confidence interval), for all families containing at least 3 signature OTUs (detected at Diffuser and all 3 Lagrangian samples). NA, data not available.

	Location				
	3	4a	4b	4c	5
11/26					
Sphingomonadaceae	5000 ± 0	4422 ± 59	3478 ± 309	NA	5318 ± 273
05/05					
Porphyromonadaceae	1900 ± 0	2131 ± 206	2209 ± 126	1889 ± 297	1337 ± 1316
05/28					
Acidobacteriaceae	2500 ± 0	2568 ± 166	2502 ± 54	2496 ± 91	2580 ± 108
Desulfobacteraceae	1900 ± 0	1785 ± 68	1756 ± 114	1792 ± 225	2353 ± 568
Enterobacteriaceae	1900 ± 0	1809 ± 167	1802 ± 112	1752 ± 194	1509 ± 774
Lachnospiraceae	2400 ± 0	2398 ± 198	2447 ± 151	2328 ± 182	2567 ± 61
06/09					
BRM*	1800 ± 0	1687 ± 20	1769 ± 75	1746 ± 171	589 ± 1155
Bradyrhizobiaceae	1600 ± 0	1529 ± 20	1624 ± 18	1753 ± 47	0 ± 0
10/13					
Clostridiaceae	2000 ± 0	1950 ± 369	1988 ± 46	2006 ± 250	1294 ± 1268

*Beijerinck/Rhodoplan/Methylocyst

Table 7.2-34. Total signature OTU counts (# sOTU), signature OTU counts at Shoreline with FI equal to zero (# sOTU(5) = 0) and fraction of signature OTUs at Shoreline equal to zero (f(sOTU)).

Sample event	# sOTU	# sOTU(5) = 0	F(sOTU)
11/26	24	3	0.13
01/28	1	0	0
05/05	22	4	0.18
05/19	6	1	0.17
05/28	74	9	0.12
06/09	21	7	0.33
07/07	4	1	0.25
10/13	24	14	0.58

7.2.11.3.5. Groups of OTUs and OTU ratios

In section 7.2.11.3.2, distinct OTUs were identified that were indicative of the Effluent based on their presence/absence patterns across all 8 sampling events. In this section, their utility for tracing the effluent plume is further investigated. This top-down approach is an alternative to the bottom-up approach presented above, based on individual OTUs.

In order to characterize bacterial families indicative of the effluent, the distinct OTUs for Effluent (site 6), Diffuser (site 3) and Offshore1000 (site 1) sites were compared (Figure 7.2-25). The percent OTUs detected were used to identify families that were indicative of either Effluent or ocean samples. The percent OTUs for *Bacillaceaea, Clostridiaceae, Enterobacteriaceae, Enterococcaceae,* and *Lachnospiraceae* in Effluent samples were higher than those in the ocean samples. The percent OTUs for *Rhodobacteraceae* was higher in the ocean samples than in the Effluent samples (Table 7.2-35). The use of indicators that incorporate both Effluent and Offshore1000 associated bacteria would capture effluent input perturbation on the whole community. From a previous whole bacterial community survey of water samples from the Mission and Laguna Channel Watersheds, Santa Barbara, the bacterial classes of *Bacilli, Bacteroidetes, Clostridia* and *Alphaproteobacteria* (BBC:A), were elucidated as potential fecal indicator ratio (Wu et al., in prep). The ratio of percent OTUs detected (at pf = 1) for *Bacillaceaea, Clostridiaceae, Enterobacteriaceae, Enterococcaceae,* and *Lachnospiraceae* to *Rhodobacteraceae* (BCEEL:R) is tested in this study as a community-based effluent indicator. Important differences between the BBC:A and BCEEL:R ratios are that the former is classified at the class phylogenetic level and is used as an indicator for fecal communities in human feces and raw sewage, whereas the latter is classified at the family level and used as an indicator for treated effluent.

The BCEEL:R ratio trends observed are generally: highest in the Effluent samples (site 6), and decreasing at the Diffuser (site 3), Lagrangian (site 4), and Offshore1000 sites (site 1) (Fig. 7.2-26). However, for most of the sampling events the Shoreline (site 5) and Offshore1000 (site 1) have slightly higher BCEEL:R than the Diffuser (site 3) samples. Only on 06/06/08 and 10/13/08 the BCEEL:R ratio at the Diffuser was at least twice the BCEEL:R ratio at the Offshore1000 sample. For the other sampling events, the BCEEL:R ratio at the Offshore1000 was very similar or even higher than the one at the Diffuser. There could be many potential explanations: 1) the minor differences between the BCEEL:R of sites could be within the range of variance observed with these samples, thus the differences may not be significant; 2) the effluent plume might not have been homogeneous, and since the same exact community is not tracked, there might have been temporal differences within the same day; 3) there might have been additional sources of bacteria, especially at the Shoreline (site 5); and 4) abnormal sampling conditions such as first sampling event (11/26) and rain event (1/28) may have affected the samples. Still, the BCEEL:R ratio could only detect a plume signal at the Diffuser on 06/09/08 and 10/13/08.

Other ratios can still be tested for their usefulness in tracing the effluent plume. In Section 7.2.11.3.3 the families *Clostriciaceae, Lachnospiraceae, Comamonadaceae* and *Enterobacteriaceae* contained most of the Diffuser/Lagrangian and Diffuser signature OTUs. Therefore ratios incorporating those families in the nominator should be tested.

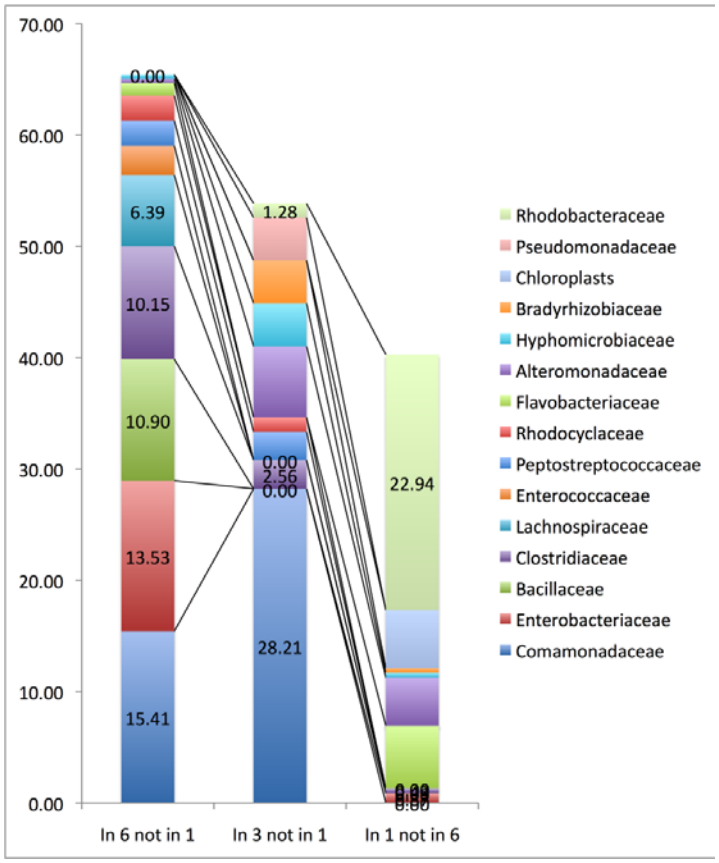


Figure 7.2-25. Percentages of OTUs detected in site 6 (Effluent) not in site 1 Offshore1000), in site 3 (Diffuser) not in site 1 and in site 1 not in site 6. Only families with percentages over 3% are graphed.

Table 7.2-35. Percentages of OTUs detected in site 6 (Effluent) not in site 1 (Offshore1000), in site 3 (Diffuser) not in site 1 and in site 1 not in site 6 in tabular form. Only families with percentages over 3% are included.

	In 6 not in 1	In 3 not in 1	In 1 not in 6
Comamonadaceae	15.41	28.21	0.00
Enterobacteriaceae	13.53	0.00	0.87
Bacillaceae	10.90	0.00	0.00
Clostridiaceae	10.15	2.56	0.43
Lachnospiraceae	6.39	0.00	0.00
Enterococcaceae	2.63	0.00	0.00
Peptostreptococcaceae	2.26	2.56	0.00
Rhodocyclaceae	2.26	1.28	0.00
Flavobacteriaceae	1.13	0.00	5.63
Alteromonadaceae	0.38	6.41	4.33
Hyphomicrobiaceae	0.38	3.85	0.43
Bradyrhizobiaceae	0.00	3.85	0.43
Chloroplasts	0.00	0.00	5.19
Pseudomonadaceae	0.00	3.85	0.00
Rhodobacteraceae	0.00	1.28	22.94

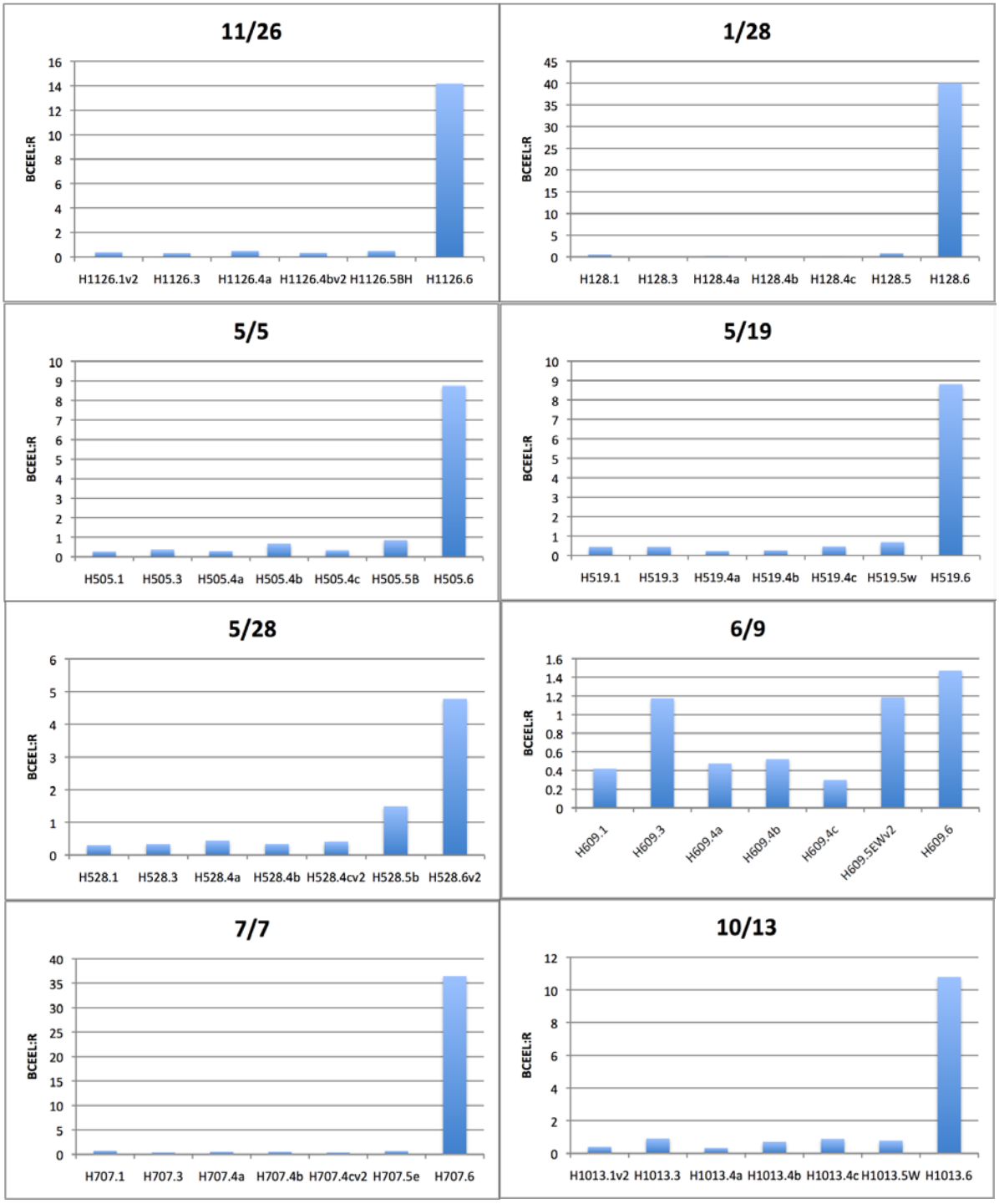


Figure 7.2-26. BCEEL:R ratios for all locations for each sampling event.

7.2.11.3.6. Lachnospiraceae as effluent tracer family

Several of the analyses presented above (Sections 7.2.10, 7.2.11.3.3, 7.2.11.3.5) suggested that OTUs belonging to the family *Lachospiraceae* are indicative of the WWTP effluent. Therefore, an in-depth analysis of the distribution of all *Lachnospiraceae* OTUs was performed.
In total 18 different *Lachnospiraceae* OTUs were detected at least once in all samples. As shown in Fig. 7.2-27 most of those OTUs are consistently present in the effluent (at least 14, except on 01/28/08), but also at the Shoreline (at least 10, except on 07/07/08). However, the number of *Lachnospiraceae* OTUs was always lower at the Offshore1000 (maximum 7). Therefore, a higher diversity of *Lachnospiraceae* is consistently added by the effluent to the ocean.
The *Lachnospiraceae* detected at the Offshore1000 were consistently limited to 7 OTUs, therefore 11 *Lachnospiraceae* OTUs were never detected at the Offshore1000 and were therefore highly indicative of the Effluent.

By plotting counts of those 11 Effluent-specific *Lachnospiraceae* OTUs (Fig. 7.2-27, bottom), a strong effluent signature (> 2 OTU) was detected at the Diffuser on 05/05/08 (9 OTUs), 06/09/08 (9 OTUs), and 10/13/08 (3 OTUs). In addition, a strong effluent signature was detected at one Lagrangian location on 05/05/08 and 06/09/08.

Interestingly, the 11 Effluent-specific *Lachnospiraceae* OTUs were very common at the Shoreline. There are two potential explanations for this observation, not mutually exclusive. First, these OTUs could originate from the Effluent and grow in the Shoreline environment. Second, they could be derived from terrestrial sources or beach sand. Without investigating the microbial community composition of beach sand and terrestrial sources it is not possible to determine the relative importance of each source of *Lachnospiraceae* OTUs.
Lachnospiraceae are strict anaerobes, and the only habitat described is the gastrointestinal tract of mammals, including ruminants and humans, where they play a role in metabolization of polymeric substrates such as hemicellulose and pectin (Cota and Forster, 2006; Dowd et al., 2008; Frank et al., 2007). In humans, they are believed to promote gastrointestinal health (Frank et al., 2007). Although *Lachnospiraceae* have been occasionally found in the environment, they are thought to be of fecal origin (Cota and Forster, 2006; Sheridan et al., 2003). Because of their typical habitat, it is not surprising to find *Lachnospiraceae* in a WWTP effluent, although their survival and growth characteristics in the environment remain unknown.

As this approach of investigating the distribution of OTUs in a family indicative of the Effluent appears very useful, this analysis should be repeated for other families, such as *Bacillaceae*, *Clostridiaceae* and some of the families belonging to the *Actinobacteria*.

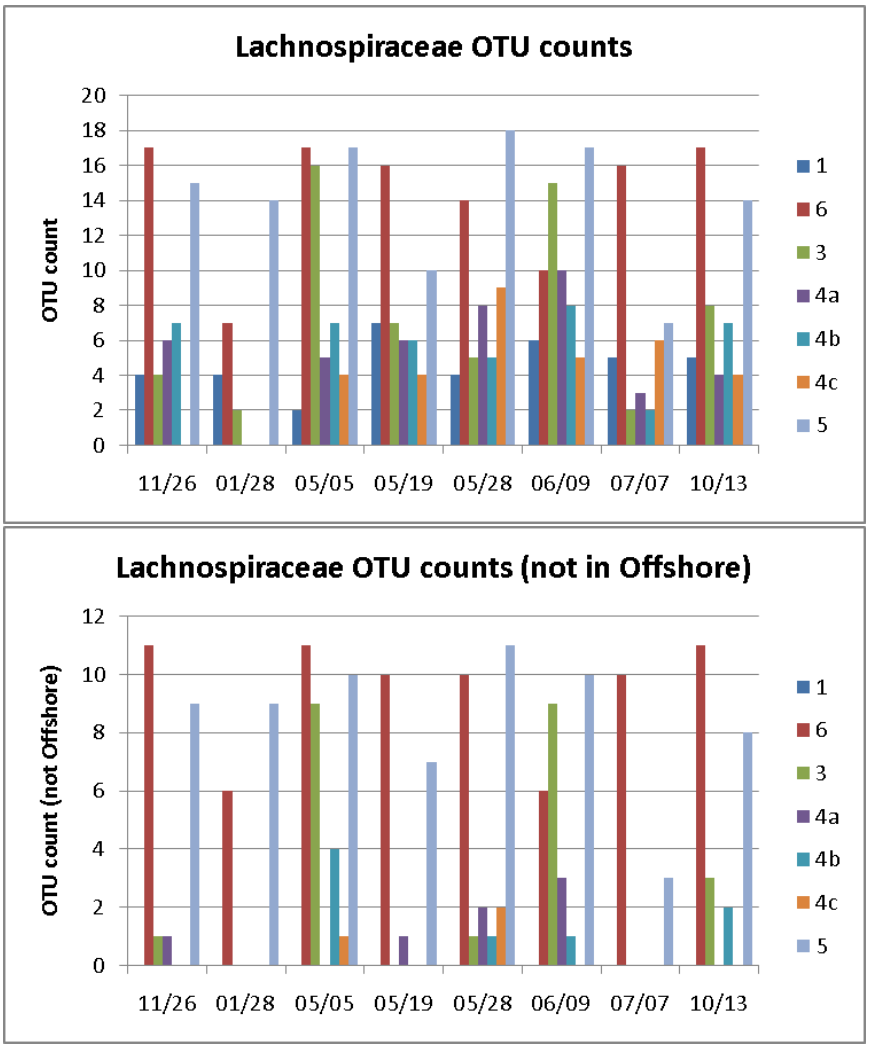


Figure 7.2-27. Distribution of *Lachnospiraceae* OTUs across all sampling events and locations (pf > 0.9). Counts of all *Lachnospiraceae* OTUs (top) as well as *Lachnospiraceae* OTUs consistently absent in the Offshore1000 samples (bottom) are presented. Locations are indicated as 1: Offshore1000, 6: Effluent, 3: Diffuser, 4a: Lagrangian 1, 4b: Lagrangian 2, 4c: Lagrangian 3, and 5: Shoreline.

7.3. Integrated Results

7.3.1. Correlations between Delta Salinity and Univariate Data

Six sampling events that had relatively large surface salinity differences between the Diffuser and Offshore1000 stations (5/5/08, 6/2/08, 6/9/08, 7/7/08, 9/29/09, 10/13/08) were selected for comparison with corresponding differences in univariate microbiological data. We examined the non-zero microbiological and chemical (single variate only) data in comparison to the delta salinity values for those six dates to determine if there were statistically significant relationships in differences between the tracers. The following microbiological characteristics were below the detection limits at the Diffuser and Lagrangian sites for those dates: *E. coli*, *Enterococcus* spp. (IDEXX), and enterovirus. Out of the remaining quantifiable characteristics, there was no apparent correlation between delta salinity values and either total coliform, biomass (by either

DNA yield or by cell count), microbial diversity (either TRFLP OTU richness or evenness), qPCR ENT, or qPCR HBM. However, for four dates (6/9/08, 7/7/08, 9/29/09, 10/13/08) of the six mentioned above, there was a significant and positive correlation ($R^2 = 0.49$, $p = 0.002$) between the "delta phosphate" concentration (defined as the difference between phosphate concentrations measured in Offshore 1000 versus Diffuser sites) and delta salinity values (Figure 7.3-1). For those same dates, there was no apparent correlation between either nitrate+nitrite or ammonia concentrations and delta salinity.

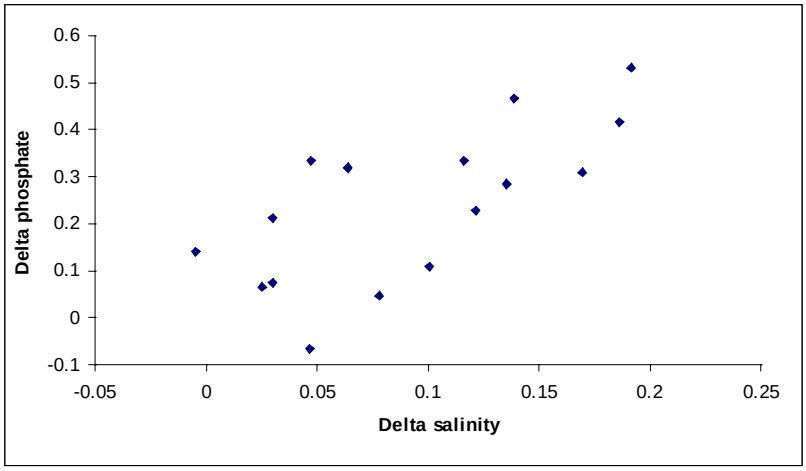


Figure 7.3-1. Delta salinity vs. delta phosphate. There was a significant and positive correlation ($R^2 = 0.49$, $p = 0.002$) between the change in salinity and the change in phosphate concentrations.

The correlation in Figure 7.3-1 represents a subset of the data for which a similar analysis can be performed to evaluate if there are more events for which phosphate and salinity deltas correspond. This analysis is ongoing.

7.3.2. Rainfall and Univariate Data

Total coliform appeared to increase in the Shoreline samples following rain events (Figure 7.3-2), suggesting that rainfall runoff across terrestrial sources, and not ocean conditions, contributed to the total coliform increases at the beach. This finding is consistent with other reports in California that show similar relationships, i.e. that FIB concentrations increase in the surf zone following a rain event.

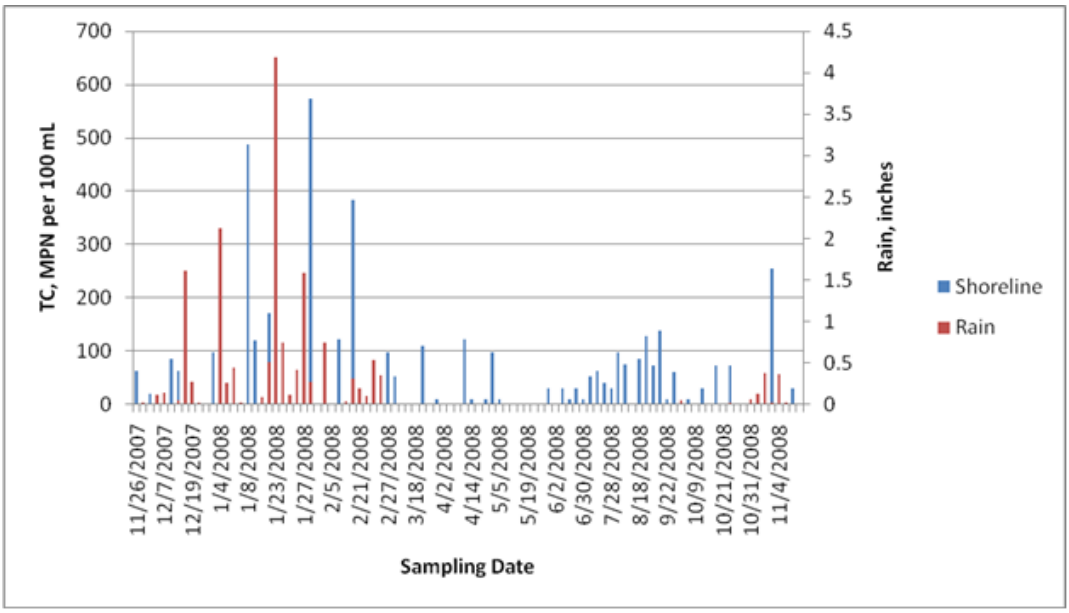


Figure 7.3-2. Total coliform versus sampling date at the Shoreline, and accumulated rainfall, showing an apparent relationship between increases in total coliform at the shoreline during rain events but also summertime variations in total coliform that are independent of rain. Note that the highest total coliform concentrations appear during rainy periods. 95% confidence intervals are not indicated, but overlap for most samples, negating statistical comparisons across dates.

7.3.3. Evaluation of PhyloChip OTUs as Tracers in Dilution

The change in FI between Effluent and Diffuser was calculated for all Diffuser plus Lagrangian signature OTUs and Diffuser signature OTUs, by subtracting FIs at site 3 from site 6 (Figure 7.3-3).

For the case of all Diffuser plus Lagrangian signature OTUs, boxplots indicate median FI differences close to zero, with 50% of the FI differences generally below 500. This roughly corresponds to changes in relative abundance of less than 1 order of magnitude, much less than estimated based on salinity or other tracers. Negative FI differences even indicate increases in relative abundance from Effluent to Diffuser samples.

For the case of Diffuser signature OTUs, the median FI differences are positive, and 50% of the FI differences were generally between 0 and 1000 or 2000, roughly corresponding to relative abundance decreases up to one or two orders of magnitude. This is closer to the estimates based on salinity or other tracers, although still lower than expected.

As discussed in Section 7.2.11.3.2, the Diffuser signature OTUs appear more specific for the Effluent than the Diffuser plus Lagrangian signature OTUs, based on their distribution across locations. The more consistent FI decreases between Effluent and Diffuser for the Diffuser signature OTUs additionally support their preferred use as effluent plume tracers.

Discrepancies of dilution estimates with PhyloChip could be due to the changes in absolute bacterial cell numbers in the Effluent and Diffuser samples. For instance, the DNA concentrations in the Effluent were usually lower than those in the ocean. Mixing low biomass effluent with higher biomass ocean water result in signature populations with lower relative abundances in the ocean than predicted based on the dilution factor alone. While this phenomenon could potentially explain larger than expected FI decreases between Effluent and Diffuser samples, it would not necessarily explain relatively constant or even increasing FI values. The discrepancies with dilution estimates based on salinity or other tracers can also be explained by the semi-quantitative nature of PhyloChip analysis. Although previous work at LBNL has validated FI using qPCR for some OTUs, this was not performed in this study. An alternative explanation for lower than expected FI decreases between Effluent and Diffuser samples is that bacteria introduced into the nearshore environment via effluent discharging continuously through the diffuser become resident in that environment. The increase in population sizes of those resident bacteria could account for the apparent lack of dilution in the PhyloChip data. While impossible to directly address in this research, this hypothesis is supported by recent research indicating altered bacterial communities due to a sewage discharge outfall in Hong Kong waters (Zhang et al., 2009). In addition, bacterial cell counts, DNA, *E. coli* and total coliform concentrations also appeared to be generally higher in the nearshore compared to Offshore1000 locations.

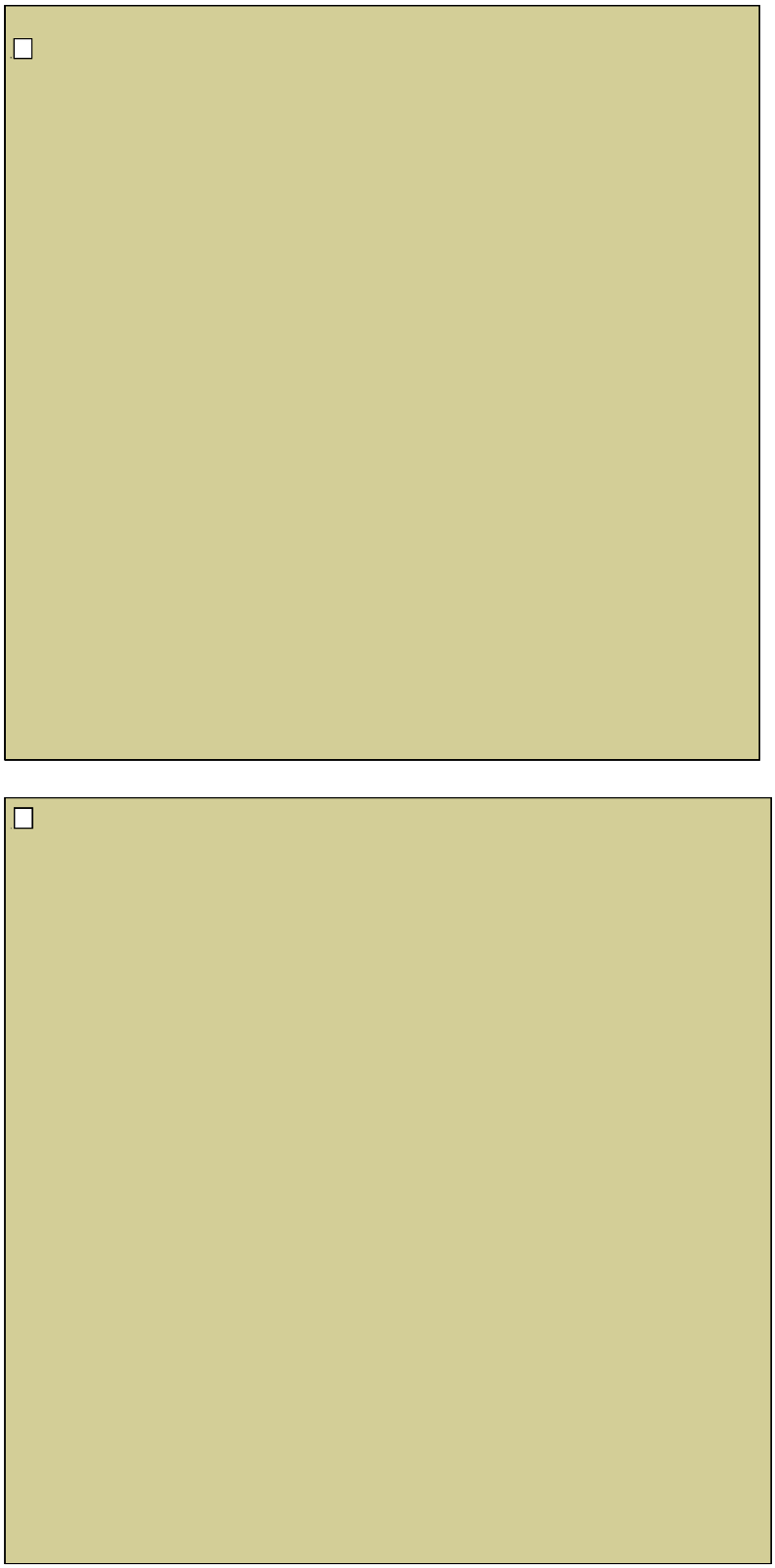


Figure 7.3-3. Box plots of FI (effluent minus diffuser) of all Diffuser/Lagrangian signature OTUs (top) and Diffuser signature OTUs (bottom), at pf > 0.9.

7.3.4. Assessment of Effluent Plume Effects on Shoreline Water Quality

Based on the drifter trajectories, for 6 out of the 8 sampling events with PhyloChip data available, the effluent plume entered the surfzone: 11/26/07, 5/05/08, 5/19/08, 5/28/08, 6/09/08 and 10/13/08 (surf zone entries, Section 7.1.3). Consequently, for sampling events on 1/28/08 and 7/07/08 there was no evidence based on the drifters that the plume entered the surfzone.

The MDS plot including only the 8 Shoreline samples did not show separation of samples based on surf zone entry (Figure 7.3-4). This is also supported by hierarchical cluster analysis (Figure 7.3-5), therefore we conclude there is no consistent relation between SZE and the overall microbial community composition at the Shoreline. In addition, most of the OTU variation in the variable OTUs at the shoreline was caused by high OTU abundances on 11/26/07 and low abundances on 07/07/08 (see Section 7.2.10). Interestingly, the high OTU abundances occurred during one sampling event with SZE, and the low OTU abundances during one sampling event with non-SZE. However, this OTU abundance pattern according to SZE or non-SZE was not observed for the other six sampling event, therefore there is no consistent effect of SZE on the most variable OTUs.
Overall, surf zone entries do not appear to significantly alter microbial community structure in the Shoreline samples.

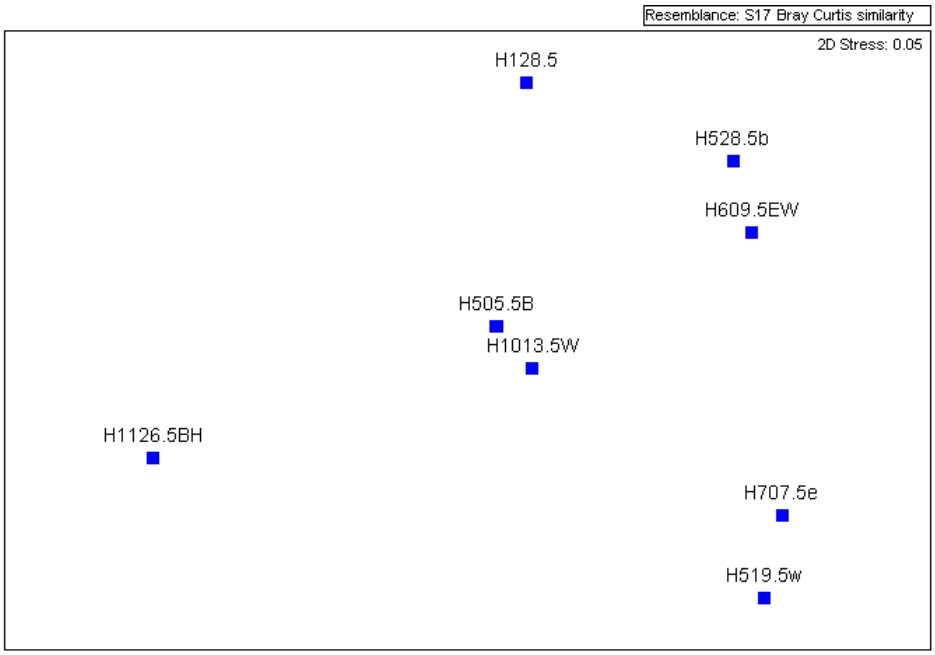


Figure 7.3-4. MDS plot of Shoreline samples based on PhyloChip fluorescence intensities.

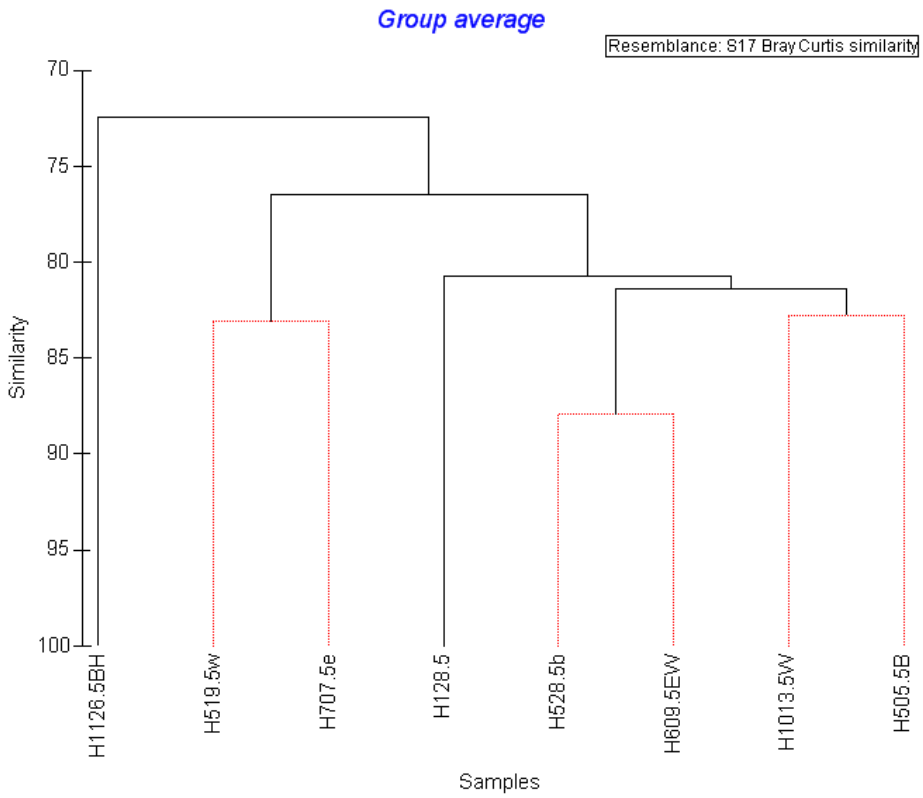


Figure 7.3-5. Hierarchical cluster analysis based on Bray-Curtis Similarity of Shoreline samples PhyloChip fluorescence intensities. Clusters in red are not significantly different based on SIMPROF analysis.

8. Conclusions

8.1. Oceanographic Conclusions

Low salinities frequently observed over the diffuser indicate that the effluent was commonly at the sea surface near the diffuser. This is consistent with numerical plume model results indicating plume surfacing within ~135 ft of the diffuser throughout the year (Figure 7.1-11).

Drifters deployed at the Diffuser station, presumably tagging plume waters, have pathways mainly in the alongshore direction away from the diffuser (both eastward and westward). The pathways also extend away from the diffuser toward shore, consistent with the effects of onshore winds and wave-driven transport. Occasional offshore pathways occur only for water parcels located offshore of the diffuser.

Currents offshore of the diffuser typically have higher velocities. Thus, offshore waters move farther alongshore during a given time interval than waters at the diffuser. Drifters released at the Offshore500 and Offshore1000 stations exhibited pronounced offshore movement on 2 of 50 sampling days.

Treated effluent is most likely to enter the surfzone inshore of the diffuser along a span of coastline 1.5 km east and west of the diffuser. This conclusion is based on the pathways for drifters released at the diffuser (Figure 7.1) and the drifter probability density functions (Figures 7.1.2.1, 7.1.2.2, and 7.1.2.3).

Median dilution estimates of the effluent plume based on salinity measured over the diffuser ranged from 250-450; the lowest dilution observed was about 100. These are consistent with near-field dilution estimates obtained from the numerical plume model. Dilution of the effluent as it enters the surf zone is estimated to be in the range 250 to about 900 corresponding to estimated dilutions at the diffuser and following the drifters.

8.2. Microbiological Conclusions

8.2.1. Univariate data Conclusions

Nutrient analyses indicated that the WWTP discharges low and rather consistent amounts of nitrate+nitrite, ammonia and phosphate. Nitrate+nitrite concentrations in the effluent, particularly for the summer months, had a pattern that was similar to concentrations in the near shore environment. However, phosphate was the only nutrient for which concentrations exceeding those in the Offshore1000 samples (delta) were related to a salinity delta. This would seem to imply that phosphate discharged from the outfall diffuser had a more distinct impact on water quality in the near shore environment than did nitrate+nitrite. Given that phosphorous is often a limiting nutrient in marine environments, this result is logical and expected.

FIB concentrations were mostly too low to draw significant conclusions about relatedness of concentrations in space and time. Still, regardless of overlapping confidence intervals, some overall assessments are possible. First, FIB were most frequently quantified in the Shoreline samples where they were also most abundant. Concentrations were comparatively very low in the effluent. This suggests that the discharge of FIB in effluent at the diffuser is not having an immediate impact on nearshore FIB concentrations. There is apparently no immediate relationship between FIB discharging from the diffuser and ankle-deep beach water concentrations. Further, the background concentration at the Offshore1000 site, at least for *Enterococcus* spp., is similar to that at the diffuser, on average. This suggests that there is little long term impact of the effluent on *Enterococcus* spp. concentrations in the nearshore environment. The *E. coli* and total coliform concentrations are lower in the Offshore1000 environment relative to either the nearshore or shoreline environments, but there is no evidence that this is caused by the shallow ocean outfall. Still FIB concentrations were the highest at the Shoreline, suggesting other sources at the Shoreline. However, it is beyond the scope of this study to determine exact sources of FIB at the Shoreline. Sources nominated in the published literature include runoff from terrestrial sources during either wet or dry weather, and colonization of beach sands. It is plausible, but not possible to determine from this study, that effluent discharging continuously into the near shore environment could contribute to beach sand inoculation of FIB.

The WWTP effluent is highly diluted at the diffusers which, for most events, resulted in insufficient concentrations of DNA at the diffuser for quantifying qPCR ENT or HBM. Only when effluent DNA concentrations were sufficiently high were we able to resolve qPCR-based indicators of the plume at the Diffuser and beyond. Biomass, by either flow cytometry or DNA yield, was slightly higher in the nearshore samples compared to the Offshore samples. Similarly as for *E. coli* and total coliform, the higher biomass concentrations in the nearshore environment cannot be directly linked to the treated wastewater effluent, as the Effluent usually contained lower biomass concentrations. Therefore, the increased biomass and FIB concentrations in the nearshore samples are either unrelated to the effluent (but perhaps related to the proximity of terrestrial sources), or increases may be caused by the noted nutrient input from the effluent.

8.2.2. TRFLP Conclusions

The significant dilution of WWTP effluent at the diffuser resulted in loss of TRFLP signal. TRFLP profiling indicated that the effluent, regardless of sample DNA content, was distinct from the ocean and shoreline samples. Thus, by TRFLP, the effluent has little microbiological impact in either the nearshore or shoreline environments.

8.2.3. PhyloChip Conclusions

PhyloChip analysis was used for two purposes. First, microbial community composition at all sampling locations was described and compared between locations. There is currently very limited information in the scientific literature about the microbial communities in secondary treated/disinfected effluent and in coastal ocean environments, including their temporal variability. Second, PhyloChip analysis was intended to identify tracers for the treated effluent plume in the ocean, using three different approaches. The first was to identify signature OTUs based on patterns observed for individual sampling events; the second was to identify a ratio of OTU abundances for selected families (BCEEL:R ratio) based on overall patterns across sampling events; the third was to select OTUs from families indicative of the effluent that were consistently absent in the Offshore1000 samples.
As treated effluent is highly diluted in the ocean by the diffuser, a very sensitive technique such as the PhyloChip is required to maximize the chance of detecting minor changes in the coastal ocean microbial communities.

PhyloChip analysis, similarly to TRFLP analysis, indicated that Effluent microbial communities were distinct from ocean microbial communities, while ocean samples were generally very similar. A highly diverse set of OTUs distinguished the Effluent from the ocean samples, with generally large differences in fluorescence intensities. Most of those distinct Effluent OTUs belonged to the families *Comamonadaceae*, *Enterobacteriaceae*, *Bacillaceae*, *Clostridiaceae* and *Lachnospiraceae*. The stable and variable OTUs in the Effluent appeared mostly phylogenetically distinct. Distinct OTUs were also found for all groups of ocean samples (Offshore1000, nearshore and Shoreline). Especially *Rhodobacteriaceae* OTUs distinguished the Offshore1000 from Effluent samples. In general, the fluorescence intensity differences of distinct OTUs between groups of ocean samples were less than between Effluent and Offshore samples, indicating more subtle differences between groups of ocean samples. Microbial communities in

the nearshore locations (Diffuser and Lagrangians) appeared to be influenced by OTUs typical for Offshore1000 and Effluent samples. Interestingly, many OTUs were identified that distinguished the Shoreline samples from the Lagrangian samples, suggesting additional inputs of microbes not related to the nearshore environment (i.e. from beach sand or terrestrial sources) or a very selective shoreline environment. A more detailed investigation of the variable and stable OTUs revealed that several OTUs belonging to the *Lachnospiraceae* were shared by the Effluent and nearshore samples, but not the Offshore1000, suggesting Effluent is the source of those OTUs.

A procedure was developed to identify signature OTUs in the ocean samples indicative of the effluent plume, based on OTU patterns for individual sampling events. The lowest numbers of signature OTUs were generally found on 01/28/08 and 07/07/08. When considering signature OTUs detected in the Effluent, Diffuser and all Lagrangian samples, the highest number of signature OTUs were detected on 05/28/08. However, when only considering signature OTUs detected in the Effluent and Diffuser, the highest number of signature OTUs were detected on 05/05/08, 05/19/08 and 06/09/08. Low numbers of signature OTUs were identified when only considering signature OTUs detected in the Effluent, Diffuser and 1 or 2 Lagrangians. Because of those unexpected trends (a decreasing number of signature OTUs is expected when including more Lagrangian samples), the specificity of signature OTUs to detect the diluted effluent in the ocean was further investigated by determining the distribution of signature OTUs across weeks. Diffuser and Lagrangian signature OTUs were commonly detected in all ocean samples for the sampling events when they were not classified at signature OTUs, indicating that their classification as Diffuser and Lagrangian signature OTUs occurred because of spatial or temporal variability at the Offshore1000. Therefore, signature OTUs detected in the Effluent, Diffuser and all Lagrangian samples were considered not specific for the Effluent. Consequently, the signature OTU approach was able to detect an effluent signal at the diffuser on 05/05/08, 05/19/08 and 06/09/08.

The BCEEL:R ratio was selected as a potentially convenient and easier-to-interpret alternative to signature OTUs, based on overall patterns of distinct OTUs identified for Effluent and Offshore1000 samples. Although this ratio was high for the Effluent samples, it did not provide sufficient discriminatory power to consistently distinguish between Offshore1000 and diluted Effluent in the ocean samples for all individual sampling events. However, an Effluent plume signal was identified at the diffuser for 06/09/08 and 10/13/08.

Finally, *Lachnospiraceae* were found to be highly specific for the Effluent, and a subset of 11 *Lachnospiraceae* OTUs (out of 18) could be identified that were consistently absent in the Offshore1000 locations, but present at the Diffuser and some Lagrangian locations on 05/05/08, 06/09/08 and 10/13/08.

Using PhyloChip OTUs as tracers in the environment is a new and promising approach in microbial ecology, and is made possible by the high sensitivity of the PhyloChip to microorganisms with low relative abundances. Because of the low number of OTUs identified as tracers compared to the total number of OTUs detected, it is important to consider if the identification of signature OTUs could have arisen by chance. Based on the PhyloChip data analysis completed in this report, using multiple approaches, microbial effluent plume tracers

were found at the Diffuser on for 4 of the 8 sampling events analyzed by PhyloChip (05/05, 05/19, 06/09 and 10/13). Also, evidence for microbial effluent plume tracers at the Diffuser was absent for sampling events 07/07/08 and 01/28/08. It is possible that additional analyses will lead to more evidence of microbial effluent plume tracers for more Lagrangian samples or for sampling events 11/26/09 and 05/28/08, although the microbial signature will be smaller than those already observed.

8.3. Integrative Conclusions

This project demonstrates that a novel sampling plan can be devised and executed combining oceanographic and microbiological approaches for assessing how an ocean outfall alters marine microbial communities in the coastal ocean environment. A first and very important success was that oceanographic modeling indicated that ocean surface water samples taken along the path of the drifters captured the diluted effluent plume.

Microbiological Effluent signatures were observed at the Diffuser and to a lesser extent in Lagrangian samples, using PhyloChip and TRFLP analysis. When observed, those signatures are a minor part of the total microbial community in the nearshore environment, therefore microbial community changes in the nearshore environment due to the effluent are very subtle. These observations make sense considering that salinity and nutrient concentrations indicate at least 100-fold dilution of the effluent at the Diffuser, and up to 900-fold before entering the surfzone. The lower than expected dilution (based on salinity) of Diffuser signature OTUs between Effluent and Diffuser suggested that Effluent-associated bacteria can grow in the nearshore environment.

The nearshore samples also had higher biomass concentrations and in some cases higher FIB concentrations compared to the Offshore samples, which could not attributed to direct inputs from the Effluent. It is possible that nutrient inputs from the effluent could have stimulated some bacteria to grow, or those bacteria could have originated from sources associated with the Shoreline.

The Shoreline microbial community composition in general was not influenced by the occurrence of surfzone entries, indicating that the effluent plume, when reaching the Shoreline, did not greatly affect microbial community composition. In addition, there appeared to be slight differences in microbial community composition between Shoreline and Diffuser and Lagrangian samples. PhyloChip analysis indicated higher abundances of distinct Shoreline OTUs, and FIB concentrations were often highest at the Shoreline. However, no direct evidence was found in this study that those microorganisms originated from the WWTP effluent. The increased abundances of selected bacteria at the Shoreline can be caused by:

- Input from terrestrial sources, such as runoff and animals dwelling on the beach, with or without additional growth in the Shoreline environment
- Input from the WWTP effluent often flowing towards the beach, followed by growth in the Shoreline environment
- Input from beach sand, inoculated with bacteria from terrestrial sources or WWTP effluent.

At this point it is not possible to conclude if one or more of those processes occur at the Shoreline.
The results of this study indicate that it is unlikely that the historical high FIB concentrations at Hammonds beach were caused by the WWTP effluent. First, FIB concentrations at the Shoreline were consistently low in this study, even following surfzone entries. Second, the drifters released at the diffuser rarely reached Hammonds beach.

8.4. Project Objective Conclusions

The proposed novel interdisciplinary sampling plan was carried out with success yielding a unique and comprehensive data set. All goals as presented indicate in Section 2.1 have been answered in previous sections of this test.

9. Recommendations for further analysis

This is a first report on a large and comprehensive dataset. Additional analyses are needed in the following areas:

- Quantification of horizontal mixing as related to dilution and plume modeling
- Evaluation of additional Effluent-specific families as effluent plume tracers using PhyloChip (similar to analysis performed for *Lachnospiraceae*), including the influence of different pf cutoff values.
- Comprehensive evaluation of interrelationships between microbiological, chemical and physical oceanographic measurements. This should include relating the detection of effluent plume tracers by TRFLP, PhyloChip and univariate microbiological metrics to oceanographic measurements.
- Evaluation of PhyloChip OTUs that are distinct to the nearshore environment, but absent in the effluent and the offshore.
- Reanalysis of microbiological and chemical data across the surfzone based on detailed ocean current conditions, including
    - Evaluation based on current direction, speed, and relative dispersion and
    - Surf zone versus last Lagrangian samples for the dates of surfzone versus non-surfzone entries.
- Detailed analysis of the three sampling events, including in the context of the WWTP data, with anomalously high DNA yield, HBM, ENT-qPCR and ammonia levels in the effluent.
- Detailed analysis of the nearshore environment to determine if it is distinctive in characteristics that can influence microbial communities.

Also, DNA extracts have been archived for possible additional PhyloChip analysis. Based on the available microbiological, chemical and oceanography data, additional samples could be selected for PhyloChip analysis to strengthen the current conclusions for a higher number of samples.

Several explanations were presented in the Conclusions sections to explain the cause of differences in microbial communities in the nearshore and Shoreline environments. Based on the

current dataset it is not possible to determine the most plausible explanation. Additional sampling with inclusion of more ocean locations (e.g. further offshore, nearshore but outside the plume) may provide a better explanation.

Additional experimentation would also be required to quantify the growth and decay kinetics of bacteria introduced into the nearshore environment with effluent discharge, and the potential effect of nutrient inputs. Such experiments would be essential to test one of our explanations that the effluent inoculates the nearshore environment, which allows for establishing a distinctive near-shore microbial community.

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