Newport Bay Fecal Indicator Bacteria Source Identification Project

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by

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Summary of Findings

As an estuary in southern California, Newport Bay provides many important ecosystem services and beneficial uses, including marine bathing and recreation, shoreline protection, water quality improvement, fisheries resources, and habitat and food for migratory animals. The health of Newport Bay is threatened by point and non-point sources of pollution, including fecal indicator bacteria (FIB), organics, metals, nutrients and sediments. Reflecting the spectrum of environmental challenges facing Newport Bay, the Santa Ana Regional Water Quality Control Board (SARWQCB) adopted for Newport Bay separate Total Maximum Daily Loads (TMDLs) for fecal coliform, nutrients, and sediment. US Environmental Protection Agency (EPA) also promulgated a technical Toxics TMDL for Newport Bay, and the SARWQCB is in the process of adopting TMDLs for separate toxic pollutants such as metals and organics.

The Fecal Coliform TMDL was adopted in 1999 with the goals of reducing FIB concentrations in the Bay, reducing public health risks, and improving water contact recreational activities. This project, which was funded by a State of California Proposition 13 Coastal Nonpoint Source Pollution Control Grant, is intended to support the Fecal Coliform TMDL by identifying and quantifying urban and natural sources of FIB in Newport Bay.

The distinction between urban and natural sources of FIB is required by the Fecal Coliform TMDL and motivated by several considerations:

- Health risk Considerations. Current water quality standards are based on epidemiology studies at beaches contaminated by point sources, such as storm drains and sewage. FIB concentrations in recreational coastal waters may not predict for adverse human health outcomes when FIB are from non-point (and likely non-human) sources, as possibly is the case in Mission Bay, San Diego, California.

- Management Considerations. Best management practices (BMPs) can be conceived to control urban sources of FIB such as sewage or storm drain inputs, whereas the same may not be true for natural sources such as bird droppings or regrowth of environmentally adapted FIB strains.

To assess the relative contribution of urban and natural sources of FIB in Newport Bay, this study reports on four complementary studies that were carried out over approximately 13 months, beginning January 1 2006:

- Field sampling studies to characterize the loading of FIB from urban and natural sources in and around the perimeter of the Upper Bay.
- Field sampling and reconnaissance studies to assess potential urban sources of FIB to Lower Bay from subsurface sewage leaks, pump-out stations, docks and wharves, wash-down activities, and storm drains.
- Microcosm and field studies to characterize the rate and magnitude of natural sources and within-Bay processes that affect the concentration of FIB in the Bay water column.
- Advanced microbial source tracking methods to assess the likely origin of FIB in Newport Bay.

The key results from these four studies are summarized below, in order of their appearance.

Chapter 1. Introduction and overview of water quality impairment of Newport Bay

Newport Bay is divided into two distinct geographic regions called Upper Bay and Lower Bay. Upper Bay is located at the head of the estuary, and is a state ecological reserve and refuge for threatened and endangered species. Lower Bay is located near the mouth of the estuary, and is a regionally important recreational area and one of the largest pleasure craft harbors in the United States. The Bay is approximately 10 km in length and has a median water volume of 16 million cubic meters, which is distributed unevenly between Upper Bay (3 million cubic meters) and Lower Bay (13 million cubic meters). The Bay's tidal prism (volume of seawater exchanged between high-high and low-low tides) is approximately 6 million cubic meters. Previous estimates for the residence time of water in Newport Bay range from approximately 2 to 30 days, depending on the methodology employed for estimating mixing times, and the location of interest within the Bay (in general, mixing time scales are longer in dead-end regions of the Bay where tidal mixing is muted).

Newport Bay can be classified geomorphically as a "Bar Built Estuary", in that it is a drowned river valley with high sedimentation rates and shallow depths. However, intensive urbanization of the land surrounding Newport Bay—most especially the islands and spits in Lower Bay—together with extensive modification of the Newport Bay shoreline for flood and sand control, imply that this estuary is far from a natural geomorphic state.

Based on the application of three different hydrodynamic classification schemes, it appears that circulation in Newport Bay depends on the magnitude of runoff flowing into the Bay from its tributaries, most particularly San Diego Creek. During dry weather, the volume of freshwater contributed by base flow from San Diego Creek is a small percentage (<1%) of Newport Bay's tidal prism. Under these conditions the water column is vertically well-mixed to partially stratified (Type 2a) in most regions of the Bay and circulation is dominated by tides and winds (barotropic circulation). During very large and sustained storms, on the other hand, the volume of freshwater contributed by San Diego Creek can nearly equal the Bay's tidal prism. When this occurs, Newport Bay experiences extreme salinity stratification, approaching that observed in salt wedge estuaries, and the Bay's circulation is likely dominated by density gradients (baroclinic circulation).

Both point and non-point sources of pollution could potentially contribute to elevated concentrations of FIB in Newport Bay. Possible non-point sources of FIB include sewage spills and overflows, illicit sewage discharges (e.g., from boats moored in the marina), bather shedding, bird and animal droppings, growth of environmentally adapted FIB strains in beach sediments or in association with decaying vegetation, and sewage-contaminated shallow groundwater. Possible point sources of FIB include tributary outlets, dry and wet weather surface runoff discharged through storm sewer drains, and treated sewage outfalls. No sewage outfalls discharge directly to Newport Bay, and the closest sewage outfall (operated by the Orange County Sanitation District, OCSD) discharges partially treated and disinfected wastewater effluent approximately 7 km offshore of Newport Bay's ocean outlet. After disinfection, the concentration of FIB in OCSD's outfall is too low, and the discharge diffuser too far offshore, to

realistically impact water quality in Newport Bay. On the other hand, storm drains in Lower Bay and tributary outlets, such as San Diego Creek, in Upper Bay appear to be important point sources of FIB in Newport Bay.

Non-conservative processes that might affect FIB concentrations in Newport Bay include physicochemical removal from the water column (adsorption, sedimentation, filtration), mobilization into the water column from substrates (sediment resuspension, tidal washing off of mudflats), die-off (including by exposure to sunlight, reactive oxygen species, and zooplankton predators), and selection and growth and recovery of injured cells.

Chapter 2. Bay-to-Ocean transect measurements of water and sediment quality in Newport Bay

To characterize the spatial and temporal variability of FIB concentration in the water and sediments of Newport Bay, twelve sites were sampled aong a Bay-to-Ocean (BTO) transect approximately 53 times over the course of 13 months, beginning January 1 2006. The twelve sampling sites were distributed as follows:

- Three sites (BTO 1 through 3) were located in three tributaries to Upper Bay—San Diego Creek (BTO 1), Santa Ana Delhi Channel (BTO 2), and Big Canyon Wash (BTO 3);
- Eight sites (BTO 4 through 11) were located in the main tidal channel of Upper Bay (BTO 4 through 7) and Lower Bay (BTO 8 through 11);
- One "offshore control" site (BTO 12) was located offshore of Newport Bay.

Altogether, 2,441 water samples and 878 sediment samples were collected from these twelve BTO sites. All water samples were analyzed for FIB (*E. coli* and enterococci bacteria, abbreviated below as "EC" and "ENT"), pH, salinity, and particle size distributions. A subset of water samples was analyzed for nutrients (ammonia, nitrate, total phosphorous, total organic carbon) and suspended particle settling velocity. All sediment samples were analyzed for FIB (EC and ENT). A subset of sediment samples was analyzed for percent organic carbon, percent water content, and grain size. In addition, 237 conductivity-temperature-depth (CTD) casts were carried out at 8 within-Bay sites (BTO 4-11). Each CTD cast generated vertical profiles of water temperature, salinity, density, and buoyancy frequency (a measure of vertical density stratification). A downward-looking Acoustic Doppler Current Profiler (ADCP) was deployed at seven sites for 24-48 hours each, to obtain high-frequency information on the nature of within-Bay circulation.

These data document both seasonal variability and down-Bay gradients in FIB concentrations, salinity, nutrients, pH, suspended particle concentration, suspended particle diameter, suspended particle settling velocity, temperature, wind speed, and bird populations.

The water column in the upper reaches of Upper Bay, near the outlet of San Diego Creek, is salinity stratified. Salinity measured at most other sites is well-mixed over the vertical, consistent with the classification of Newport Bay as a Type 2a, or well-mixed, estuary (see Chapter 1 summary above). During storms, however, salinity stratification can extend into Lower Bay.

There is relatively little salinity variation evident in the transverse direction (i.e., from left-to-right bank).

The log-mean concentration of EC and ENT, and percent of samples exceeding marine bathing water standards, decline with distance down the Bay. Tributary sites had the highest concentrations and single-sample exceedence rates (between 16 and 86% of samples, depending on the tributary and FIB group), followed by sites in the upper reaches of Upper Bay (BTO 4 and 5, exceedence rates between 18 and 38%), followed by sites in the lower reaches of Upper Bay (BTO 6 and 7, exceedence rates between 7 and 17%), followed by sites in Lower Bay (BTO 9-11, exceedence rates between 0 and 8%), followed by the offshore control site (BTO 12, exceedence rate of 0%). At the offshore control site all but one sample had FIB concentrations below the lower limit of detection of 10 MPN/100 mL.

Among Lower Bay sites, samples collected from BTO 8 had relatively high concentrations and exceedence rates for both EC and ENT (7 and 12%, respectively). This particular site is located in West Newport Bay, in a region with relatively poor tidal mixing and significant sources of dry and wet weather urban runoff (e.g., from Arches Drain).

A subset of water samples collected during the BTO studies were analyzed for nutrients, including nitrate, ammonia, total phosphorous, and total organic carbon. Generally speaking, nutrient concentrations were highest in samples collected from one or more tributaries, followed by samples collected from within the main tidal channel of Upper Bay, followed by samples collected from Lower Bay, followed by samples collected from the offshore control site. The exception is ammonia, for which the concentrations measured in the upper reaches of Upper Bay are generally higher than the concentrations measured in the tributary sites.

Among the tributary sites, San Diego Creek has the highest average nitrate concentration (26 mg/L or 420 μM), Santa Ana Delhi Channel has the highest average ammonia concentration (0.13 mg/L or 7.6 μM), and Big Canyon Wash has the highest average total phosphorous (1.1 mg/L or 36 μM) and TOC (9.4 mg/L) concentrations. Among within-Bay sites, without exception the upper reaches of Upper Bay had the highest nutrient concentrations. Average nutrient concentrations reported here for Upper Bay sites are similar to values published previously for this wetland. The average nitrate (3.1 mg/L or 50 μM) and total phosphorous (0.36 mg/L or 11 μM) concentrations are at the high end of measurements reported for estuaries on both the east and west coasts of the United States.

FIB concentrations in Newport Bay are positively and significantly correlated with the concentration of several nutrients. In Upper Bay, FIB are significantly and positively correlated with ammonia (EC, ENT), total organic nitrogen (EC), nitrate (EC), Total P (EC), and TOC (ENT). In Lower Bay, the only significant correlations are negative: both EC and ENT are negatively correlated with Total P. Because FIB are significantly and positively correlated with nutrients only in Upper Bay, which is impacted by runoff from the San Diego Creek and Santa Ana Delhi Channel, it is likely that these correlations are associative not causal; i.e., both FIB and nutrients are sourced by the tributaries to Upper Bay, particularly during storms.

During wet weather, EC concentrations are highest in sediment collected from one or more tributaries (BTO 1 through 3), followed by sediment collected from Upper Bay (BTO 4 through 7), followed by sediment collected from Lower Bay (BTO 8 through 11). During dry weather, EC concentrations are higher in sediments collected from the tributaries, and very low in most sediment samples collected from within-Bay sediments.

ENT concentrations are highest in sediments collected from Lower Bay (BTO 8 through 10), followed by sediments collected from Upper Bay and the three tributaries (BTO 1 through 7), followed by the site nearest the ocean outlet (BTO 11). Apart from the tributaries, which have relatively low sample ("N") values (typically N<10), the spatial pattern just described applies to both dry weather and wet weather periods. Spearman rank correlations were carried out to determine if FIB in the sub-tidal sediments of Newport Bay are correlated with other sediment parameters, including organic carbon content, water content, and average grain size. FIB in the sediment tends to be positively correlated with organic-rich, fine-grained sediments with elevated water content. The positive correlations with organic carbon and water content are particularly strong in the case of EC (Spearman Rank Correlations of Sp= 0.5 and 0.6, respectively).

Additional Spearman rank correlations were run to determine if FIB concentrations in the sediment were correlated with FIB concentration in the overlying water column. At Santa Ana Delhi channel site (BTO 2), ENT measured in the sediment is significantly ($p=0.00$) and positively ($Sp = 0.6$) correlated with ENT measured in the overlying water column. For a significance of $p<0.01$, FIB concentrations in the sediment are not significantly correlated with FIB in the overlying water column at any within-Bay sampling sites.

To determine if FIB in the water column are attached to suspended particles, a subset of the water samples collected in Newport Bay were assayed for FIB before and after filtration through a 5 or 10 micron filter. In the majority of cases, filtration of water samples through a 5-micron filter did not significantly alter the concentration of EC and ENT. Hence, within the resolution of the measurement method used, most of the FIB in Newport Bay are present as free-living single cells, or are associated with particles smaller than 5 microns in diameter (note that the volume-averaged diameter of EC and ENT is 1.2 microns). This conclusion is consistent with a number of studies that indicate marine and estuarine waters are dominated by free-living (planktonic) cells. There are several noteworthy exceptions to this rule. Passage through a 5-micron pore sized filter did significantly lower the EC concentration in water samples collected from Upper Bay water during the dry season, suggesting that EC cells may be particle-associated in this specific case. Also, passage of Santa Ana Delhi samples through the 5 micron pore-sized filter appeared to increase the concentration of FIB, perhaps due to the removal of an (unknown) substance that interferes with the EC assay, and/or because the filtration process disrupts EC-laden flocs.

Chapter 3. Speciation and molecular fingerprinting of Newport Bay *Enterococcus* Isolates

In this and the next chapter, advanced microbial source tracking (MST) methods were utilized to obtain additional information on urban and natural sources of FIB in Newport Bay. The focus of

Chapter 3 is on using MST methods to assess sources of enterococci bacteria (ENT). The focus of Chapter 4 is on using MST methods to assess sources of *Escherichia coli* (EC).

Two MST methods were used to obtain information on potential sources of ENT in Newport Bay: (1) speciation of ENT isolates cultured from Newport Bay sediment and water samples; and (2) pulsed field gel electrophoresis (PFGE) typing of speciated isolates. Some species of ENT (such as *Enterococcus faecium* and *E. faecalis*) are commonly associated with fecal waste, while other species (such as *E. casseliflavus* and *E. mundtii*) are not. Thus, the species distribution of ENT isolates could potentially provide insight into the relative sources of these organisms. PFGE typing goes a step further by helping to discern the degree to which ENT isolates belonging to a single species are clonal (i.e., genetically identical at the level of discrimination afforded by the PFGE method). Presumably, if ENT were growing in the sediments and water column of Newport Bay, a large percentage of the isolates would not only belong to the same species, but also would be clonal.

A total of 103 samples were included in the speciation study, including 69 water samples and 34 sediment samples. These 103 samples were collected from Newport Bay as part of the BTO study described in Chapter 2 and the Diurnal Intertidal Sediment (DITS) study described in Chapter 6. A total of 349 isolates were cultured from the 103 samples, including 249 and 100 isolates from water and sediment samples, respectively. Because ENT concentrations were generally higher at the tributary and Upper Bay sites, most ENT isolates were cultured from these regions. However, a more even distribution of isolates across the Bay was achieved by binning the isolates as follows: (1) San Diego Creek (BTO 1), N=53 isolates; (2) Santa Ana Delhi Channel (BTO 2), N=54 isolates; (3) Big Canyon Wash (BTO 3), N=35 isolates; (4) BTO 4, N=78 isolates; (5) BTO 5 through 7, N=87 isolates; (6) BTO 8 through 12, N=42 isolates. An analysis of the species breakdown in each of these site groupings can be summarized as follows:

- In both tributary and Upper Bay sites, ENT is dominated by *E. casseliflavus* and *E. mundtii,* and to a lesser extent by the *E. faecalis*, *E. faecium*, and *E. hirae*.
- With distance down-Bay, the species distribution appears to shift toward a more even distribution of (*E. faecalis, E. faecium, and E. hirae*) and (*E. casseliflavus and E. mundtii*) species.

It is important to keep in mind that the concentration of *Enterococcus* also declines with distance down-Bay (see discussion of Chapter 2 results above), so the down-Bay change in species composition could reflect differential die-off of certain strains in the Bay, and/or predominance of different sources of enterococci bacteria in Upper and Lower Bay. In summary, a very large percentage (between 45 and 65%, depending on location) of the enterococci bacteria in Newport Bay and its tributaries are species (e.g., *E. casseliflavus and E. mundtii*) that are potentially from non-fecal sources, such as growth on decaying plant material.

Four *Enterococcus* species were selected for molecular typing by PFGE. *E. faecalis* was chosen because it was frequently isolated from water and sediment samples from Newport Bay, and it has been typed in previous environmental studies in Orange County and thus a large database of types exist. *E. casseliflavus, E. mundtii* and *E. faecium* were also frequently isolated from water and sediment samples from Newport Bay, but these species have not been previously typed in

Orange County environmental studies by PFGE fingerprinting. Altogether, 232 isolates from 77 samples were typed, including 85 *E. casseliflavus,* 63 *E. mundtii*, 40 *E. faecalis* and 44 *E. faecium*.

Most PFGE types (or fingerprint patterns) were detected only once, consistent with the idea that there are many different strains of each *Enterococcus* species present in the water and sediment of Newport Bay. Not only are there many different types, these types are relatively heterogeneous, in that the dendrograms of the PFGE patterns reveal no meaningful grouping or sub-grouping at any level of relatedness by sampling site, sample type or date taken. Overall the number of pattern matches, indicating either inter-sample or intra-sample clonality, was low for the number of isolates analyzed. Of the samples where ENT clones were detected, there were relatively numerous PFGE type matches between isolates cultured from San Diego Creek and isolates cultured from either Upper Bay or Lower Bay. One interpretation of these results is that San Diego Creek is the source of these clonal types. However, it is also possible that clonal types found at different sites in the Bay were not transported there from San Diego Creek, but rather originated from a widely distributed source that releases ENT throughout the Bay (e.g., birds, animals, plants or soil).

None of the *E. faecalis* types detected in this study matched *E. faecalis* types isolated from Dana Point Harbor, Lower Santa Ana River and Huntington Beach from 2003 to 2006. Thus, within the geographic resolution of the existing database, *E. faecalis* strains measured in Newport Bay may be unique.

The overall effect of regrowth of *Enterococcus* in sediments on the levels of bacterial pollution in Newport Bay could not be determined with the limited data from this study. However, a moderate percentage of sediment and water samples contained clonal isolates representing several species suggest that regrowth may occur, consistent with the observations of high ENT concentrations in the sub-tidal sediments of Newport Bay (see discussion of Chapter 2 above).

Chapter 4. Phenotyping and Gene Array Analysis of Newport Bay *E. coli* isolates

In this chapter, presumptive *E. coli* (EC) isolates cultured from water and sediment in Newport Bay were assayed using an identification system called API 20E, a commercially available biochemical substrate test kit designed to identify enteric bacteria. Results from the API 20E tests serve to confirm that presumptive EC isolates are in fact EC. Further, different strains of EC may be distinguished by their API biotypes (a unique numerical code that is assigned the isolate based on the scoring of biochemical reactions). The API 20E biotypes have considerably less discriminatory power than the PFGE method used to evaluate *Enterococcus* clonality in Chapter 3. The limited discriminatory power of API 20E means that its use to evaluate the diversity of EC strains in Newport Bay should be viewed as exploratory. Indeed, the dominant EC biotype present in Newport Bay can have many different sources, based on a literature review and API 20E analysis of EC isolated from fecal samples from different animals. Finally, an experimental method was employed—called a gene expression assay—to assess if EC strains isolated from Newport Bay are uniquely adapted to grow in the estuarine environment of Newport Bay.

The main findings of these studies are:

- 177 and 24 EC isolates were cultured from Newport Bay water and sediment samples, respectively.
- EC in Newport Bay is dominated by a single biotype, API code 5144572.
- The low biotype diversity of EC isolates in Newport Bay is surprising, but its significance cannot be evaluated without further analysis of the isolates belonging to this biotype, perhaps using more discriminatory molecular methods such as PFGE and/or rep-PCR.
- The predominance of a single EC biotype at both Bay and tributary sites suggest that the tributaries are the primary source of these organisms in the Bay. However, the finding is inconclusive without the application of a more discriminatory typing method, as noted above. Indeed, EC belonging to the single dominant biotype reported here (API code 5144572) can be cultured from a variety of fecal sources, based on experiments reported in this chapter and a survey of the literature.
- This study did not detect differences in the global gene expression patterns of EC isolates from Newport Bay and from human sewage, bird and cattle feces, but that may be the result of the experimental design.

It is interesting to note that, based on the BTO data presented in Chapter 2 and the loading analysis presented in Chapter 8, EC in Newport Bay may have two primary sources that dominate at different times of the year. The loading studies in Chapter 8 suggest that, during wet weather, San Diego Creek and, to a lesser extent Santa Ana Delhi Channel, are the primary sources of EC in Upper Bay. Further, based on the particle association studies in Chapter 2, during wet weather EC in the water column do not appear to be associated with particles greater than 5 microns in diameter, and their concentrations are well-mixed over the water column at each site. During dry weather, on the other hand, loading of EC from San Diego Creek and Santa Ana Delhi channel is not sufficient to account for EC concentrations observed in Upper Bay, based on modeling results presented in Chapter 8. Further, during dry weather, EC concentrations are higher at the bottom of the water column in Upper Bay, appear to be associated with particles greater than 5 microns in diameter, but are not present at high concentrations in sub-tidal sediments (Chapter 2). In future analysis of these data, an attempt will be made to integrate the biotyping results presented in this chapter, and the temporal and spatial patterns and loading studies presented in Chapters 2 and 8, respectively.

Chapter 5. Microcosm studies to characterize the rate and magnitude of natural sources and within-Bay processes that affect the concentration of FIB in Newport Bay

This chapter describes the results from 64 separate laboratory and field microcosm experiments. These microcosm experiments were carried out to characterize the input of FIB to Newport Bay from a variety of natural (bird droppings, debris mats, sediment) and urban (runoff) sources, and to elucidate the influence of environmental variables (sunlight, synergistic interactions between various substrates) on FIB persistence in Newport Bay. Microcosm studies were conducted in the laboratory to isolate the influence of specific variables, and in the field to more realistically simulate conditions in Newport Bay. Supplemental field investigations were also carried out, including a synoptic study of FIB concentrations in the Upper Bay "Unit 1 Basin" during a

macroalgal bloom, and field observations of debris mat formation in Upper and Lower Bay during and following storm events.

The main conclusions from these studies can be summarized as follows:

- FIB from San Diego Creek are present at higher concentrations, longer-lived, and more resilient to changes in salinity, than FIB from Upper Bay.
- Consistent with the literature, bird feces harbor very high concentration of FIB, but after entering the Bay bird-derived FIB die-off at a rate that depends on solar irradiation levels (faster die-off in the presence of sunlight, slower die-off in the dark). However, even in the presence of sunlight, FIB from bird feces can take several days to achieve 90% die-off.
- In laboratory microcosms, the addition of macroalgae significantly increases the survival of FIB from bird feces. However, the protective effects of macroalgae on FIB survival and/or regrowth were not replicated in field experiments, perhaps due to different redox conditions (oxic in the case of field microcosms, and hypoxic in the case of laboratory microcosms).
- A synoptic study of FIB in Upper Bay during a macroalgae bloom found significant positive correlations between EC and other nutrients (TKN, ammonia, TOC, and nitrate), perhaps signaling the presence of contaminated runoff from San Diego Creek. By contrast, ENT was not correlated (either positively or negatively) with salinity, which could be interpreted in several ways: (1) San Diego Creek is not the only source of ENT in the Unit 1 Basin; or (2) San Diego Creek is a significant source of ENT in the Unit 1 Basin, but these organisms die-off rapidly once they enter the Bay. The second hypothesis is not supported by the results of the Bay/Creek Microcosms studies (see first bullet above), which indicate that Creek ENT are relatively stable when diluted into Bay waters. A strong positive correlation was noted between ENT concentration and total phosphorous, which might indicate that bird droppings contribute to ENT concentrations along the shore in the Unit 1 Basin.
- There is some evidence that debris mat material may aid in survival of FIB in creek and Bay water. In principle, both debris mat material and macroalgae could provide nutrients and organic carbon to the water column as they decay, which FIB can potentially use as a substrate for maintenance or regrowth. Macroalgae and debris mat material can also provide shading from the germicidal action of UV radiation.
- Bay sediments contribute ENT to the water phase of the microcosms, and the presence of sediments in the microcosms appears to reduce the die-off rate of these organisms. This result was observed in both laboratory and field experiments. Translation of these microcosm results into the field had mixed result. In the case of intertidal sediments along the shoreline, it appears that ENT are mobilized from sediments into the water column during rising tides (Chapter 6). However, sub-tidal sediments do not appear to be a significant source of ENT in the water column of the main tidal channel in either Upper or Lower Bay (Chapter 2). From a monitoring perspective, the intertidal results may be more significant, because water samples for routine monitoring are typically collected from the water's edge, where resuspension of intertidal sediments is likely to occur.
- Bay sediments appear to contribute relatively little EC to the water phase, but may aid in their survival. As noted in Chapter 2, during dry weather EC concentrations are low in

sub-tidal sediments, but particle-associated EC appear to accumulate near the sediment bed in Upper Bay water column (Chapter 2).

- It is also possible that turbidity contributed by sediments provide UV shading, thus reducing sunlight-induced die-off of FIB. Sediments can also contribute nutrients and organic carbon to the water-column, particularly if the sediment is fine grained, which, in turn, can be used by FIB to sustain their populations.
- FIB in dry weather runoff behave relatively conservatively when diluted into high salinity water from Lower Bay; i.e., runoff-derived FIB do not precipitously die-off when mixed into high salinity Newport Bay waters.
- The Single-Hit-Model for UV mediated die-off of FIB generally underestimates the survival of runoff-derived FIB in the high salinity waters of Newport Bay.

Chapter 6. Diurnal Intertidal Sediment (DITS) Studies

A number of recent studies have implicated beaches—either the beach itself, or shallow groundwater discharge through the beach face—as the source of FIB impairment in ankle depth waters along the shore. Based on the microcosm results presented in the last chapter, FIB might originate from the surface of the beach in at least two ways: (1) deposition of bird feces on the surface of the beach, which mobilize into the water column during rising tides; (2) growth of environmentally adapted strains of FIB in the beach sand and/or in decaying vegetation (e.g., macroalgae or wrack lines) concentrated along the shore.

As a first step toward evaluating the beach as a potential source of FIB, we carried out a field sampling effort in which water and sediment samples were collected along two cross-shore transects in Upper Bay, near sites BTO 4 and 5. In the results presented below, the cross-shore transect located near BTO 4 is called "DITS 1", while the cross-shore transect located near BTO 5 is called "DITS 2". Sampling campaigns were carried out a total of four times (twice during wet weather, and twice during dry weather). During each sampling campaign, water and sediment samples were collected from a set of sites at DITS 1 and 2 every 3 hours for 12 or 24 hours (depending on the study). Sediment samples were analyzed for FIB, organic carbon, water content, and grain size distribution. Water samples were analyzed for FIB, and a subset of water samples were analyzed for nutrients.

Not surprisingly, FIB concentrations and exceedence rates were much higher during the wet weather sampling, compared to the dry weather sampling. However, in both cases the mudflats appear to be a source of FIB in the very shallow waters along the shoreline in Upper Bay. While the study design adopted here cannot definitively determine the source of FIB on the mudflats, several observations point to the deposition of bird feces:

1. Birds are present at high numbers along the mudflats during both wet and dry weather studies. The physical location of the birds (near the water's edge) coincides with the location where FIB concentrations in both water and sediment are highest.
2. Along the banks of the tidal channel, in many cases FIB concentrations are higher in sediment collected above the waterline, and lower in sediment collected below the waterline, even during periods of time when the tide is falling. This suggests that there is fast replenishment of FIB on the exposed mudflat as the tide recedes, consistent with our

photographic observations that birds congregate, and presumably defecate, right at the water's edge. If regrowth in the sediment was the dominant source of FIB on the mudflat, it would presumably take some time to regenerate FIB in the sediments after they were washed by the tide, as was observed during a recently published study of ENT regrowth in intertidal sediments at Lover's Point in northern California.

3. Water samples collected during the dry weather studies had lower FIB exceedence rates compared to water samples collected during the wet weather studies. Suspended particle concentrations were generally higher wet weather (ca 100 ppm) compared to dry weather (ca., 50 ppm). As noted in a recent article, suspended particles can reduce the deleterious effects of sunlight on FIB survival in estuarine environments, which would be at least superficially consistent with FIB exceedence rates measured during the dry and wet weather DITS studies.

Bird Census. Estimates of the number of birds present on the mudflats during the DITS 1 and 2 studies suggest that bird populations in Upper Bay are highly variable in space and time. Despite this variability, it can be said that large numbers of birds congregate on the mudflats in Upper Bay (frequently >300 birds could be counted in the images), and more birds tend to loaf on the right bank, compared to the left bank, of the main tidal channel at DITS 1 and 2. Some diurnal variability was also noted; e.g., in several cases, birds seemed to prefer to congregate on the mudflats during falling tides in the early morning or late evening.

Nutrient Concentrations in the Water Column. Water samples from the thalweg of the tidal channel were analyzed for a suite of nutrients. These data generally confirm the results presented in Chapter 2. Namely, San Diego Creek appears to be a significant source of nitrate and total phosphorous. San Diego Creek may also be an important source of ammonia, TKN, and TOC. However, within-Bay processes appear to be a source of both ammonia and TOC, perhaps from the microbially mediated conversion of nitrate to ammonia under anaerobic conditions, and the fixation of organic carbon by macro- and micro-algae present in the Bay.

Chapter 7. Urban sources of FIB in Lower Newport Bay

This chapter summarizes efforts to characterize the input of FIB into Newport Bay from urban sources located in Lower Bay, including storm drains, sewer lines, and shallow groundwater.

Sewage Contamination of Shallow Groundwater. A series of exploratory investigations were carried out to identify regions of the Lower Bay where bathing water quality might be impacted by the efflux of sewage-contaminated shallow groundwater. The main findings are:

1. Sewer collections lines in Lower Bay vary in age and composition. Older sewer lines, some of which were laid in the early 1920s, are composed primarily of vitrified clay pipe (VCP) with cement joints. Newer sewer lines are composed primarily of polyvinyl chloride (PVC) with rubber-gasket wedge-lock joints. The City of Newport Beach is implementing programs to replace the older sewer lines as new development and/or significant remodeling occurs.
2. Five sites in Lower Bay were selected for shallow groundwater testing, based on their proximity to sewer lines of various age and material composition, proximity to

recreational beaches, and accessibility. Samples of shallow groundwater from these sites had oceanic salinities.

3. EC concentrations in the shallow groundwater were below the California single-sample standard for marine bathing waters at all five shallow groundwater testing sites. ENT concentrations, on the other hand, were above the California single-sample standard for marine bathing waters at two of the shallow groundwater testing sites. One site (at 9th street on the Balboa Peninsula, ENT = 292 MPN/100 mL) is near a sewer main constructed of VCP and installed in 1931. The other site (at Alvarado Place on the Balboa Peninsula, ENT= 1118 MPN/100 mL) is located near a sewer main constructed of PVC and installed in 1985.

Given that ENT concentrations were somewhat elevated at one site (9th Street) and significantly elevated at another site (Alvarado Place), and given that 2 of 5 shallow groundwater sites had elevated FIB concentrations, further testing of the shallow groundwater in Lower Bay may be warranted.

Synoptic Study of Lower Newport Bay. The goal of the synoptic survey was to measure water quality at 80 to 90 locations along the shoreline in Lower Bay during a dry weather period in the summer, and to determine how water quality there is modulated by: (1) tide conditions (high tide vs. low tide), and (2) cross-shore location (water's edge, 100 feet bay-ward). The main findings are:

1. For a fixed tide condition (i.e., low or high tide), water samples collected closer to shore have lower salinity, lower pH, and higher EC and ENT concentrations.
2. For a fixed cross-shore location (i.e., water's edge or 100 feet bay-ward), samples collected at low tide have lower salinity, lower pH, and higher EC and ENT concentrations.
3. During low tide, more samples exceed the single-sample marine bathing water standards for EC and ENT. Regions prone to water quality exceedences include: (1) Turning Basin in West Newport (near the Arches Drain); (2) north-east shore of Lido Isle; (3) north-east shore of Balboa Peninsula; (4) east shore of Balboa Island (near the El Paseo Drain); and (5) Rhine Channel.
4. During low tide, the same four regions of Lower Bay have relatively low salinity.
5. During high tide, few samples exceeded single-sample standards for EC and ENT.
6. FIB contamination along the shoreline in Lower Bay may be affected by the episodic discharge of runoff to the Bay by tidal flow in storm drains.

These results demonstrate that water quality along the shoreline in Lower Bay is strongly modulated by the tides—with higher FIB concentrations (and more frequent FIB standard exceedences) occurring during low tides, and lower FIB concentrations (and less frequent FIB standard exceedences) occurring during high tides. This tidal signature, together with the association between elevated FIB concentrations and depressed salinity, suggest that runoff flowing into storm drains in Lower Bay may adversely impact water quality along the shoreline in Lower Bay.

OCHCA's ENT Monitoring Data for Newport Bay. The Orange County Health Care Agency (OCHCA) collects water samples weekly from 35 sites in Newport Bay and analyzes the samples for TC, FC, and ENT concentrations. These data were acquired from OCHCA and analyzed with two goals in mind: (1) to identify regions of the Bay where water quality is consistently poor during dry and/or wet weather, and (2) to determine if water quality is modulated by the tides at storm drain impacted sites. The main findings are:

1. During dry weather, log-mean ENT concentrations are highest at the following sampling sites: Western Newport Bay (BNB09, BNB11, BNB35), Big Canyon Creek (CNBBC), Vaughn's Launch (BNB25), Santa Ana Delhi Channel (CNBSA), San Diego Creek (CNBCD), a drain in the Dunes Recreation area (CNBND), and Harbor Patrol Beach (BNB33).
2. During storm advisories, the log-mean ENT concentration shifts upward at most sites by approximately 0.5 log units, although some sites experience more of an increase than others. Sites with the greatest storm-related increase in ENT concentration include: BNB23 (Rocky Point Beach), BNB10 (38th Street Beach), BNB30 (De Anza, near the PCH bridge), BNB24M (Middle Station at Newport Dunes), and the San Diego Creek (CNBCD).
3. During dry weather, ENT concentrations are significantly and modestly negatively correlated with tide levels at 6 sites in Newport Bay, including three sites in Western Newport Bay and Rhine Channel (BNB35, BNB11, and BNB12), a beach site and drain in the Dunes Recreation area (BNB24W and CNBND), and a site near the Harbor Entrance (BNB 22). Negative correlations between tide level and ENT concentrations are also observed at BNB 22 and CNBND. The Dunes Recreation area (BNB24W), on the other hand, exhibits a positive correlation between ENT and tide level.
4. During rain advisories, none of the sampling stations exhibit a significant correlation between ENT and either tide level or change in tide level. This lack of significant correlation may be due to the relatively small sample size for the FIB measurements.

ENT concentrations are higher during low tides at several OCHCA monitoring sites in Newport Bay. These sites tend to be located in "dead-end" regions of the Bay—such as Western Newport Bay, Rhine Channel, and the Dunes Recreation area—with limited tidal mixing, long water residence times, and significant sources of runoff. Change in tide level (i.e., whether the tide is rising or falling at the time a sample is collected), on the other hand, does not appear to be a predictor of water quality at most sites.

Irrigation Runoff and Storm Drains. From the results of the synoptic study and the historical analysis of OCHCA data described above, it appears that dry and wet weather runoff can be a significant source of FIB in the nearshore waters of Lower Bay. To better quantify the magnitude of this FIB source, 21 storm drains in Lower Bay were singled out for detailed study. Two samples were collected at each storm drain site: (1) a mixture of runoff and Bay water collected from near, or at, the outlet of the drainpipe (called near-drain sites); and (2) irrigation runoff collected from the curb of the nearby street. The entire study was conducted twice, once during a dry weather period and once during a wet weather period. The main findings are:

1. A relatively large percentage of samples of both irrigation water and near-drain water had EC and/or ENT concentrations above the California single-sample marine bathing water standards (and US EPA's marine bathing water criteria).
2. The percentage of samples with ENT concentrations above water quality standards decreased in the following order: wet weather irrigation runoff (76%) > wet weather near-drain samples (71%) > dry weather irrigation runoff (67%) > dry weather near-drain samples (57%).
3. The percentage of samples with EC concentrations above water quality standards decreased in precisely the same order: wet weather irrigation runoff (62%) > wet weather near-drain runoff (38%) > dry weather irrigation runoff (29%) > dry weather near-drain runoff (24%).
4. Nearly all storm drain samples with relatively low salinity (<25) also exceeded both the EC and ENT standards, implying that low salinity is a good predictor of poor bathing water quality near storm drain outlets in Lower Bay.
5. The main result described here—that low salinity is a risk factor for poor water quality around the outlet of storm drains in Lower Bay--is consistent with a conceptual model developed in this study for how runoff from storm drains impacts near shore water quality in Lower Bay.
6. The water quality exceedences occurred across all of Lower Bay (i.e., there was no single " hot spot" in the Bay which accounted for the majority of water quality exceedences).

From these data, it appears that dry and wet weather runoff is a significant source of FIB in the near shore waters of Lower Bay, as evidenced by (1) the high concentrations of FIB measured in samples of irrigation runoff; (2) the high concentrations of FIB measured in samples of Bay water collected from near the outlet of storm drain during both dry and wet weather; and (3) the fact that, in samples of Bay water, elevated FIB concentrations are frequently associated with low salinity. In addition to implicating dry weather runoff from storm drains in Lower Bay as a source of FIB pollution in near shore waters, these results also suggest that salinity may be a good (i.e., reliable, fast, and cheap) proxy for FIB contamination.

The following runoff drains that outlet to Newport Bay currently have low-flow diversions in place:

1. Back Bay Drive Diversion (operated by the City of Newport Beach).
2. Four small plug type diversions for the storm drains in the Newport Dunes parking lot (operated by the Newport Dunes maintenance staff).

Chapter 8. FIB Loading from urban and natural sources and impact on Newport Bay water quality

This chapter provides first-order estimates of the impact that urban and natural sources of FIB have on shoreline water quality in Newport Bay. These estimates are intended to guide the assignment of Waste Load Allocations (WLAs) as part of the Fecal Coliform TMDL management plan. One difficulty encountered when developing WLAs for FIB is the fact that water quality exceedences—a critical condition of interest—occur in the very shallow (*ca*., ankle depth) waters along the shoreline. As described earlier in Chapter 1, water quality along the

shoreline is affected by both Bay-wide events (e.g., the impact of storm flow from the San Diego Creek on water quality over the entire Bay, see Chapter 2), and highly localized and transient shore-based non-point sources of FIB from storm drains, bird droppings and environmental growth on wrack lines and in beach sediments (see microcosm results and DITS study, Chapters 5 and 6).

To address the complexity just noted, in this chapter we evaluate the water quality impact of FIB loads from urban and natural sources at three distinct scales:

1. Bay Scale: Bay-wide water quality impact of FIB loading from San Diego Creek and Santa Ana Delhi Channel.
2. Beach Scale: Local water quality impact (in ankle depth waters) of FIB loading from distributed non-point sources along the shore.
3. Drain Scale: Local water quality impact (in ankle depth waters) of FIB loading from individual storm drains.

For each of these three scales, water quality impacts are evaluated using a two-step process. First, FIB loading rates are estimated for the specific FIB source of interest. In some cases this is a straightforward calculation, involving the multiplication of measured concentrations by measured flow rates (e.g., FIB loading from San Diego Creek). In other cases, FIB loading rates are estimated based on a model of how FIB are mobilized into the water column from a particular source (e.g., FIB loading from resuspension of contaminated sediment along the shoreline). Second, once the FIB loading rate associated with a specific source has been estimated, simple mass balance models are used to predict the consequent water quality impacts at an appropriate spatial scale. While more sophisticated models could be employed to translate FIB loads into shoreline FIB concentrations, the approach adopted here (of focusing on three distinct scales separately, and employing simple analytical models to convert loading estimates to FIB concentrations) conforms to EPA's Principle of Parsimony; namely, that the optimal WLA model is the simplest approach that captures "all the important estuarine phenomena affecting water quality".

The measurements and modeling studies documented in this chapter support the following conclusions.

Bay Scale. A simple mathematical model of tidal transport and dilution in Newport Bay was developed and used to investigate the impact of San Diego Creek and Santa Ana Delhi channel on water quality in Upper and Lower Bay. During wet weather, the model accurately predicts FIB concentrations measured in the Bay during the BTO studies (Chapter 2), provided that we use as model input the FIB loading from both San Diego Creek and Santa Ana Delhi channel. Thus, during wet weather, it appears that FIB concentrations in the main tidal channel of Newport Bay are controlled by the magnitude of FIB loading from these two tributaries.

During dry weather, the model accurately predicts measured ENT concentrations in the Bay, but only when we use as model input the FIB loading from San Diego Creek. When dry weather ENT loading from Santa Ana Delhi channel is used as input to the model, predicted ENT concentrations are significantly (10 to 100 times) greater than measured FIB concentrations in

the Bay. Taking these model results at face value, they suggest that dry weather concentrations of ENT in the main tidal channel of Newport Bay are lower than would be expected, given the very high loading of ENT measured at our sampling site on the Santa Ana Delhi channel. This observation could have several possible explanations:

1. One or more assumptions in the simple 1D model used here may render the model predictions inaccurate during dry weather.
2. The load of ENT from the Santa Ana Delhi channel may be attenuated between where the loading rate was measured on the Santa Ana Delhi channel (i.e., site BTO 2) and the main tidal channel of Newport Bay (where model predictions were compared to ENT measurements at BTO 4 through 11).

The first explanation will be evaluated as the model is vetted through peer review, and possibly followed up with more sophisticated (multi-dimensional) modeling efforts. To evaluate the second explanation, and obtain more information about how bacteria from the Santa Ana Delhi channel make their way into Newport Bay, several TAC members, led initially by Dr. Jack Skinner, conducted a field reconnaissance of the site. From this field reconnaissance, the following can be stated:

(1) Before runoff from the Santa Ana Delhi channel flows into Newport Bay's main tidal channel, it must first travel along a 1 km incised tidal mudflat channel referred to here as the "Santa Ana Delhi Tidal Channel".
(2) Tidewaters intrude a significant distance up the Santa Ana Delhi Tidal Channel, including upstream of our Santa Ana Delhi sampling site (BTO 2), consistent with the brackish salinities that were measured in water samples collected from BTO 2 during the summer of 2006.
(3) Water in the Santa Ana Delhi Tidal Channel does not appear to overflow its banks and spill into the surrounding mudflat. During a high tide on 5/29/09, for example, water in the Santa Ana Delhi Tidal Channel was well below the height of the channel levee (personal observation, Ray Hiemstra). A photograph of the lower reaches of the Santa Ana Delhi Channel during a high tide indicates that water in the channel is confined between the banks.
(4) In the upper reaches of the Santa Ana Delhi channel, just downstream of our Santa Ana Delhi channel sampling site (BTO 2), the channel bottom forms a set of basins separated by shallow sills. While the depth of the basins and sills varies throughout the tidal cycle, when observed during a low tide, one basin documented in the report was >2 m deep.

Taken together, these field observations suggest that the dry weather residence time of water in the tidally influenced portion of the Santa Ana Delhi channel is likely to be long (i.e., multiple tidal cycles) because: (1) the basins and sill topography of the channel bottom will act to retard drainage of the channel during ebb tides, and (2) flow in the channel will probably be dominated by tidal action during dry weather, when the volume of freshwater from the Santa Ana Delhi channel is low. The long residence time of water in the Santa Ana Delhi Tidal Channel may act to attenuate the summertime loading of ENT from BTO 2, as our modeling suggests, by increasing the dilution of runoff with (relatively clean) Bay water, and increasing the time over which bacteria in the runoff die-off in the brackish waters of the tidal channel.

It is important to note that, even if the above scenario is correct, changes in the topography of the tidal channel (e.g., elimination of the basin and sills by scouring of the channel bottom during storms), could alter the residence time of water in the channel, and thus alter the loading of FIB to the main tidal channel of Newport Bay. Indeed, it appears that dry weather water quality in Newport Bay has improved over the last several years, which might be attributable to changing bottom topography in the Santa Ana Delhi channel and possibly the recent construction of detention basins in the lower reaches of the San Diego Creek (efforts are ongoing to determine the timing of alterations to the lower reach of San Diego Creek).

Model predictions also indicate that dry weather loading of EC from San Diego Creek and Santa Ana Delhi channel is not sufficient to explain measured EC concentrations in the Bay, suggesting that another source—perhaps growth in a near-bed layer as suggested in previous chapters (see Chapter 2) —dominates during dry weather periods.

Model predictions were also carried out to obtain a region of impact (ROI)—defined as the region of the Bay where FIB concentrations are predicted to exceed marine bathing water standards—attributable to loading from San Diego Creek and Santa Ana Delhi channel. These ROI calculations indicate that, for all but the largest 90th percentile of storms, water quality violations attributable to FIB loading from the two tributaries are confined to Upper Bay.

Beach Scale. The water quality impact of shore-based non-point sources of FIB—including bather shedding, bird droppings, and tidal washing of contaminated sediments—was also investigated using measured data and a simple (integrated) form of Fick's Law of Diffusion. The results indicate that bather shedding is unlikely to cause water quality exceedences along the shoreline in Newport Bay. Tidal washing of contaminated sediments and bird droppings, on the other hand, are very likely to cause at least transient exceedences of marine bathing water standards in ankle depth waters.

Drain Scale. An inventory of storm drains in Newport Bay was conducted, and the following attributes were determined: drainage area, drain diameter, outfall location, and estimated dry weather flow rates. From these data, probability distributions were generated of dry weather FIB loadings from the many small and large drains in Newport Bay. The results suggest that the cumulative dry weather loading of FIB to the Newport Bay shoreline from all storm drains is (1) dominated by five major storm drains, and (2) comparable in magnitude to the median dry weather loading rates measured in San Diego Creek and Santa Ana Delhi channel. However, the FIB loading from drains is distributed around the perimeter of Newport Bay, and thus should have only localized impact on shoreline water quality; i.e., with a few exceptions (such as Arches Drain), discharge of dry weather runoff from storm drains is unlikely to contaminate large regions of Newport Bay. Conversion of these loading rates into FIB concentrations along the shoreline is complicated by a number of issues, related to the timing of the discharges, the geometry of the site, and the nature of mixing and transport that occurs after discharge.

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[1]Chapter 1. Introduction and Overview of Water Quality Impairment of Newport Bay

1.1. Introduction

Newport Bay is the second largest tidal embayment in southern California. As a critical transition zone between freshwater and the sea, Newport Bay provides many important recreational and ecosystem services, including marine bathing and recreation, shoreline protection, water quality improvement, fisheries resources, and habitat and food for migratory animals[2]. Newport Bay is divided into two distinct geographic regions called Upper Bay and Lower Bay. Upper Bay is located at the head of the estuary, and is a state ecological reserve and refuge for threatened and endangered species. Lower Bay is located near the mouth of the estuary, and is a regionally important recreational area and one of the largest pleasure craft harbors in the United States.

The health of Newport Bay is threatened by point and non-point sources of pollution, including fecal indicator bacteria, organics, metals, nutrients and sediments. Nutrient input from the surrounding 400 km^2 watershed triggers frequent macroalgae blooms and intermittent hypoxia in Upper Bay[3,4,5]. A recent study concluded that Upper Bay is a "highly eutrophic estuary"[6]. Rapid urbanization of the surrounding San Diego Creek watershed has accelerated the input of sediment to Newport Bay, necessitating its periodic dredging[7,8]. Widespread and persistent pesticide[9] and heavy metal[10] contamination

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[1] This chapter was written by Dr. S. Grant with the assistance of Ms. Lin Ho.

[2] Levin, L.A., D.F. Boesch, A. Covich et al (2001) "Function of marine critical transition zones and the importance of sediment biodiversity" *Ecosystems* **4**: 430-451.

[3] Nezlin, N.P., K. Kamer, E.D. Stein (2007) "Application of color infrared aerial photography to assess macroalgal distribution in an eutrophic estuary, Upper Newport Bay, California" *Estuaries and Coasts* **30**:855-868.

[4] Kennison, R., K. Kamer, P.Fong "Nutrient dynamics and macroalgal blooms: comparison of five southern California estuaries" *Southern California Coastal Water Research Project Technical Report 416*, November 2003.

[5] Boyle, K.A., K. Kamer, P. Fong (2004) "Spatial and temporal patterns in sediment and water nutrients in a eutrophic southern California estuary" *Estuaries* **27**:378-388.

[6] Kamer, K., K.A. Boyle, P. Fong. (2001) "Macroalgal bloom dynamics in a highly eutrophic southern California estuary" *Estuaries* **24**:623-635.

[7] Trimble, S.W. (1997) "Contribution of stream channel erosion to sediment yield from an urbanizing watershed" *Science* **278**:1442-1444.

[8] Trimble, S.W. (2003) "Historical hydrographic and hydrologic changes in the San Diego Creek watershed, Newport Bay, California" *J. Historical Geography* **29**:422-444.

[9] Bondarenko, S., J.Y. Gan, D.L. Haver, J.N. Kabashima (2004) "Persistence of selected organophosphate and carbamate insecticides in waters from a coastal watershed" *Environmental Toxicology and Chemistry* **23**:2649-2654.

[10] Lee, G.F., S. Taylor (2001) "Results of heavy metal water quality analysis conducted during 2000 in Upper Newport Bay Orange County, CA watershed, Including recommended regulatory approaches" A Report Prepared by G. Fred lee and Associates and RBF Consultants.

contributes toxicity to Newport Bay's water and sediments[11]. Fecal indicator bacteria—including total coliform, fecal coliform, *Escherichia coli*, and enterococci bacteria—are frequently measured at elevated concentrations along the Newport Bay shoreline, with the result that swimming beaches in the Bay can be posted as unfit for recreational contact. For example, during the four year period from 2001 through 2005, >20% and >50% of water samples collected at some shoreline sites in Newport Bay during dry and wet weather, respectively, had FIB concentrations in excess of California and US Environmental Protection Agency (EPA) marine bathing water criteria for enterococci bacteria (see Chapter 7 of this report).

Reflecting the above environmental challenges, the EPA and Santa Ana Regional Water Quality Control Board (SARWQCB) adopted for Newport Bay separate Total Maximum Daily Loads (TMDLs) for fecal coliform, nutrients, sediment, and toxic pollutants[12,13,14]. The Fecal Coliform TMDL was adopted in 1999 to reduce FIB concentrations in the Bay, reduce public health risks, and improve water contact recreational activities. This project, which was funded by a State of California Proposition 13 Coastal Nonpoint Source Pollution Control Grant, is intended to assist the Fecal Coliform TMDL by identifying urban and natural sources of FIB in Newport Bay.

<u>*Stakeholder Groups*</u> A Technical Advisory Committee (TAC) was formed to review scientific and programmatic issues related to the Newport Bay Fecal Coliform TMDL and the Newport Bay/San Diego Creek watershed. The TAC includes members from the SARWQCB, the County of Orange Health Care Agency, Orange County Public Works (OCPW), Orange County Coastkeeper, University of California Irvine (UCI), City of Newport Beach, Orange County Sanitation District and various environmental groups.

<u>*Previous Studies of Newport Bay*</u> A number of investigations of FIB pollution in Newport Bay have been completed in recent years, or are presently ongoing. A listing of ongoing monitoring efforts is provided in Appendix 1A; studies directly relavent to the current effort include:

(1) Identification of FIB hot spots and analysis of historical trends based on ongoing water quality monitoring data collected by the Orange County Health Care Agency[15]

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[11] Konar, B., M.D. Stephenson (1995) "Gradients of subsurface water toxicity to oyster larvae in bays and harbors in California and their relation to mussel watch bioaccumulation data" *Chemosphere* **30**:165-172.
[12] CA RWQCB 1999: A Resolution Amending the Water Quality Control Plan for the Santa Ana River Basin to Establish a Total Maximum Daily Load for Fecal Coliform Bacteria in Newport Bay. Resolution, 99–10. State of California, California, USA.
[13] Zheng, Y. A. A. Keller (2008) "Stochastic watershed water quality simulation for TMDL development—a case study in the Newport Bay watershed" *J. American Water Resources Association* **44**: 1397-1410.
[14] French, C., L. Wu, T. Meixner, D. Haver, J. Kabashima, W.A. Jury (2006) "Modeling nitrogen transport in the Newport Bay/San Diego Creek watershed of southern California" *Agricultural Water Management* **81**:199-215.
[15] Pednekar, A., Grant, S.B., Jeong, Y., Poon, Y., Oancea, C. (2005) "Influence of climate change, tidal mixing, and watershed urbanization on historical water quality in Newport Bay, a saltwater wetland and tidal embayment in southern California", *Environmental Science and Technology*, **39**:9071-9082.

(2) Evaluation of illicit boat discharges as a source of FIB in Newport Bay[16]
(3) A public health risk assessment for recreational contact from exposure to FIB concentrations in the Bay[17,18];
(4) A swimmer shedding study at the Dunes beach[19];
(5) A shell fish and sediment study, that reported on bivalve mullusk species diversity and abundance in Newport Bay, and FIB and F+ coliphage concentrations in sediments and mullusk tissue[20]; and
(6) Relationships between the occurrence of FIB, F+ coliphage viruses, and human pathogenic viruses[21,22].

Collectively these studies suggest that FIB occurrence patterns in the Bay are controlled by a combination of land-use, season, storm water inputs, and mixing processes driven primarily by tidal exchange of water between the Bay and the ocean. Consistent with investigations at other coastal sites in southern California[23], the occurrence patterns of FIB do not necessarily conform to the occurrence patterns of fecal indicator viruses, including F+ coliphage and human viruses.

This study was formulated with the goal of delineating the relative importance of urban and natural sources of FIB in Newport Bay. Such delineation addresses several issues critical to the Newport Bay Fecal Coliform TMDL:

- Health risk considerations. Current water quality standards are based on epidemiology studies at beaches contaminated by point sources, such as storm drains[24] and sewage[25]. FIB concentrations in recreational coastal waters may not predict for adverse human

[16] Jeong, Y., Grant, S.B., Ritter, S., Pednekar, A., Candelaria, L., Winant, C. (2005) "Identifying pollutant sources in tidally mixed systems: case study of fecal indicator bacteria from marinas in Newport Bay, southern California" *Environmental Science and Technology*, **39**:9083-9093.
[17] Public Health Risk Assessment for the Newport Bay Watershed: Recreational Contact and Microbiological Risk Final Report. September 2001; EOA, Inc., RMA, UCB School of Public Health.
[18] Soller, J.A., Eisenberg, J.N.S., DeGeorge, J.F., Cooper, R.C., Tchobanoglous, G., Olivieri, A.W. *J. Water and Health*, 2006, **4**:1-19.
[19] Jiang, S.C. (2005) "Swimmer Shedding Study in Newport Dunes, California" Final report to Regional Water Quality Control Board.
[20] "Final Report Newport Bay Shellfish Harvesting Assessment" August 2004, Kinnetic Laboratories, Inc and EOA, Inc.
[21] Jiang, S.C. (2005) "Fecal Indicator Bacteria and Viruses in Newport Bay, California", Final Report to Regional Water Quality Control Board.
[22] Jiang, S.C., W. Chu, J.W. He (2007) "Seasonal detection of human viruses and coliphage in Newport Bay, California" *Appl. Environ. Microbiol*. **73**:6468-6474.
[23] Boehm, A.B.et al., *Environ. Sci. Technol.* (2003) **37**: 673-680. Jiang et al., *Appl. Environ. Microbiol*. (2001) **67**: 179-184.
[24] Haile, R.W., J.S. Witte, M. Gold et al. (1999) "The health effects of swimming in ocean water contaminated by storm drain runoff" *Epidemiology* **10**:355-363.
[25] Cabelli, V.J. et al., *Am. J. Epidemiology* 1982, **115**, 606-616

health outcomes when FIB are from non-point (and likely non-human) sources, as appears to be the case in Mission Bay, San Diego, California[26].

- Management considerations. Best management practices (BMPs) can be conceived to control urban sources of FIB such as sewage or storm drain inputs, whereas the same may not be true for natural sources such as bird droppings or regrowth of environmentally adapted FIB strains.

1.2. Objectives and Project Design.

It is likely that both natural and urban sources contribute, to varying degrees and at different times, to FIB impairment of Newport Bay. The challenge is in defining the relative contribution of these different sources so that watershed managers can make scientifically justifiable and cost effective decisions regarding which sources to target, and how best to mitigate them.

To better understand the relative contribution of urban and natural sources, and prioritize the water quality impacts of identified sources, four tasks were carried out as part of this project:

> Task 2.1.1: Field sampling studies to characterize the loading of FIB from urban and natural sources in and around the perimeter of the Upper Bay.
> Task 2.1.2: Field sampling and reconnaissance studies to assess potential urban sources of FIB to Lower Bay from subsurface sewage leaks, pump-out stations, docks and wharves, wash-down activities, and storm drains.
> Task 2.1.3: Microcosm and field studies to characterize the rate and magnitude of natural sources and within-Bay processes that affect the concentration of FIB in the Bay water column.
> Task 2.1.4: Advanced tracking methods to assess the likely origin of FIB in Newport Bay.

Field sampling was carried out with a 17-foot research boat custom built for this project (Fig. 1-1). The boat has a small draft (which allowed sampling in the shallow regions of Upper Bay) and a relatively fast cruising speed (suitable for sampling of an offshore control site). The boat was outfitted with a downward looking acoustic Doppler current profiler (ADCP) for in situ measurements of water currents, conductivity-temperature-depth (CTD) profiler, sediment collection system, and a weather station for measuring wind speed, air temperature, dew point, and humidity (see Appendix 1A for photographs of instrumentation on the boat, and aerial photograph of Newport Bay).

Microcosm studies were carried out both in the laboratory (to control for temperature and light) and in the field (to better approximate actual conditions in the Bay), and included

[26] Colford, J.M., T.J. Wade, K.C. Schiff, C.C. Wright, J.F. Griffith, S.K. Sandhu, S. Burns, M. Sobsey, G. Lovelace, S.B. Weisberg (2007) "Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination" *Epidemiology* **18**:27-35.

mixtures of creek and Bay FIB under different salinity regimes and in the presence of different amendments (bird feces, macroalgae, sediment, and debris mats).

To better understand the ecology of FIB in the Bay *Enterococcus* isolates were speciated and typed using pulsed field gel electrophoresis (PFGE); *E. coli* isolates were typed using biochemical substrate utilization assays and global gene expression patterns.

1.3. Report Organization.

Below is a summary of the main topics covered in each chapter of the report. The sub-tasks in the Prop 13 contract addressed in each chapter are shown in parentheses.

Chapter 1: Motivation and overview of project, physical properties of Newport Bay, and literature review of FIB contamination of estuaries (Task 2.1.1.1.)

Chapter 2: Field data collected during Bay-to-Ocean (BTO) transect studies of Newport Bay (Tasks 2.1.1.1, 2.1.1.2, 2.1.1.3, 2.1.1.4, 2.1.3.5, and 2.1.3.7).

Chapter 3: Speciation and DNA fingerprinting of Newport Bay *Enterococcus* isolates (Tasks 2.1.4.1, 2.1.4.2, 2.1.4.3)

Chapter 4: Substrate utilization phenotyping and gene array analysis of Newport Bay *Escherichia coli* isolates (Tasks 2.1.4.1, 2.1.4.2, 2.1.4.3)

Chapter 5: Field and microcosm studies of natural sources of FIB, and the growth and decay of FIB in Newport Bay and its tributaries (Tasks 2.1.3.1, 2.1.3.2, 2.1.3.3, 2.1.3.6, 2.1.3.7)

Chapter 6: Field data collected during the diurnal intertidal sediment studies (DITS) (Tasks 2.1.3.4 and 2.1.3.7)

Chapter 7: Assessment of urban sources of FIB in Lower Newport Bay (Tasks 2.1.2.1, 2.1.2.2, 2.1.2.3, 2.1.2.4, and 2.1.2.5)

Chapter 8: Estimates for the loading of FIB from urban and natural sources in Newport Bay (Tasks 2.1.1.4, 2.1.2.4).

The remainder of this chapter provides background information on tidal mixing in Newport Bay, and factors that might affect FIB impairment of the Bay.

1.4. Mixing Time Scales in Newport Bay

Newport Bay is approximately 10 km in length and has a median water volume of 16 million cubic meters, which is distributed unevenly between Upper Bay (3 million cubic meters) and Lower Bay (13 million cubic meters) (Fig. 1-2). The Bay's tidal prism (volume of seawater exchanged between high-high and low-low tides) is approximately 6

Figure 1-1. UCI research boat used to conduct fieldwork in Newport Bay. In this photograph graduate student Ms. Lin Ho is measuring tidal currents in Upper Bay using a downward looking acoustic Doppler current profiler (ADCP) located below the water surface at the end of the vertical alumninum pole on the port side of the boat.

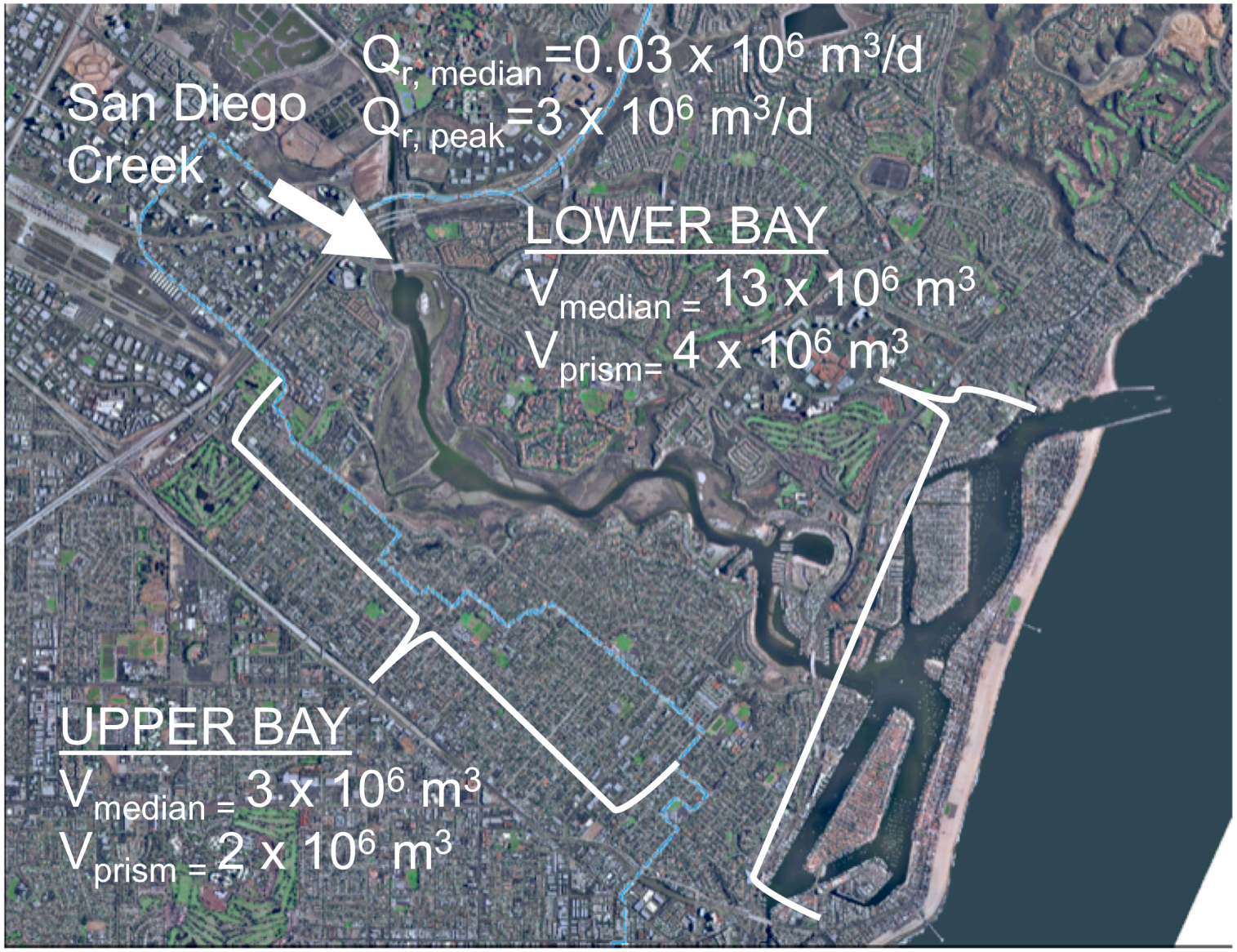

Figure 1-2. Median volumes and tidal prisms in Upper and Lower Newport Bay, compared to median and peak flows of freshwater into the Bay from San Diego Creek.

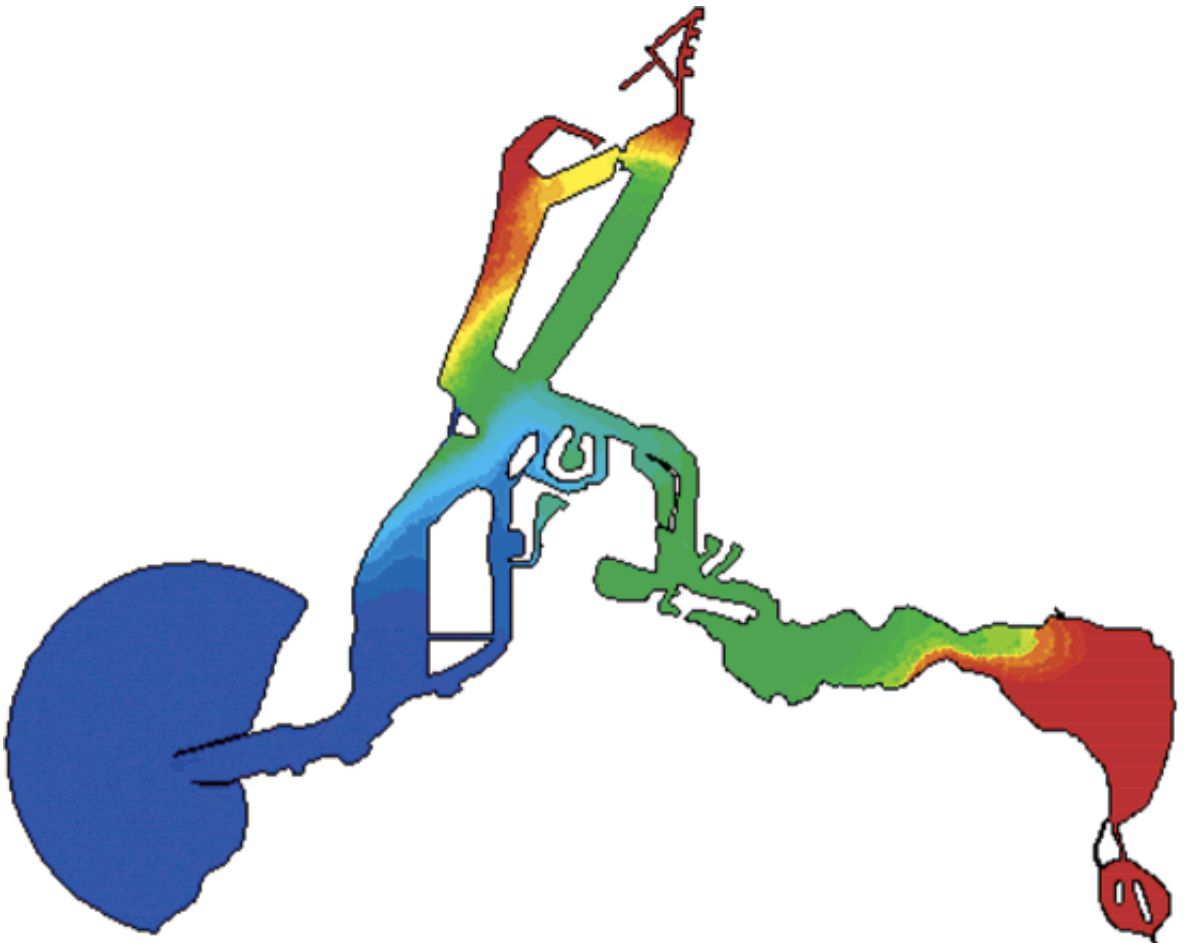

Figure 1-3. Flushing time scales estimated for different regions of Newport Bay (taken from Pednekar et al., 2005).

million cubic meters (Fig. 1-2). These volume estimates are based on an average 1.2 m tide, and are calculated using pre-2006 bathymetric data. Recent dredging activities may affect the median volume and tidal prism of the Upper and Lower Bay.

The Bay water volumes described above can be compared with the volume of freshwater added by Newport Bay's largest tributary, San Diego Creek, which drains 80% of the surrounding 400km^2 watershed (Fig. 1-2). For the 2006 calendar year (the year this study was carried out), median and peak flows in San Diego Creek were 0.03 and 3 million cubic meters per day. The median volumetric flow rate of 0.03 million cubic meters per day reported for San Diego Creek is very close to the base (dry weather) flow rate in this tributary.

Mixing time scales for water parcels in Newport Bay can be estimated in a several different ways. Pednekar et al[27] report flushing time scales for different regions of Newport Bay based on hydrodynamic simulations of the time it takes a conservative tracer to dilute by tidal mixing. The resulting map of flushing time scales, reproduced in Fig. 1-3, predicts flushing times ranging over 30 days in the dead-end regions of the Bay (Western Newport Bay and the at the head of Upper Bay) to approximately 1 week in the mid-region of the Bay, to zero days near the ocean outlet.

Nezlin et al[28] recently estimated a mean residence time of 1.8 days for Upper Newport Bay based on the application of a freshwater fraction model to continuous measurements of salinity at three locations in Upper Newport Bay. A mixing time scale of 1.8 days for the entire Newport Bay was obtained by one of the authors (SBG) of this report using a tidal prism model and volume estimates shown in Fig. 1-2[29]:

$$\tau = \frac{V}{Q_{FW} + Q_{FL}} \qquad (1)$$

where V is the median volume of the estuary, Q_{FW} is the flow of freshwater into the estuary over a tidal period (12 hours), and Q_{FL} is seawater inflow on the flood tide. The freshwater mixing model used by Nezlin et al and the tidal prism model (eq. 1), assume that the estuary is well-mixed (i.e., a single mixing time scale can be applied to the Bay as a whole). Further, eq. 1 assumes no gravitational circulation (baroclinic, see later) and no ebb return[29]. Because Newport Bay exhibits strong longitudinal gradients in salinity (see Chapter 2 of this report), these last two mixing time scales probably underestimate the true mixing time of water parcels in Newport Bay. A more realistic 1 dimensional (1D) model of tidal mixing in Newport Bay is presented in Chapter 8 of this report.

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[27] Pednekar, A.M., S.B. Grant, Y. Jeong, Y. Poon, C. Oancea (2005) "Influence of climate change, tidal mixing, and watershed urbanization on historical water quality in Newport Bay, a saltwater wetland and tidal embayment in southern California" *Environmental Science and Technology* **39**:9071-9082.

[28] Nezlin, N.P., K. Kamer, J. Hyde, E.D. Stein (2009) "Dissolved oxygen dynamics in a eutrophic estuary, Upper Newport Bay, California" Estuarine, Coastal, and Shelf Science 82:139-151.

[29] Sheldon, J.E., M.Alber (2006) "The calculation of estuarine turnover times using freshwater fraction and tidal prism models: a critical evaluation" *Estuaries and Coasts* **29**:133-146.

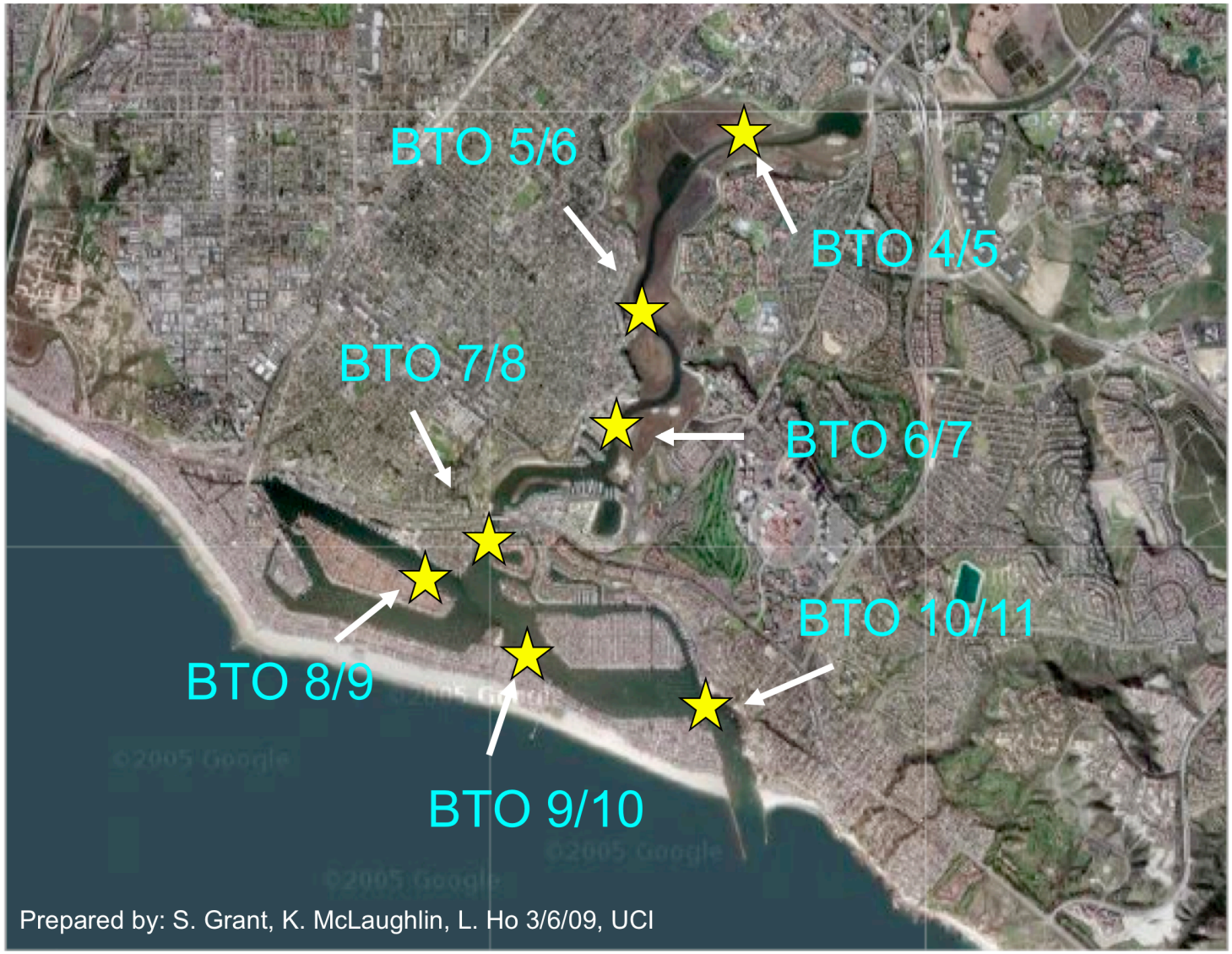

Figure 1-4. Map of Newport Bay showing the location of ADCP deployments and CTD casts to assess the dominant circulation patterns in the Bay.

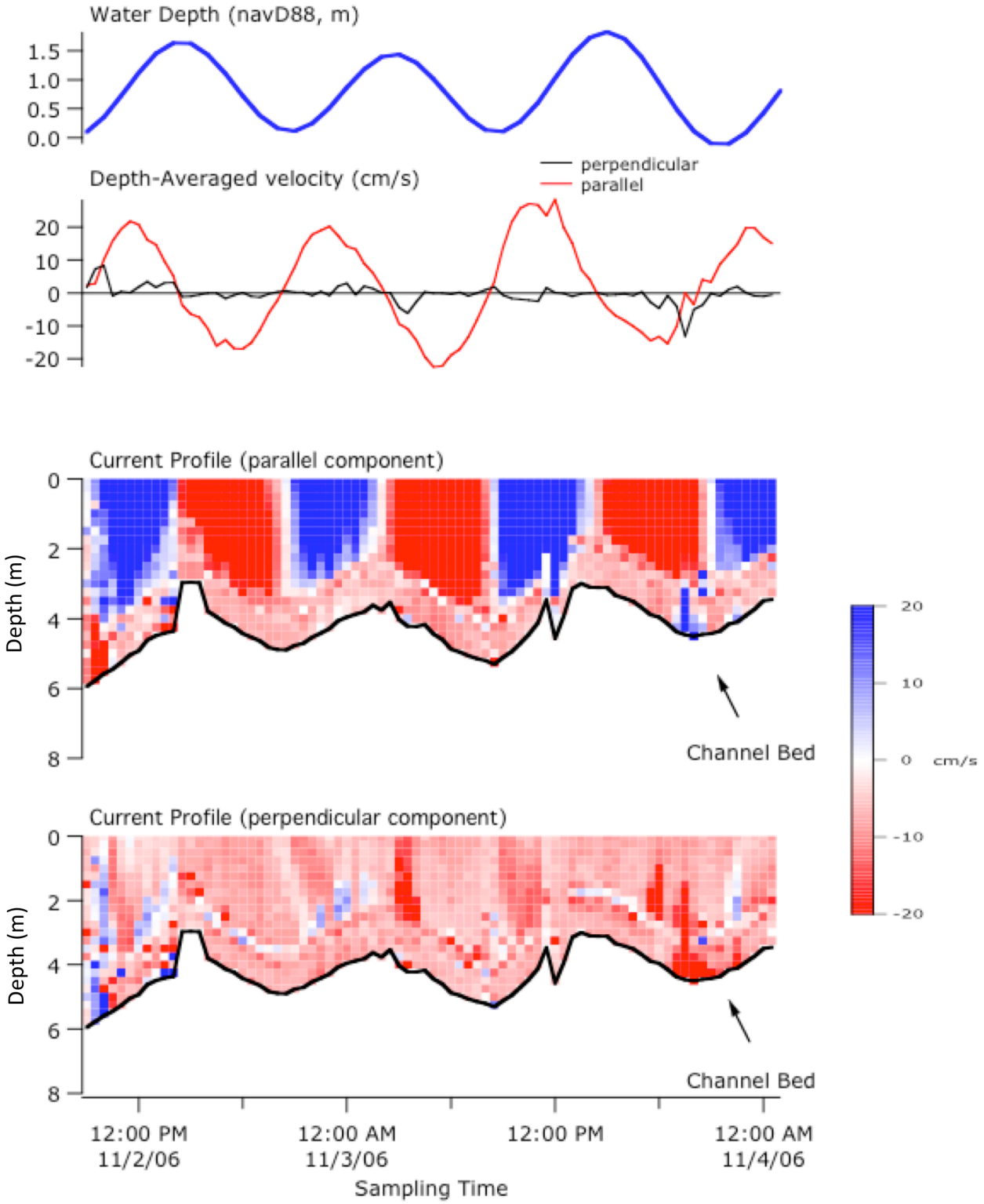

Figure 1-5. ADCP records measured during November 2006 at site BTO 9/10 in Lower Newport Bay (see map in Fig. 1-4 for location of sampling site). Shown are predicted tide level (top panel), depth averaged current velocity in the channel-parallel and channel-perpendicular direction (middle panel), and vertical profiles of the current velocity in the channel-parallel and channel-perpendicular direction.

1.5. Estuarine Classification of Newport Bay

Estuaries can be classified based on their geomorphology and hydrodynamics[30]. The geomorphology of Newport Bay can be described as a "Bar Built Estuary", in that it is a drowned river valley with high sedimentation rates and shallow depths. However, intensive urbanization of the land surrounding Newport Bay—most especially the islands and spits in Lower Bay—together with extensive modification of the Newport Bay shoreline for flood and sand control, imply that this estuary is far from a natural geomorphic state.

Hydrodynamic classifications focus on whether circulation in an estuary is dominated by density gradients resulting from the partial mixing of fresh and salt waters (baroclinic circulation) or by circulation that does not involve density gradients, such as tides and winds (barotropic circulation). Below we apply three different estuarine classification schemes to Newport Bay, with the goal of finding where the Bay fits in the broader spectrum of the world's estuaries.

Flow Ratio Classification This classification compares the volume of freshwater flowing into an estuary over a tide cycle relative to the estuary's tidal prism. The ratio of these two volumes, or "flow ratio", provides an indication of the circulation regime likely to prevail in a given estuary[31]. Based on the volumes reported in Fig. 1-2, during dry weather the flow ratio for Newport Bay ranges from 0.003 (if Upper and Lower Bay tidal prisms are included) to 0.008 (if only the Upper Bay tidal prism is included). The corresponding hydrodynamic classifications range from a slightly stratified estuary (flow ratios 0.005 to 0.1) to a vertically mixed estuary (flow ratios <0.005).

During wet weather, and assuming that the peak wet weather flow of 3 million cubic meters is sustained for a full tidal cycle, the flow ratio increases to between 0.5 (if Upper and Lower Bay tidal prisms are included) to 1.5 (if only the Upper Bay tidal prism is included). The corresponding hydrodynamic classifications range from a highly stratified estuary (flow ratios 0.1 to 1) to a salt wedge estuary (flow ratios >1).

Based on these simple calculations, it appears that Newport Bay can experience significant changes in its circulation over the course of a year—from a vertically mixed estuary dominated by barotropic flows during dry weather to a highly stratified estuary dominated by baroclinic flows during sustained storms.

Estuarine Richardson Number The Estuarine Richardson Number is a dimensionless number that represents the ratio of river input of buoyancy per unit width of tidal channel to the mixing power available from the tide[32]. The magnitude of the Estuarine

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[30] Pritchard, D.W. (1952) "Estuarine hydrography" *Advances in Geophysics* **1**: 243-280.
[31] Tomczak, M. "Shelf and Coastal Oceanography" v. 1.0 (1998) http://www.es.flinders.edu.au/~mattom/ShelfCoast/chapter11.html
[32] Fischer, H.B., E. J. List, R.C.Y. Koh, J. Imberger, N.H. Brooks "Mixing in Inland and Coastal Waters" 1979, Academic Press.

Table 1-1. Locations and deployment periods for ADCP measurements in Newport Bay.

Sites	***Latitude***	***Longitude***	***Study Period***
BTO 4/5	33°38'51.23" N	117°52'41.57" W	10/5/06 - 10/7/06
BTO 5/6	33°38'13.20" N	117°53'22.58" W	8/30/06 - 9/2/06
BTO 6/7	33°37'29.80" N	117°53'30.02" W	9/14/06 - 9/16/06
BTO 7/8	33°36'54.85" N	117°54'16.64" W	9/28/06 - 9/30/06
BTO 8/9	33°36'42.45" N	117°54'38.98" W	10/12/06 - 10/14/06
BTO 9/10	33°36'19.91" N	117°54'03.58" W	11/2/06 - 11/4/06
BTO 10/11	33°36'01.30" N	117°52'56.46" W	11/9/06 - 11/11/06

Table 1-2 Estuarine Richardson Number calculated from current measurements and density profiles at select sites in Newport Bay

Sites	***R***	***Interpretation***
BTO 4/5	0.03	well-mixed
BTO 5/6	0.04	well-mixed
BTO 6/7	0.2	partially mixed
BTO 7/8	0.4	partially mixed
BTO 8/9	6	strongly stratified
BTO 9/10	0.02	well-mixed
BTO 10/11	0.03	well-mixed

Richardson Number indicates whether the estuary is strongly salinity stratified and thus likely to be dominated by baroclinic (density-driven) circulation ($R > 0.8$) or well-mixed over the vertical and thus likely to be dominated by barotropic (tide and wind driven) circulation ($R < 0.08$).

To estimate the Richardson Number for Newport Bay, currents were monitored during a series of dry weather studies at seven sites in Newport Bay (Fig. 1-4). Water currents were measured using a downward looking acoustic Doppler current profiler (ADCP) deployed from the UCI research boat (see Fig. 1-2 for a picture of the ADCP deployed at site BTO 5/6). Coincident with the ADCP measurements, hourly conductivity-temperature-depth (CTD) casts were carried out to characterize vertical profiles of temperature and salinity. The dates and location of the seven deployments are summarized in Table 1-1.

An example ADCP record for one of the Lower Bay sites (BTO 9/10) is included in Fig. 1-5. Water circulation at this particular site is strongly dominated by the tides, with full flow reversals occurring throughout the water column. Estimated values for the Estuarine Richardson Numbers are documented for the seven Newport Bay sites in Table 1-2. Four of the seven sites have values of R<0.08, implying that Newport Bay is a weakly

Table 1-3 Estuarine classification types determined for Newport Bay based on salinity profiles and tidally averaged surface currents measured during dry weather at seven different sites in Upper and Lower Newport Bay.

Sites	***Stratification parameter***	***Circulation parameter***	***Type***
BTO 4/5	0.05	89	2b
BTO 5/6	0.07	12	2a
BTO 6/7	0.04	5.2	2a
BTO 7/8	0.02	4.1	2a
BTO 8/9	0.02	34	2a
BTO 9/10	0.008	39	2a
BTO 10/11	0.007	190	2b

stratified estuary dominated by barotropic circulation. Two sites have somewhat larger values of R (0.2 to 0.4) consistent with partial salinity stratification. One site, located on the main channel connecting West Newport Bay to Lower Bay, has a relatively large value of R=6. This large value of R appears to arise from relatively weak tidal currents measured during the 24-hour deployment of the ADCP at this site.

<u>*Stratification-Circulation Classification*</u> The last estuary classification scheme evaluated here utilizes two features of an estuary: (1) a stratification parameter equal to the difference in salinity at the top and bottom of the water column divided by the depth-averaged salinity; (2) a circulation parameter calculated from the ratio of the surface current averaged over a full tide cycle to the mean freshwater velocity through the tidal channel's cross-section. These two parameters were calculated from the ADCP records and CTD casts carried out at the seven sites indicated in Table 1-1. The resulting values for these two parameters, together with the corresponding estuary stratification, are indicated in Table 1-3.

Five out of the seven sites correspond to a Type 2a estuary, which is described by Hansen and Rafftray as well-mixed estuaries where "salinity stratification is slight, the net flow reverses at depth, and both advection and diffusion contribute to the upstream salt flux."[33] The remaining two sites are classified as Type 2b, which is defined as the same as Type 2a, except there is "appreciable stratification". One of these Type 2b sites, BTO 4/5, is near the outlet of San Diego Creek where stratification was evident during both dry and wet weather periods (see Chapter 2). It is not immediately apparent why site BTO 10/11 is salinity stratified, although this site is located near the outlet of several large runoff drains, including Carnation, El Paseo, and Bayside drains (see Chapter 7 of this report).

<u>*Summary*</u> Based on the three hydrodynamic classification studies described above, it appears that the nature of circulation in Newport Bay depends on the magnitude of runoff flowing into the Bay from its tributaries. During dry weather, the volume of freshwater

[33] Hansen, D.V., M.Rattray (1966) "New dimensions in estuary classification" *Limnology and Oceanography*,**11**:319-326.

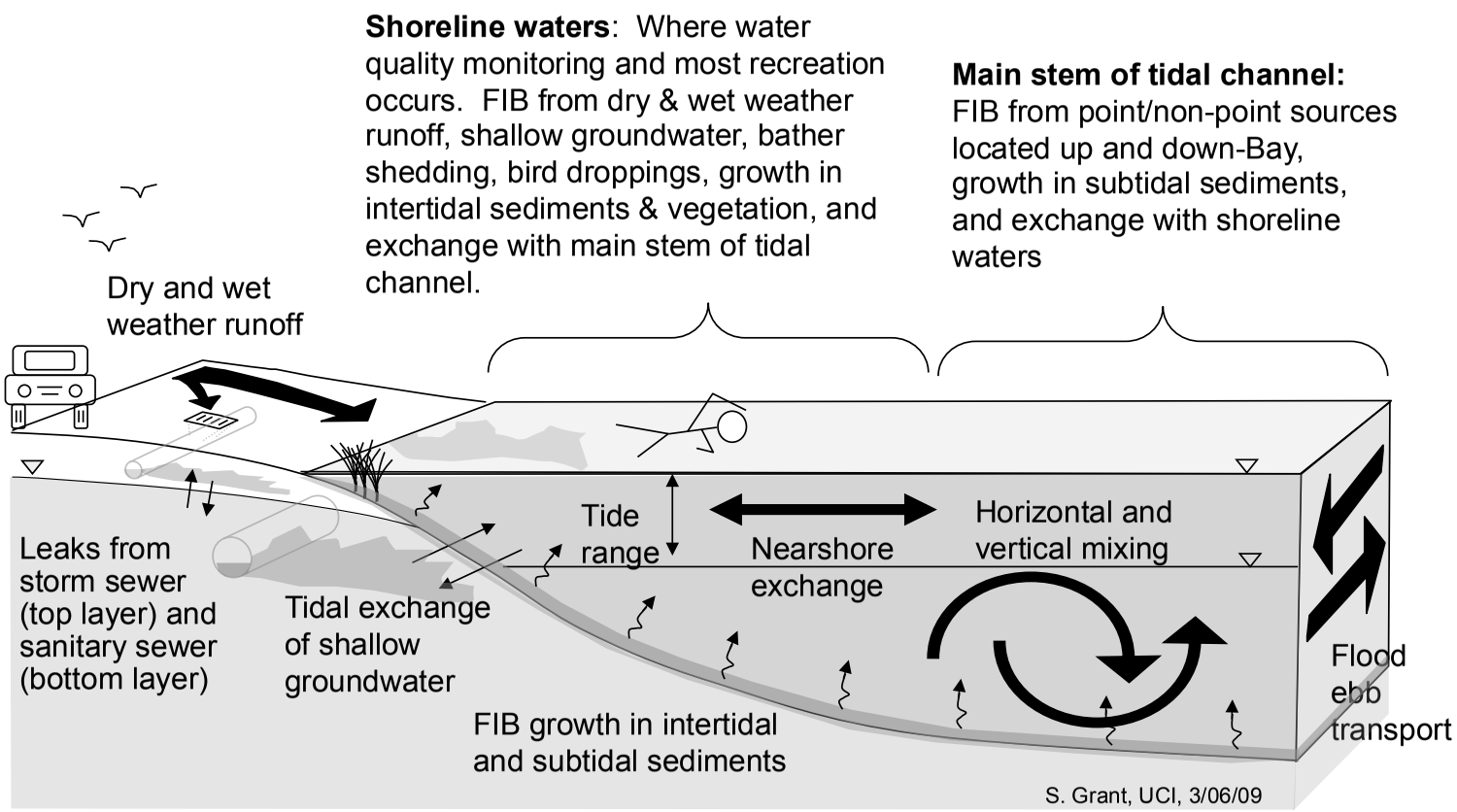

Figure 1-6. Proposed "beachshed" for FIB contamination of the tidal channels and beaches in Newport Bay.

contributed by base flow from San Diego Creek is small compared to Newport Bay's tidal prism. Under these conditions most sites in Newport Bay are vertically well-mixed to partially stratified (Type 2a) and circulation is likely dominated by tides and winds (barotropic flows). During large storms, on the other hand, the volume of freshwater contributed by San Diego Creek can approach the same order as the Bay's tidal prism. When this occurs, Newport Bay will experience significant salinity stratification, approaching that observed in salt wedge estuaries, and its circulation is likely to be dominated by density gradients (baroclinic circulation).

1.6. Literature Review: A Beachshed Approach to Water Quality Management

Whitman and co-workers[34] argue that water quality impairment of shoreline waters should be evaluated from the perspective of a "beachshed", which includes all non-point and point sources of FIB and pathogens that could potentially affect shoreline water quality, coupled with the biological and physical processes that amplify or diminish their environmental concentrations. As noted by these authors:

> *Determining the components, fluxes, and interactions of a dynamic conceptual model or simply an [FIB] "budget" will help define the anticipated pathogen concentration at a given beach and the real health consequences. Understanding the relationship between the factors that ultimately lead to the net [FIB] concentrations at bathing beaches will help optimize monitoring approaches and define remediation strategies for using indicators that reflect actual, not apparent, fecal pollution*.

The beachshed approach described by Whitman et al is consistent with the goals of this project; namely, to quantify the relative contribution of urban and natural sources of FIB in Newport Bay. In this brief literature review, a conceptual description of the beachshed for Newport Bay is presented, with the goal of framing the field and laboratory studies described later in the report.

Beachshed Model for Newport Bay A beachshed model for Newport Bay is illustrated schematically in Fig. 1-6. At any location in the Bay, the tidal channel can be divided into two regions: (1) shoreline waters, and (2) center region, or "main stem", of the tidal channel. The center of the tidal channel is generally the deepest portion of the tidal channel, is mixed by vertical and horizontal turbulence and eddies, and receives water and associated FIB from sources up and down Bay by tidal action. Shoreline waters are generally the shallowest portion of the tidal channel cross-section where water quality monitoring and most recreational contact occurs.

Water in the center of the tidal channel receives FIB and pathogens from up or down Bay on the tides, diffusion out of subtidal sediments, and diffusive and advective exchange

[34] Whitman, R.L., Nevers, M.B., Byappanahalli M.N. (2006) "Examination of the watershed-wide distribution of *Escherichia coli* along Southern Lake Michigan: an integrated approach" *Appl. Environ. Microbiol*. **72**:7301-7310.

with shoreline waters. Shoreline waters receive FIB from non-point sources located along the shoreline (runoff and sewage, bather shedding, bird droppings), growth of FIB in intertidal sediments, growth of FIB in subtidal sediments, and diffusive and advective exchange with the center of the tidal channel. Below, FIB sources are considered in detail.

Non-point sources of runoff and sewage Non-point source runoff can contribute terrestrial flux of FIB into shallow shoreline waters. During dry weather, non-point source runoff originates from activities in the watershed that generate excess water, such as over irrigation and car washing. Runoff that does not evaporate or infiltrate, flows through a network of gutters, curbs, and underground pipes (collectively referred to as the storm sewer system) to the beach, where it discharges without treatment through small drains distributed around the perimeter of the Bay. In general, the volume of runoff flowing out of any single storm drain is small and intermittent. Nevertheless, dry weather runoff from drains can potentially affect local water quality because of the high concentration of FIB carried in runoff and the siting of drain outlets close to the waterline (see Chapter 7).

While most FIB in runoff is probably not of human fecal origin (see later), sewage spills or sewage overflows can contaminate the storm sewer system, intertidal and subtidal sediments[35], and/or shallow groundwater[36] leading to high nearshore FIB concentrations, obvious human health risk from fecal-oral pathogens, and acute hypoxia and fish kills[37]. Tide level can affect the rate and timing of FIB discharges from both the storm sewer drains and shallow groundwater, by acting as a tide gate at the end of submerged drains (see Chapter 7) and changing the hydraulic gradient that drives the exchange of water between shallow groundwater and the Bay[38,39].

In the absence of sewage spills or other illicit inputs of sewage, FIB in urban runoff can have myriad origins[40,41,42,43], including bird and animal feces, regrowth of

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[35] Sinigalliano, C.D., M.L. Gidley, T. Shibata, D. Whitman, T.H. Dixon, E. Laws, A. Hou, D. Bachoon, L. Brand, L. Amaral-Zettler, R.j. Gast, G.F. Steward, O.d. Nigro, R.Fujioka, W.Q. Betancourt, G. Vithanage, J. Mathews, L.E. Fleming, and H.M. Solo-Gabriele (2007) "Impacts of hurricanes Katrina and Rita on the microbial landscape of the New Orleans area" Proceedings of the National Academy of Sciences (PNAS) **104**:9029-9034.

[36] In 2004, sewage spills and overflows were implicated in 25% of beach closing/advisory days in the U.S. where a cause of water quality impairment was identified (Natural Resources Defense Council (NRDC), "Testing the Waters 2005", available online at www.nrdc.org).

[37] Mallin, MA, L.B. Cahoon, B.R. Toothman, D.C. Parsons, M.R. McIver, M.L. Ortwine, R.N. Harrington (2007) "Impacts of a raw sewage spill on water and sediment quality in an urbanized estuary" *Marine Poll. Bull*. **54**:81-88.

[38] Michael, H.A., A.E. Mulligan, C.F. Harvey (2005) "Seasonal oscillations in water exchange between aquifers and the coastal ocean" *Nature* **436**: 1145-1148

[39] De Sieyes, N.R., Yamahara, K.M., Layton, B.A., Joyce, E.H., Boehm, A.B. "Submarine discharge of nutrient-enriched fresh groundwater at Stinson Beach, California is enhanced during neap tides" *Limnology and Oceanography*, in press.

[40] Jiang, S.C., W. Chu, B.H. Olson, J-W He, S. Choi, J. Zhang, J.Y. Le, P.B. Gedalanga (2007) "Microbial source tracking in a small southern California urban watershed indicates wild animals and growth as a source of fecal bacteria" *Environ. Biotechnol*. **76**:927-934.

environmentally adapted FIB strains in ponded water and soil, soil amendments containing indicator bacteria, decaying food, and human fecal matter from homeless populations.

<u>*Bather Shedding*</u> Elmir et al[44] estimate that the average marine bather sheds 300,000 enterococci bacteria in the first 15 minutes of immersion. Less enterococci bacteria are shed with subsequent immersions, and sand attached to the skin of bathers does not appear to be the source of FIB. The shedding pattern observed by Elmir et al is consistent with a previous study by Gerba and co-workers that concluded most FIB released by bathers is associated with fecal matter[45].

<u>*Bird and Animal Droppings, growth in beach sand and on decaying vegetation*</u> Bird and animal droppings, as well as decaying vegetation (wracklines, phytoplankton, and macroalgae) and foreshore sands, are implicated as non-point sources of FIB in estuarine systems[46,47]. Bird droppings and wracklines on the beach can mobilize into the water column during high tides, perhaps explaining why bathing water quality at many California beaches is consistently worse during spring tides when the tide range is large[48]. Beach sand can also be a source of FIB[49], and harbor genetic markers for human fecal contamination (enterococci bacteria with the human *esp* gene, and human specific *Bacteroidales*)[50]. Potential sources of FIB in beach sands include human and animal feces, runoff, spilled sewage, and/or the growth of environmentally adapted FIB strains.[51]

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[41] Reeves, R.L., S.B. Grant, R.D. Mrse, C.M. C. Oancea, B.F. Sanders, A.B. Boehm (2004) "Scaling and management of fecal indicator bacteria in runoff from a coastal urban watershed in southern California" *Environ. Sci. Technol*. **38**:2637-2648.

[42] He, L-M, Lu, J. Shi, W. (2007) "Variability of fecal indicator bacteria in flowing and ponded waters in southern California: implications for bacterial TMDL development and implementation" *Water Research* **41**:3132-3140.

[43] Byappanahalli, M.N. and R.S. Fujioka (1998) "Evidence that tropical soil environment can support the growth of *Escherichia coli*" *Water Sci. Technol*. **38**:171-174.

[44] Elmir, S.M., M.E. Wright, A. Abdelzaher, H.M. Solo-Gabriele, L.E. Fleming, G.Miller, M. Rybolowik, M.P. Shih, S.P. Pillai, J.A. Cooper, E.A. Quaye (2007) "Quantitative evaluation of bacteria released by bathers in a marine water" *Water Res*. **41**:3-10.

[45] Gerba, C.P. (2000) "Assessment of enteric pathogen shedding by bathers during recreational activity and its impact on water quality" *Quant. Microbiol***.** **2**:55-68.

[46] Hartel,P.G., L.C. Gentit, C.N. Belcher, K.W. Gates, K. Rodgers, J.A. Fisher, K.A. Smith, K. A. Payne (2006) "Identifying sources of fecal contamination inexpensively with targeted sampling and bacterial source tracking" *J. Environ. Qual.* **35**:889-897.

[47] Grant, S.B., B.F. Sanders, A.B. Boehm, J.A. Redman, J.H. Kim, R.D. Mrse, A.K. Chu, M. Gouldin, C.D. McGee, N.A. Gardiner, B.H. Jones, J. Svejkovsky, G.V. Leipzig, A. Brown (2001) "Generation of enterococci bacteria in a coastal saltwater marsh and its impact on surf zone water quality" *Environmental Science and Technology* **35**:2407-2416.

[48] Boehm, A.B., S.B. Weisberg (2005) "Tidal forcing of enterococci at marine recreational beaches at fortnightly and semidiurnal frequencies" *Environ. Sci. Technol*. **39**:5575-5583.

[49] Lee, C.M., T.Y. Lin, C-C Lin, G.A. Kohbodi, A. Bhatt, R. Lee, J.A. Jay (2006) "Persistence of fecal indicator bacteria in Santa Monica Bay beach sediments" *Water Research* **40**:2593-2602.

[50] Yamahara, K.M., B.A. Layton, A.E. Santoro, A.B. Boehm (2007) "Beach sands along the California coast are diffuse sources of fecal bacteria to coastal waters" *Environ. Sci. Technol*. **41**:4515-4521.

[51] Pianetti, A., f. Bruscolini, L. Sabatini, P. Colantoni (2004) "Microbial characteristics of marine sediments in bathing area along Pesara-Gabicce coast (Italy): A preliminary study. *J. Appl. Microbiol*. **97**:682-689.

A recent study found high FIB concentrations in intertidal sediments collected from several coastal areas in Orange County, including the Santa Ana River and a beach in Dana Point Harbor[52]. Interestingly, the relative abundance of different *Enterococcus* species (which was dominated by either *E. faecalis* or *E. faecium*) was not statistically different in shoreline water samples with low or high FIB concentrations. The study's authors speculate that water quality violations might occur when environmentally adapted strains of FIB growing in the intertidal sediments are suspended off the bottom by wind, waves, and/or tides. Consistent with the studies just described, microcosm experiments described in Chapter 5 of this report indicate that FIB survive, and in some cases grow, when water from San Diego Creek is exposed to filter sterilized water from Newport Bay, with or without amendment with macroalgae, bird droppings, floating debris, and sand. However, in contrast with these previous studies, a large percentage (>50%) of the *Enterococcus* isolates cultured from Newport Bay and its tributaries (San Diego Creek and Santa Ana Delhi channel) are species not typically associated with fecal sources, including *E. casseliflavus* and *E. mundtii* (Chapter 3)

<u>*Point sources of runoff and sewage*</u> Point sources of runoff and sewage can impact water quality in tidal embayments by two pathways: (a) direct discharge into the Bay, and/or (b) discharge outside of the Bay (i.e., in the coastal ocean), followed by import into the Bay by tides and currents. No sewage outfalls discharge directly to Newport Bay, and the closest sewage outfall (operated by the Orange County Sanitation District, OCSD) discharges partially treated and disinfected wastewater effluent approximately 7 km offshore of Newport Bay's ocean outlet. After disinfection, the concentration of FIB in OCSD's outfall is too low, and the discharge diffuser too far offshore, to realistically impact water quality in Newport Bay. Further, as noted in Chapter 2 of this report, FIB concentrations decrease with distance down-Bay, making it unlikely that either sewage or river outfalls on the ocean side of the Newport Bay outlet are responsible for water quality impairment in the Bay.

San Diego Creek, on the other hand, discharges to the head of Newport Bay, and appears to be a primary cause of Bay-wide water quality impairment during storm events (see salinity measurements in Chapter 2 and loading studies in Chapter 8). During wet weather periods, peak flow rates in San Diego Creek can exceed 3×10^6 m^3/day, and thus in a single day[53] the creek can discharge a volume of fresh water roughly equal to the tidal prism and median volume of water in Upper Bay and somewhat less than the tidal prism and median volume of water in Lower Bay (see above Flow Ratio calculations in *Section 1.5* above). During dry weather, the flow rate in San Diego Creek drops to 0.03×10^6 m^3/day, or roughly 1% (or less) of the median volume and tidal prism in Upper Bay and Lower Bay. This "back of the envelope" calculation explains why brackish

[52] Ferguson, D.M., D.F. Moore, M.A. Getrich, M. Zhowandai (2005) "Enumeration and speciation of enterococci found in marine and intertidal sediments and coastal water in southern California" *J. Appl. Microbiol.* **99**:598-608.

[53] Typically, the peak flow rate quoted here for San Diego Creek would not be sustained for a full day, but this number is still a useful order-of-magnitude estimate for the volume of water added by San Diego Creek to Newport Bay during a large storm.

(mesohaline) salinities extend deep into Lower Bay during wet weather, but are confined to a region fairly close to the creek outlet during dry weather (Chapter 2 of this report). Based on the classification results presented earlier in this chapter, the water column in Newport Bay is relatively well-mixed over the vertical, at least during dry weather periods. Thus, FIB discharged from San Diego Creek will be transported down-Bay by longitudinal advection and tidal dispersion[54], and undergo cross-shore mixing into shoreline waters by advection and turbulent diffusion[55,56].

The volume of freshwater discharged from Santa Ana Delhi Channel is less than the discharge from San Diego Creek, by a factor of 3 to 4 during dry weather and a factor of 10 during wet weather (Chapter 8). Thus, to a first approximation, San Diego Creek is the dominant point source of runoff in Newport Bay during wet and dry weather periods. However, as documented in Chapter 8, Santa Ana Delhi channel can be a significant source of enterococci bacteria during dry weather.

Non-conservative Processes In addition to the sources described above, and their associated transport pathways, there are a number of additional processes that potentially affect FIB concentrations in the Bay. These include physicochemical removal from the water column (adsorption, sedimentation, filtration), mobilization into the water column from substrates (sediment resuspension, tidal washing off of mudflats)[57,58], die-off[59] (including by exposure to sunlight[60], reactive oxygen species[61], and zooplankton predators[62]), selection and growth[34] and recovery of injured cells[63]. Non-conservative processes affecting FIB concentrations in Newport Bay are explored with microcosm studies in Chapter 5.

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[54] See salinity transect measurements reported in Chapter 2 of this report.
[55] Clarke, L.B., D. Ackerman, J. Largier (2007) "Dye dispersion in the surf zone: Measurements and simple models" *Continental Shelf Res*. **27**:650-669.
[56] Bogucki, D.J., B.H. Jones, M-E Carr (2005) "Remote measurements of horizontal eddy diffusivity" *J. Atmospheric and Oceanic Technol*. **22**:1373-1380.
[57] Mallin, MA, L.B. Cahoon, B.R. Toothman, D.C. Parsons, M.R. McIver, M.L. Ortwine, R.N. Harrington (2007) "Impacts of a raw sewage spill on water and sediment quality in an urbanized estuary" *Marine Poll. Bull*. **54**:81-88.
[58] Kay, D., C.M. Stapleton, M.D. Wyer, A.t. McDonald, J. Crowther, N. Paul, K. Jones, C. Farancis, J. Watkins, J. Wilkinson, N. Humphrey, B. Lin, L. Yang, R.A. Falconer, S. Gardner (2005) "Decay of intestinal enterococci concentrations in high-energy estuarine and coastal waters: towards real-time T90 values for modeling faecal indicators in recreational waters" *Water Research* **39**:655-667.
[59] Bartram, J. and G. Rees eds (2000) *Monitoring Bathing Waters* pp 175-179. New York, NY: E & FN Spon.
[60] Sinton, L.W., R. K. Finlay, P.A. Lynch (1999) "Sunlight inactivation of fecal bacteriophages and bacteria in sewage polluted seawater" *Applied and Environ. Microbiol*. **65**:3605-3613.
[61] Clark, C.D., W.J. De Bruyn, S.D. Jakubowski, S.B.Grant (2008) "Hydrogen peroxide production in marine bathing waters: Implications for fecal indicator bacteria mortality" *Marine Poll. Bull*. **56**:397-401.
[62] Hartke, A., S. Lemarinier, V. Pichereau, Y. Auffray (2002) "Survival of *Enterococcus faecalis* in seawater microcosms is limited in the presence of bacterivorous zooflagellates" *Current Microbiology* **44**:329-335.
[63] Bolster, C.H., J.M. Bromley, S.H. Jones (2005) "Recovery of chlorine-exposed *Escherichia coli* in estuarine microcosms" *Environ. Sci. Technol*. **39**: 3083-3089.

[1]Chapter 2. Bay-to-Ocean transect measurements of water and sediment quality in Newport Bay

2.1 Introduction and Overview

This chapter describes field studies designed to characterize the spatial and temporal variability of water and sediment quality in Newport Bay. Twelve sites were sampled across a Bay-to-Ocean (BTO) transect approximately 54 times over the course of 13 months. Each BTO transect included three sites in the creeks and tributaries to Upper Bay, eight sites in the tidal channels of Upper and Lower Bay, and one "offshore control" site located on the ocean side of the Newport Bay outlet (Fig. 2-1). At Upper and Lower Bay sites, water and sediment samples were collected from five different locations in the cross-section of the tidal channel.

All water samples were measured for FIB (*E. coli* and enterococci bacteria), particle size distribution, pH, and salinity. A subset of water samples was assayed for ammonium, nitrate, total phosphorous, and total organic carbon. Approximately 10% of the water samples were filtered through 5 and 10 micron pore filters and re-assayed for FIB to determine if FIB were associated with specific particle size classes. All sediment samples were analyzed for FIB; a subset of sediment samples was analyzed for grain size distributions, water content, and organic carbon content. Enterococci bacteria isolated from the water and sediment samples were speciated and typed using pulsed-field gel electrophoresis. *E. coli* isolates were typed using substrate utilization tests and gene expression patterns. Microbial source tracking results are presented in Chapters 3 and 4.

Digital photographs were collected at the within-Bay site to assess the number of birds present at the time of sampling. Field measurements at the within-Bay sites included air temperature, humidity, wind speed, and conductivity-temperature-depth (CTD) casts. Currents were measured with a downward looking acoustic Doppler current profiler (ADCP) mounted on the research boat; however, these spot current measurements proved too noisy to assess the direction and magnitude of tidal currents. Consequently, a separate study was conducted in which the ADCP was deployed continuously for 48 hours at seven sites in Upper and Lower Newport Bay. This chapter partially satisfies sub-task 2.1.1.4 of the Proposition 13 project, by reporting on work conducted under the following tasks:

- Task 2.1.1.1. Bay-To-Ocean Transect Studies
- Task 2.1.1.2. Bird Census within the Bay
- Task 2.1.3.3. Solar Irradiance Data

[1] This chapter was written by S. Grant with the assistance of L. Ho, R. Litton, and Drs. B. Sanders, K. McLaughlin, J. Ahn, N. Nezlin, and L. Candelaria.

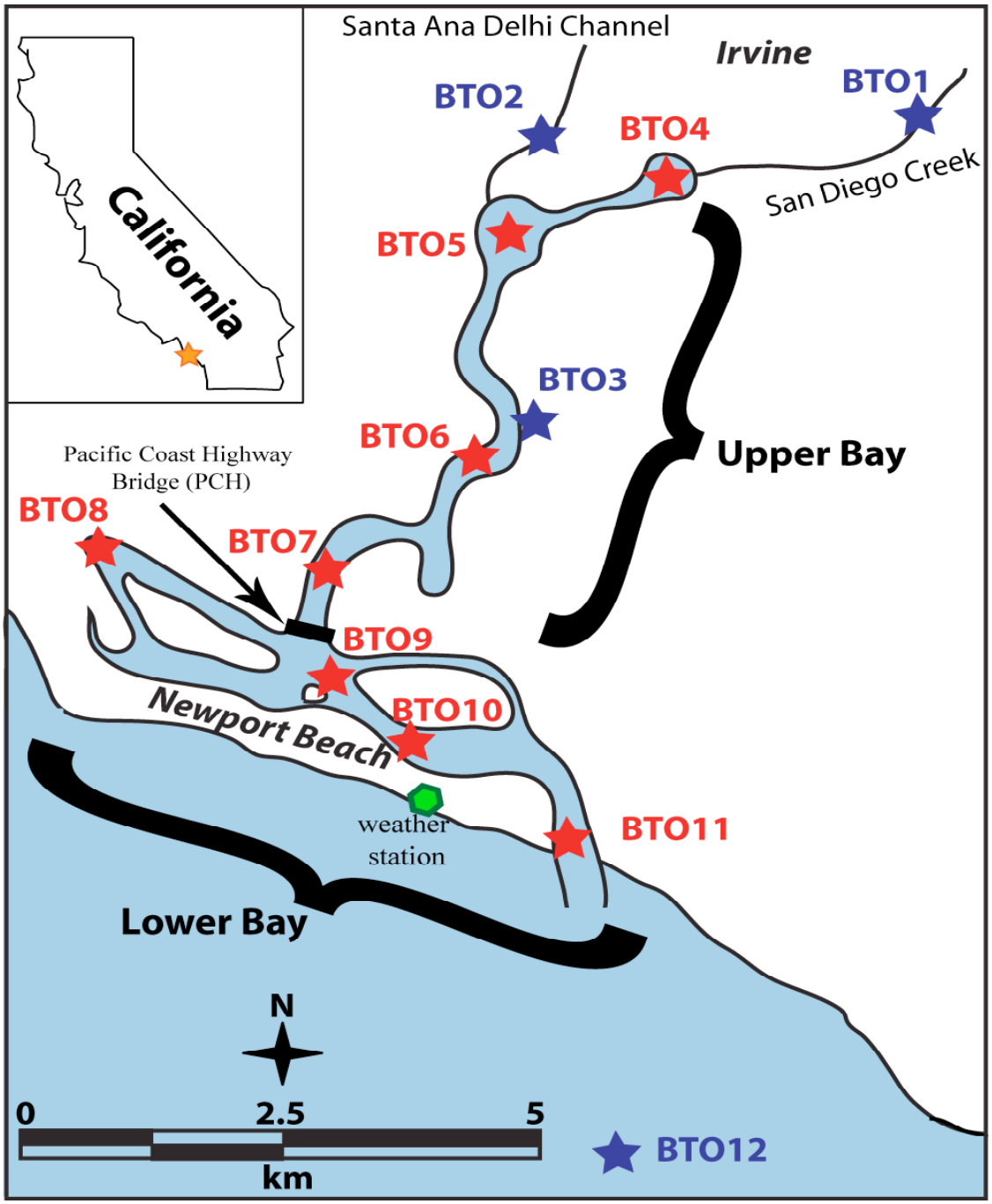

Figure 2-1. Map of Newport Bay showing the location of the twelve Bay-to-Ocean (BTO) transect sites in Upper and Lower Newport Bay.

Table 2-1. Location of the three creek/tributary sites (BTO 1-3), nine within Bay sites (BTO 4-11) and one offshore control site (BTO 12).

Station	***Description***	***Latitude***	***Longitude***
BTO 1	San Diego Creek	33° 39.342N	117° 50.761W
BTO 2	Santa Ana Delhi Channel	33° 39.213N	117° 53.025W
BTO 3	Big Canyon Wash	33° 38.601N	117° 50.019W
BTO 4	Unit 1 Basin	33° 39.033N	117° 52.138W
BTO 5	Unit 2 Basin	33° 38.634N	117° 53.241W
BTO 6	North Star Beach	33° 37.416N	117° 53.573W
BTO 7	Pacific Coast Highway Bridge	33° 37.029N	117° 54.284W
BTO 8	Arches Drain/West Newport Bay	33° 37.183N	117° 55.642W
BTO 9	Pavillion	33° 36.496N	117° 54.220W
BTO 10	Harbor Patrol	33° 36.223N	117° 53.832W
BTO 11	Newport Bay outlet	33° 35.668N	117° 52.809W
BTO 12	Offshore Control (approximate location)	33° 33.964N	117° 54.602W

2.2 Methods

2.2.1 Water and sediment sampling

2.2.1.1 Location of BTO Sampling Sites

The locations of Bay-to-Ocean (BTO) sampling sites are shown in Fig. 2-1. The sampling sites fall into three categories:

- Creek and tributary sites: San Diego Creek (BTO 1), Santa Ana Delhi Channel (BTO 2), and Big Canyon Wash (BTO 3)
- Within-Bay sites in Upper Bay (BTO 4-7) and Lower Bay (BTO 8-11)
- Offshore control site (BTO 12)

The latitudes and longitudes for the twelve BTO sites are listed in Table 2-1; note that the latitude and longitude of the offshore control site (BTO 12) is approximate, because the distance the boat went offshore varied by sampling run, depending on weather conditions, wave conditions, and time of day.

2.2.1.2 Schedule and sampling frequency

Tables 2-2 and 2-3 summarize sampling events carried out for the BTO study. Altogether, 53 sampling events were carried out over the course of 13 months[2], including

[2] For comparison, a total of 52 transects were budgeted in the Prop 13 project (1 year*1 transect per week).

46 transect studies (Table 2-2) and 7 high frequency sampling events associated with the tidal current study (Table 2-3). As described above, the 7 high frequency sampling events were necessitated by the failure of the ADCP to provide useful tidal current data during the routine BTO transect studies. Several points should be noted:

1. Tributary/creek sites (BTO 1, 2, and 3) were sampled on a near-daily basis during most of the 13 month study
2. Within-Bay sites (BTO 4-11) were sampled on a near weekly basis from 1/16/06 through 11/31/06, except for two week-long gaps when the boat underwent required maintenance (1/23/06 through 1/27/06, and 6/26/06 through 6/30/06), and a three-week gap (7/05/06 through 8/02/06) when the TAC required a preliminary analysis of the BTO transect data to determine if measurements were impacted by dredging operations in the Bay, and a two-month gap toward the end of the field sampling effort (12/06 through 1/07).
3. Sampling gaps were balanced by shorter periods of high frequency sampling at some or all of the BTO sites, including:
    a. A storm event in February-March 2006, during which water and sediment samples were collected from BTO 7 every three to four hours over three days (2/27/06 through 3/2/06);
    b. A storm event in March-April 2006, during which water and sediment samples were collected from all BTO sites on a near-daily frequency for five days;
    c. A sequence of storm events in February 2007, during which water and sediment samples were collected every other day for approximately three weeks;
    d. In conjunction with the diurnal intertidal sediment (DITS) studies (see Chapter 6), water samples were collected from BTO 1, 2, 4, and 5 every three hours for twenty hours during dry weather on four separate occasions (3/10/06, 3/24/06, 10/19/06, 10/26/06).
    e. In conjunction with in situ current velocity measurements, water samples were collected hourly for 24 hours at BTO sites 4 through 10.

2.2.1.3 Water and sediment sampling from tributaries to Newport Bay (BTO 1-3)

Tributary sites include San Diego Creek (BTO 1), Santa Ana Delhi channel (BTO 2), and Big Canyon Wash (BTO 3). Tributary sites were accessed by car and on foot. Water samples were collected on a near daily basis from the surface of the water column, capped, placed on ice, and transported back to the laboratory at UCI for analysis. Each time a water sample was collected, the temperature of the water surface was recorded with an infrared gun (Raynger-ST, Raytek, Santa Cruz, CA). Sediment samples were collected approximately once per month from the same three creek sites using 50 mL conical tubes (Fischer Scientific, Pittsburg, PA) affixed to the end of a telescoping pole. The conical tube was forced into the bottom sediments to a depth of approximately 10 cm. After collection, sediment samples were capped, placed on ice in the dark, and transported to UCI where they were analyzed for FIB.

Table 2-2. Sampling events completed for Task 2.1.1.1.

#	Date	Stations	[b]Water Collected	[c]Sediment Collected	[d]Water Analyses	Sediment Analyses	FIB Isolates	CTD (y/n)	[a]Metdata
1	January 16-18, 2006	BTO 1-12	RS, LS, MS, MI	Upper Bay Sediment only	3XFIB, PSD,pH	FIB, TOC, grain size, moisture	N	Y	N
2	January 30-31, 2006	BTO 1-12	RS, LS, MS, MI, MB	Upper Bay Sediment only	3XFIB, PSD, pH	FIB, TOC, moisture	N	Y	Y
3	February 2-3, 2006	BTO 1-12	RS, LS, MS, MI, MB	No sediment	3XFIB, PSD, pH	None	Y	Y	Y
4	February 8-10, 2006	BTO 1-12	RS, LS, MS, MI, MB	Upper Bay Sediment only	3XFIB, PSD, pH	FIB, TOC, moisture	N	Y	Y
5	February 13-16, 2006	BTO 1-12	RS, LS, MS, MI, MB	Upper Bay Sediment only	3XFIB, PSD, pH	FIB, TOC, grain size, moisture	N	Y	Y
6	February 22-24, 2006	BTO 1-12	RS, LS, MS, MI, MB	Upper Bay Sediment only	3XFIB, PSD, pH	FIB, TOC, moisture	Y	Y	Y
7	March 1-3, 2006	BTO 1-12	RS, LS, MS, MI, MB	Upper Bay Sediment only	3XFIB, PSD, pH	FIB, TOC, moisture	N	Y	Y
8	March 7, 2006	BTO 1-12	MS, MB	No sediment	1XFIB, PSD, pH	None	N	Y	Y
9	March 14-16, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	3XFIB, PSD, pH	FIB, TOC, moisture	Y	Y	Y
10	March 28, 2006	BTO 8-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH	FIB, TOC, moisture	N	N	N
11	March 29, 2006	BTO 1-12	MS, MI, MB	M	2XFIB, PSD, pH	FIB, TOC, moisture	Y	Y	Y
12	March 30, 2006	BTO 1-12	MS, MI, MB	M	2XFIB, PSD, pH	FIB, TOC, moisture	N	Y	Y
13	March 31, 2006	BTO 1-12	MS, MI, MB	M	2XFIB, PSD, pH	FIB, TOC, moisture	N	Y	Y
14	April 3, 2006	BTO 1-12	MS, MI, MB	M	2XFIB, PSD, pH	FIB, TOC, moisture	N	Y	Y
15	April 7, 2006	BTO 1-12	MS, MI, MB	M	2XFIB, PSD, pH	FIB, TOC, moisture	N	Y	Y
16	April 12-13, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, grain size, moisture	N	Y	Y
17	April 26-27, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, moisture	Y	Y	Y
18	May 2-3, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	Y	Y
19	May 8-10, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, grain size, moisture	N	Y	Y
20	May 15-17, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	Y	Y

Table 2-2. Sampling events completed for Task 2.1.1.1 (cont.)

#	Date	Stations	[b]Water Collected	[c]Sediment Collected	[d]Water Analyses	Sediment Analyses	FIB Isolates	CTD (y/n)	[a]Metdata
21	May 22-24, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, moisture	Y	Y	Y
22	June 1-2, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	Y	Y
23	June 6-7, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	1XFIB, PSD, pH, salinity	FIB, TOC, grain size, moisture		N	Y
23	June 20-21, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, moisture	Y	N	Y
24	July 5-7, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, grain size, moisture	Y	Y	Y
25	August 1-3, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, moisture, moisture	Y	N	Y
26	August 10-11, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	1XFIB, PSD, pH, salinity	FIB, TOC, grain size, moisture	N	N	Y
27	August 15-17, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	1XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	N	Y
28	August 22-24, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	1XFIB, PSD, pH, salinity	FIB, TOC, moisture	Y	N	Y
29	August 28-29, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	1XFIB, PSD, pH, salinity	FIB, TOC, moisture	Y	Y	Y
30	September 6-7, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, moisture	Y	Y	Y
31	September 11-12, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, grain size, moisture	Y	Y	Y
32	September 18-19, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	N	Y
33	September 25-26, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	1XFIB, PSD, pH, salinity	FIB, TOC, moisture	Y	Y	Y
34	October 9-10, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, grain size, moisture	Y	Y	Y
35	October 16-17, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	1XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	Y	Y
36	November 6-7, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	1XFIB, PSD, pH, salinity	FIB, TOC, grain size, moisture	Y	Y	Y
37	November 28-30, 2006	BTO 1-12	RS, LS, MS, MI, MB	R, L, M	2XFIB, PSD, pH, salinity	FIB, TOC, grain size, moisture	N	Y	Y
38	February 8, 2007	BTO 1-12	RS, LS, MS, MI, MB	M	1XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	Y	Y

Table 2-2. Sampling events completed for Task 2.1.1.1 (cont.)

#	Date	Stations	[b]Water Collected	[c]Sediment Collected	[d]Water Analyses	Sediment Analyses	FIB Isolates	CTD (y/n)	[a]Metdata
39	February 14, 2007	BTO 1-12	RS, LS, MS, MI, MB	M	1XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	N	Y
40	February 16, 2007	BTO 1-12	RS, LS, MS, MI, MB	M	1XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	N	Y
41	February 18, 2007	BTO 1-12	RS, LS, MS, MI, MB	M	1XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	N	Y
42	February 20, 2007	BTO 1-12	RS, LS, MS, MI, MB	M	1XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	Y	Y
43	February 22, 2007	BTO 1-12	RS, LS, MS, MI, MB	M	1XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	N	Y
44	February 24,2007	BTO 1-12	RS, LS, MS, MI, MB	M	1XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	Y	Y
45	February 26,2007	BTO 1-12	RS, LS, MS, MI, MB	M	1XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	Y	Y
46	February 28,2007	BTO 1-12	RS, LS, MS, MI, MB	M	1XFIB, PSD, pH, salinity	FIB, TOC, moisture	N	N	Y

[a]Meteorology measurements, including wind speed, humidity, and air temperature.

[b]RS and LS, samplees collected from the surface of the water column at the right and left banks of the channel (with back to the ocean); MS, sample collected from the surface of the water column in the middle (thalweg) of the channel; MI, depth-integrated sample collected from the middle (thalweg) of the channel; MB, sample collected from the bottom of the water column at the middle (thalweg) of the channel.

[c]Sediment collected from the right bank (R), left bank (L), and middle (thalweg-M) of the channel, with back to the ocean.

[d]3XFIB: FIB measurements carried out on water samples after processing three different ways: no filtration, filtration through a 5 micron filter, and filtration through a 10 micron filter; 2XFIB: FIB measurements carried out on water samples after processing two different ways: no filtration, and filtration through a 5 micron filter; 1XFIB: FIB measurements carried out only on unfiltered water samples. PSD, suspended particle size distributions measured with a Sequoia LISST 100.

Table 2-3. ADCP sampling events completed for Task 2.1.1.1.

Number	***Date***	***Stations Sampled***	***Analyses Performed***
47	August 30-September 1, 2006	BTO 5/6	48 hours: ADCP measurements every second. First 24 hours only: Hourly FIB, Particle Size Distribution, pH, Salinity, Weather Measurements, 1mL preserved for microscopy. Half-hourly CTD casts
48	September 14-16, 2006	BTO 6/7	48 hours: ADCP measurements every second. First 24 hours only: Hourly FIB, Particle Size Distribution, pH, Salinity, Weather Measurements,1mL preserved for microscopy. Half-hourly CTD casts
49	September 28-30, 2006	BTO 4/5	48 hours: ADCP measurements every second. First 24 hours only: Hourly FIB, Particle Size Distribution, pH, Salinity, Weather Measurements, 1mL preserved for microscopy. Half-hourly CTD casts
50	October 5-7, 2006	PCH Bridge (BTO 7)	48 hours: ADCP measurements every second. First 24 hours only: Hourly FIB, Particle Size Distribution, pH, Salinity, Weather Measurements, 1mL preserved for microscopy. Half-hourly CTD casts
51	October 12-14, 2006	BTO 8/9	48 hours: ADCP measurements every second. First 24 hours only: Hourly FIB, Particle Size Distribution, pH, Salinity, Weather Measurements, CTD cast, 1mL preserved for microscopy.
52	November 3-5, 2006	BTO 9/10	48 hours: ADCP measurements every second. First 24 hours only: Hourly FIB, Particle Size Distribution, pH, Salinity, Weather Measurements, CTD cast, 1mL preserved for microscopy.
53	November 10-12, 2006	BTO 10/11	48 hours: ADCP measurements every second. First 24 hours only: Hourly FIB, Particle Size Distribution, pH, Salinity, Weather Measurements, CTD cast, 1mL preserved for microscopy.

2.2.1.4 Water and sediment sampling from the within-Bay sites (BTO 4-11)

Within-Bay sites were sampled using a 17-foot sampling boat designed and built for this study (see Chapter 1). Water and sediment samples were collected approximately weekly during the first nine-months of the study (except for boat maintenance and TAC reviews of data, see Section 2.2.1.2). A series of special studies were also undertaken in which one or more within-Bay sites were sampled for a shorter duration but at a higher sampling frequency; the most notable of these was every other day sampling of all BTO sites during a sequence of four storms in February 2007.

Each time the boat went out to sample a particular within-Bay site, water and sediment samples were collected from multiple locations in the tidal channel cross-section. Samples collected from the tidal cross-section at within-Bay sites include:

- A surface water sample, a bottom water sample, and a depth-integrated water sample collected from the deepest point (thalweg) in the tidal channel
- Surface water samples collected from the channel's left and right edges (in approximately 1m deep water).
- A sediment sample collected from the tidal channel thalweg.

- Sediment samples collected from the channel's left and right edges (in approximately 1m deep waters).

In the nomenclature used here, the edge samples are designated as "left" or "right", assuming the observer's back is to the ocean. Because the sampling boat could not operate in water shallower than 1m, water and sediment samples collected from the right and left banks of the tidal channel were typically sampled in water that was approximately 1 m deep. Thus, in most cases, the sediments collected were sub-tidal (i.e., never exposed to air at low tide).

Surface water samples were collected with 500 mL sterile polypropylene bottles lowered over the side of the boat, rinsed twice with ambient water, filled, capped, and placed on ice in the dark.

Bottom water samples were collected using a Van Doren Bottle, which was emptied into a 500 mL polypropylene bottle, capped and stored on ice in the dark.

Depth-integrated water samples were collected by lowering a weighted Tygon tube over the side of the boat. When the weighted end of the tube reached the bottom, the top of the tube was crimped, and the entire tube was raised to the deck of the boat and emptied into a 500 mL Nalgene polypropylene bottle, which was then capped and stored on ice in the dark.

Sediment samples were collected using a Wildco Ponar Petite Grab lowered over the side of the boat with a mechanical davit. Sediment grab samples were emptied into a plastic tray on the deck of the boat, and sub-sampled with a 50 mL sterile plastic centrifuge tube (Fisher Scientific). The 50 mL tube was immediately capped and placed on ice in the dark.

2.2.1.5 Water sampling from the offshore control site (BTO 12)

Water samples were collected from the surface of the water column at the offshore control site, BTO 12. This sample was collected in a 500 mL sterile polypropylene bottle lowered over the side of the boat, rinsed twice with ambient water, filled, capped, and placed on ice in the dark.

2.2.2. Measurement of Solar Irradiance in San Joaquin Marsh

Total incoming solar irradiance was recorded every 30 minutes using a CM3 Pyranometer (Kipp and Zonen, Delft, The Netherlands) deployed in the San Joaquin marsh, located inland of Newport Bay (33.66º, -117.85º)

2.2.3 Boat Observations of Air Temperature, Humidity, Wind Speed

Meteorological measurements were collected from the boat, including air temperature, relative humidity, and wind speed (RM Young Model 85000 Ultrasonic Anemometer and RM Young 26700 series Translator, Traverse City, MI).

2.2.4. Bird Census

Digital photographs (Konica Minolta Dynax Maxxum 5D, Malaysia) were taken to provide a 360° degree view of each site at the time of sampling. Photographs were downloaded onto a computer, and the number of birds present on each photograph recorded. These data were reported as the total number of birds present at a given site during a particular sampling event.

2.2.5 CTD Casts

Conductivity-Temperature-Depth (CTD) casts were conducted at the thalweg of BTO sites to provide information on the vertical structure of water column temperature, salinity, and density (SeaBird 19 Plus CTD Profiler). CTD downcasts were captured and processed with custom programming routines written and implemented in the data analysis program Igor v. 6,1 (WaveMetric Inc., Oswego, OR). Data products generated from Igor include depth-averages and two-dimensional (depth and time) contour plots of (1) temperature, (2) salinity, (3) density, and (4) Buoyancy Frequency. Buoyancy Frequency (N) is a measure of water column stratification, and is calculated from the density profile data using the following equation:

$$N = \left| \frac{g}{\rho} \frac{\partial \rho}{\partial z} \right|^{0.5}$$

where g is gravitational acceleration, ρ_0 is the water density averaged over all CTD measurements collected during a particular deployment, and $\partial\rho/\partial z$ is the local vertical density gradient. Values of the Buoyancy Frequency $N > 0.2$/s indicate a stratified water column; values <0.02/s indicate weak to no water column column stratification.[3]

2.2.6 Water Sample Analyses

All or some of the water samples were analyzed for:

- FIB using IDEXX Colilert-18 (*E. coli*) and Enterolert (enterococci bacteria) defined substrate tests implemented in a 96 well quantitray format.

[3] Mikkelsen, O.A., Milligan, T.G., Hill, P.S., Chan, R.J., Jago, C.F., Jones, S.E., Krivtsov, V., Mitchelson-Jacob, G. (2008) "The influence of schlieren on in situ optical measurements used for particle characterization" *Limnology and Oceanography: Methods* **6**:133-143.

- Conductivity (Model 162A, Thermo Orion, Waltham, MA) subsequently converted to salinity using the Practical Salinity Scale.
- A suite of nutrients including ammonium, nitrate, total phosphorous, and total organic carbon. Nutrient analyses were carried out on a subset of the water samples, and were conducted by a third party lab under contract with Orange County Public Works.
- Particle size distributions using a LISST-100 small angle light diffraction instrument (Sequoia Scientific, Inc., Bellvue, WA)
- Approximately 1.5 mL of each water sample was transferred to an epi tube, and archived at -85°C for later analyses by optical microscopy.

2.2.7 Water Analyses: Size Fractionation of FIB

Filtration tests were run on a subset of water samples to determine if FIB are associated with specific particle size classes. To carry out these tests, 30 mL of a water sample was passed through a TMTP filter (Millipore, Ireland) of known pore size (either 5 or 10 micron nominal diameter). The filtrate was tested for FIB using the IDEXX Colilert and Enterolert defined substrate systems. The FIB concentrations before and after filtration were then compared.

2.2.8 Sediment Analyses: FIB Measurements

Sediment samples were analyzed for FIB using a procedure published by Craig et al[4], but optimized for Newport Bay sediments by the Orange County Health Care Agency. Specifically, 10 g (wet weight) of sediment was added to a 150 mL sterilized bottle (IDEXX, Westbrook, ME) resuspended in 100 mL of 1% sodium metaphosphate (10g sodium metaphosphate + 1000mL sterile DI water), hand shaken for 1 min., and sonicated for 30 seconds using a Branson Sonifier 450 (Danbury, CT). The sediment suspension was allowed to settle for 10 minutes, and 10 mL of the supernatant was collected and analyzed for FIB using the Colilert and Enterolert defined substrate tests (IDEXX, Westbrook, ME) as described above for the water phase samples. The concentration of FIB measured in the 10 mL of supernatant was converted to the sediment FIB concentration (units of most probable number (MPN)/100g dry sediment). To compute a dry-to-wet weight ratio for the sediment, approximately 10 g of wet sediment from each sediment sample was weighed out and dried overnight in an oven at 110 $^{\circ}$C and re-weighed. Approximately 100g of each sediment sample was archived at -85°C for future grain size analysis.

2.2.9 Sediment Analyses: Grain Size Distributions

The grain size distributions were measured on a subset of sediment samples as follows. Archived sediment samples were retrieved from the -85°C freezer and defrosted overnight in the refrigerator. Approximately 30 g of refrigerated sediment sample was

[4] Craig, D. L., Fallowfield, H. J., Cromar, N. J. (2002) "Enumeration of faecal coliforms from recreational coastal sites: evaluation of techniques for the separation of bacteria from sediments" *J. Applied Microbiology* **93**, 557-565..

suspended in 30 mL of 30% (v/v) hydrogen peroxide and placed in the fume hood overnight to remove the organic fraction. Each sample was then centrifuged at high speed for 10-15 minutes, filtered through a 53-µm sieve to remove coarse fraction, diluted into DI water, and analyzed using a LISST-100 particle size analyzer (Sequoia Scientific, Inc., Bellevue, WA) in batch mode.

2.2.10 Sediment Analyses: Water Content and Organic Carbon

The sediment water content was calculated by taking the difference of the wet and dry weights after drying sediment sample in the oven at 60°C overnight, and the sediment organic carbon content was determined by burning off all organic carbon in the dried sediment sample in the furnace at 400°C overnight.

2.3 Results and Discussion

2.3.1. Environmental Conditions during the BTO Study

To streamline the analysis of this large dataset[5], and to identify seasonal trends, the BTO transect data were parsed into the following four time periods:

- **All BTO Data**: Data collected over the entire study period, from January 16 (2006) through February 28 (2007)
- **Dry Season (2006)**: Data collected during a dry weather period between July 1 and November 1 (2006).
- **Storm Season (2006)**: Data collected during a wet weather period between March 1 and May 1 (2006)
- **February Storm (2007):** Data collected during a sequence of four storms, from February 8 through February 28 (2007)

Environmental conditions during these four time periods are summarized in Fig. 2-2 and Table 2-4. The BTO transect study was conducted during a year with somewhat less than the long-term average annual precipitation (total precipitation for the water year beginning January 1 2006 was 7.22 inches, compared to the long-term annual average of 11.17 inches[6]). A total of 7.64 inches of precipitation fell during the 13 months of the BTO study (from January 16 2006 to February 28 2007). Most of this rain (56%, or 4.26 inches) fell during the three-month "Storm Season (2006)" period, from March 1 2006 through May 1 2006.

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[5] During the BTO transect study, a total of 2,441 water samples and 878 sediment samples were collected from Newport Bay and its tributaries. All water samples were analyzed for FIB (*E. coli* and enterococci bacteria), pH, salinity, and particle size distributions. A subset of water samples was analyzed for nutrients (ammonia, nitrate, total phosphorous, TOC) and particle settling velocity. All sediment samples were analyzed for FIB (*E. coli* and enterococci bacteria). A subset of sediment samples was analyzed for % organic carbon, % water content, and grain size. In addition, 237 conductivity-temperature-depth (CTD) casts were carried out in Newport Bay, each of which generated vertical profiles of water temperature, salinity, density, and buoyancy frequency.

[6] See historical data for Newport Beach Harbor rain gauge (046175) on the Western Regional Climate Center website: http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?ca6175 .

Referring to Table 2-4, the Storm Season (2006) is characterized by relatively high total and daily average precipitation (4.26 inches and 0.07 +/- 0.15 inches per day), elevated and variable flow rates from the San Diego Creek (75 +/- 232 cfs), lower solar irradiance (497 +/- 232 W/m^2), lower air temperature (14.2 +/- 1.4^oC), and lower water temperature (15.3 +/- 2.2^oC). The Dry Season (2006) is characterized by lower total and daily average precipitation (0.33 inches and 0.0026 +/- 0.018 inches per day), base flow from San Diego Creek (9.8 +/- 6 cfs), higher solar irradiance (560 +/- 255 W/m^2), higher air temperature (19.8 +/- 2.1^oC), and higher water temperature (23.0 +/- 3.2^oC). Environmental conditions during the February 2007 storm sequence are similar to those recorded during the Storm Season (2006). The timing of sampling events at all 12 BTO sites are shown graphically for these four time periods in Appendix 2A.

2.3.2. Water and Sediment Sampling Times

The time of day when water and sediment samples were collected from Newport Bay is presented in Fig. 2-3. The four different panels in the figure correspond to the four time periods described above. Samples from within Bay (BTO 4-11) and offshore (BTO 12) sites were typically collected in the morning and early afternoon, while samples from the tributary sites (BTO 1-3) were typically collected in the late afternoon. Additional trends are evident during specific periods of time. For example, during dry season sampling (upper right panel, Fig. 2-3), sampling at within-Bay sites occurred over two consecutive days, with one day focused on sample collection from Upper Bay sites (BTO 4 to BTO 7), and the other day focused on sample collection from Lower Bay and offshore sites (from BTO 8 to BTO 12). During the sequence of storms in February 2007 (lower left panel, Fig. 2-3), all within-Bay and offshore sites were sampled in a single day, from BTO 4 in the morning to BTO 12 in the late afternoon.

2.3.3. Tide levels during BTO sampling

Predicted tide levels for sampling events in Newport Bay are presented graphically in Fig. 2-4. With one exception, there are no obvious down-Bay trends in the distribution of tide levels predicted for sampling events in Newport Bay. The one exception is the February Storm study (lower left panel, Fig. 2-4), during which the median tide level progressively decreased as the boat transited down-Bay. Also, the median tide level appears to be somewhat higher (ca., 0.3 m) during the dry season sampling compared to the wet season sampling (compare upper and lower right panels, Fig. 2-4).

2.3.4. Measurement of Solar Irradiance in San Joaquin Marsh

Solar irradiance measured during BTO sampling in Newport Bay is presented graphically in Fig. 2-5. Generally speaking, down-Bay trends in the median solar irradiance are weak, with two exceptions. During the Dry Season (upper right panel, Fig. 2-5), median solar irradiance was generally higher for samples collected closer to noon, and lower for samples collected in the morning (compare sample collection times and solar irradiance, upper right panels, Figs. 2-3 and 2-5). Median and peak solar irradiance were generally

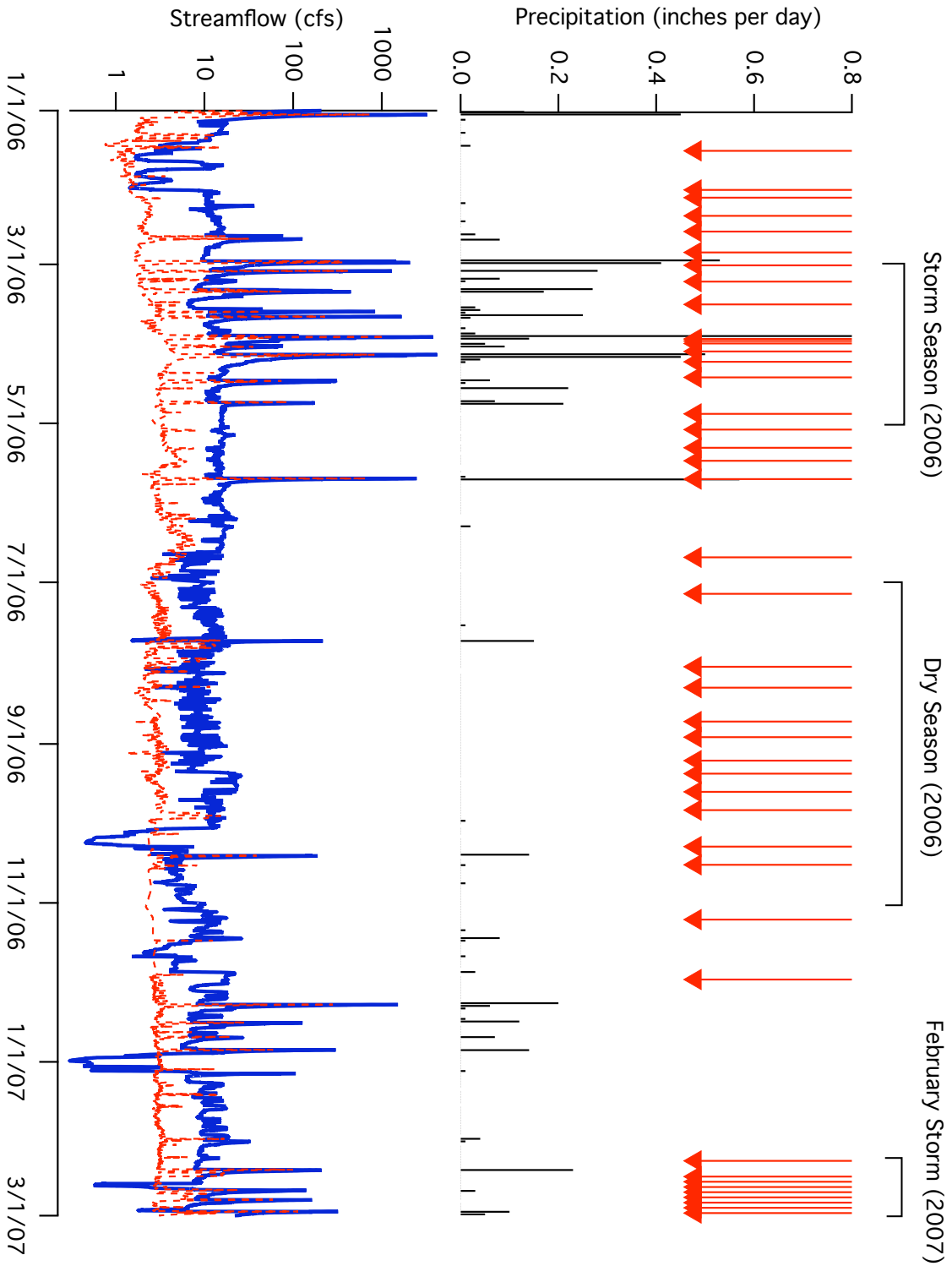

Figure 2-2. Precipitation (black vertical lines), stream flow in San Diego Creek (blue curve) and Santa Ana Delhi channel (red dashed curve), and within-Bay sampling events (red vertical arrows) during the BTO study.

Table 2-4. Environmental Conditions during the four BTO sampling periods

BTO Sampling Period	***Number of BTO Transects***	***[a]Total Rainfall, Average Daily Rainfall***	***[b]San Diego Creek Stream Flow***	***[c]Number of Storm Runoff Events***	***[d]Solar Irradiance***	***[e]Air Temperature***	***[f]Water Temperature***
All BTO Data (423 days)	53	7.64 inches, 0.018 +/- 0.08 inches/day	25 +/- 80 cubic feet per second (cfs)	22	528 +/- 228 W/m^2	17.5 +/- 13.1 ºC	19.5 +/- 4.1 ºC
Storm Season (2006) (63 days)	10	4.26 inches, 0.07 +/- 0.15 inches/day	75 +/- 166 cubic feet per second (cfs)	9	497 +/- 232 W/m^2	14.2 +/- 1.4 ºC	15.3 +/- 2.2 ºC
Dry Season (2006) (125 days)	13	0.33 inches, 0.0026 +/- 0.018 inches/day	9.8 +/- 6 cubic feet per second (cfs)	2	560 +/- 255 W/m^2	19.8 +/- 2.1 ºC	23.0 +/- 3.2ºC
February 2007 Storm Sequence (21 days)	9	0.36 inches, 0.017 +/- 0.05 inches/day	22 +/- 36 cubic feet per second (cfs)	4	529 +/- 166 W/m^2	14.0 +/- 1.9 ºC	15.4 +/- 0.4 ºC

[a]Total rainfall is the sum of all precipitation recorded during the time interval; average daily rainfall is the total rainfall divided by the time interval in days
[b]Streamflow at the campus crossing of San Diego Creek averaged over the time period
[c]Storm runoff events defined by flow in San Diego Creek exceeding ten times base flow, or >100 cfs
[d]Average solar irradiance measured in San Joaquin Marsh during transect sampling events over the time period.
[e]Average air temperature measured from the research boat during transect sampling events over the time period
[f]Average water temperature measured from the research boat during the time period

higher during sampling events in the dry season (upper right panel, Fig. 2-5), and lower during sampling events in the wet season (bottom two panels, Fig. 2-5).

2.3.5. Boat Measurements
2.3.5.1. Air Temperature

Air temperature measured from the boat is plotted in Fig. 2-6. Median air temperatures exhibit very little in the way of down-Bay trends. As expected, median air temperatures were higher during the dry season (ca., 20°C, upper right panel, Fig. 2-6), and lower during the two storm studies (ca., 12 to 15°C, lower two panels, Fig. 2-6).

2.3.5.2. Relative Humidity

Relative humidity measured from the boat is plotted in Fig. 2-7. Relative humidity exhibits some seasonal variability, varying from 70-90% during the dry season, to 50-80% during the two storm periods (compare upper right and bottom two panels, Fig. 2-7). These data are consistent with historical trends, which indicate that relative humidity in coastal southern California is moderate to high throughout the year[7].

[7] See http://www.wrcc.dri.edu/narratives/CALIFORNIA.htm

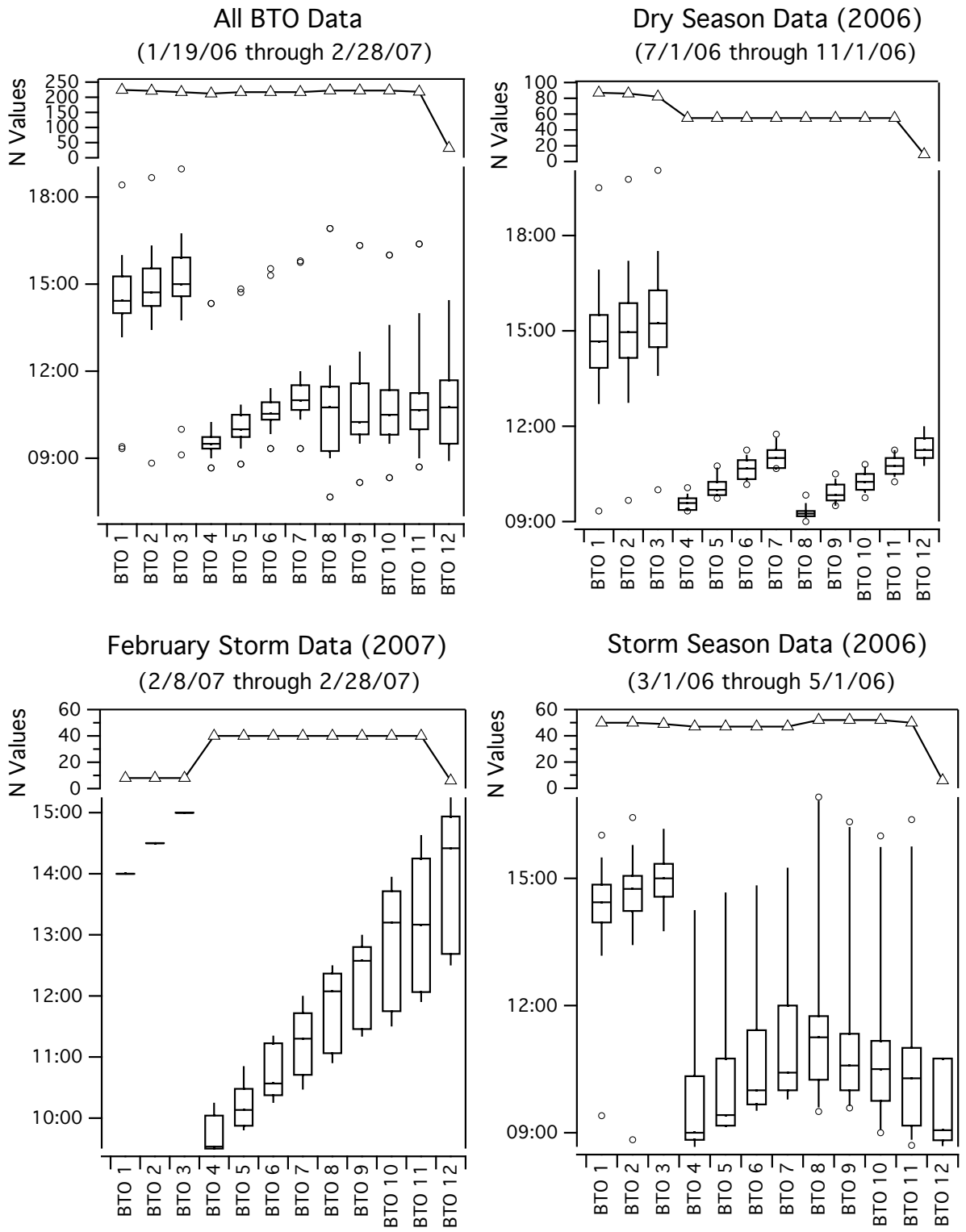

Figure 2-3. Sampling times at all BTO sites, separated into the four different time periods described in the text. Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of samples collected at each site during the time period indicated

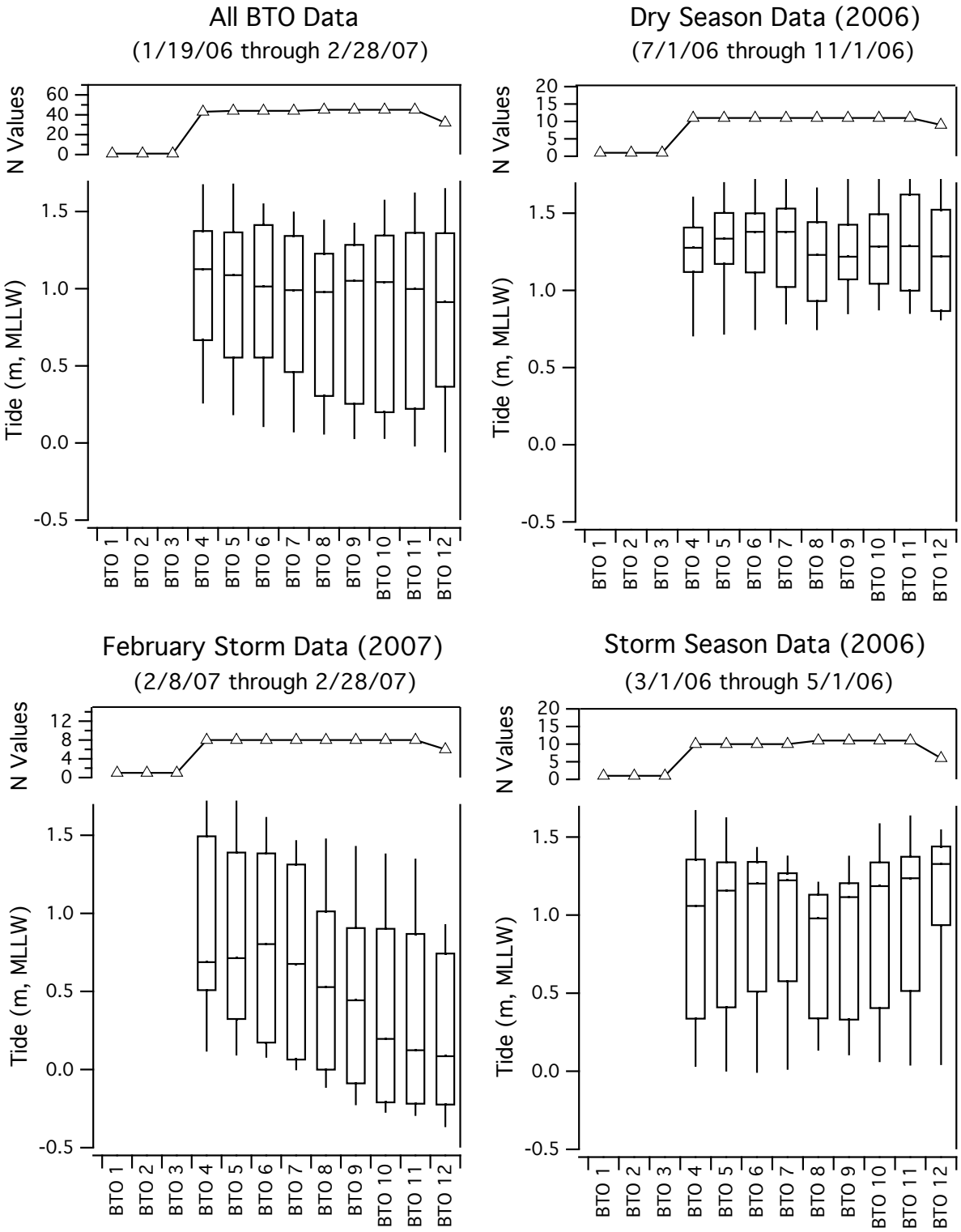

Figure 2-4. Predicted tide levels during BTO sampling events, separated into the four different time periods described in the text. Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of samples collected at each site during the time period indicated.

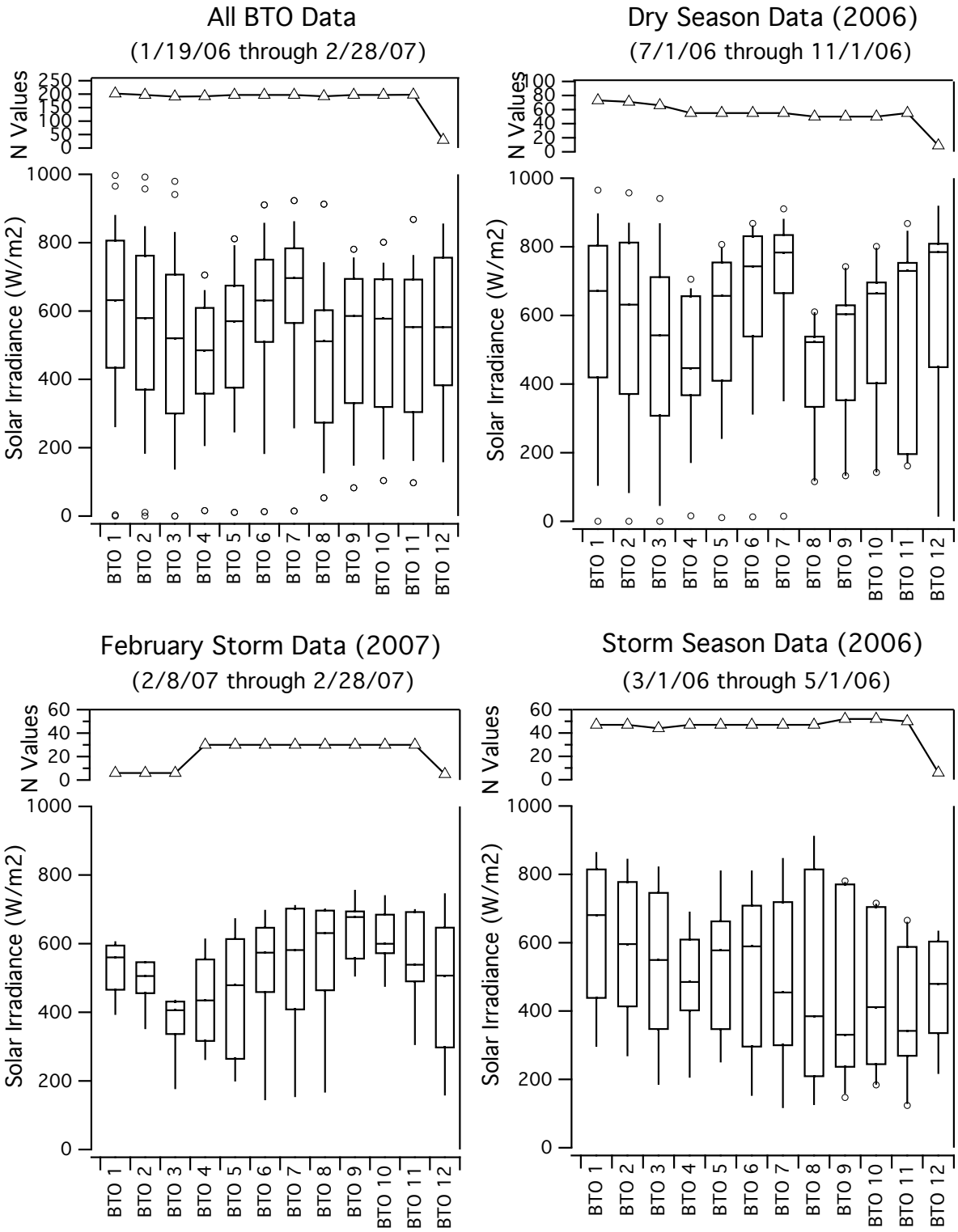

Figure 2-5. Solar irradiance measured during BTO transect sampling, separated into the four different time periods described in the text. Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of samples collected at each site during the time period indicated.

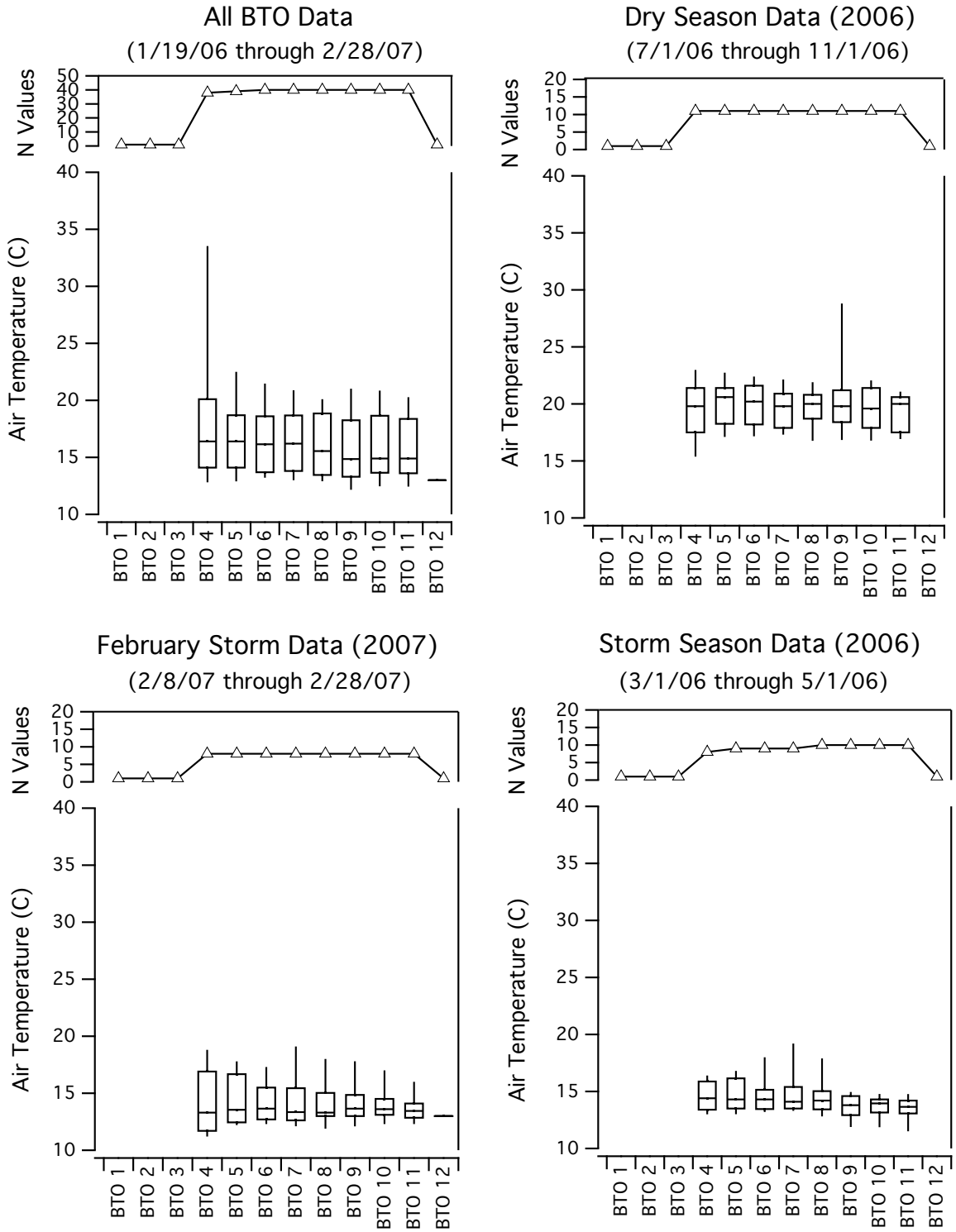

Figure 2-6. Air temperature measured from the research boat during BTO transect sampling, separated into the four different time periods described in the text. Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

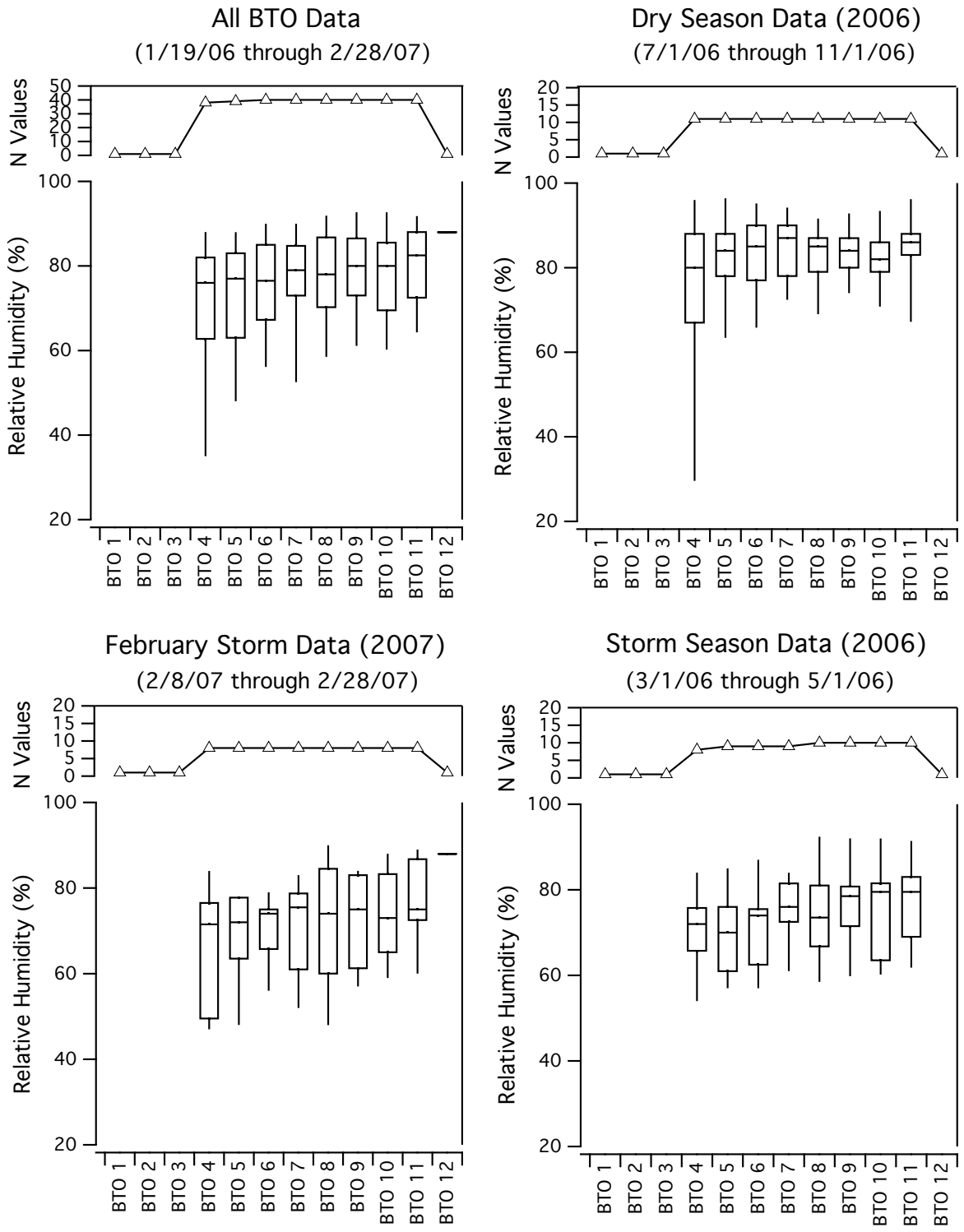

Figure 2-7. Relative humidity measured from the research boat during BTO transect sampling, separated into the four different time periods described in the text. Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

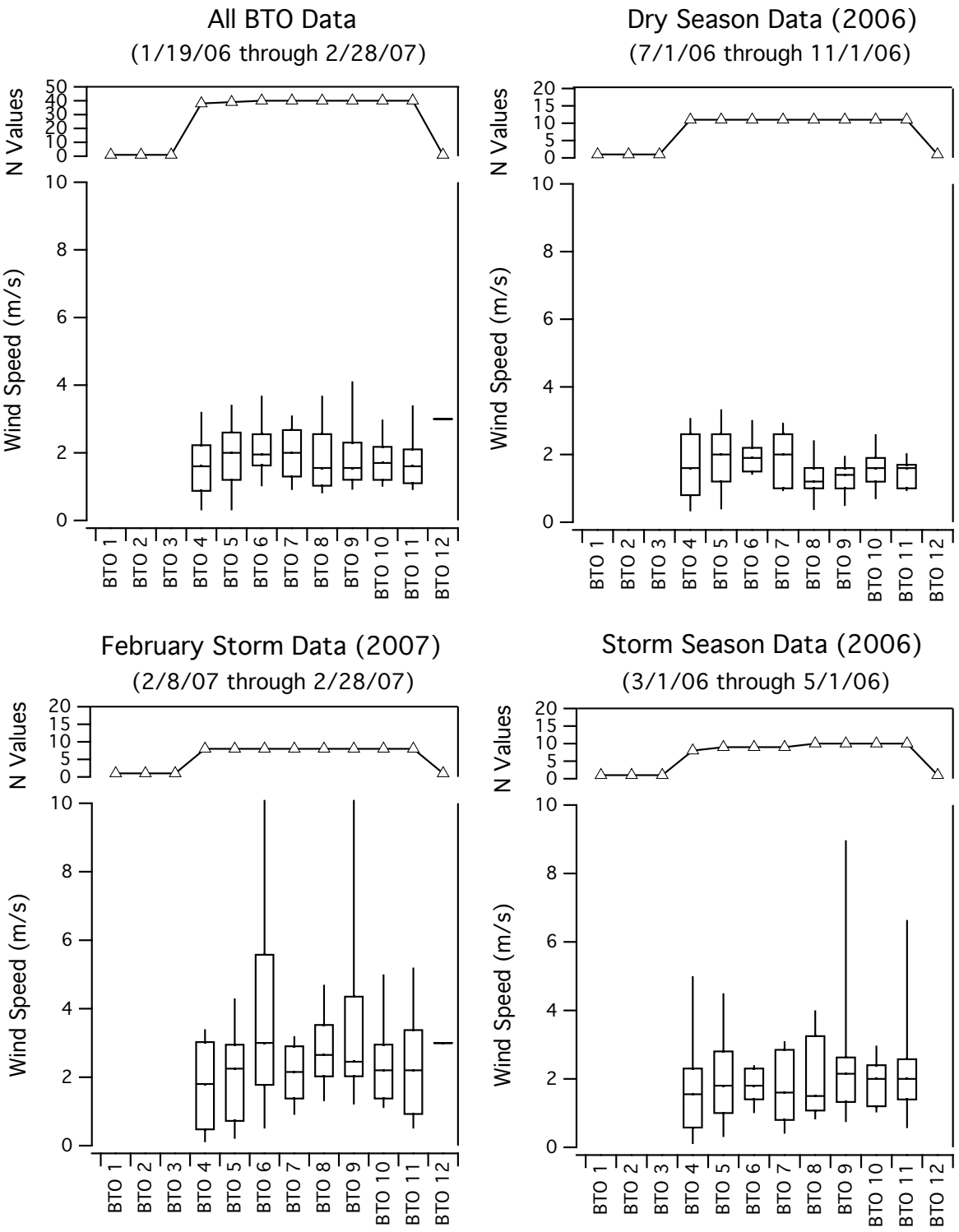

Figure 2-8. Wind speed measured from the research boat during BTO transect sampling, separated into the four different time periods described in the text. Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

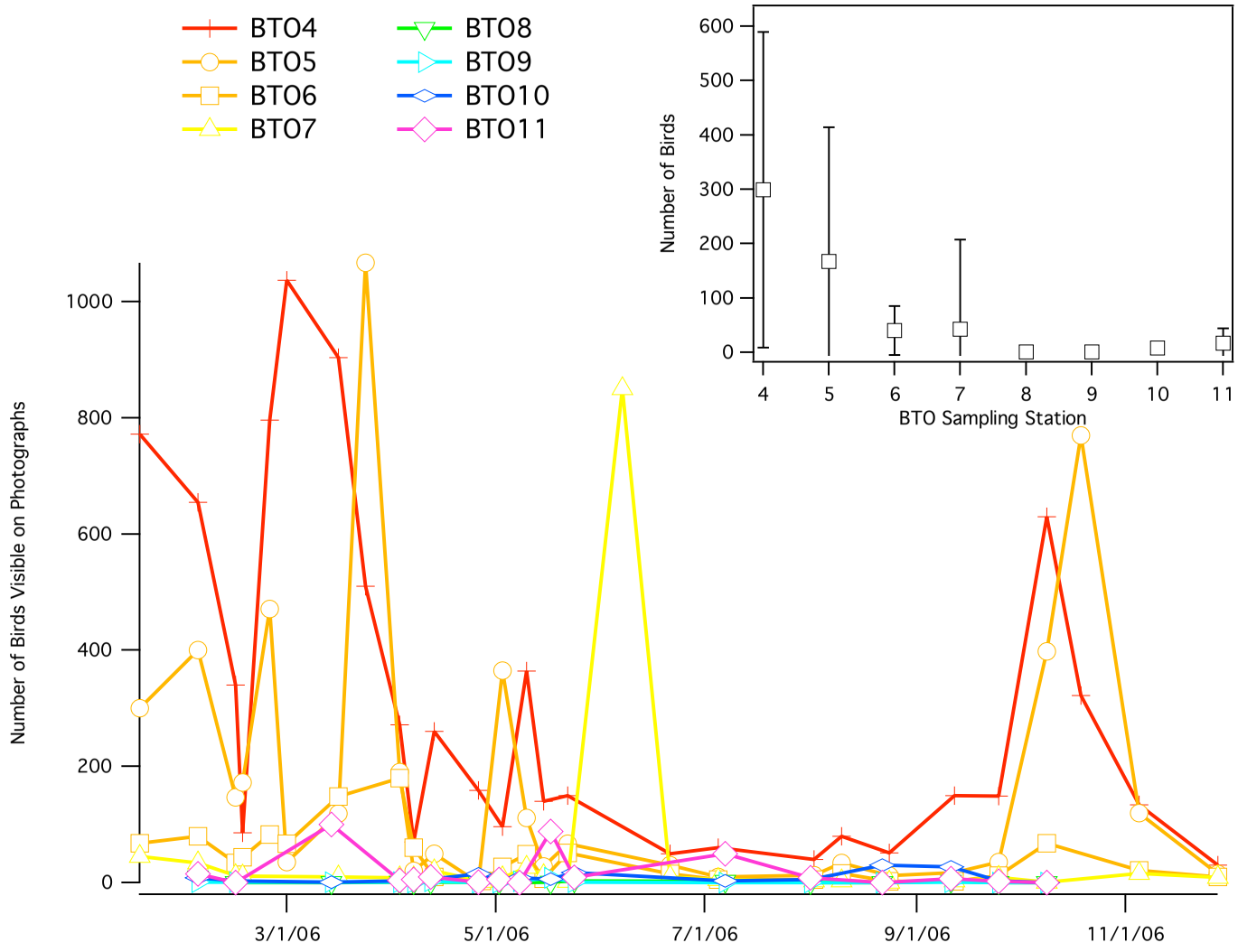

Figure 2-9. Bird census conducted from the research boat during the BTO study. Larger plot shows time series of bird counts measured at each BTO station. Small inset plot shows the average (and standard deviation) of birds observed at each BTO station over the entire 13 month study.

2.3.5.3. Wind Speed

Wind speeds measured from the boat are plotted in Fig. 2-8. During the dry season, median wind speeds were generally higher (ca., 2 m/s) and more variable at Upper Bay sites and lower (ca., 1 m/s) and less variable at Lower Bay sites (upper right panel, Fig. 2-8). Wind speeds were most variable during the two storm periods, with 25% of wind measurements exceeding 8 m/s at some sites (bottom two panels, Fig. 2-8).

2.3.5.4. Bird Census

Birds were most numerous at Upper Bay stations (BTO sites 4-7) during the spring (January through June) and fall (after October) migrations (Fig. 2-9). Bird counts were lower during summer months (June through September), and lower at Lower Bay sites (BTO sites 8-11). The inset in Fig. 2-9 displays the number of birds counted at each site averaged over the 13-month study. The average number of birds counted increased with distance inland, from an average of <5 birds per sampling event at all Lower Bay sites (BTO 8-11) to an average of approximately 300 birds per sampling event at the most inland site (BTO 4). Among the Lower Bay sites, birds are slightly more numerous near the Newport Bay outlet (BTO sites 10 and 11). Based on the bird census carried out by Sea and Sage Audubon during 2006, the most numerous bird species sighted in Upper Newport Bay included geese and ducks, pelicans and cormorants, herons and egrets, gallinules and coots, and sandpipers (see Appendix 2G).

2.3.6. CTD Casts

2.3.6.1. Depth-Averaged Water Temperature

Water temperatures obtained by depth-averaging CTD temperature profiles are plotted in Fig. 2-10 (BTO 4-11). Temperatures at tributary sites, which were measured with an infrared gun, are also presented in the figure (BTO 1-3). At within-Bay sites, median depth-averaged water temperature decreased modestly with distance down Bay, decreasing < 3°C from BTO 4 to BTO 12. Median depth-averaged temperatures are approximately 10°C higher during the dry period compared to the storm periods (compare upper right and lower right panels, Fig. 2-10). During dry weather, median surface water temperatures at tributary sites are a couple of degrees warmer than depth-averaged median temperatures measured at BTO 4 and 5, suggesting that runoff from the creeks can be a source of relatively warm water in Upper Bay (see CTD cross section results, presented later in Section 2.3.6.5).

2.3.6.2. Depth-Averaged Salinity

Water salinities obtained by depth-averaging CTD salinity profiles are plotted in Fig. 2-11. Depth-average median salinity ranges from brackish in Upper Bay (15 to 30) to oceanic near the ocean outlet (32 to 34). In Upper Bay, depth-averaged salinity is generally lower and more variable during the storm season (15 to 28), and higher and less variable during the dry weather season (28 to 32) (compare upper right and lower right

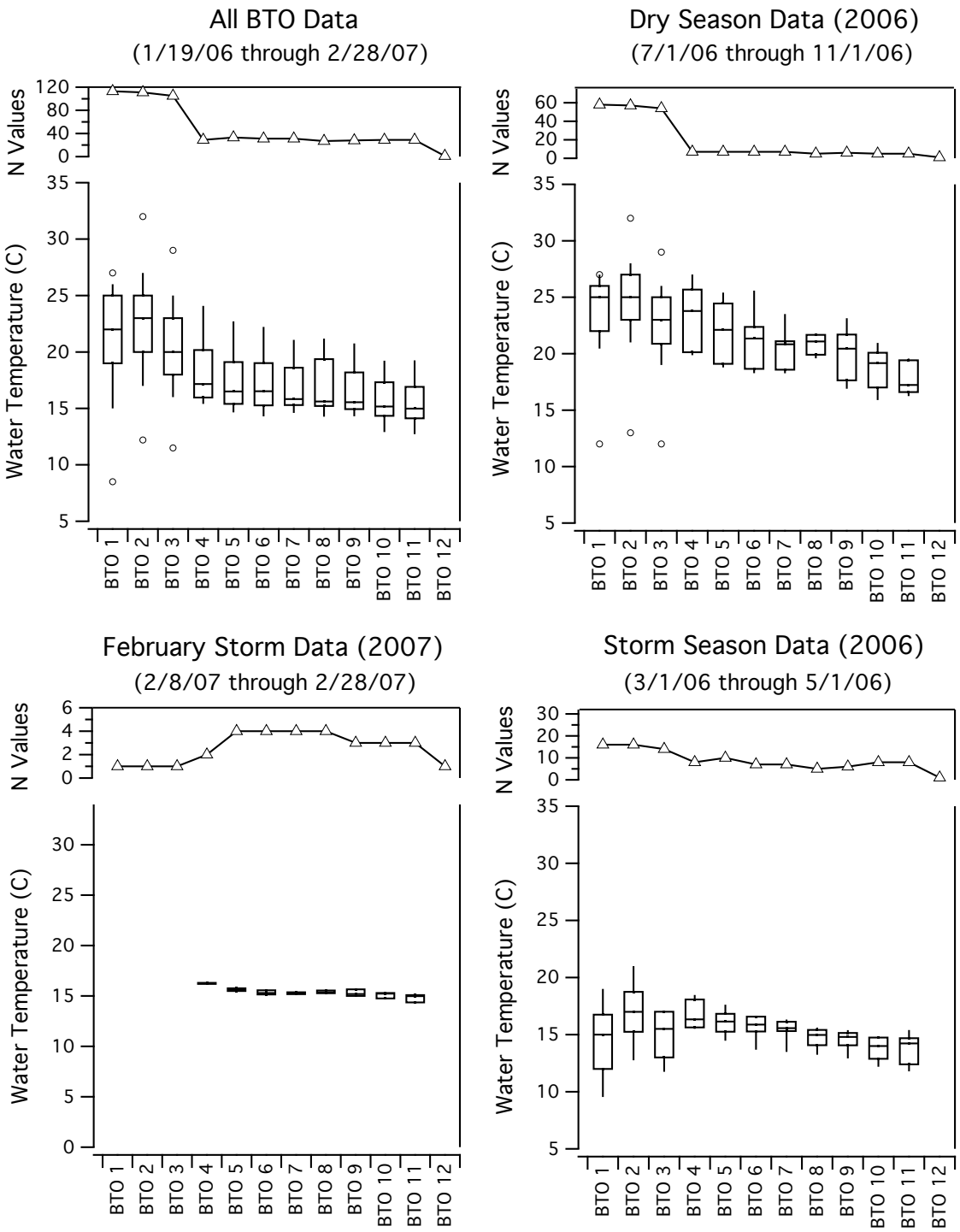

Figure 2-10. Depth-averaged water temperature calculated from CTD casts at within-Bay sites (BTO sites 4-11) and measurements of water temperature with an infrared gun at tributary sites (BTO 1-3). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

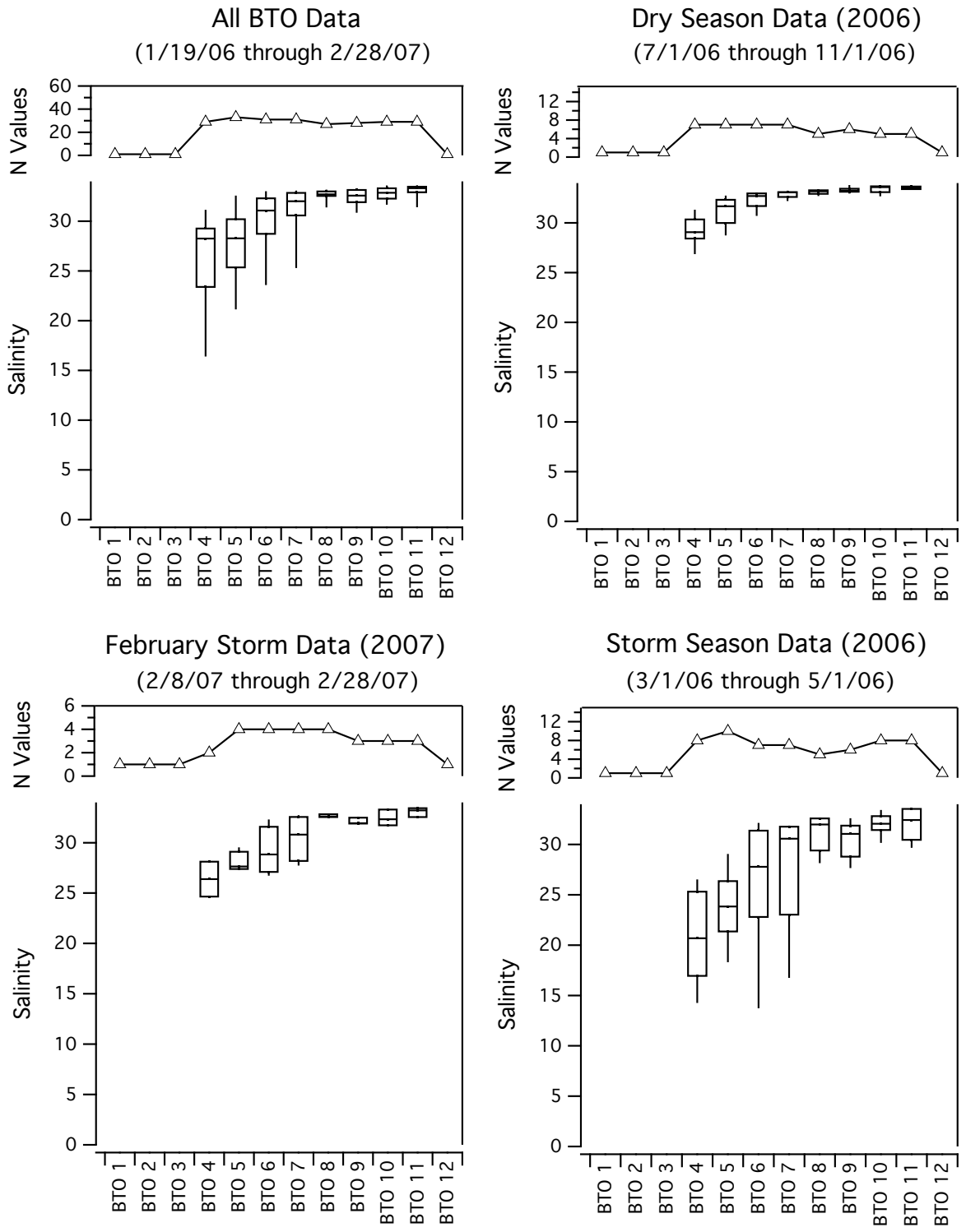

Figure 2-11. Depth-averaged water salinity calculated from CTD casts at within-Bay sites (BTO sites 4-11). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

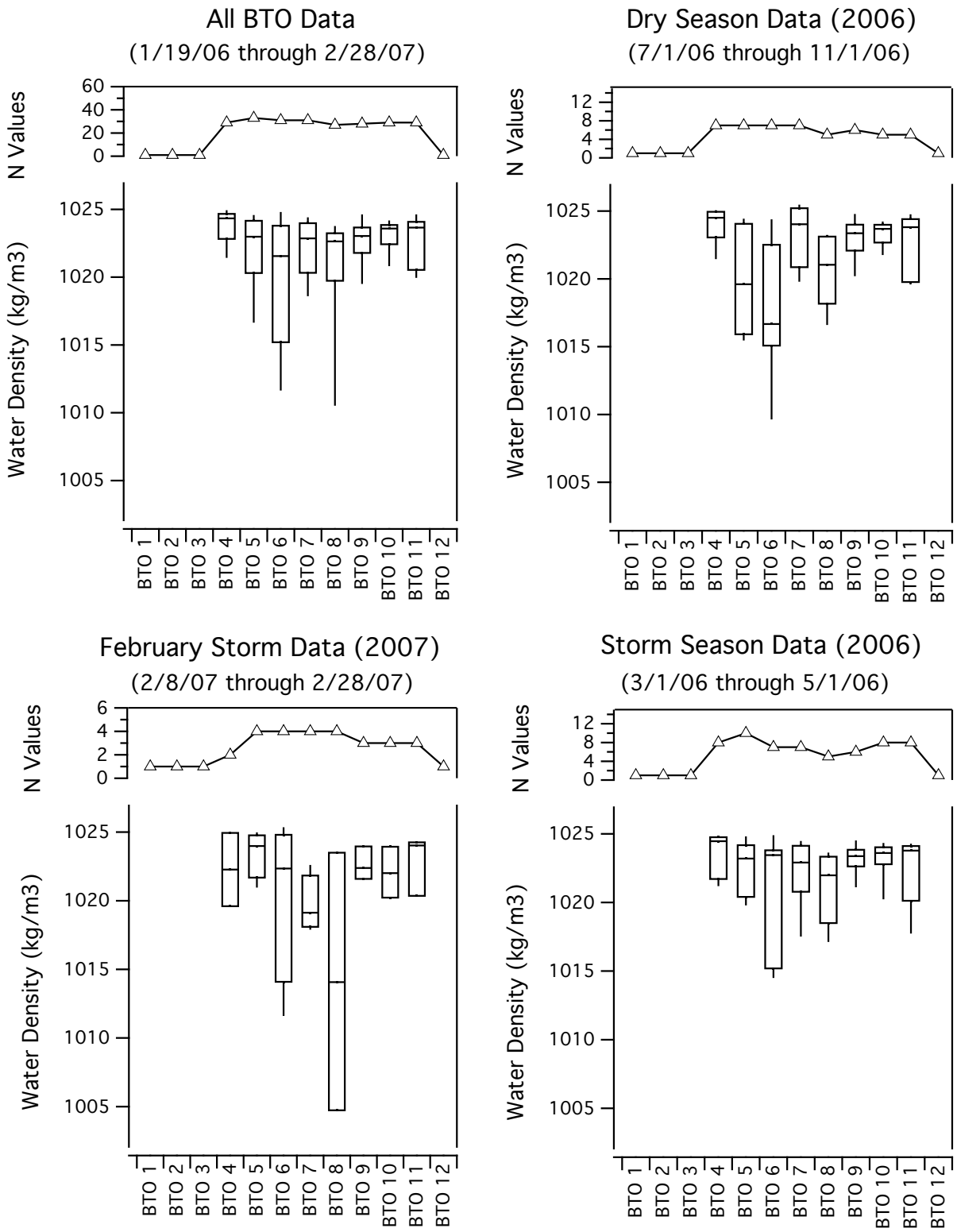

Figure 2-12. Depth-averaged water density calculated from CTD casts at within-Bay sites (BTO sites 4-11). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

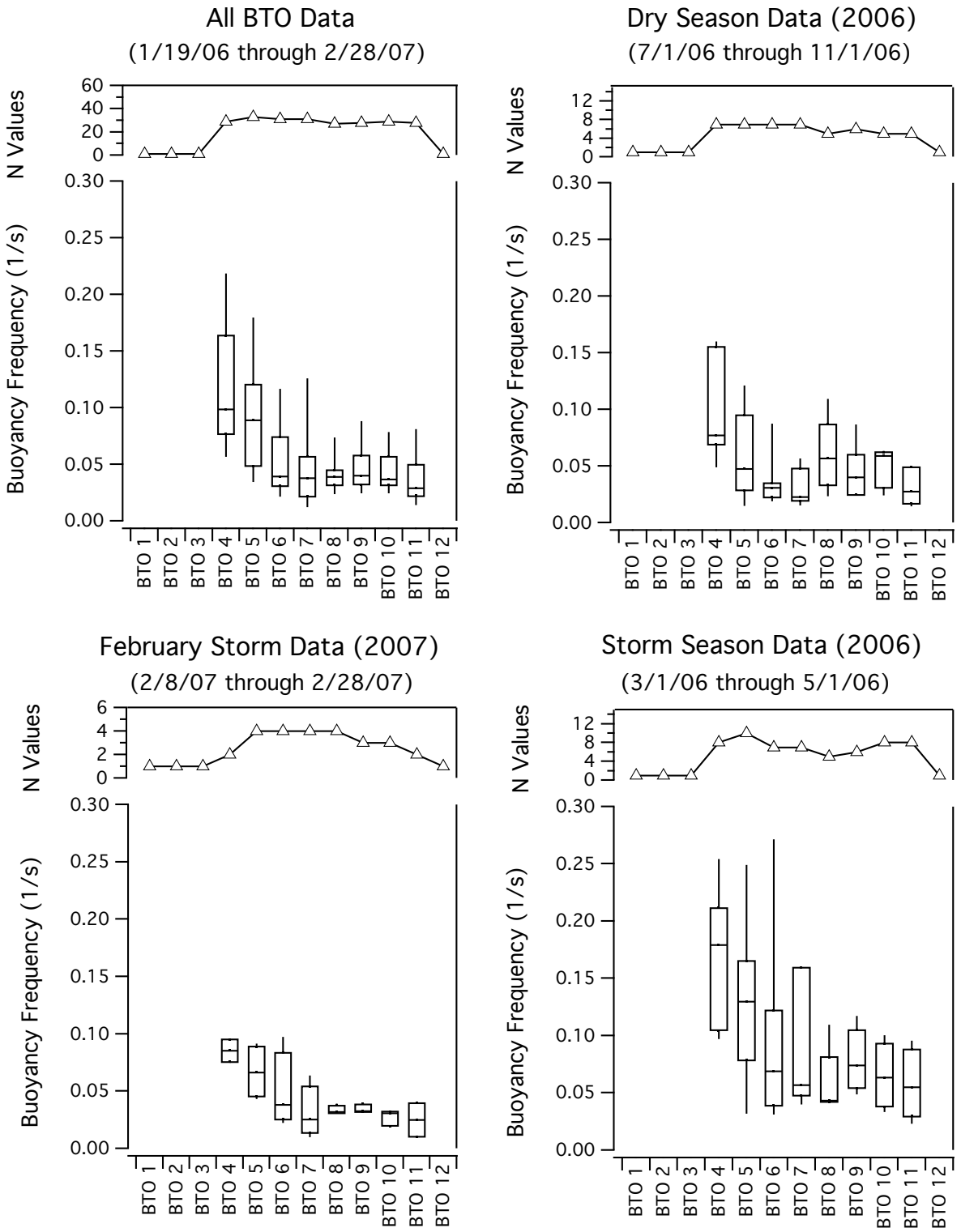

Figure 2-13. Depth-averaged buoyancy frequency calculated from CTD casts at within-Bay sites (BTO sites 4-11). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

panels, Fig. 2-11). The brackish salinities measured in Upper Bay appear to be modulated by storm runoff from San Diego Creek and Santa Ana Delhi channel.

2.3.6.3. Depth-Averaged Water Density

Water densities obtained by depth-averaging CTD density profiles are plotted in Fig. 2-12. Median depth-averaged water density varied from 1005 to 1027 kg/m^3; by way of comparison, the average density of ocean water is 1024 kg/m^3. Water density tends to exhibit more within site variability (particularly at BTO 5-8), than across site variability. Despite the fact Upper Bay is generally warmer and more brackish than Lower Bay (see above), there is not a statistically significant difference in the depth-averaged water density measured in these two regions of the Bay.

2.3.6.4. CTD Casts: Depth-Averaged Buoyancy Frequency

Depth-averaged buoyancy Frequencies are plotted in Fig. 2-13. As noted in the methods section, values of the Buoyancy Frequency $N > 0.2$/s indicate a density stratified water column; values <0.02/s indicate weak density stratification. The highest and most variable Buoyancy Frequencies occur in the upper reaches of Upper Bay, at sites BTO 4 and 5. These two sites are located near the San Diego Creek and Santa Ana Delhi channel outlets and, as will be shown later, are subject to significant temperature and salinity stratification. The highest Buoyancy Frequencies occur in the storm season, reflecting the influence of storm runoff on the vertical structure of the water column in Newport Bay, particularly Upper Bay. During dry weather periods, Buoyancy Frequencies are relatively low across all BTO sites (median values less than 0.1/s). The upper reaches of Upper Bay are density stratified during most of the year, while the rest of the Bay experiences moderate to significant stratification only during storms.

2.3.6.5. CTD Casts: Salinity, Temperature, Density, and Buoyancy Frequency Cross-Sections through Newport Bay

Data from the CTD casts in Newport Bay were used to create cross-sections of salinity, temperature, density, and Buoyancy Frequency. The results, which are presented in Appendix 2B, include 10 cross-sections through the entire Bay (from BTO 4 through BTO 11), 24 cross-sections through Upper Bay (from BTO 4 through BTO 7), and 22 cross-sections through Lower Bay (from BTO 8 through BTO 11). Here we present a few of these cross-sections to provide a first-order assessment of the vertical structure of the Newport Bay water column. A deeper analysis of the full data set will be carried out at a later date.

Salinity and temperature stratification in Newport Bay varies with location and season. During dry weather, base flow from San Diego Creek equals approximately 1% of Upper Newport Bay's tidal prism of 3×10^6 m^3 (see discussion in Chapter 1). This base flow leads to the formation of a relatively warm and low salinity plume at the surface of the

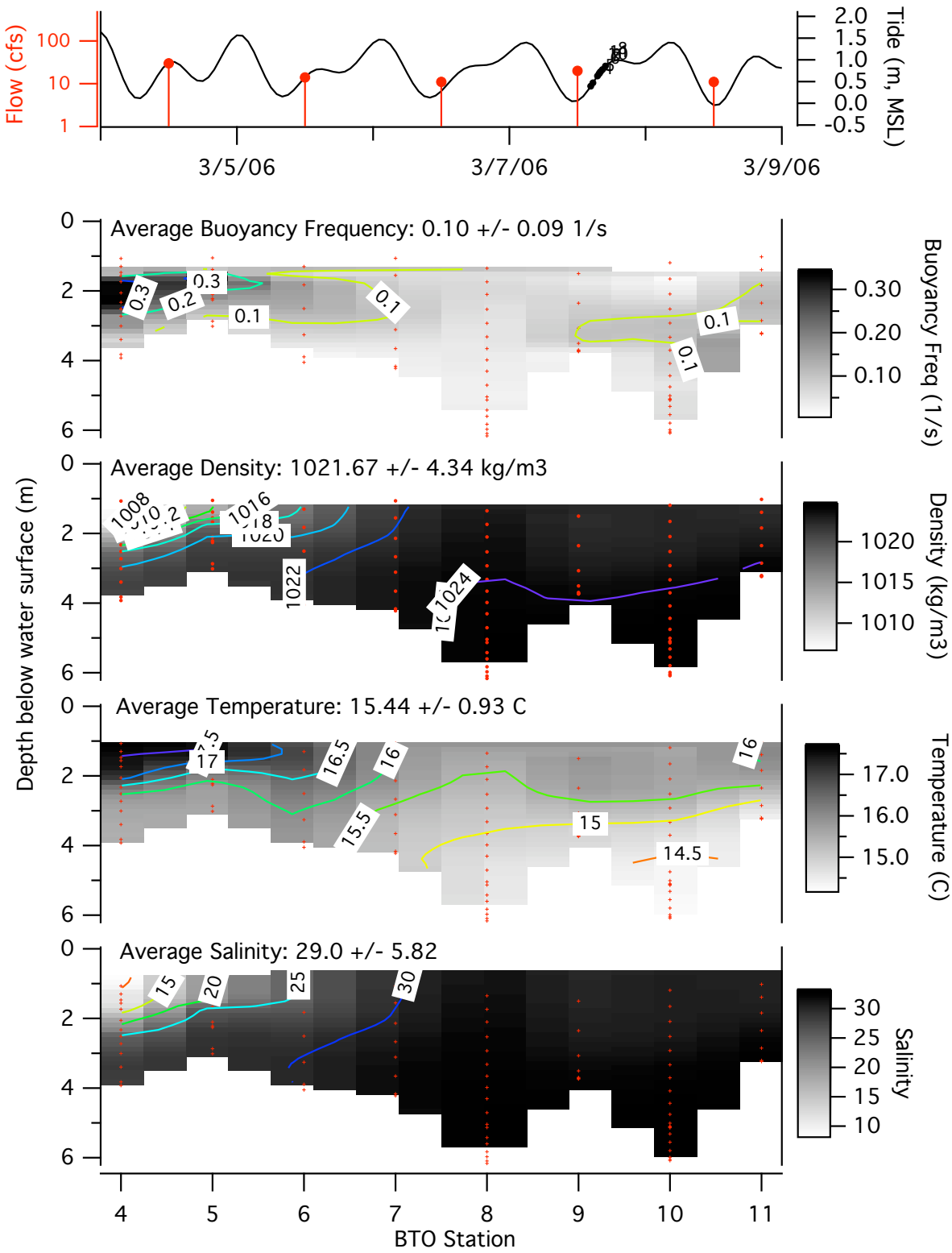

Figure 2-14. Cross-section showing vertical and longitudinal variation in Buoyancy Frequency, Density, Temperature, and Salinity in Newport Bay on 3/7/06. Top panel shows the predicted tide level at the time of sampling (filled circles on tide curve correspond to individual sampling events, and the adjacent numbers indicate the sequence of sampling events). Also shown is the daily-average flow from San Diego Creek (red sticks with balls). Base (dry weather) flow from San Diego Creek is approximately 10 cfs.

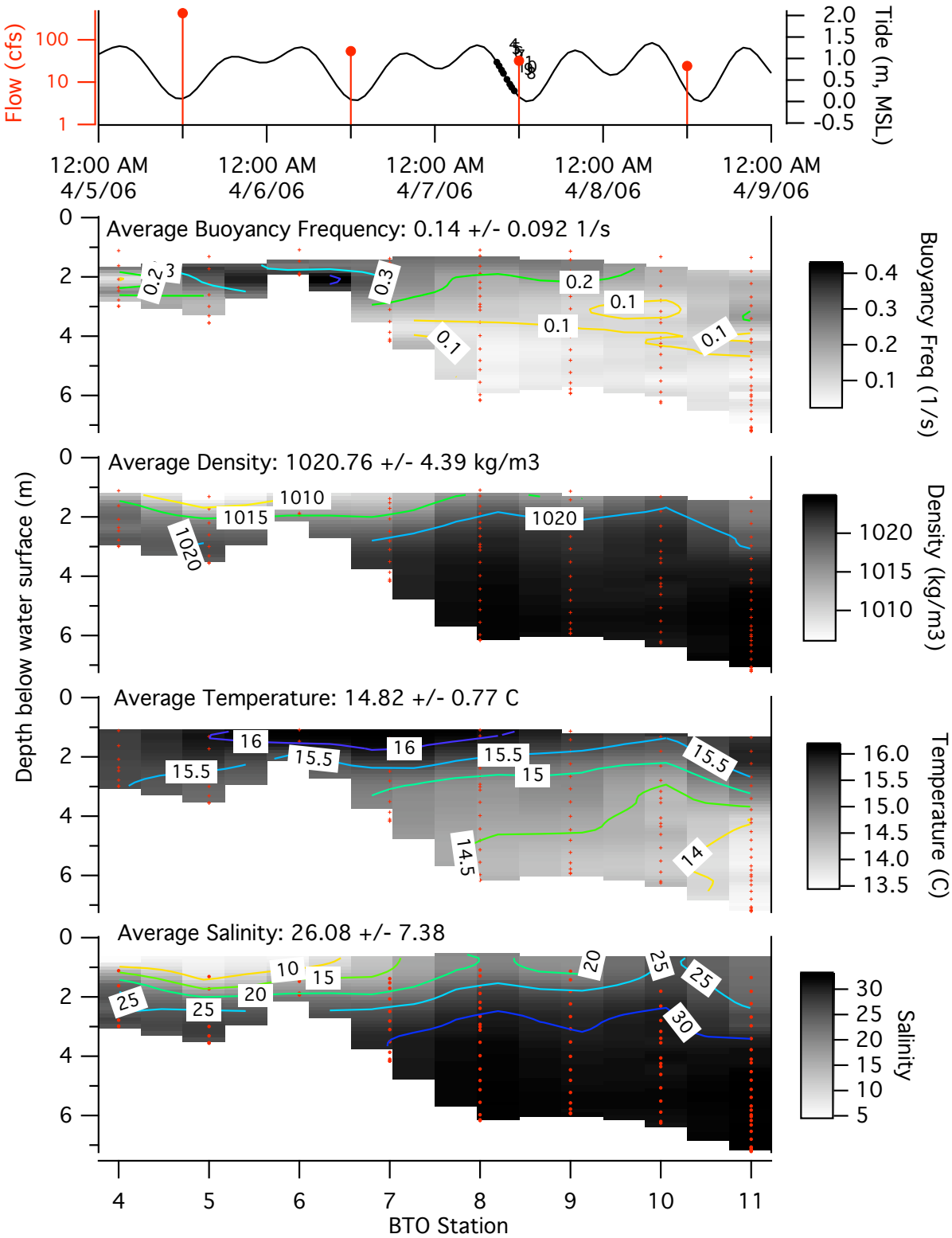

Figure 2-15. Cross-section showing vertical and longitudinal variation in Buoyancy Frequency, Density, Temperature, and Salinity in Newport Bay on 4/7/06. Top panel shows the predicted tide level at the time of sampling (filled circles on tide curve correspond to individual sampling events, and the adjacent numbers indicate the sequence of sampling events). Also shown is the daily-average flow from San Diego Creek (red sticks with balls). Base (dry weather) flow from San Diego Creek is approximately 10 cfs.

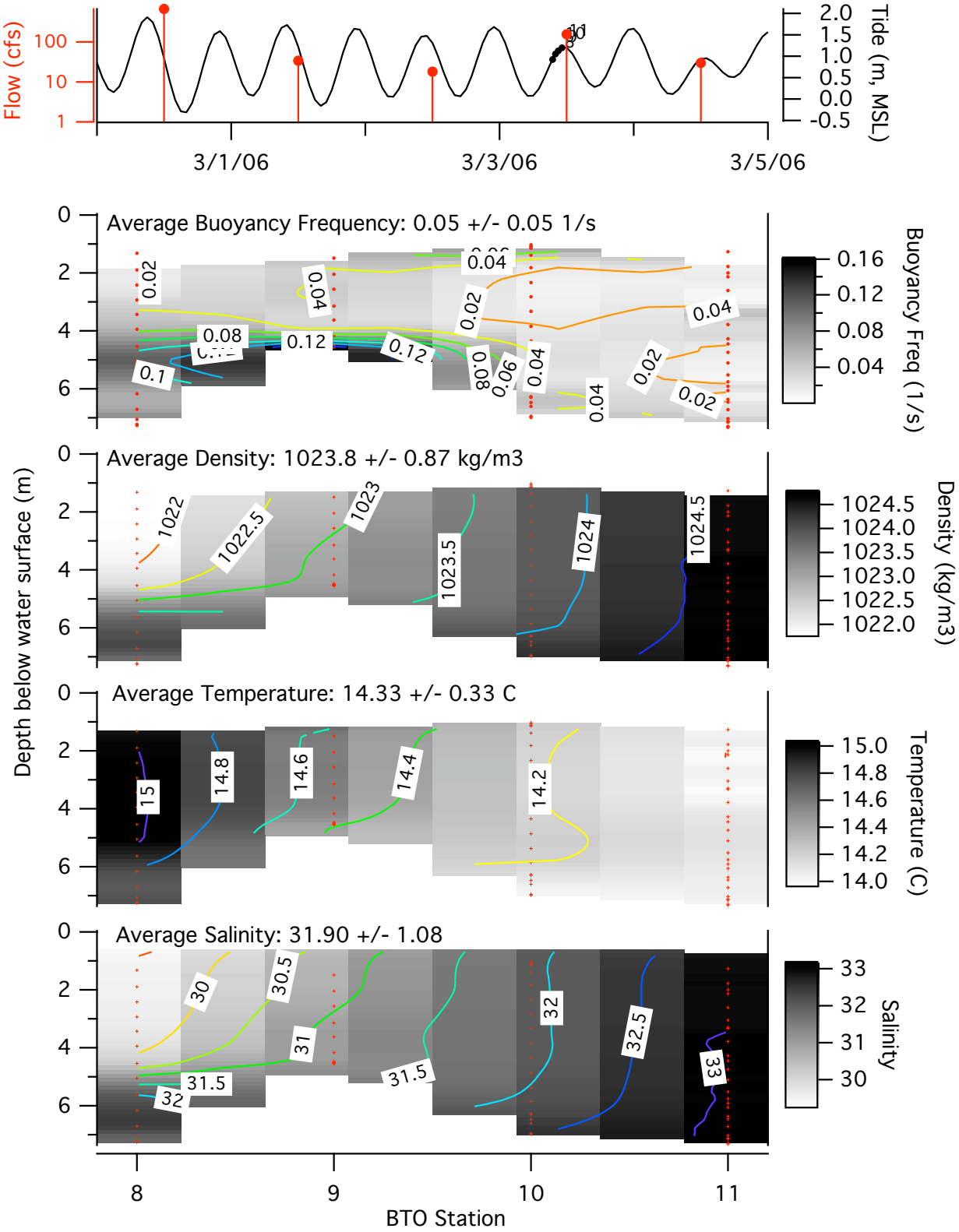

Figure 2-16. Cross-section showing vertical and longitudinal variation in Buoyancy Frequency, Density, Temperature, and Salinity in Lower Newport Bay on 3/3/06. Top panel shows the predicted tide level at the time of sampling (filled circles on tide curve correspond to individual sampling events, and the adjacent numbers indicate the sequence of sampling events). Also shown is the daily-average flow from San Diego Creek (red sticks with balls). Base (dry weather) flow from San Diego Creek is approximately 10 cfs.

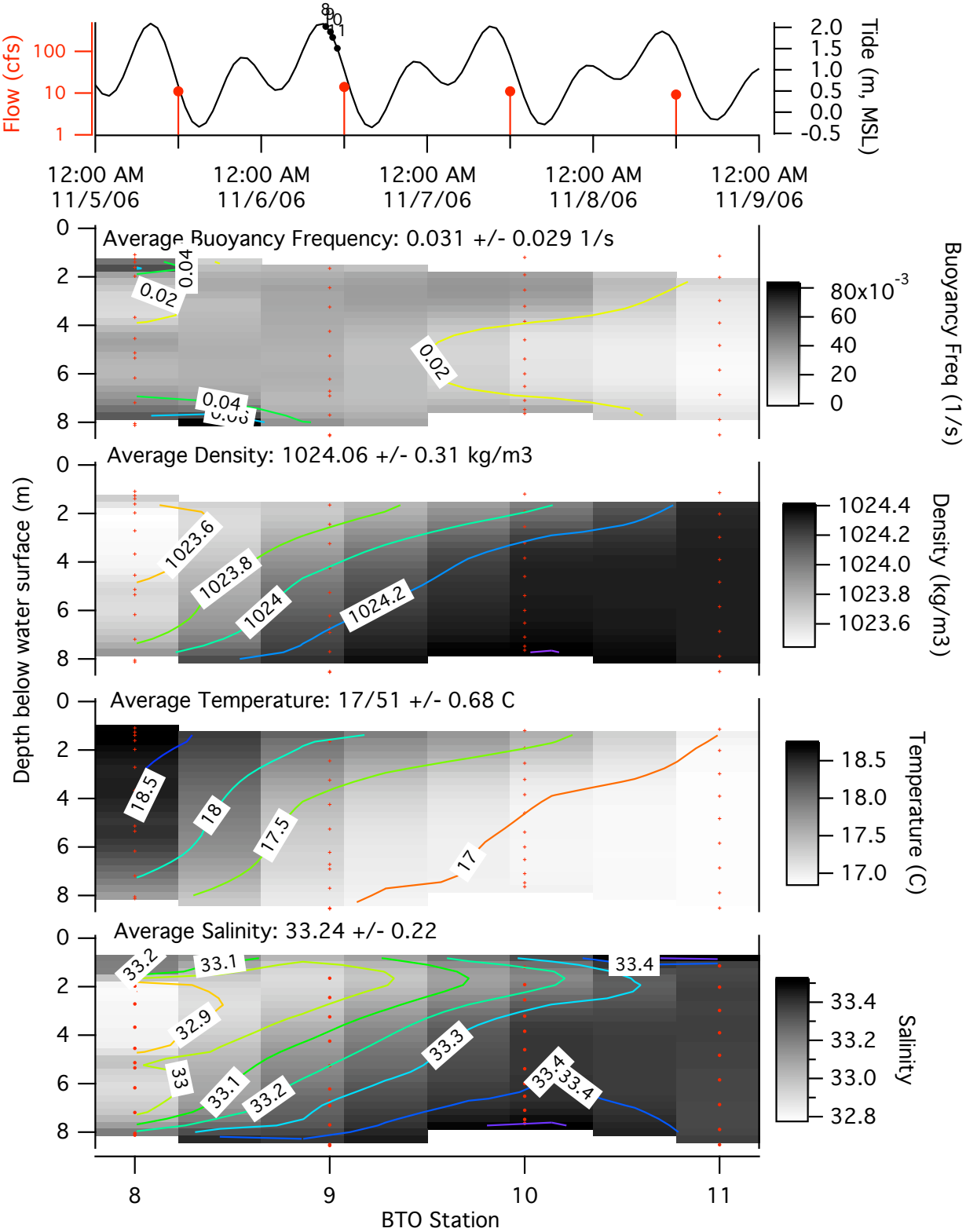

Figure 2-17. Cross-section showing vertical and longitudinal variation in Buoyancy Frequency, Density, Temperature, and Salinity in Lower Newport Bay on 11/6/06. Top panel shows the predicted tide level at the time of sampling (filled circles on tide curve correspond to individual sampling events, and the adjacent numbers indicate the sequence of sampling events). Also shown is the daily-average flow from San Diego Creek (red sticks with balls). Base (dry weather) flow from San Diego Creek is approximately 10 cfs.

water column at sites BTO 4 and 5 that persists most of the year (e.g., cross-sections measured on 3/7/06, Fig. 2-14). The persistent salinity stratification at BTO 4 and 5 contradicts previous assessments that Upper Bay is rarely stratified[8]. During storm events, the volume of runoff entering the Bay from San Diego Creek can be a substantial fraction of Upper Bay's tidal prism; indeed, at the highest flow rates, the volume of runoff discharged from San Diego Creek in a single day approximately equals Upper Bay's tidal prism (see discussion in Chapter 1). Storm water runoff results in the formation of a transient but spatially extensive warm and low salinity plume, which is present at the surface of the water column at most sampling sites in Upper and Lower Bay (e.g., the section measured approximately one day after a large storm on 4/7/06, Fig. 2-15).

On the Lower Bay sections, a brackish plume is evident at BTO 8, either at the surface of the water column (e.g., see section measured during a sequence of storms on 3/3/06, Fig. 2-16) or submerged approximately 1 to 2 m below the surface (e.g., see section measured during dry weather on a high tide on 11/06/06, Fig. 2-17). The brackish plume at BTO 8 may be caused by discharge of runoff from Arches Drain, which is located near BTO 8. Arches Drain is submerged below the Bay's water surface during high tides[9], which may account for the fact that the brackish plume near BTO 8 is sometimes submerged below the water surface, particularly when the tide is high (Fig. 2-17). Interpretation of these Lower Bay sections is complicated by the fact that ebb flow from Upper Bay flows into the sections around BTO 9.

2.3.7. Water Sample Analyses: Down Bay and Seasonal Trends

2.3.7.1. FIB Concentrations

The next two tables summarize statistical data on the log-transformed concentration of *E. coli* (EC, Table 2-5) and enterococci bacteria (ENT, Table 2-6) measuring during the BTO studies.[10,11] These tables document a significant down-Bay decrease in the log-mean

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[8] U.S. Army Corps of Engineers. 1993. Reconnaissance Report Upper Newport Bay Orange County, California. Numerical modeling of hydrodynamics and transport, February 1993. Los Angeles District, California.

[9] Personal communication, Robert Stein, City of Newport Bay

[10] To generate the average and standard deviations reported in Tables 2-5 and 2-6 the following analysis was carried out. All measurements performed on samples collected at a particular site and during one of the four periods of time (All Data, Dry Season 2006, Storm Season 2006, February Storm Data 2007) were pooled, and the following statistics were calculated: (1) mean and standard deviation of the log-transformed FIB concentrations, (2) the percent of samples that had bacterial concentrations above the California marine bathing water single-sample or geometric mean standards. Note that a log-mean is reported in the tables, instead of an arithmetic mean, because FIB monitoring data collected in Orange County (and elsewhere) are approximately log-normally distributed. It is, of course, possible that the FIB data collected during the BTO study do not conform to a log-normal distribution (in which case log-means would also be an invalid indicator of central tendency).

[11] Over the nine-month BTO study, the total number of samples was relatively similar from site-to-site (n=192 to 220, depending on the site), with the exception of BTO 12 where relatively few samples (n=34) were collected due to logistical constraints associated with sampling offshore. The tributary sites (BTO 1-3) were sampled on a near-daily basis, but only one sample from the middle of the channel was collected during a sampling event. The within-Bay sites, on the other hand, were sampled less frequently

Table 2-5. Summary statistics for E. coli measurements on water samples collected from BTO sampling sites in Newport Bay.

Sampling Site	***Study Period[1]***	***N***	***Log-mean (std)[2]***	***Samples above SSS[3]***	***Samples above GM[4]***	***Minimum (MPN/100 mL)[5]***	***Maximum (MPN/100 mL)[6]***
BTO 1	All Data	220	2.0 (0.94)	46 (21%)	65 (29%)	1	24192
	2006 Dry Season	88	1.9 (0.91)	13 (15%)	20 (23%)	1	24192
	2006 Wet Season	51	2.5 (0.80)	20 (39%)	26 (51%)	20	20000
	2007 Feb. Storm	9	2.7 (0.87)	5 (56%)	5 (56%)	41	4600
BTO 2	All Data	220	2.5 (0.74)	91 (41%)	135 (61%)	1	24192
	2006 Dry Season	87	2.8 (0.48)	56 (64%)	78 (90%)	63	24192
	2006 Wet Season	51	2.1 (0.81)	9 (18%)	17 (33%)	10	24192
	2007 Feb. Storm	9	2.6 (0.70)	5 (56%)	6 (67%)	30	2900
BTO 3	All Data	217	2.1 (0.55)	34 (16%)	66 (30%)	1	14000
	2006 Dry Season	83	2.0 (0.49)	7 (8%)	17 (20%)	1	9800
	2006 Wet Season	50	2.3 (0.55)	12 (24%)	22 (44%)	20	4900
	2007 Feb. Storm	9	2.8 (0.21)	6 (67%)	9 (100%)	292	1000
BTO 4	All Data	192	1.7 (1.1)	36 (19%)	48 (25%)	1	24192
	2006 Dry Season	55	1.6 (0.70)	2 (6%)	8 (15%)	1	960
	2006 Wet Season	35	2.2 (1.4)	17 (49%)	18 (51%)	1	24000
	2007 Feb. Storm	44	1.9 (1.2)	12 (27%)	16 (36%)	1	6100
BTO 5	All Data	194	1.8 (1.0)	34 (18%)	47 (24%)	1	24192
	2006 Dry Season	55	1.7 (0.54)	1 (2%)	7 (13%)	1	540
	2006 Wet Season	35	2.0 (1.4)	13 (37%)	16 (46%)	1	24000
	2007 Feb. Storm	45	2.1 (0.9)	14 (31%)	18 (40%)	1	3900
BTO 6	All Data	195	1.4 (0.98)	22 (11%)	32 (16%)	1	9800
	2006 Dry Season	55	1.6 (0.69)	2 (4%)	6 (11%)	1	760
	2006 Wet Season	36	1.7 (1.1)	11 (31%)	11 (31%)	1	3400
	2007 Feb. Storm	44	1.6 (0.9)	4 (9%)	10 (23%)	1	1900
BTO 7	All Data	202	1.2 (0.98)	14 (7%)	25 (12%)	1	4100
	2006 Dry Season	54	1.6 (0.60)	1 (2%)	5 (9%)	1	1200
	2006 Wet Season	37	1.4 (1.1)	4 (11%)	9 (24%)	1	2500
	2007 Feb. Storm	44	1.3 (1.0)	4 (9%)	6 (14%)	1	1200
BTO 8	All Data	209	1.1 (0.95)	12 (6%)	19 (9%)	1	4900
	2006 Dry Season	54	1.7 (0.61)	4 (7%)	8 (15%)	1	4900
	2006 Wet Season	41	1.0 (1.0)	4 (10%)	5 (12%)	1	2400
	2007 Feb. Storm	45	0.62 (0.7)	0 (0%)	0 (0%)	1	160
BTO 9	All Data	208	0.95 (0.9)	10 (5%)	15 (7%)	1	2100
	2006 Dry Season	54	1.6 (0.59)	2 (4%)	3 (6%)	1	1300
	2006 Wet Season	42	0.77 (1.0)	3 (7%)	4 (10%)	1	670
	2007 Feb. Storm	44	0.90 (0.8)	1 (2%)	3 (7%)	1	400
BTO 10	All Data	208	0.7 (0.8)	4 (2%)	9 (4%)	1	810
	2006 Dry Season	55	1.1 (0.61)	0 (0%)	0 (0%)	1	110
	2006 Wet Season	42	0.57 (0.9)	1 (2%)	5 (12%)	1	810
	2007 Feb. Storm	43	0.56 (0.8)	0 (0%)	1 (2%)	1	260
BTO 11	All Data	206	0.4 (0.64)	0 (0%)	3 (1%)	1	300
	2006 Dry Season	55	0.56 (0.6)	0 (0%)	0 (0%)	1	63
	2006 Wet Season	40	0.39 (0.3)	0 (0%)	1 (3%)	1	300
	2007 Feb. Storm	44	0.25 (0.5)	0 (0%)	0 (0%)	1	20
BTO 12	All Data	34	0.02 (0.1)	0 (0%)	0 (0%)	1	6
	2006 Dry Season	9	0.08 (0.2)	0 (0%)	0 (0%)	1	6
	2006 Wet Season	6	0 (0)	0 (0%)	0 (0%)	1	1
	2007 Feb. Storm	6	0 (0)	0 (0%)	0 (0%)	1	1

(approximately once weekly), but five samples were collected from the cross-section during a sampling event (two from each of the banks, and three from the channel thalweg).

[1]Denotes samples collected over the following time intervals. All Data: 1/19/06 through 2/28/07; 2006 Dry Season: 7/1/06 through 11/1/06; 2006 Wet Season: 3/1/06 through 5/1/06; 2007 Feb. Storm: 2/8/07 through 2/28/07.
[2]Mean and standard deviation of the log-transformed *E. coli* concentration, in units of MPN/100 mL. Note that censored data were included in the mean and standard deviation calculations (see definition of censored data in footnotes 5 and 6).
[3]Number of samples (and percentage of samples) that had *E. coli* concentrations in excess of the AB411 single-sample standard for marine bathing waters for fecal coliform of 400 MPN/100 mL.
[4]Number of samples (and percentage of samples) that had *E. coli* concentrations in excess of the AB411 geometric mean standard for marine bathing waters of 200 MPN/100 mL
[5]Minimum concentration of *E. coli* measured in samples collected from a particular site over a particular time. Note that the number "1 MPN/100 mL" was used as the censored value for samples whose *E. coli* concentration fell below the detection limit of 10 MPN/100 mL.
[6]Maximum concentration of *E. coli* concentration measured in samples collected from a particular site over a particular time. Note that the number "24,192 MPN/100 mL" was used as the censored value for samples whose *E. coli* concentration fell above the detection limit of 24,192 MPN/100 mL.

Table 2-6. Summary statistics for Enterococci bacteria measurements on water samples collected from BTO sampling sites in Newport Bay.

Sampling Site	***Study Period[1]***	***N***	***Log-mean (std)[2]***	***Samples above SSS[3]***	***Samples above GM[4]***	***Minimum (MPN/100 mL)[5]***	***Maximum (MPN/100 mL)[6]***
BTO 1	All Data	217	2.31 (0.75)	149 (69%)	189 (87%)	1	24192
	2006 Dry Season	85	2.47 (0.67)	72 (85%)	80 (94%)	1	24192
	2006 Wet Season	50	2.29 (0.93)	28 (56%)	41 (82%)	1	24192
	2007 Feb. Storm	9	2.78 (0.91)	6 (67%)	8 (89%)	31	24192
BTO 2	All Data	213	3.19 (0.99)	183 (86%)	201 (94%)	10	24192
	2006 Dry Season	84	4.00 (0.56)	82 (98%)	84 (100%)	74	24192
	2006 Wet Season	50	2.41 (0.74)	35 (70%)	44 (88%)	10	13000
	2007 Feb. Storm	9	3.37 (0.96)	8 (89%)	8 (89%)	31	24192
BTO 3	All Data	210	1.94 (0.70)	101 (48%)	164 (78%)	1	20000
	2006 Dry Season	80	2.10 (0.60)	44 (55%)	72 (90%)	1	20000
	2006 Wet Season	49	1.80 (0.75)	16 (33%)	34 (69%)	1	2300
	2007 Feb. Storm	9	2.40 (0.48)	7 (78%)	9 (100%)	75	3000
BTO 4	All Data	193	1.84 (1.25)	73 (38%)	107 (55%)	1	24192
	2006 Dry Season	55	1.63 (0.76)	14 (25%)	35 (64%)	1	4900
	2006 Wet Season	35	2.40 (1.45)	19 (54%)	22 (63%)	1	24192
	2007 Feb. Storm	45	2.47 (1.30)	31 (69%)	32 (71%)	1	24192
BTO 5	All Data	194	1.59 (1.33)	59 (30%)	82 (42%)	1	24192
	2006 Dry Season	55	1.33 (0.92)	10 (18%)	19 (35%)	1	3400
	2006 Wet Season	35	2.02 (1.46)	16 (46%)	18 (51%)	1	24192
	2007 Feb. Storm	45	2.34 (1.42)	24 (53%)	31 (69%)	1	24192
BTO 6	All Data	195	1.08 (1.27)	34 (17%)	53 (27%)	1	24192
	2006 Dry Season	55	0.63 (0.68)	1 (1.8%)	5 (9.1%)	1	180
	2006 Wet Season	36	1.50 (1.37)	13 (36%)	19 (53%)	1	20000
	2007 Feb. Storm	44	1.70 (1.55)	15 (34%)	21 (48%)	1	24192
BTO 7	All Data	204	0.97 (1.12)	31 (15%)	48 (23%)	1	20000
	2006 Dry Season	55	0.86 (0.66)	1 (1.8%)	9 (16%)	1	110
	2006 Wet Season	37	1.32 (1.16)	10 (27%)	16 (43%)	1	7700
	2007 Feb. Storm	45	1.31 (1.40)	15 (33%)	16 (36%)	1	20000
BTO 8	All Data	208	0.79 (0.93)	25 (12%)	43 (21%)	1	20000
	2006 Dry Season	54	1.02 (0.94)	10 (19%)	13 (24%)	1	4600
	2006 Wet Season	41	0.79 (0.92)	5 (12%)	10 (24%)	1	1700
	2007 Feb. Storm	45	0.72 (0.90)	5 (11%)	7 (16%)	1	2200
BTO 9	All Data	208	0.67 (0.87)	16 (7.7%)	28 (13%)	1	3900
	2006 Dry Season	54	0.75 (0.68)	3 (5.6%)	6 (11%)	1	630
	2006 Wet Season	42	0.73 (1.02)	5 (12%)	9 (21%)	1	2900
	2007 Feb. Storm	44	0.89 (1.11)	6 (14%)	9 (20%)	1	3900
BTO 10	All Data	209	0.50 (0.80)	12 (5.7%)	19 (9.1%)	1	3900
	2006 Dry Season	55	0.36 (0.65)	1 (1.8%)	2 (3.6%)	1	3900
	2006 Wet Season	42	0.53 (0.92)	5 (12%)	6 (14%)	1	1000
	2007 Feb. Storm	44	0.78 (1.08)	6 (14%)	9 (20%)	1	2100
BTO 11	All Data	206	0.25 (0.56)	6 (2.9%)	8 (3.9%)	1	600
	2006 Dry Season	55	0.15 (0.47)	1 (1.8%)	2 (3.6%)	1	290
	2006 Wet Season	40	0.42 (0.80)	5 (13%)	5 (12%)	1	600
	2007 Feb. Storm	44	0.35 (0.55)	0 (0%)	0 (0%)	1	31
BTO 12	All Data	34	0.03 (0.17)	0 (0%)	0 (0%)	1	10
	2006 Dry Season	9	0 (0)	0 (0%)	0 (0%)	1	1
	2006 Wet Season	6	0 (0)	0 (0%)	0 (0%)	1	1
	2007 Feb. Storm	6	0 (0)	0 (0%)	0 (0%)	1	1

[1]Denotes samples collected over the following time intervals. All Data: 1/19/06 through 2/28/07; 2006 Dry Season: 7/1/06 through 11/1/06; 2006 Wet Season: 3/1/06 through 5/1/06; 2007 Feb. Storm: 2/8/07 through 2/28/07.
[2]Mean and standard deviation of the log-transformed Enterococcus bacteria concentration, in units of MPN/100 mL. Note that censored data were included in the mean and standard deviation calculations (see definition of censored data in footnotes 5 and 6).
[3]Number of samples (and percentage of samples) that had enterococci bacteria concentrations in excess of the AB411 single-sample standard for marine bathing waters of 104 MPN/100 mL.
[4]Number of samples (and percentage of samples) that had enterococci bacteria concentrations in excess of the AB411 geometric mean standard for marine bathing waters of 35 MPN/100 mL
[5]Minimum concentration of enterococci bacteria measured in samples collected from a particular site over a particular time. Note that the number "1 MPN/100 mL" was used as the censored value for samples whose enterococci concentration fell below the detection limit of 10 MPN/100 mL.
[6]Maximum concentration of enterococci bacteria measured in samples collected from a particular site over a particular time. Note that the number "24,192 MPN/100 mL" was used as the censored value for samples whose enterococci concentration fell above the detection limit of 24,192 MPN/100 mL.

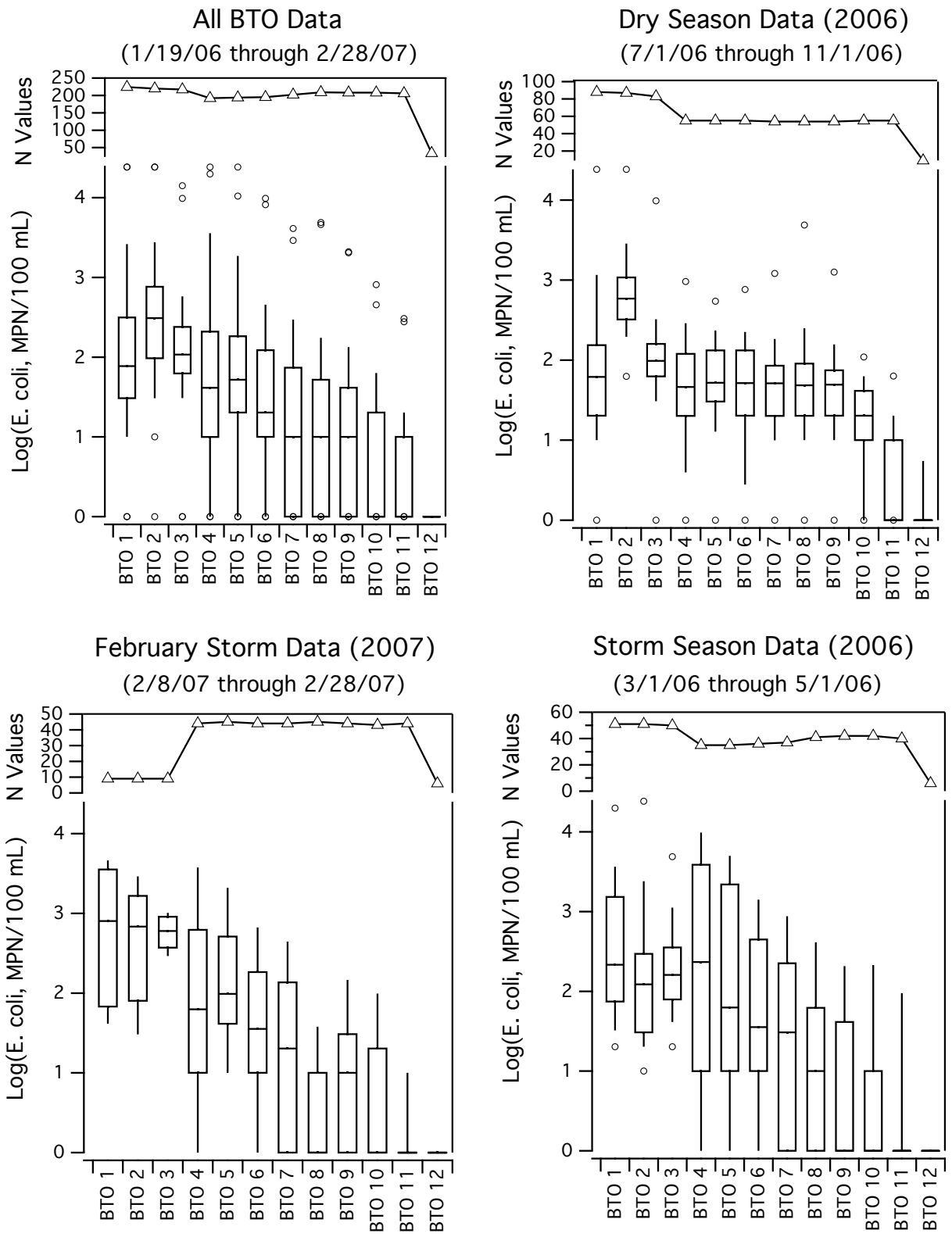

Figure 2-18. *E. coli* concentrations measured in water samples collected from tributary (BTO 1-3), within-Bay sites (BTO sites 4-11), and the offshore control site (BTO 12). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

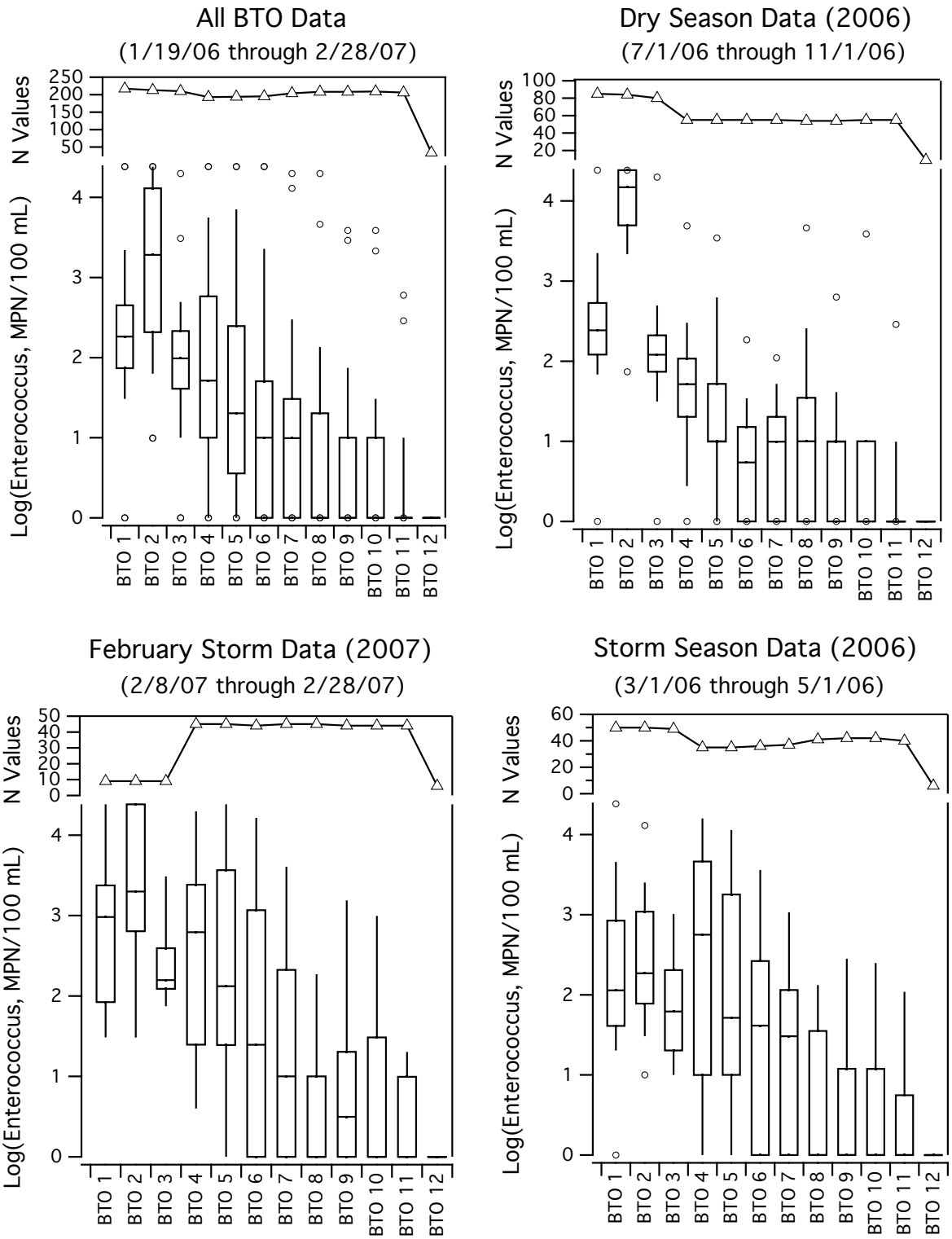

Figure 2-19. Enterococci bacteria concentrations measured in water samples collected from tributary (BTO 1-3), within-Bay sites (BTO sites 4-11), and the offshore control site (BTO 12). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

concentration of FIB and percent of samples exceeding single-sample and geometric mean standards. Tributary sites have the highest log-means and exceedence rates, followed by Upper Bay (BTO 4 through 7), followed by Lower Bay (BTO 9-11), followed by the offshore control site (BTO 12). At the offshore control site all but one sample had FIB concentrations below the lower limit of detection of 10 MPN/100 mL.

Seasonal FIB patterns are often site specific. In the Santa Ana Delhi channel (BTO 2) and near the outlet of Arches Drain In West Newport Bay (BTO 8) the median concentrations of EC and ENT are 10 to 100 times higher during dry weather compared to wet weather (compare upper right and lower right panels, Figs. 2-18 and 2-19). This seasonal pattern was also observed for total coliform measurements in West Newport Bay, based on an analysis of >20 years of shoreline monitoring data[12]. At most other within-Bay sites (BTO 4-7 and 9-11), FIB concentrations are higher during wet weather and lower during dry weather, consistent with the expected impact that storm water runoff has on Newport Bay water quality (compare upper and lower right panels, Figs. 2-18 and 2-19).

2.3.7.2. Salinity: Down Bay and Seasonal Trends

Water salinities measured in grab samples collected from Newport Bay and its tributaries are plotted in Fig. 2-20. The salinity data plotted in Fig. 2-20 are separate from the data presented in Fig. 2-10 in that the latter are calculated from depth-averaging CTD measurements (see Section 2.3.6.2), while the former are obtained by measuring the salinity of water in grab samples collected from each BTO site. Compared to the CTD data plotted in Fig. 2-10, salinity measured in grab samples is frequently more variable and has lower median values, particularly at Upper Bay sites. This is not surprising, because three of the five grab samples collected at each BTO site are taken from the surface of the water column (practically, the upper 5 cm), where a thin lens of freshwater is often present, particularly near the tributary outlets (i.e., BTO 4 and 5).

The salinity data presented in Fig. 2-20 provides a relatively complete picture of the salinity distribution across the entire Newport Bay-tributary system. As expected, salinity measured on samples from the tributaries tends to be very low (<2), with the exception of BTO 2 (Santa Ana Delhi channel) where salinities range from 0 to 5 during the wet season, and from 0 to 10 during the dry season (with one or two samples reaching salinities of 17, Fig. 2-20). The higher salinities measured in the Santa Ana Delhi channel indicates that this sampling site is occasionally under tidal influence, as explained in more detail in Chapter 8. Salinities measured in Upper Bay are generally intermediate between the low salinities measured in the tributaries and the high salinities measured in Lower Bay. Salinities are generally lower, as reflected by both median and extreme values, during wet weather, and higher during dry weather.

2.3.7.3. pH: Down Bay and Seasonal Trends

[12] Pednekar, A.M., Grant, S.B., Jeong, Y., Poon, Y., Oancea, C. (2005) "Influence of climate change, tidal mixing and watershed urbanization on historical water quality in Newport Bay, a saltwater wetland and tidal embayment in southern California" *Environmental Science and Technology* **39**:9071-9082.

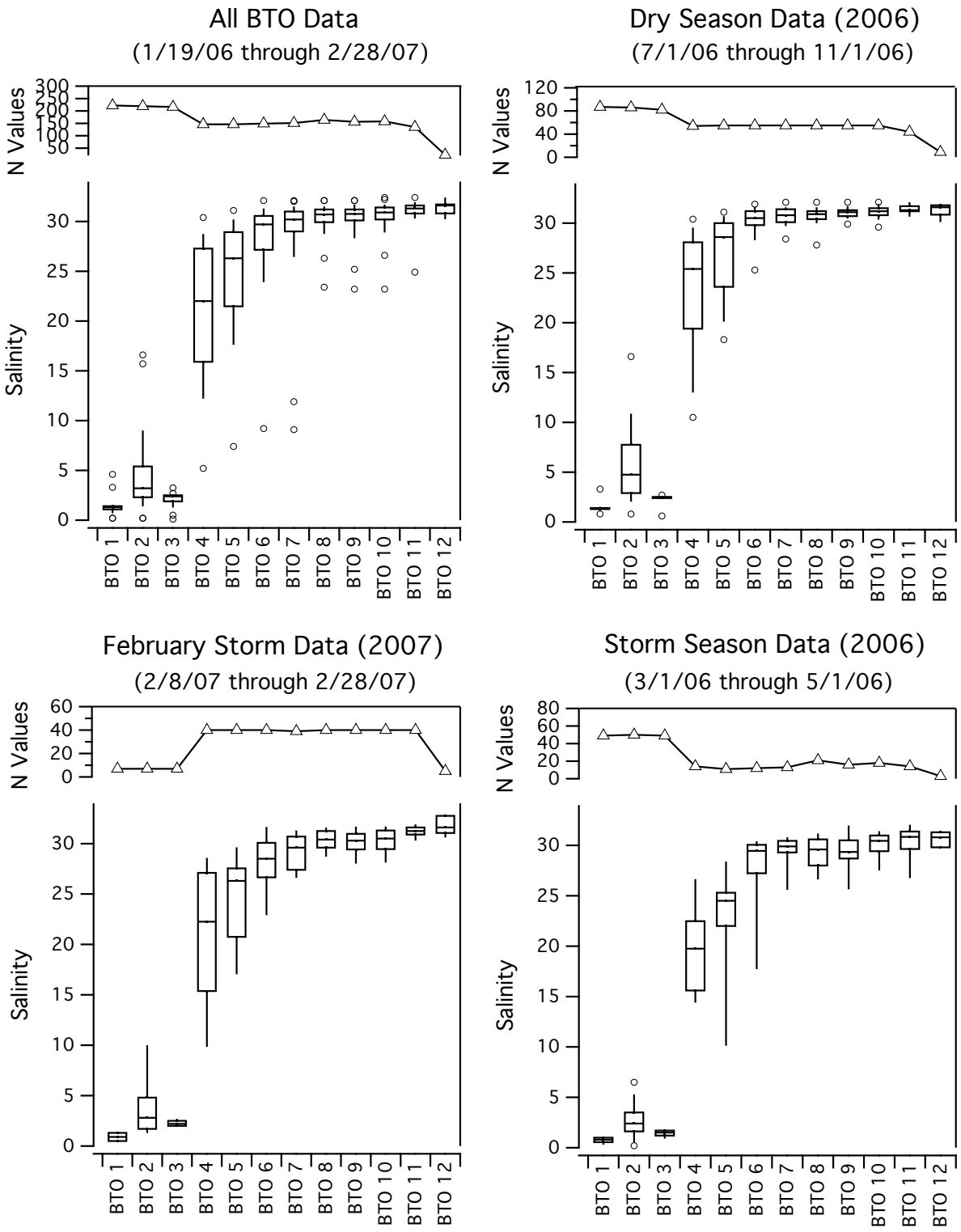

Figure 2-20. Salinity measured in water samples collected from tributary (BTO 1-3), within-Bay sites (BTO sites 4-11), and the offshore control site (BTO 12). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

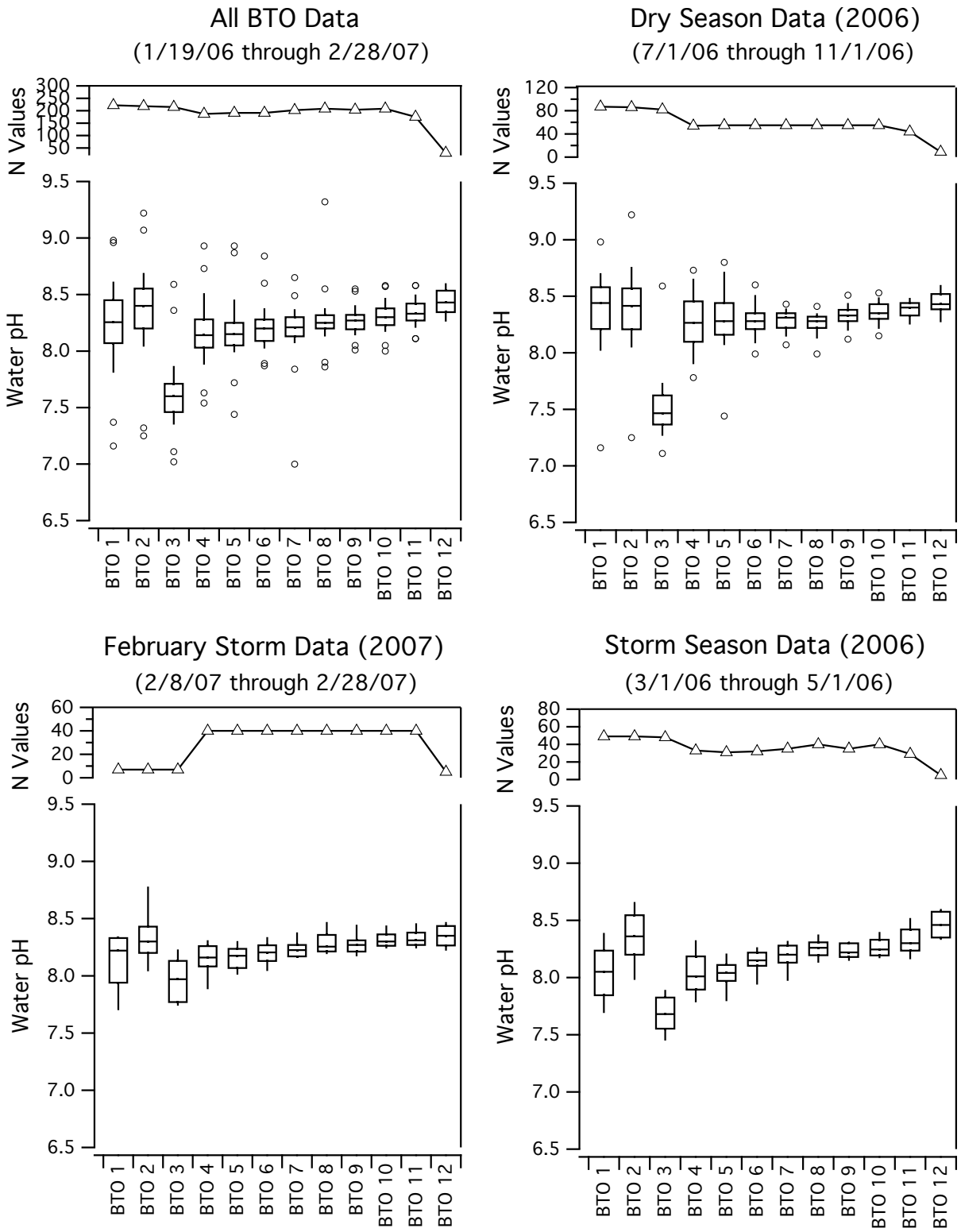

Figure 2-21. pH measured in water samples collected from tributary (BTO 1-3), within-Bay sites (BTO sites 4-11), and the offshore control site (BTO 12). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

The pH of water samples collected from Newport Bay and its tributaries is generally confined to a fairly narrow range of values, between 7 and 9 (Fig. 2-21). Among the tributary sites, water collected from San Diego Creek and Santa Ana Delhi channel tend to be more alkaline (median pH between 8 and 8.5), while water collected from Big Canyon is closer to neutral (median pH between 7 and 7.5). Within the Bay, there is a subtle but progressive increase in pH along the transect, from a median of around 8 at BTO 4, to a median of around 8.5 at the offshore control site (BTO 12). The pH of the Bay is also slightly lower (by about 0.5 pH units) during the wet season compared to dry season. These variations in pH could originate from many different processes, including the mixing of different water parcels (e.g., creek water with ocean water), generation or consumption of acidity associated with microbial metabolism in the marsh sediments, and changes in pH of the coastal ocean, to name a few.

2.3.7.4. Nitrate, Ammonia, Total Organic Carbon (TOC), and Total Phosphorous

Approximately 80 water samples collected from Newport Bay and its tributaries were assayed for a suite of nutrients, including nitrate, ammonia, total organic carbon (TOC), and total phosphorous. The small sample size of this effort precludes a meaningful separation of data into wet and dry weather periods, and thus only the combined data set is presented here (Fig. 2-22).

Nitrate concentrations exhibit a significant down-Bay trend (upper left panel, Fig. 2-22). Among the tributary sites, San Diego Creek has the highest nitrate concentration (median concentration of 32 mg/L), followed by Santa Ana Delhi Channel (median of 16 mg/L), and Big Canyon Wash (<0.1 mg/L). The low nitrate concentrations in Big Canyon Wash may indicate a limited nitrate sources in that sub-drainage, and/or the removal of nitrate from runoff (e.g., by denitrification) in a small freshwater wetland located upstream of BTO 3. Within the Bay, nitrate concentrations decline steadily through Upper Bay, approaching the lower limit of detection (0.1 mg/L) at most Lower Bay sites.

Ammonia also exhibits significant spatial variability (upper right panel, Fig. 2-22). Among the tributary sites, the concentration of ammonia is moderate in San Diego Creek and Santa Ana Delhi (0.1 to 0.15 mg/L), and below the detection limit at Big Canyon Wash (<0.05 mg/L). Ammonia concentrations in the upper reaches of Upper Bay (i.e., sites BTO 4-6) are on par, or higher, than ammonia concentrations in the tributaries. The dissimilative reduction of nitrate to ammonia in anoxic Upper Bay sediments (i.e., ammonification)[13] may be responsible for the relatively high concentrations of ammonia observed in Upper Bay. Ammonia concentrations drop off gradually with distance down Bay, falling below the detection limit at BTO 8 (near Arches drain), at the ocean outlet (BTO 11), and at the offshore control site (BTO 12).

The spatial distributions of TOC and total phosphorous are similar to the distribution described above for nitrate (bottom two panels, Figs. 2-22). Both TOC and total

[13] Takeuchi, J. (2006) "Habitat segregation of a functional gene encoding nitrate ammonification in estuarine sediments" *Geomicrobiology* J. 23:75-87.

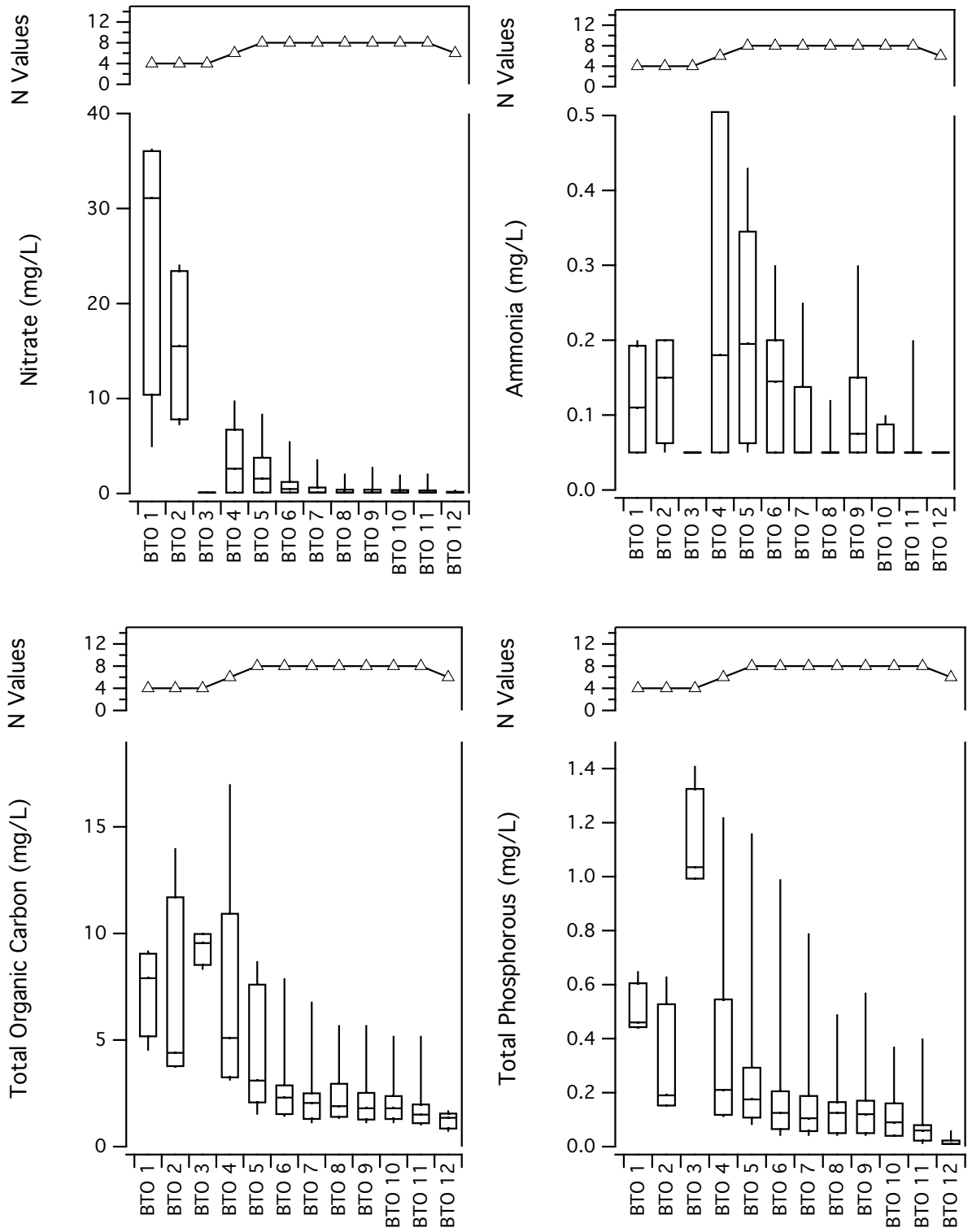

Figure 2-22. Nutrients measured in water samples collected from tributary (BTO 1-3), within-Bay sites (BTO sites 4-11), and the offshore control site (BTO 12). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

phosphorous are present at relatively high concentrations at tributary sites, and progressively decrease with distance down Bay, approaching the lower limit of detection at the offshore control site. While BTO 3 (Big Canyon Wash) has low nitrate and ammonia concentration (see above), it has relatively high phosphorous concentration (median 1.03 mg/L). Average nutrient concentrations reported here for Upper Bay sites (BTO 4-7) are similar to values published previously for this wetland[14]. The average nitrate (3.1 mg/L or 50 μM) and total phosphorous (0.36 mg/L or 11 μM) concentrations are at the high end of measurements reported for estuaries on both the east[15] and west[16] coasts of the United States.

2.3.7.5. Measurement of Particles: Sequoia LISST-100

The LISST-100 uses laser diffraction technology to estimate the concentration and size distribution of suspended particles in water. The LISST-100 can be operated in situ (the acronym LISST stands for Laser In Situ Scattering and Transmissometry), but here it was used in the laboratory to analyze suspended particle size distributions in water samples collected from Newport Bay. The LISST measures two quantities: laser light transmitted through a water sample (optical transmission, T), and laser light forward scattered by suspended particles in a water sample. The optical transmission is converted directly into a measure of water clarity called beam-c. The forward scattering light is used to estimate the size distribution, volume concentration (volume of particles per volume of water), particle number concentration (number of particles per volume of water), and average diameter of suspended particles in a sample[17].

2.3.7.5.1 LISST-100: Particle Size Distribution

Every water sample collected in Newport Bay was analyzed with the LISST-100. This instrument reports the concentration of particles (in microliters of particles per liter of water, or parts per million, or ppm) in 32 size bins, ranging in diameter from 2.73

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[14] Boyle, K.A., Kamer, K., and Fong, P. (2004) "Spatial and temporal patterns in sediment and water column nutrients in a eutrophic southern California estuary" *Estuaries* 27:378-388.

[15] Pruell, R.J., Taplin, B.K., Lake, J.L., Jayaraman, S. (2006) "Nitrogen isotope ratios in estuarine biota collected along a nutrient gradient in Narragansett Bay, Rhode Island, USA" *Marine Pollution Bulletin* 52:612-620.

[16] Sigleo, A.C., Mordy, C.W., Stabeno, P., Frick, W.E. (2005) "Nitrate variability along the Oregon coast: Estuarine-coastal exchange" *Estuarine, Coastal and Shelf Science* 64:211-222.

[17] To estimate the particle size distribution, the LISST instrument measures the angle and intensity of forward scattered light, and uses an inversion routine to convert that information into estimates of particle volume concentration in 32 separate size bins (each size bin corresponds to a different light detector, and a different forward scattering angle). While light scattering is relatively insensitive to particle composition (as opposed to light absorption and light reflection which are strongly dependent on particle composition), the inversion of scattering data is sensitive to the shape of the particle, and researchers continue to publish improved inversion methods that account for particle irregularity. Despite these limitations (indeed, all particle sizing methods have limitations), particle size distributions measured with the LISST are being widely adopted as the method of choice for investigating particle dynamics in a wide range of systems, including oceans, estuaries, lakes, and drinking water.

microns to 462 microns.[18] These data, together with measurements of particle sedimentation rates, are described in a draft manuscript that is currently in revision for the journal *Environmental Science and Technology* (see Appendix 2C). In short, it appears that particle size distributions in Newport Bay detect at least three particle populations: relatively large particles (perhaps associated with phytoplankton blooms) that are carried into the Bay from the ocean on flood tides, particles transported to the Bay in storm water runoff from the surrounding watershed, and particles resuspended off of the Newport Bay sediment bed during dredging operations.

2.3.7.5.2 LISST-100: Particle Volume Concentration

Particle Volume Concentration. Particle volume concentrations measured in water samples collected from Newport Bay and its tributaries are presented in Fig. 2-23. Among tributary sites, particle concentrations are highest in San Diego Creek (median 44 ppm), followed by Big Canyon Wash (median 16 ppm), and the Santa Ana Delhi Channel (median 7 ppm). The median particle concentration is approximately the same during dry and wet weather in San Diego Creek (BTO 1), higher during dry weather in the Santa Ana Delhi channel (BTO 2), and higher during wet weather in Big Canyon Wash (BTO 3) (top right and bottom right panels, Fig. 2-23).

Within the Bay, particle concentrations are typically higher in Upper Bay (BTO 4 through 7) and lower in Lower Bay (BTO 8 through 12). In Upper Bay, median particle concentrations are approximately 20 ppm during wet and dry seasons. In Lower Bay, median particle concentrations are closer to 10 ppm, with the exception of BTO 9 where median particle concentration reaches 20 ppm during dry weather. Offshore at BTO 12, median particle concentrations are lower (4 ppm) during dry weather and higher (10 ppm) during wet weather. The range of particle concentrations measured in the ocean is similar to that measured with the same instrument along the open coastline in Orange County (0 to 10 ppm)[19]

2.3.7.5.3 LISST-100: Particle Number Concentration[20]

The particle number concentrations measured in water samples collected from Newport Bay and its tributaries are presented in Fig. 2-24. Among tributary sites, median particle number follows the same trend just described in Section 2.3.7.5.2. Namely, the highest particle numbers are recorded in San Diego Creek (median of approximately 10^9 particles per liter), followed by Big Canyon Wash (median of 10^8 particles per liter), and the Santa Ana Delhi Channel (median ranges between 10^7 and 10^8 particles per liter). Particle numbers are higher during wet weather in San Diego Creek, and higher during dry weather in Santa Ana Delhi Channel (compare upper and lower right panels, Fig. 2-24).

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[18] The particle diameter of each bin is 1.18 times larger than the diameter of the next smallest bin.
[19] Ahn, J.H. and S.B. Grant (2007) "Size distribution, sources, and seasonality of suspended particles in southern California marine bathing waters" *Environmental Science and Technology* v. 41:695-702.
[20] Particle number concentration and particle volume concentration are both measures of particle abundance. However, particle number is more sensitive to the presence of small particles, because the same amount of particle volume will divide into fewer large particles and more smaller particles.

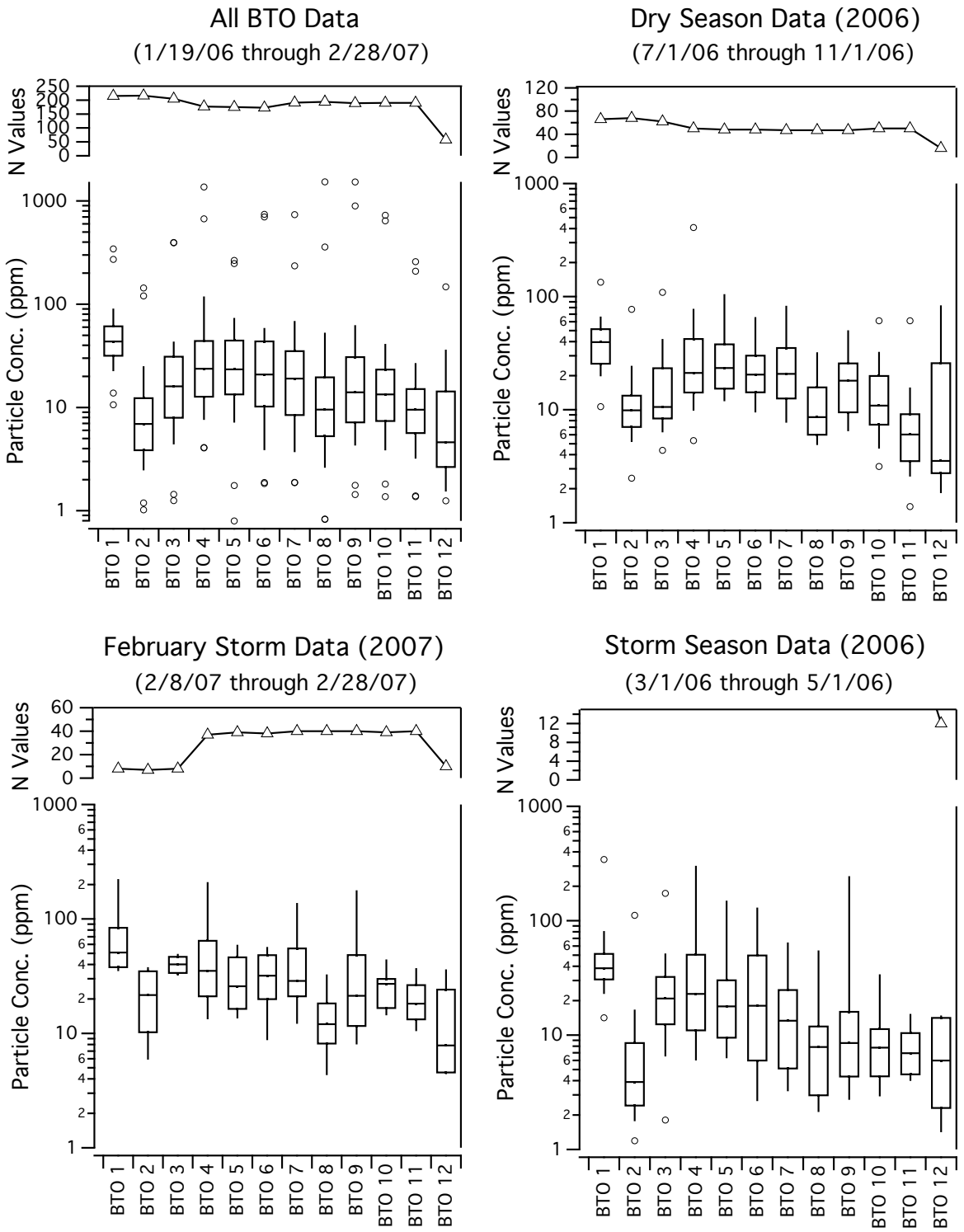

Figure 2-23. Particle concentration measured with the LISST-100 in water samples collected from tributary (BTO 1-3), within-Bay sites (BTO sites 4-11), and the offshore control site (BTO 12). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

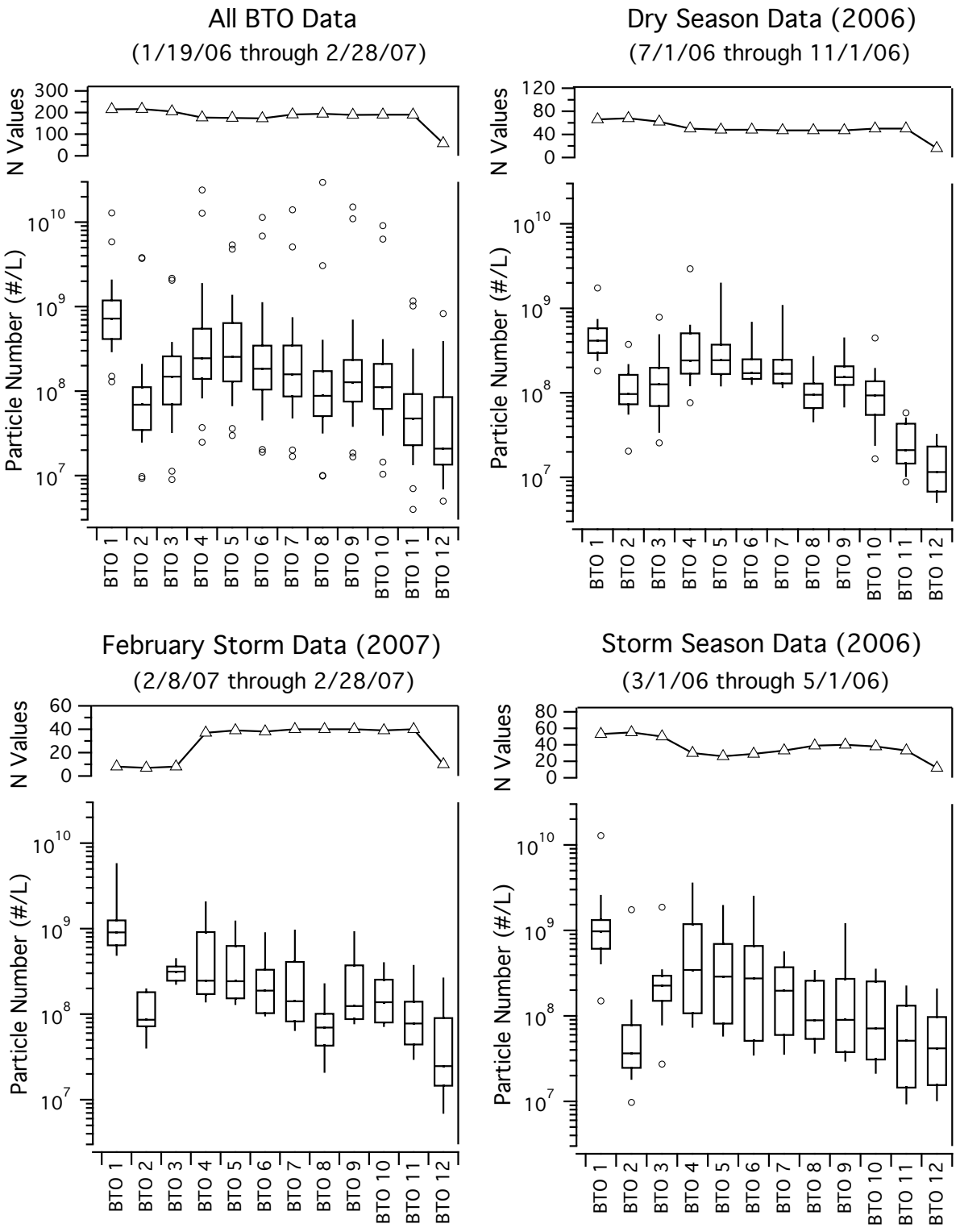

Figure 2-24. Particle number measured with the LISST 100 in water samples collected from tributary (BTO 1-3), within-Bay sites (BTO sites 4-11), and the offshore control site (BTO 12). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

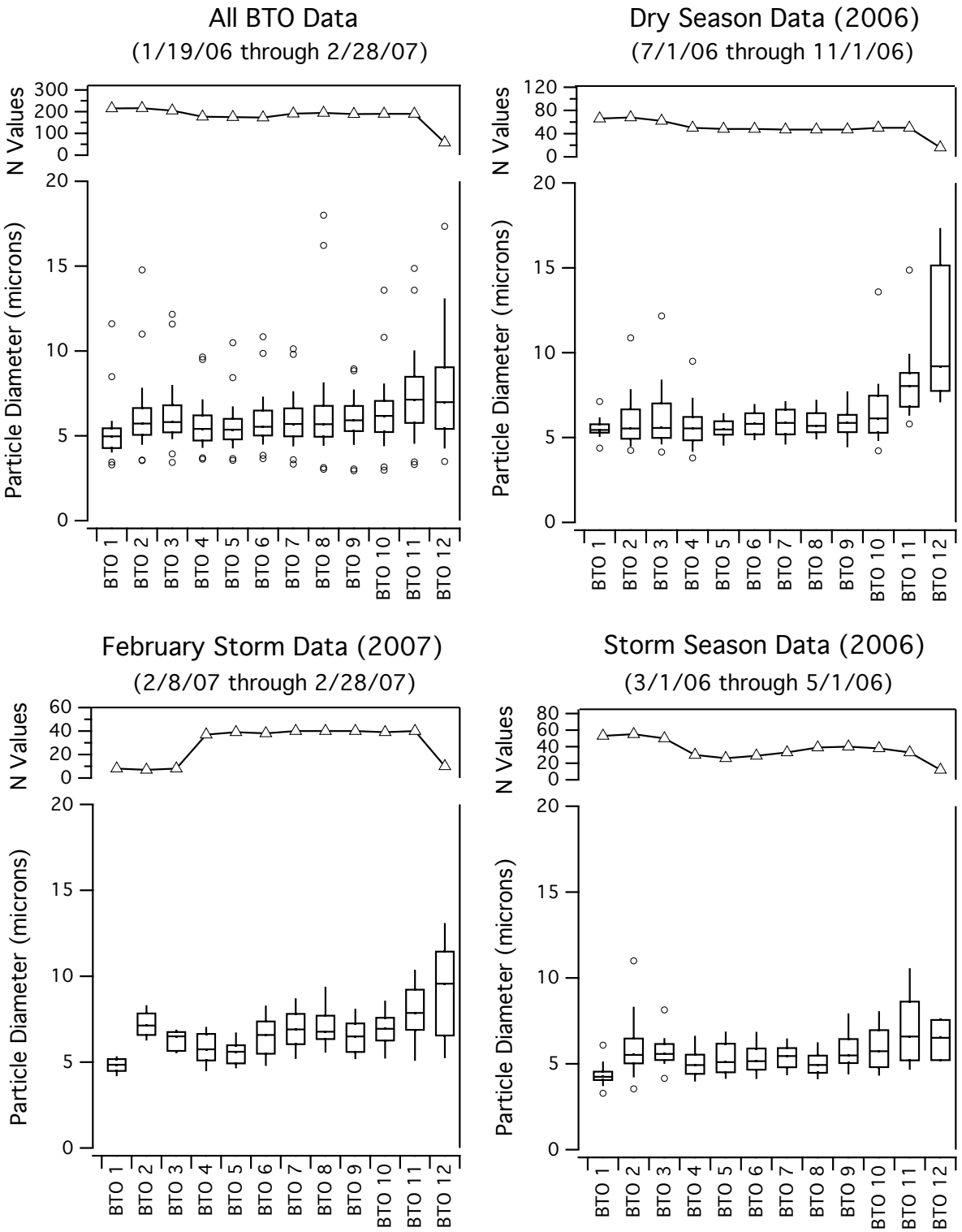

Figure 2-25. Average particle diameter measured with the LISST 100 in water samples collected from tributary (BTO 1-3), within-Bay sites (BTO sites 4-11), and the offshore control site (BTO 12). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

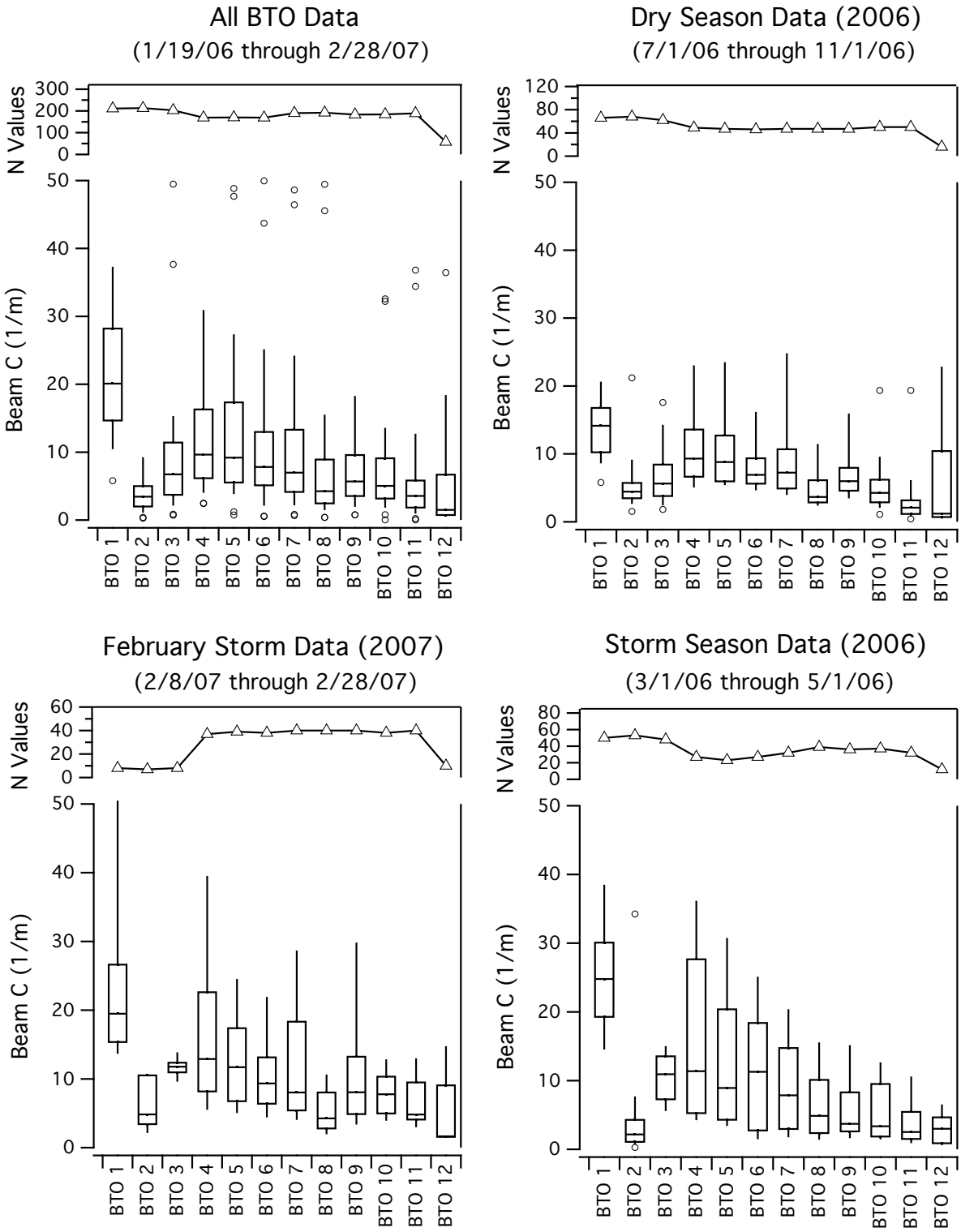

Figure 2-26. Beam-C measured with the LISST 100 in water samples collected from tributary (BTO 1-3), within-Bay sites (BTO sites 4-11), and the offshore control site (BTO 12). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

Particle numbers measured at Big Canyon Wash are, within error, the same during dry and wet weather.

Particle number concentrations measured at within-Bay sites exhibit down-Bay and seasonal variability. During dry weather (upper right panel, Fig. 2-24), the median particle number is relatively constant across most of the Bay (BTO 4 through 9, approximately 10^8 particles per liter), dropping off steeply at the ocean outlet (BTO 10 through 12). During storms, the median particle number is highest in Upper Bay, followed by Lower Bay, followed by the ocean (lower right panel, Fig. 2-24).

2.3.7.5.4 LISST-100: Average Particle Diameter

The average particle size measured in water samples collected from Newport Bay and its tributaries are presented in Fig. 2-25. During dry weather, the median average diameter is similar across the three tributary sites (ca., 6 microns). During wet weather, the median particle diameter in San Diego Creek drops to about 4 microns. The median diameter is relatively constant, at about 5 microns, across most within-Bay sites (BTO 4 through 9). The median particle diameter increases markedly oceanward of BTO 10, particularly during dry weather (compare upper and lower right panels, Fig. 2-25). Within the Bay, the median particle diameter is slightly larger during dry weather (ca., 6 microns), compared to wet weather (ca., 5 microns).

2.3.7.5.5. LISST-100: Beam-C

The optical beam coefficient, or "beam-c", is a measure of water clarity (i.e., how far light penetrates water). The higher the beam-c value, the less transparent the water is to light. In relatively turbid systems like Newport Bay, beam-c is affected primarily by the size and concentration of the suspended particles.[21] Beam c measurements in Newport Bay and its tributaries are plotted in Fig. 2-26. Among the tributary sites, median beam c values decrease in the order San Diego Creek, Big Canyon Wash, and Santa Ana Delhi Channel. Within the Bay, beam c measurements exhibit down-Bay and seasonal variability. The highest median beam c values were recorded in Upper Bay, followed by Lower Bay, followed by the ocean. This down-Bay trend is especially evident during wet weather (lower right panel, Fig. 2-26), when the median beam c values in Upper Bay are higher and more variable.

2.3.8. Water Sample Analyses: Time Series of Cross-Sectional Average and Cross-Sectional Deviations

Each time the boat visited a within-Bay site, water samples were collected from five locations in the channel cross section: grab samples from the surface of the water column

[21] Beam c is proportional to particle concentration, and inversely proportional to particle diameter, see Fugate, D.C. and Friedrichs, C.T. (2002) "Determining concentration and fall velocity of estuarine particle populations using ADV, OBS, and LISST" *Continental Shelf Res*. v. **22**:1867-1886.

near the left and right banks[22], a grab sample from the surface of the water column at the deepest point in the tidal channel (thalweg), a grab sample of bottom water at the thalweg, and a depth-integrated sample at the thalweg (see Methods for details). To determine if the concentration of an analyte was consistently high or low at any particular location in the cross section, "deviations" from the cross-sectional average were calculated as follows:

$$\text{Deviation}_i = C_i - (\text{XS Average})$$
$$(\text{XS Average}) = \frac{1}{5}\left[C_{LS} + C_{MS} + C_{RS} + C_B + C_{DI}\right]$$

where the variables are:

C_i concentration of analyte at one of the five cross-section sampling points
C_{LS} concentration of analyte in the left surface sample
C_{MS} concentration of analyte in the middle surface sample
C_{RS} concentration of analyte in the right surface sample
C_B concentration of analyte in the bottom sample at the thalweg
C_{DI} concentration of analyte in the depth-integrated sample at the thalweg

If the deviation is positive, then the concentration at a particular location is greater than the cross-section average. Conversely, if the deviation is negative, then the concentration at a particular location is lower than the cross-section average. The results of this analysis are summarized in a series of 64 figures (see Appendix 2D), one each for every combination of the eight within-Bay sites (BTO 4 through 11) and eight analytes (EC, ENT, salinity, pH, particle concentration, particle number, average particle size, and beam-c). Here, we present results for a subset of BTO sites representative of the three major geographical regions of the Bay: BTO 5 or 6 for Upper Bay, BTO 8 for West Lower Bay, and BTO 10 for East Lower Bay.

2.3.8.1. Cross-Section Average and Deviations: Upper Bay (BTO 5 or 6)

Salinity and pH in Upper Bay. Salinity is consistently lower at the surface of the water column and higher at the bottom of the water column (Fig. 2-27). All three surface water samples (left surface, middle surface, and right surface) have similar salinities, indicating that the tidal channel is well-mixed in the transverse (cross-channel) direction. pH is slightly lower in samples collected from the bottom of the water column, but all samples collected from the surface of the water column have similar pH values (pH data not shown, see pg. 32, Appendix 2D).

E. coli Concentration in Upper Bay. Surface and bottom samples frequently had different EC concentrations, but the sign of that difference (i.e., whether the surface samples had higher or lower concentrations than bottom samples) depends on the season. During

[22] The designation "left" and "right" banks assumes that the observer is facing up-Bay (i.e., the direction the flood tide flows).

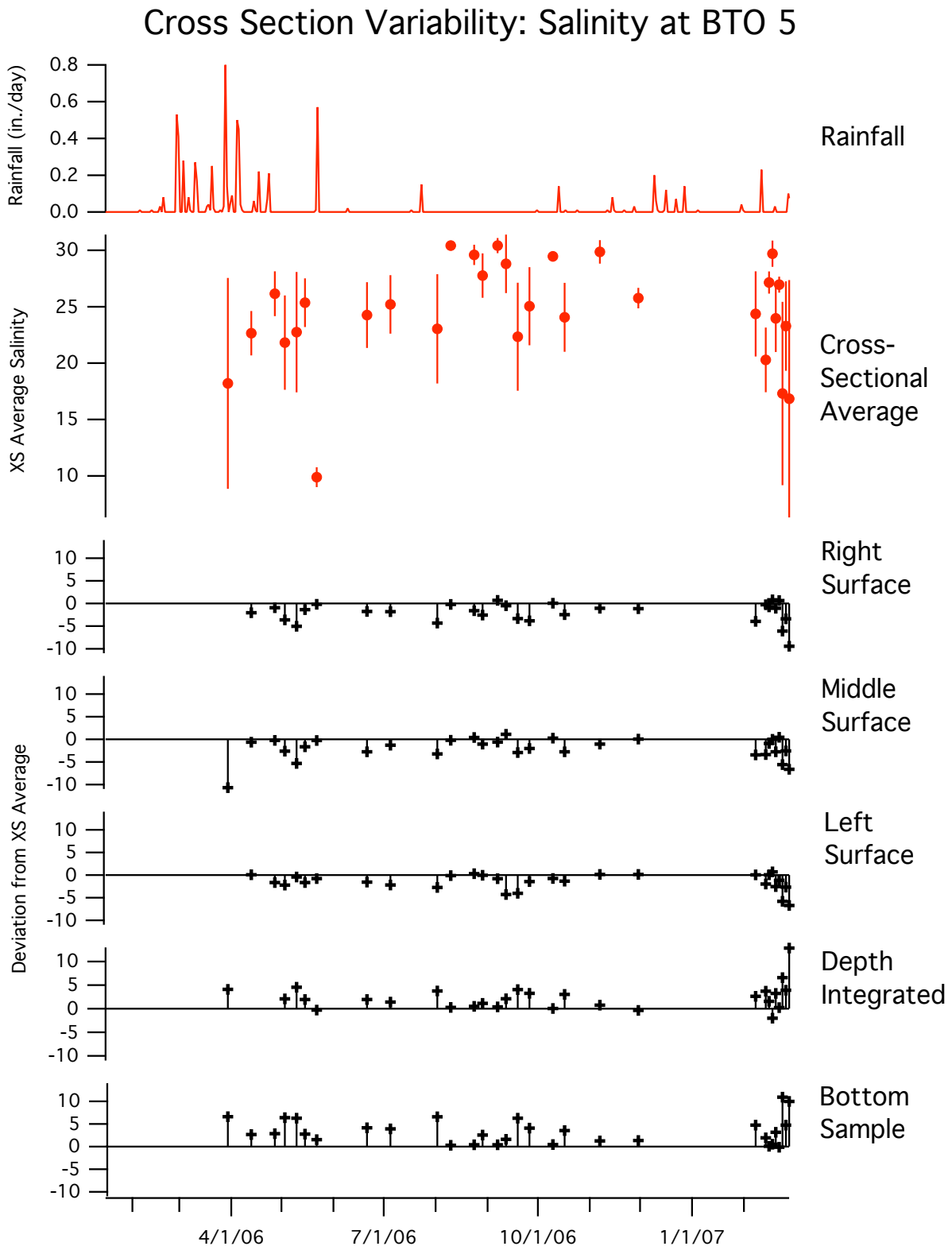

Figure 2-27. Variation of salinity across the cross-section at BTO 5. Shown are rainfall history (top panel), cross-sectionally averaged salinity (second panel), and the deviation of salinity from the cross-sectional average at each of five cross-section locations (bottom five panels).

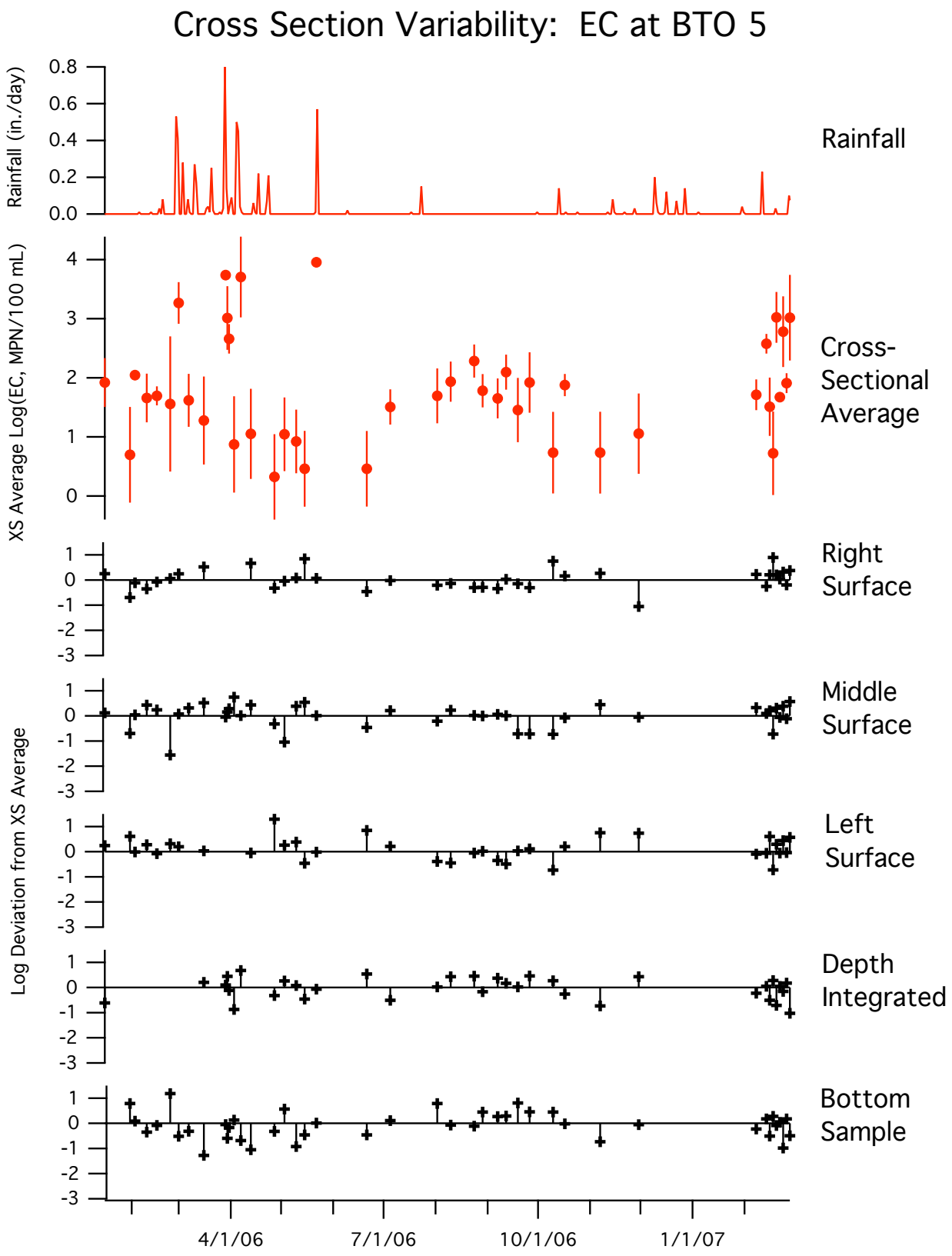

Figure 2-28. Variation of EC across the cross-section at BTO 5. Shown are rainfall history (top panel), cross-sectionally averaged EC (second panel), and the deviation of EC from the cross-sectional average at each of five cross-section locations (bottom five panels).

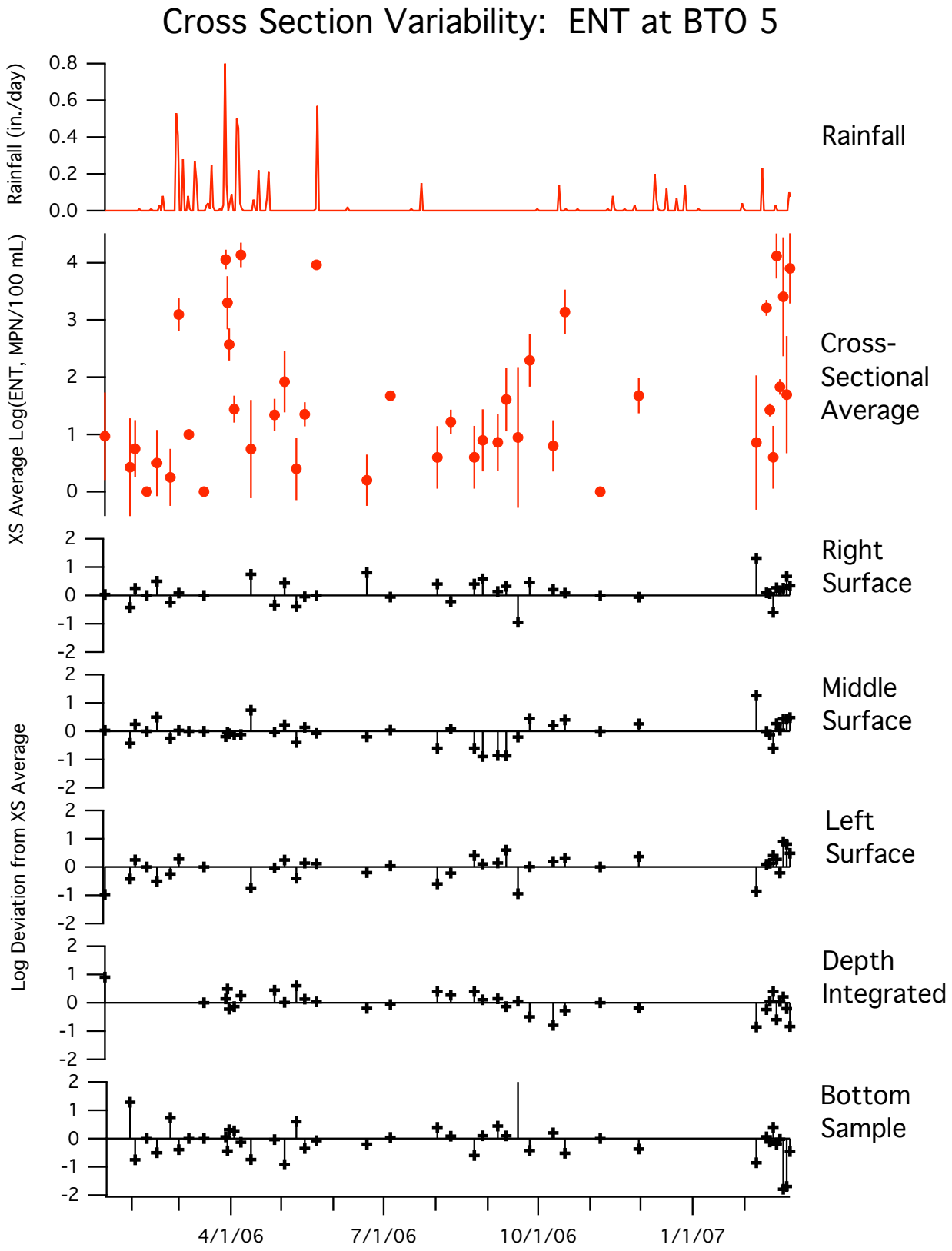

Figure 2-29. Variation of ENT across the cross-section at BTO 5. Shown are rainfall history (top panel), cross-sectionally averaged ENT (second panel), and the deviation of ENT from the cross-sectional average at each of five cross-section locations (bottom five panels).

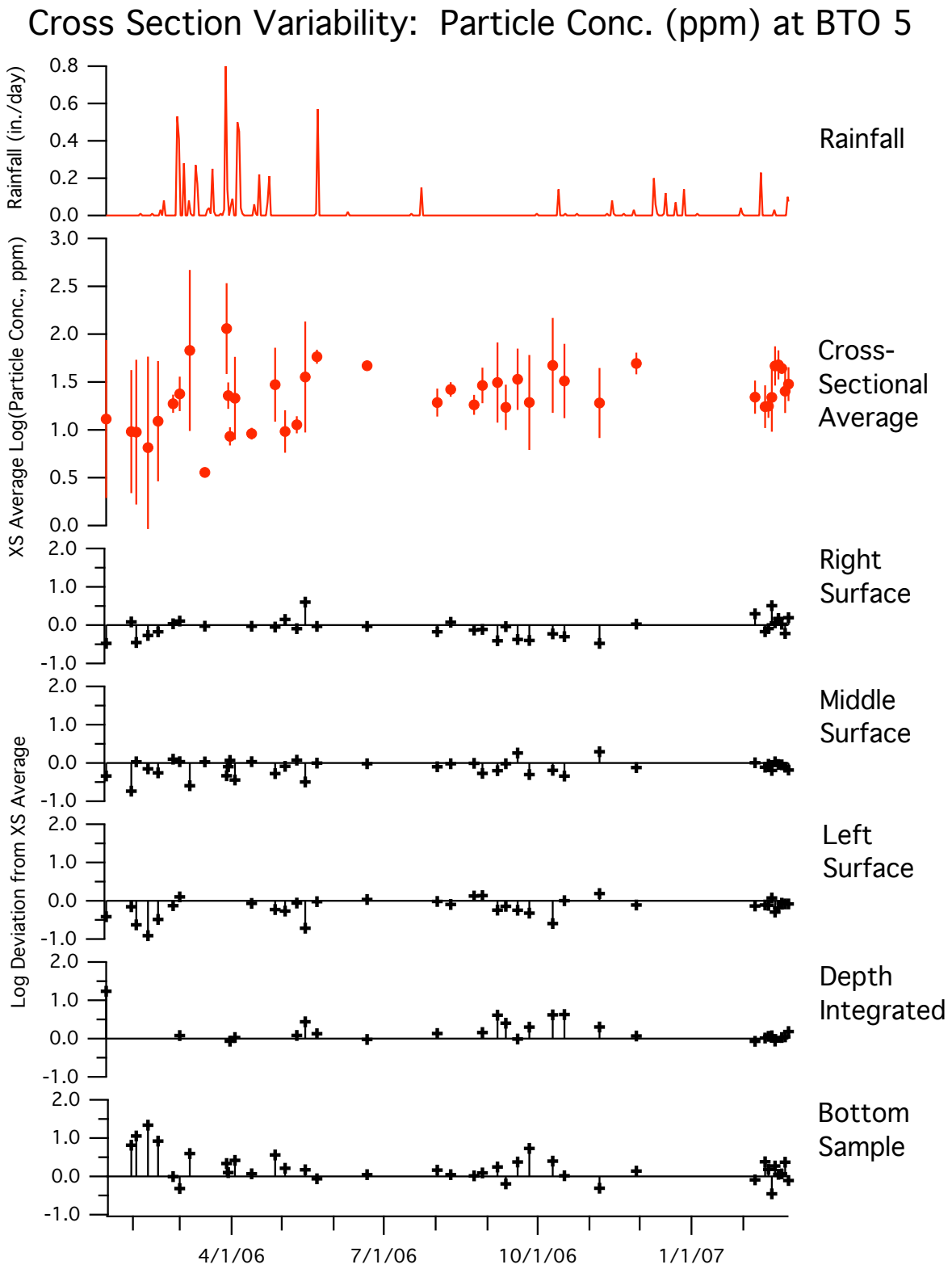

Figure 2-30. Variation of particle concentration across the cross-section at BTO 5. Shown are rainfall history (top panel), cross-sectionally averaged particle concentration (second panel), and the deviation of particle concentration from the cross-sectional average at each of five cross-section locations (bottom five panels).

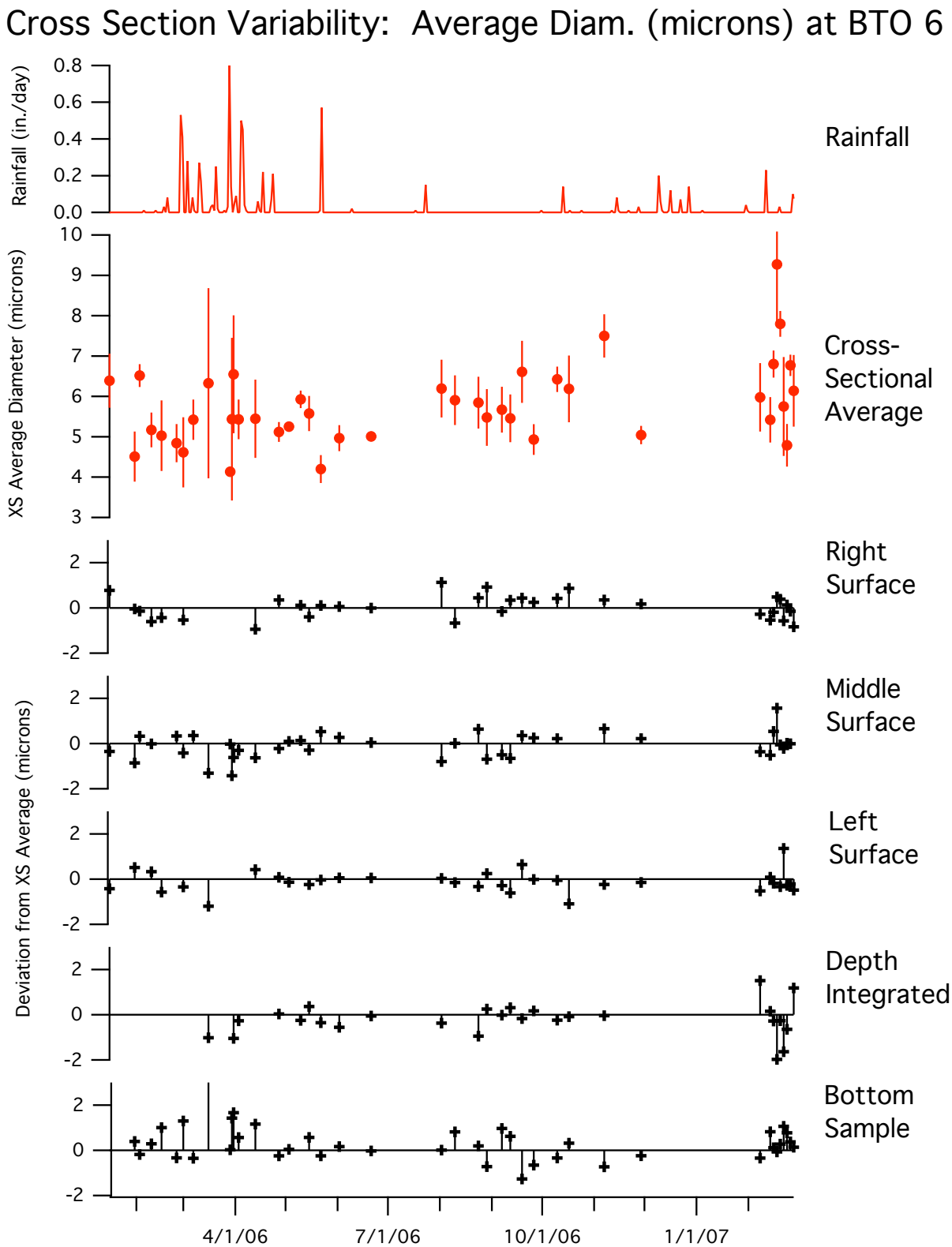

Figure 2-31. Variation of average particle diameter across the cross-section at BTO 6. Shown are rainfall history (top panel), cross-sectionally averaged average particle diameter (second panel), and the deviation of average particle diameter from the cross-sectional average at each of five cross-section locations (bottom five panels).

storms, EC concentrations are generally higher at the surface of the water column and lower at the bottom of the water column (refer to sequence of storms in March 2006, and in February 2007, Fig. 2-28). During dry weather, EC concentrations are generally higher at the bottom of the water column and lower at the surface of the water column (refer to dry weather period from August through October 2006). This pattern may reflect the seasonal variability of different sources of EC; perhaps storm water runoff during wet weather and bottom sediments during dry weather.

Enterococcus in Upper Bay. No consistent cross-sectional trends are obvious in the measurement of ENT in Upper Bay (Fig. 2-29).

Particle Volume Concentration, Particle Number Concentration, Average Particle Size, and Beam-C in Upper Bay. Particle concentrations are consistently higher at the bottom of the water column, and lower at the surface of the water column (Fig. 2-30). Beam c measurements are also higher at the bottom of the water column and lower at the surface of the water column (particle number concentration and beam c data not shown, see pages 50 and 68, Appendix 2D). While there is some variability by site, generally speaking the average diameter of particles is also higher at the bottom of the water column and lower at the surface of the water column, particularly during the wet season (Fig. 2-31).

2.3.8.2. Cross-Section Average and Deviations: West Lower Bay (BTO 8)

Salinity and pH in West Lower Bay. Salinity is frequently higher in samples collected from the bottom of the water column, and lower in samples collected from the right bank of the tidal channel near the outlet of Arches Drain (Fig. 2-32). There are no consistent cross-sectional trends in pH (pH data not shown, see pg. 35, Appendix 2D).

E. coli in West Lower Bay. The concentration of EC is frequently lower in samples collected from the bottom of the water column, and higher in samples collected from the left and right banks (Fig. 2-33). Cross-sectional average EC concentrations in the water column at BTO 8 are frequently higher during dry weather, and lower during wet weather— precisely opposite the seasonal pattern observed for sampling sites in the rest of the Bay (see upper panel, Fig. 2-33).

Enterococcus in West Lower Bay. No consistent cross-sectional trends are obvious in the measurement of ENT in West Lower Bay (Fig. 2-34).

Particle Concentration, Particle Number, Average Particle Size, and Beam-C. Similar to the results reported in Upper Bay, particle concentrations are frequently higher in depth-integrated samples and/or water samples collected from the bottom of the water column, and lower in samples collected from the surface of the water column; e.g., during sampling in February and March 2006 (Fig. 2-35). No obvious cross-sectional trends are evident for measurements of water clarity (beam c) or average particle size (see pgs. 62 and 71, Appendix 2D).

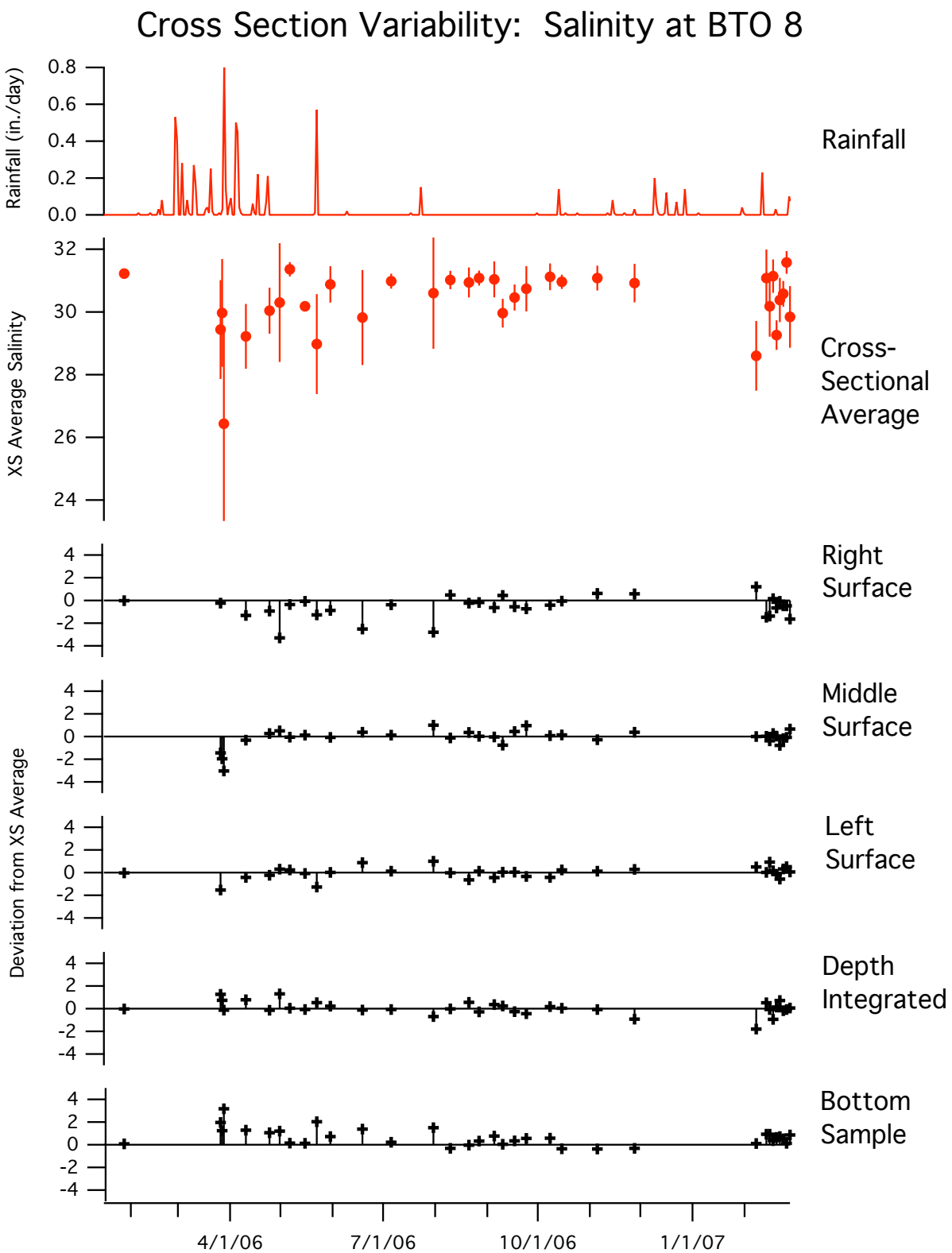

Figure 2-32. Variation of salinity across the cross-section at BTO 8. Shown are rainfall history (top panel), cross-sectionally averaged salinity (second panel), and the deviation of salinity from the cross-sectional average at each of five cross-section locations (bottom five panels).

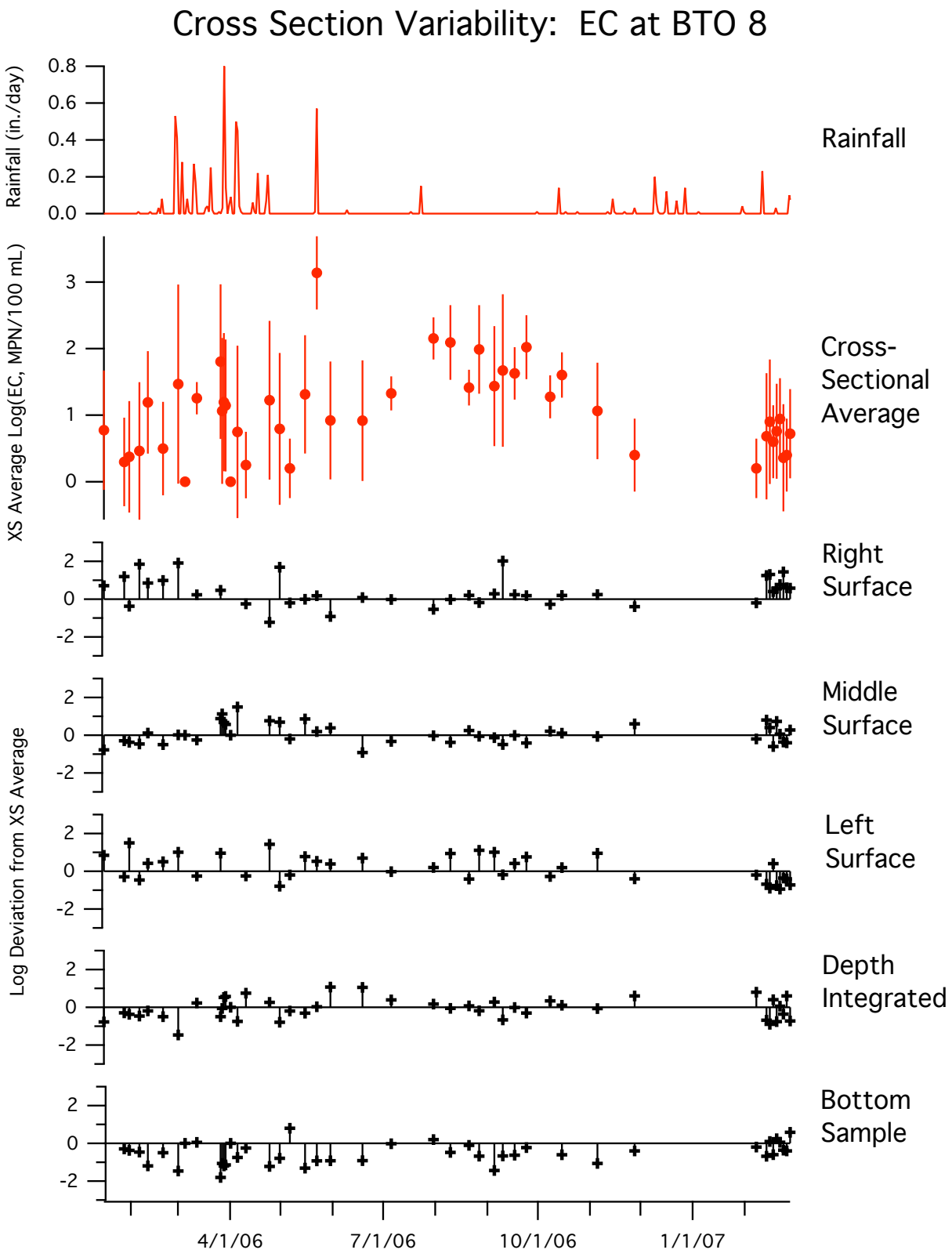

Figure 2-33. Variation of EC across the cross-section at BTO 8. Shown are rainfall history (top panel), cross-sectionally averaged EC (second panel), and the deviation of EC from the cross-sectional average at each of five cross-section locations (bottom five panels).

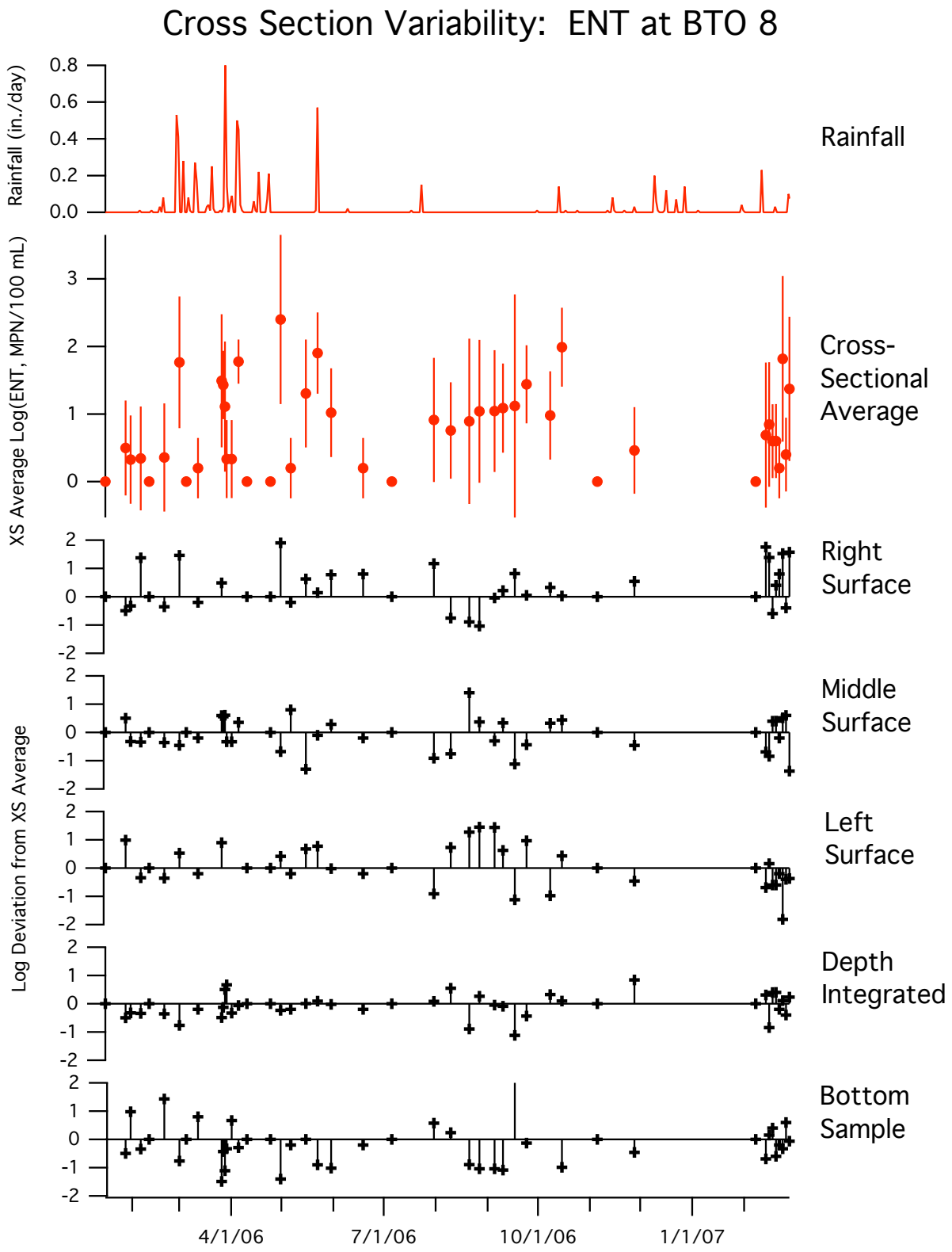

Figure 2-34. Variation of ENT across the cross-section at BTO 8. Shown are rainfall history (top panel), cross-sectionally averaged ENT (second panel), and the deviation of ENT from the cross-sectional average at each of five cross-section locations (bottom five panels).

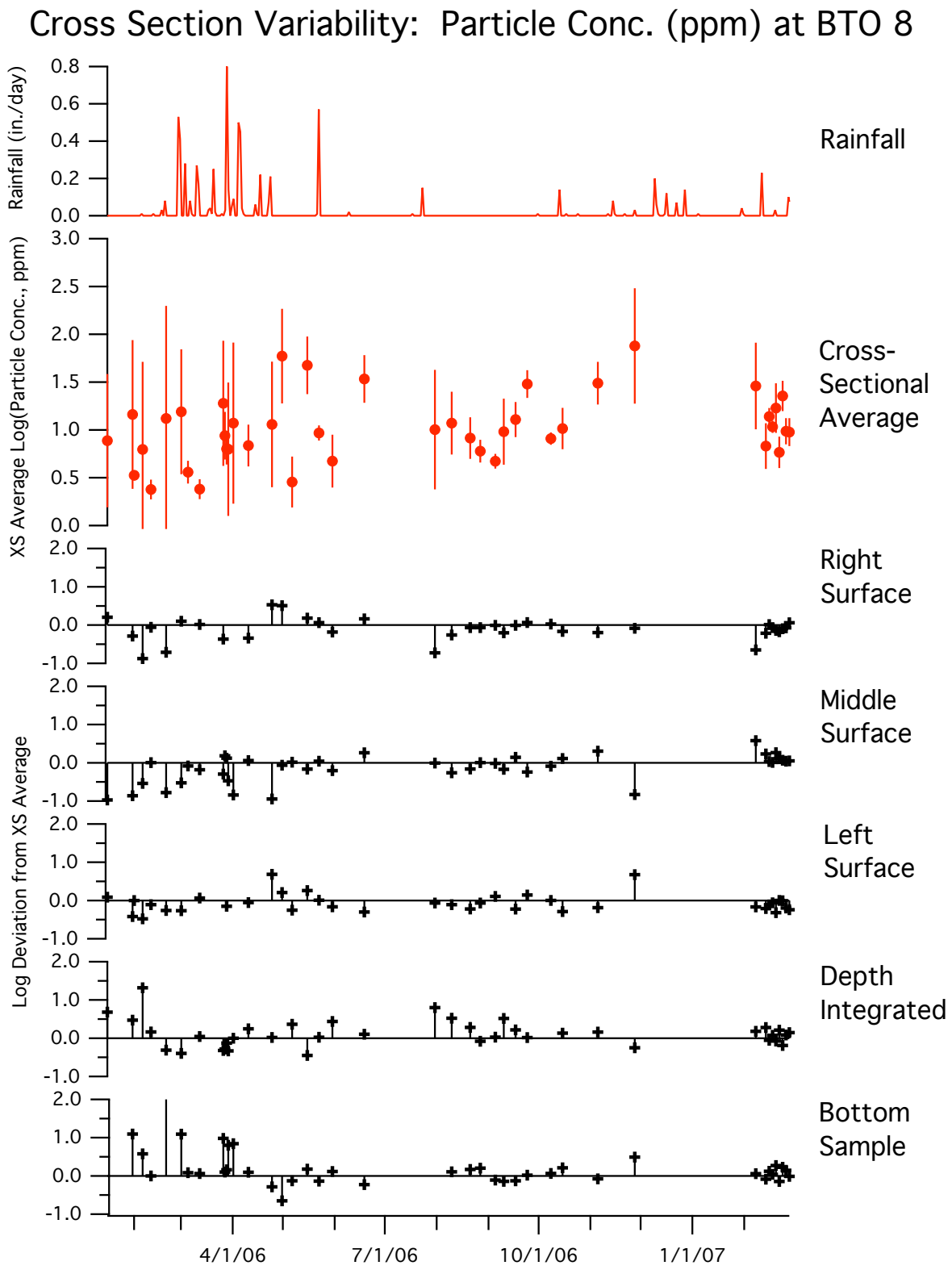

Figure 2-35. Variation of particle concentration across the cross-section at BTO 8. Shown are rainfall history (top panel), cross-sectionally averaged particle concentration (second panel), and the deviation of particle concentration from the cross-sectional average at each of five cross-section locations (bottom five panels).

2.3.8.3. Cross-Section Average and Deviations: East Lower Bay (BTO 10)

Salinity and pH in East Lower Bay. During large storms, salinity is higher at the bottom of the water column and lower at the surface of the water (e.g., see storm events in late May 2006 and late February 2007, Fig. 2-36). Otherwise, salinity is well-mixed over the cross-section. pH also appears to be relatively well-mixed over the cross-section (see pg. 37 of Appendix 2D for pH).

E. coli and Enterococcus in East Lower Bay. No consistent cross-sectional trends are obvious in the measurement of EC and ENT in East Lower Bay (Fig. 2-38 and 2-39). Based on the cross-sectionally averaged data plotted in these two figures, the concentration of both EC and ENT tends to exhibit two patterns: (1) concentrations are high during storm events in the winter; and (2) concentrations are high during dry weather periods in October 2006.

Particle Concentration, Particle Number, Average Particle Size, and Beam-C in East Lower Bay. During storm events, particle concentration is frequently higher at the bottom of the water column during storms (e.g., Fig. 2-39 and 2-40). The average particle size is consistently 2 to 4 microns larger in water samples collected from the bottom of the water column, compared to water samples collected from the surface of the water column (Fig. 2-41). Beam c measurements exhibit relatively little cross-sectional variability, with the exception of a few sampling events during the wet season when beam c is larger at the bottom of the water column (see pg. 73, Appendix 2D).

2.3.9. Water Sample Analyses: Optical micrographs of suspended particles

Optical micrographs of particles and flocs in archived Newport Bay water samples are included in Appendix 2E. As expected, there is considerable variability in the nature of particles observed under the microscope. In some cases the particles appear to be inorganic in nature (e.g., they appear solid and have fractured edges), and in other cases they appear to be organic in nature (e.g., they are intact phytoplankton cells or pieces of small animals). In still other cases, the particles appear to be aggregates of inorganic and organic particles.

2.3.10. Particle-Association of FIB in Newport Bay

One of the objectives of the Prop 13 project was to assess if FIB in the water column of Newport Bay are associated with suspended particles. The particle-association of FIB can affect FIB fate and transport in a number of ways. Particle-association could alter the rate at which FIB die-off in the environment, for example, by providing a micro-environment favorable to FIB survival or growth.[23] Further, assays used to measure FIB in water samples do not distinguish between the presence of single free-living cells (i.e.,

[23] Craig, D.L., Fallowfield, J.H., Cromar, N.J. (2004) Persistence of *E. coli* in recreational coastal water and sediment *J. Appl. Microbiol*. **96**:922-930; see also the microcosm results presented in this document, Chapter 4.

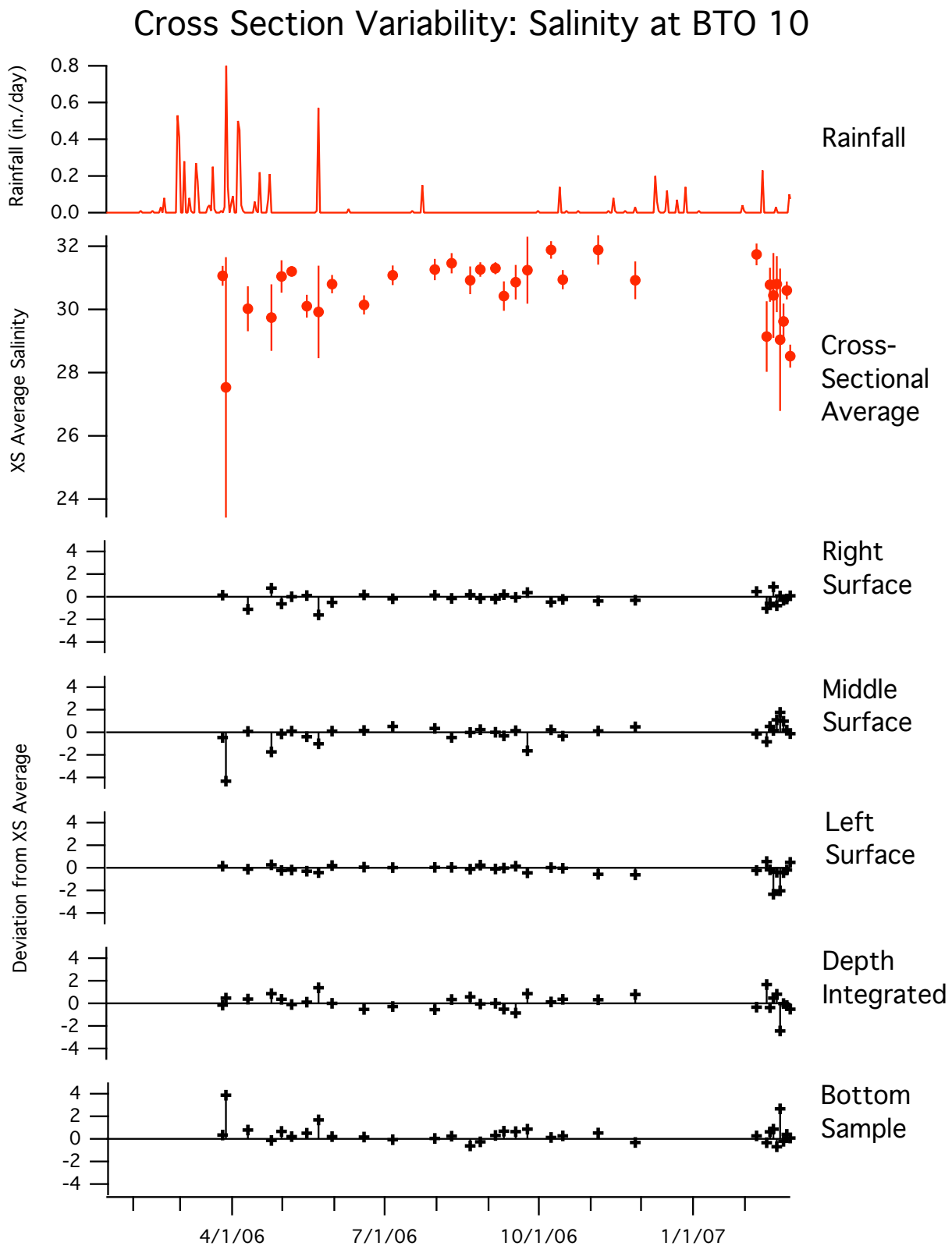

Figure 2-36. Variation of salinity across the cross-section at BTO 10. Shown are rainfall history (top panel), cross-sectionally averaged salinity (second panel), and the deviation of salinity from the cross-sectional average at each of five cross-section locations (bottom five panels).

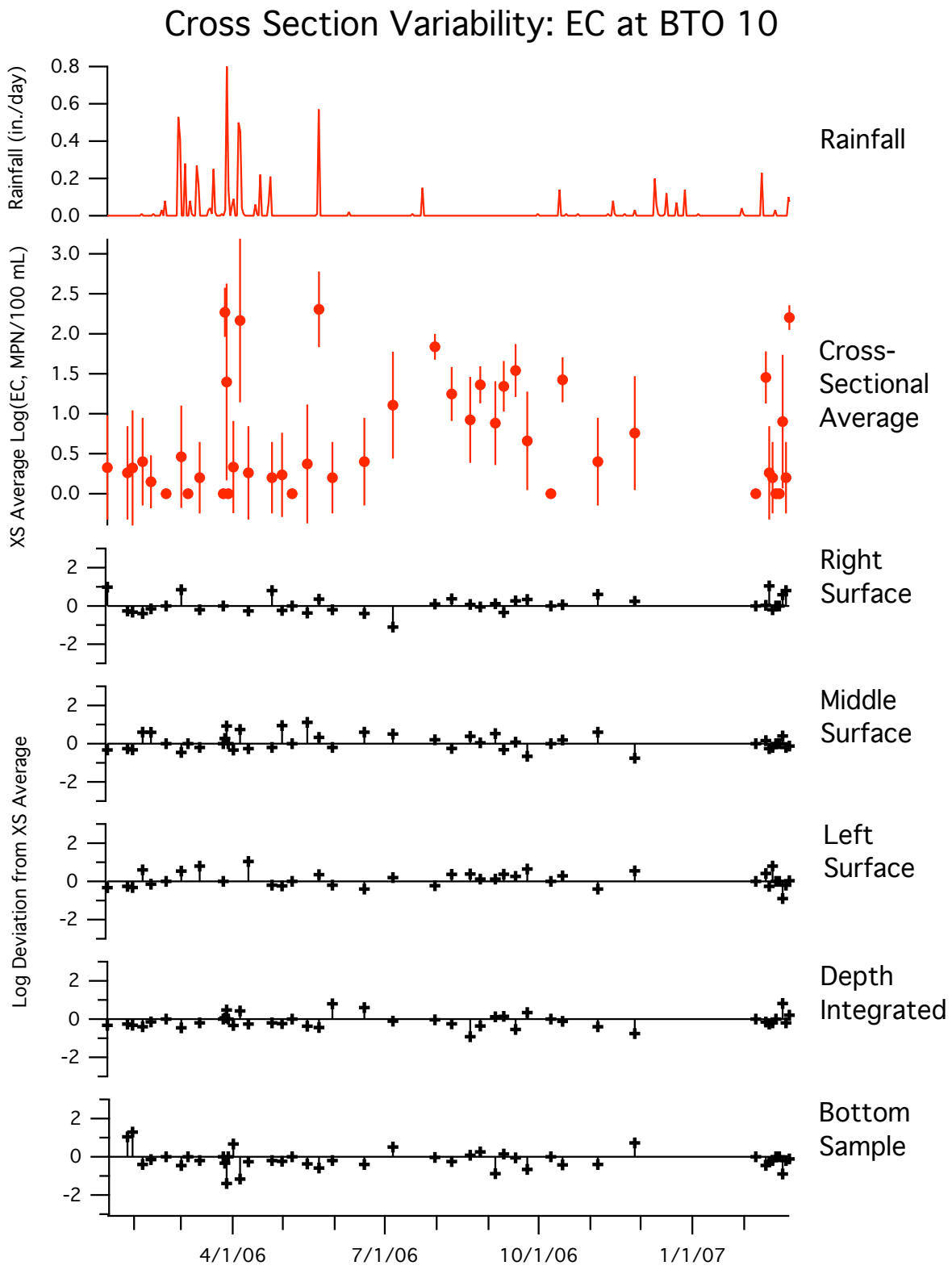

Figure 2-37. Variation of EC across the cross-section at BTO 10. Shown are rainfall history (top panel), cross-sectionally averaged EC (second panel), and the deviation of EC from the cross-sectional average at each of five cross-section locations (bottom five panels).

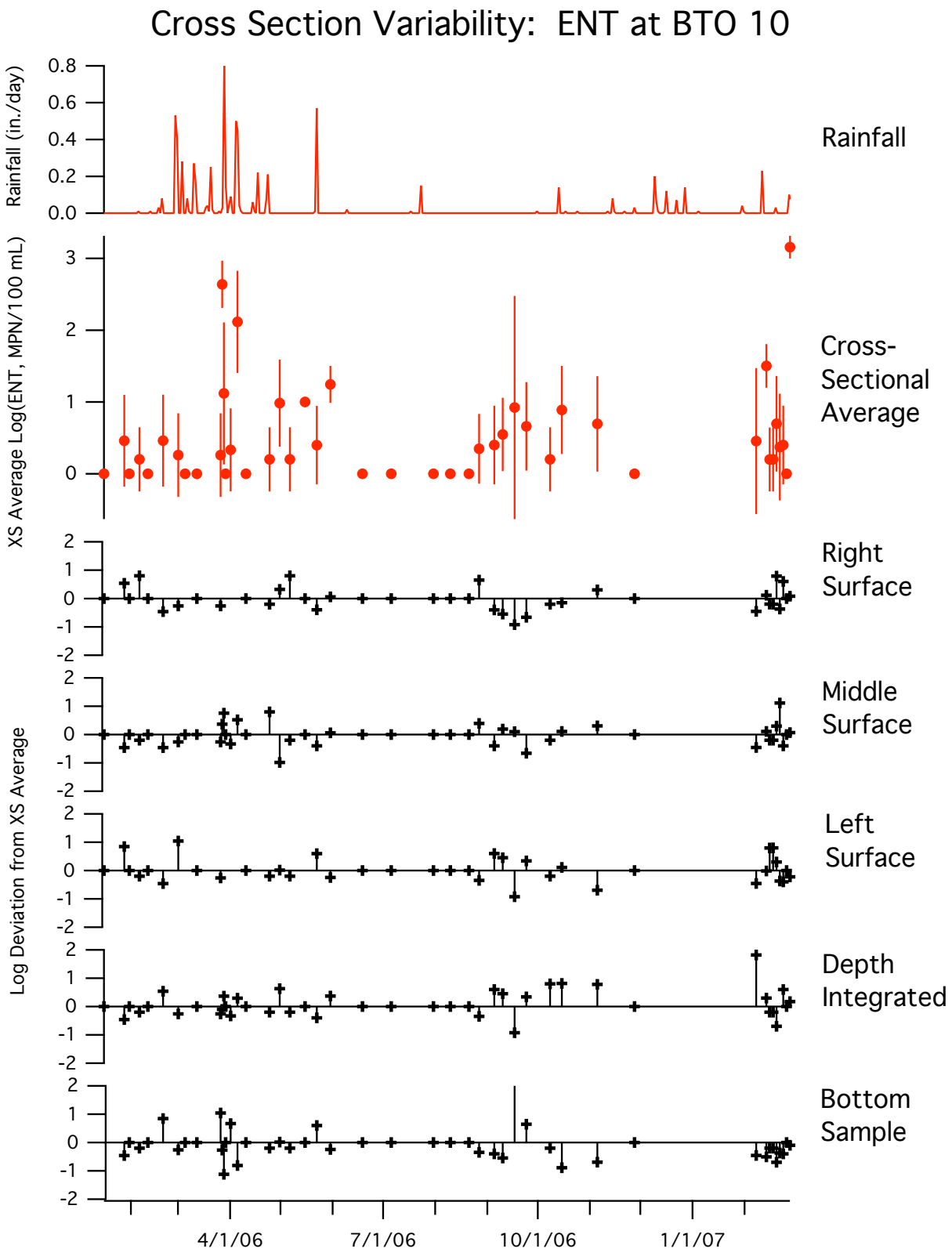

Figure 2-38. Variation of ENT across the cross-section at BTO 10. Shown are rainfall history (top panel), cross-sectionally averaged ENT (second panel), and the deviation of ENT from the cross-sectional average at each of five cross-section locations (bottom five panels).

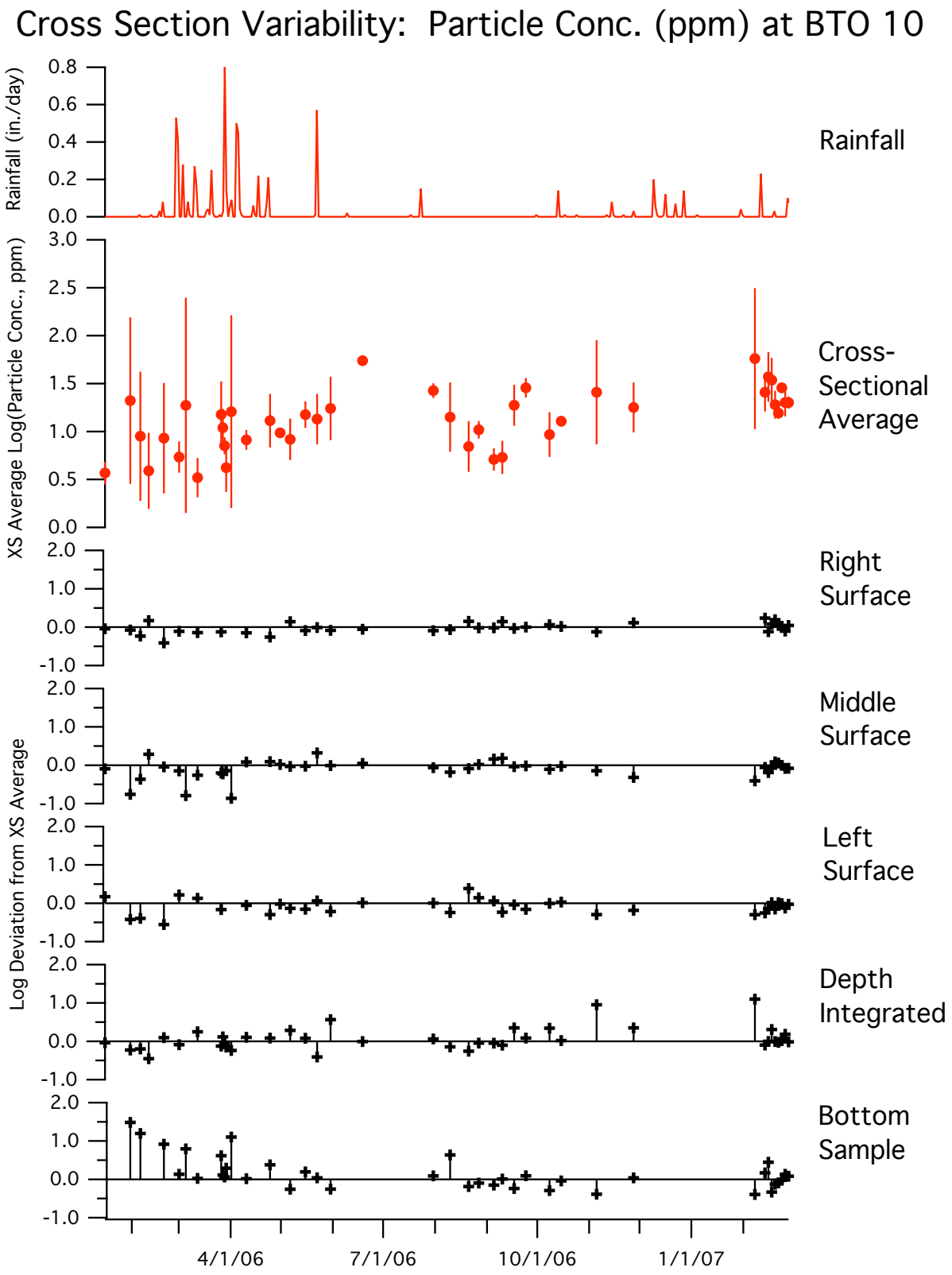

Figure 2-39. Variation of particle volume concentration across the cross-section at BTO 10. Shown are rainfall history (top panel), cross-sectionally averaged particle concentration (second panel), and the deviation of particle concentration from the cross-sectional average at each of five cross-section locations (bottom five panels).

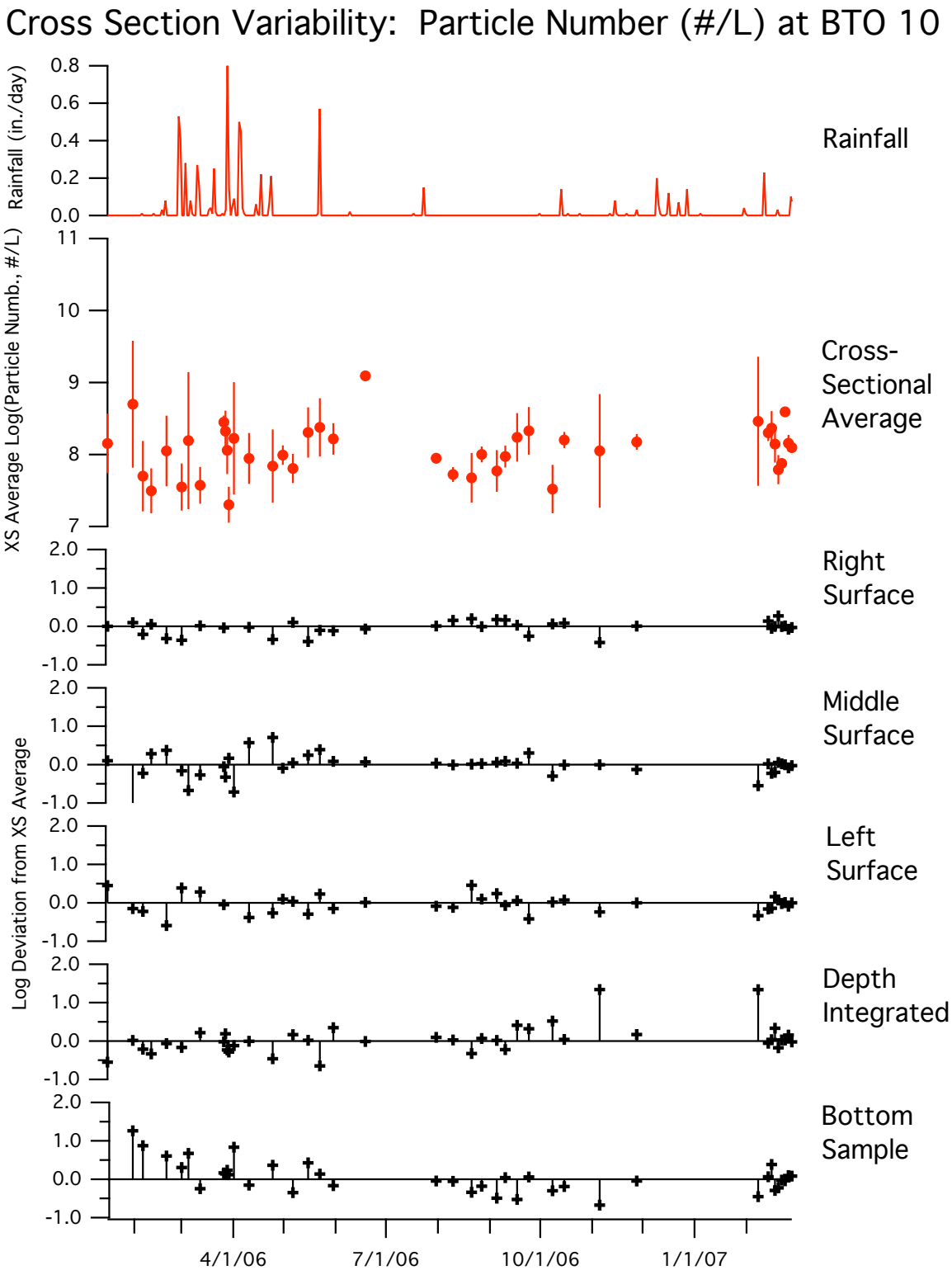

Figure 2-40. Variation of particle number concentration across the cross-section at BTO 10. Shown are rainfall history (top panel), cross-sectionally averaged particle number (second panel), and the deviation of particle number from the cross-sectional average at each of five cross-section locations (bottom five panels).

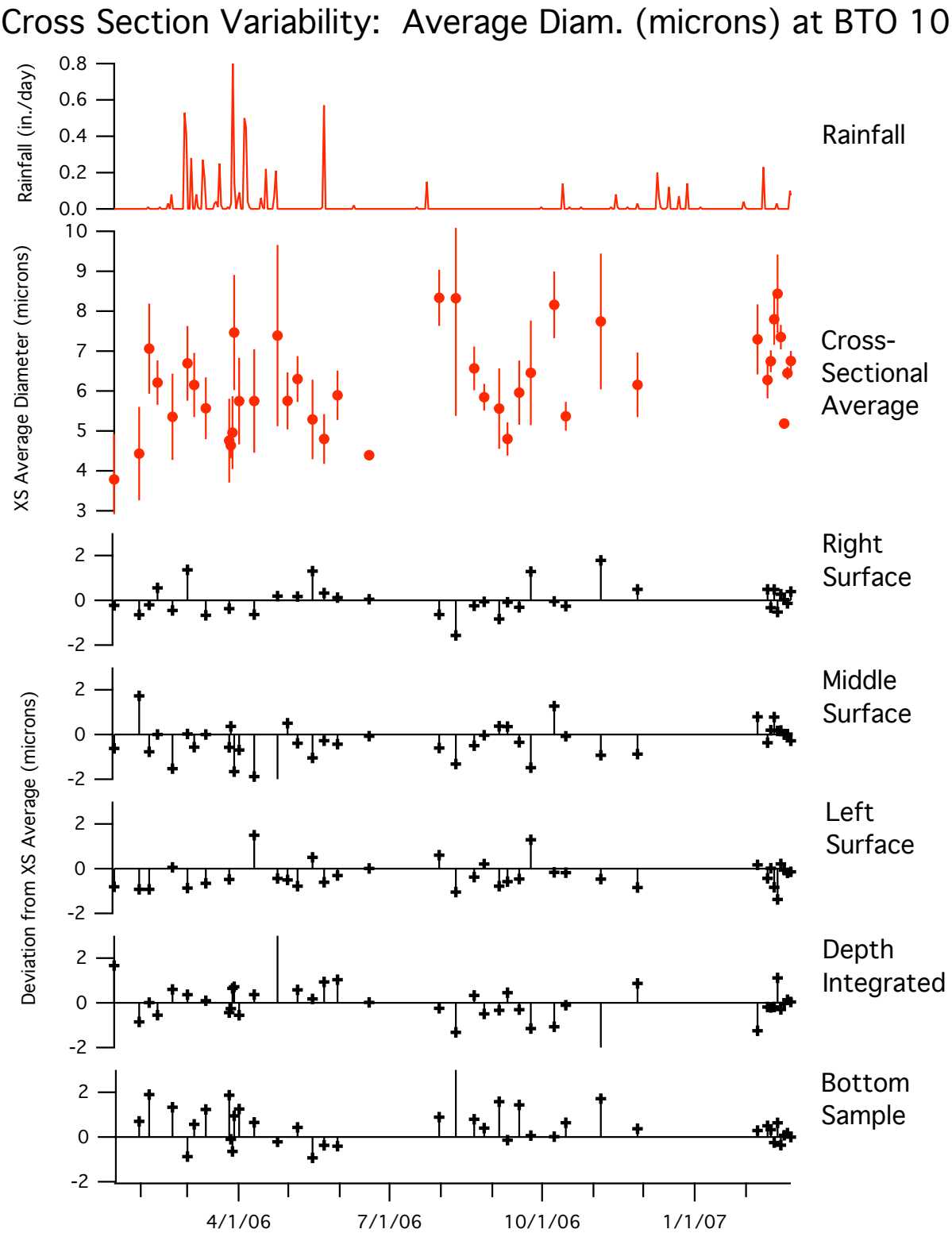

Figure 2-41. Variation of average particle diameter across the cross-section at BTO 10. Shown are rainfall history (top panel), cross-sectionally averaged particle diameter (second panel), and the deviation of particle diameter from the cross-sectional average at each of five cross-section locations (bottom five panels).

ones that are not particle-associated), and a particle that harbors many FIB cells. In both cases, the standard assays register a "single" FIB[24]. One potential consequence is that FIB associated with particles may appear to die-off more slowly, because all FIB cells associated with the particle must die before the particle no longer tests positive for FIB.

2.3.10.1. Particle-Association Can Accelerate the Removal of FIB by Gravitational Sedimentation.

Particle-association can affect the fate and transport of FIB in the Bay by altering the rate at which FIB settle through the water column. To illustrate this point, sedimentation times for EC and ENT in Newport Bay are calculated for different degrees of particle association. If the FIB are not particle associated and not aggregated (i.e., they are present as single free-living cells), then Stokes' Law yields the following range of settling velocities (V_s) and times to settle one meter (T_{1m}):

EC: $V_s = 5 \text{ to } 10 \times 10^{-8}$ m/s; $T_{1m} = 120 \text{ to } 240$ days

ENT: $V_s = 7 \text{ to } 12 \times 10^{-8}$ m/s; $T_{1m} = 95 \text{ to } 173$ days

For these calculations it is assumed that both EC and ENT have a volume-averaged radius of 0.62 microns, that the density of ENT is greater than that of EC[25], and that the density and water temperature (which affects the dynamic viscosity[26]) are in the range measured during the BTO study; i.e., 1005 to 1025 kg/m^3 for water density and 10 to 30°C for water temperature (see Figs. 2-10 and 2-12). To put these settling times in perspective, the flushing time[27] of water in Newport Bay is estimated to be, at most, 30 days, based on a numerical simulations performed by Dr. Ying Poon.[28] Hence, single

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[24] Borst, M., Selvakumar, A. (2003) Particle-associated microorganisms in stormwater runoff. *Water Res.* **37**, 215–223; Note that this artifact is not unique to the Colilert and Enterolert assays used here, but would also occur with other more conventional assays, such as the MTF and CFU methods.

[25] The density and size of the two FIB of interest here—*E. coli* and enterococci bacteria—can be determined from the literature. *E. coli* is rod-shaped Gram-negative cell, with a diameter of 0.8 $\times 10^{-6}$ m, length of 2.0 $\times 10^{-6}$ m, volume-averaged radius of 0.62 $\times 10^{-6}$ m, and density of $\rho_{E.coli}$ = 1105 kg/m^3. The cell density of *E. coli* is relatively insensitive to different exponential growth rates and cell ages. Enterococci bacteria is a spherical (cocci) Gram-positive cell, with a volume averaged radius of 0.62 $\times 10^{-6}$ m, and an approximate density of 1130.9 kg/m^3. In contrast to *E. coli*, the density of enterococci bacteria (in particular, *Streptococcus faecium*) varies with cell cycle stage, although the range of density values measured was relatively limited (from 1130.1 to 1131.2 kg/m^3). For comparison, the density of solid quartz (a common mineral in inorganic Bay sediment) is 2643 kg/m^3.

[26] The water temperature, in turn, affects the dynamic viscosity of the saltwater, which was assumed to range between 0.87 (for 30C) and 1.30 x10^{-3} (for 10 C) Ns/m^2.

[27] The flushing time is the time required for a uniformly distributed conservative tracer to decay 1/e of its initial concentration. The flushing time of 30 days is for the most inland reaches of Newport Bay, including portions of Upper Bay and West Newport Bay.

[28] Pednekar, A.M., S.B. Grant, Y. Jeong, Y. Poon, C. Oancea (2005) "Influence of climate change, tidal mixing, and watershed urbanization on historical water quality in Newport Bay, a saltwater wetland and tidal embayment in southern California" *Environmental Science and Technology*, v. **39**: 9071-9082.

FIB cells entering the Bay from, for example, watershed runoff, will either die-off or be flushed into the ocean long before they are removed from the water column by gravitational sedimentation.

The situation changes markedly if FIB attach to suspended particles. Particle settling velocities measured in water samples collected from Newport Bay (see manuscript in Appendix 2C) range from 6 x 10^{-6} m/s for particles 1.7 microns in diameter, to 9 x 10^{-5} m/s for particles 93 microns in diameter. If FIB were attached to these particles, the time to settle 1 m would be reduced to T_m = 2 days (for a settling velocity of 6 x 10^{-6} m/s) and T_m = 3 hours (for a settling velocity 9 x 10^{-5} m/s). Because these settling times are shorter than the flushing time of water in the Bay (in the latter case the settling time is shorter than a full tidal cycle), it is very likely that a fraction of particle-associated FIB would partition to the bottom of the Bay, where they could be resuspended by tidal action, storm events, or other sources of turbulent shear (e.g., boat prop wash). This effect would likely be especially important in areas with long flushing times, like Upper Bay and West Newport Bay.

2.3.10.2. Experimental Investigation of Particle-Association of FIB in Newport Bay.

Approach. There are a number of approaches described in the literature for assessing the degree to which bacteria are particle-associated, including (1) direct microscopic examination, (2) settling experiments, (3) homogenization studies, and filtration studies[29]. In principle, filtration experiments are straightforward to interpret, and were utilized here. In our implementation of the filtration approach, particle-association of FIB is evaluated by comparing the log-transformed concentration of ENT or EC before (C_b) and after (C_a) filtration through a 5 or 10 micron filter (see methods). To provide a quantitative measure of particle-association, we calculated the relative percent difference (RPD) of the FIB concentration before and after filtration:

$$\mathrm{RPD}_{\mathrm{filter}} = \frac{(C_a - C_b)}{\frac{1}{2}(C_b + C_a)} \times 100$$

If FIB in a water sample are mostly particle-associated, then $\mathrm{RPD}_{\mathrm{filter}}$ should be large in magnitude and negative in sign. If FIB in a water samples are not particle-associated, then $\mathrm{RPD}_{\mathrm{filter}}$ should be close to zero. However, even if all FIB are not particle-associated, $\mathrm{RPD}_{\mathrm{filter}}$ will not, in general, exactly equal zero, because the FIB assay itself is a source of variability. To quantify this assay variability (i.e., assay precision), we performed negative control experiments in which water samples were assayed for FIB twice without filtration. A relative percent difference for the negative control was then calculated from the two independent measurements of log-transformed FIB concentration, referred to here as C_{b1} and C_{b2}:

[29] Plancherel, Y.; Cowen, J.P. (2007) "Towards measuring particle-associated fecal indicator bacteria in tropical streams" *Water Res*. **41**:1501-1515.

$$\mathrm{RPD}_{\mathrm{control}} = \frac{(C_{b,1} - C_{b,2})}{\frac{1}{2}(C_{b,1} + C_{b,2})} \times 100$$

In all, N=1,175 sample pairs were analyzed with the 5 micron pore-size filter, N=344 sample pairs were analyzed with the 10 micron pore-size filter, and N=92 sample pairs were treated as negative controls. In a large percent of cases, one or both samples in each sample pair yielded FIB concentrations below the lower-limit of detection of 10 MPN/100 mL. All such pairs had to be excluded from the RPD analysis, because the inclusion of censored data would artificially bias the RPD calculation toward zero. Thus, in calculating RPD, sample pairs were included in our analysis only if both C_a and C_b (or $C_{b,1}$ and $C_{b,2}$) were above the lower limit of detection (as will be shown shortly, more EC than ENT measurements met this criteria).

Results: In most cases, the median RPD values were very close to zero (Figs. 2-42 through 2-45). This observation applies to both EC and ENT and most BTO sites and time periods analyzed. In cases where the median RPD deviates from zero, the N values are typically very small (<10) and the distribution of RPD values is not statistically significant (e.g., see the RPD distribution for ENT at BTO 2 during the Storm Season 2006, lower right panel, Fig. 2-45). However, as described below, there are two cases where the median RPD does appear to be significantly different from zero.

When water samples collected from BTO 2 are passed through a 5-micron pore-size filter, the concentration of EC increases relative to the concentration measured in the unfiltered sample (i.e., the median RPD is significantly greater than zero, upper left panel, Figs. 2-42). The reason for this unexpected result is not clear. One possibility is that runoff at Santa Ana Delhi Channel (BTO 2) contains a filterable substance that interferes with the defined substrate tests (IDEXX Colilert) used to measure EC. Alternatively, if EC at this site was associated with delicate flocs, it is possible that shear forces created during the filtration step disrupt, instead of remove, the flocs, increasing the concentration of EC in the filtered sample. Interestingly, ENT measured on the same set of samples showed no bias before and after filtration; i.e., the median RPD was zero (upper left panel, Fig. 2-44). Thus, whatever process is responsible for the positive RPD values observed at BTO 2, it applies only to EC.

Median RPD values also deviate from zero for samples from the within the Bay during dry weather (upper right panel, Fig. 2-42). In this case, the median RPD values are consistently negative across most within Bay sites (with the exception of BTO 8), consistent with the idea that EC in these samples are associated with particles that can be removed by passage through a 5 micron filter. For filtration experiments carried out with the 10 micron pore size filter, the N values were generally too small and/or the variability of RPD values too large to determine if the median RPD is significantly different from zero (Figs. 2-43 and 2-45).

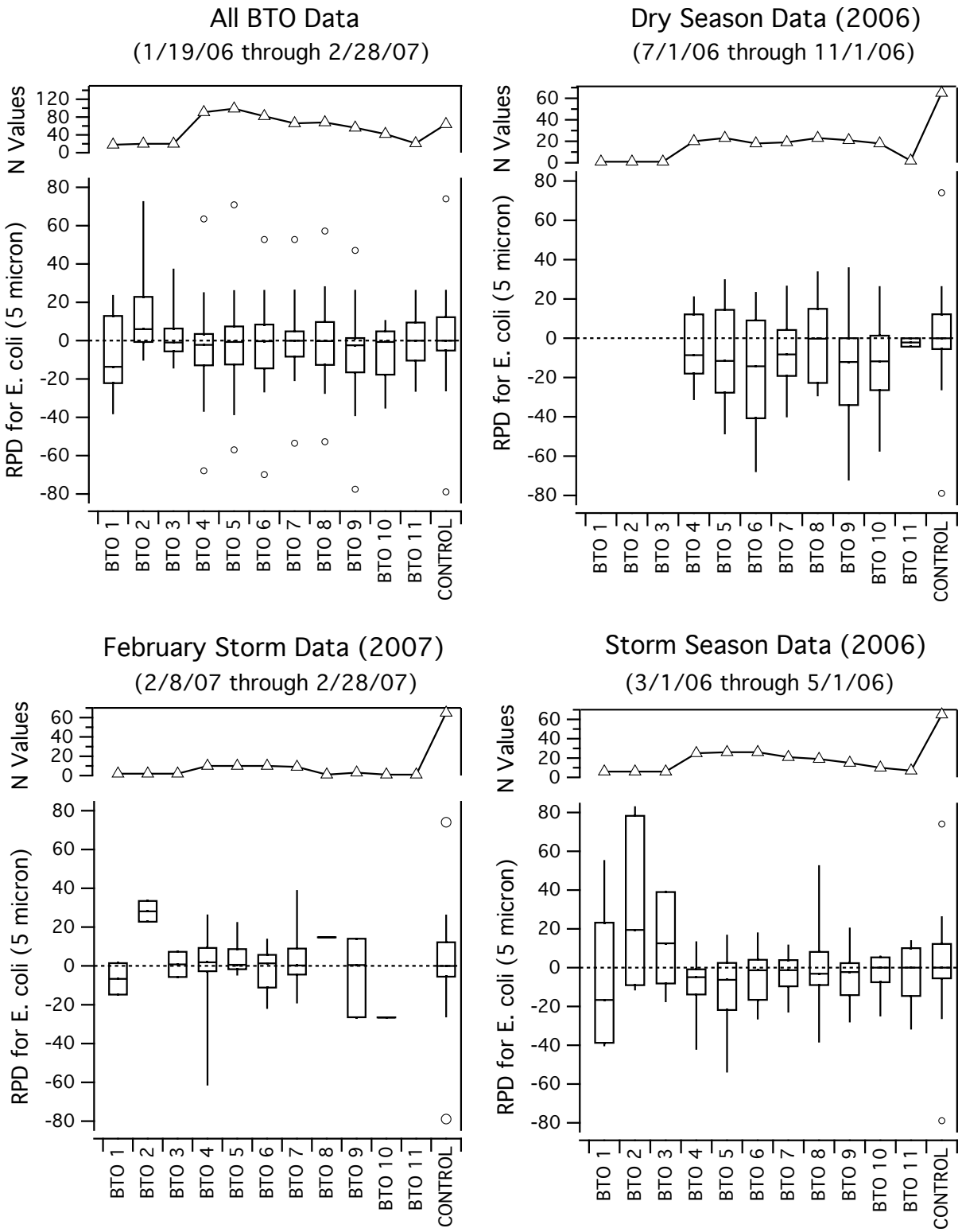

Figure 2-42. Relative percent difference (RPD) calculated from EC measurements on samples before and after filtration with a 5 micron pore-size filter. Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown are the RPD values for the negative control (see text).

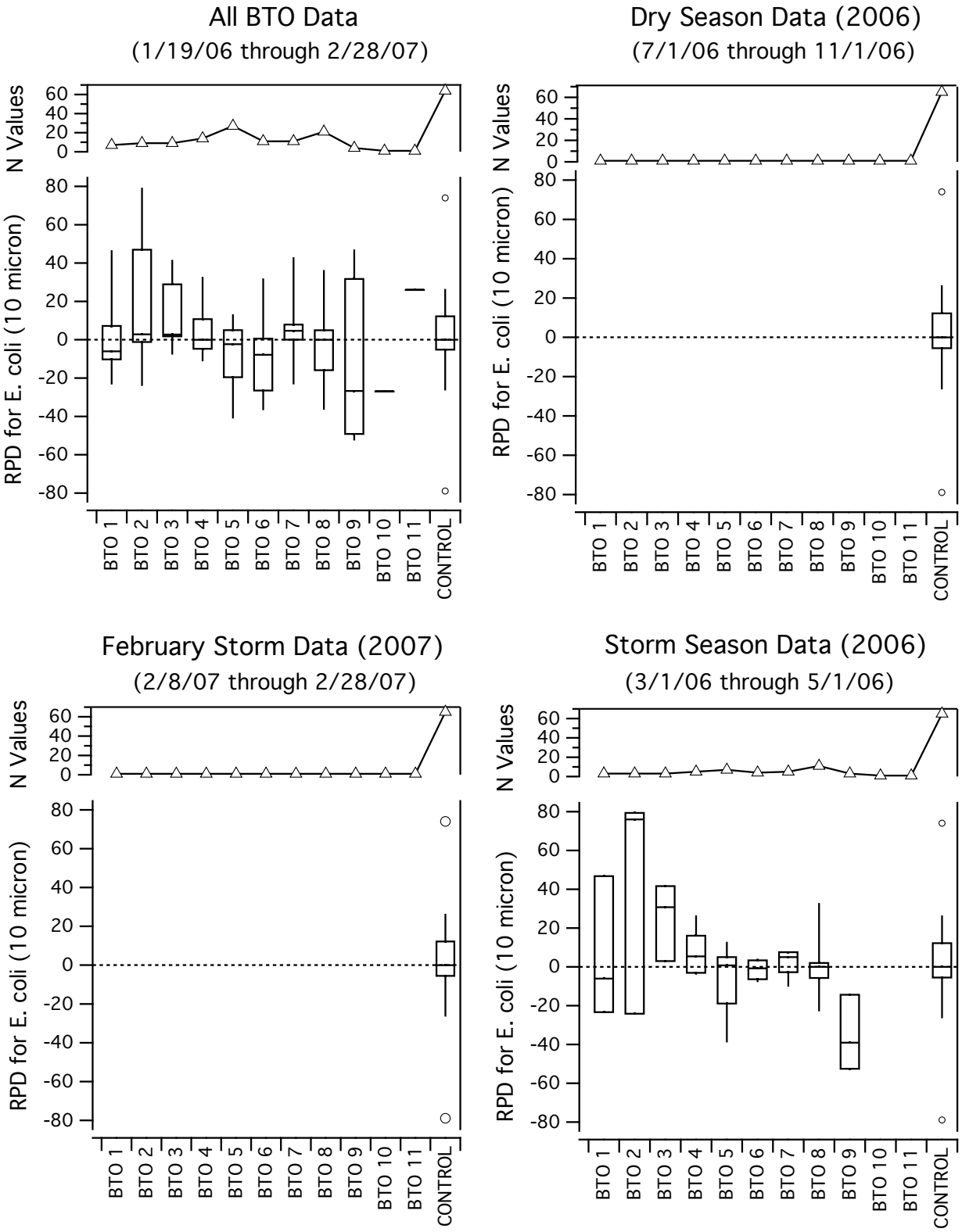

Figure 2-43. Relative percent difference (RPD) calculated from EC measurements on samples before and after filtration with a 10 micron pore-size filter. Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown are the RPD values for the negative control (see text).

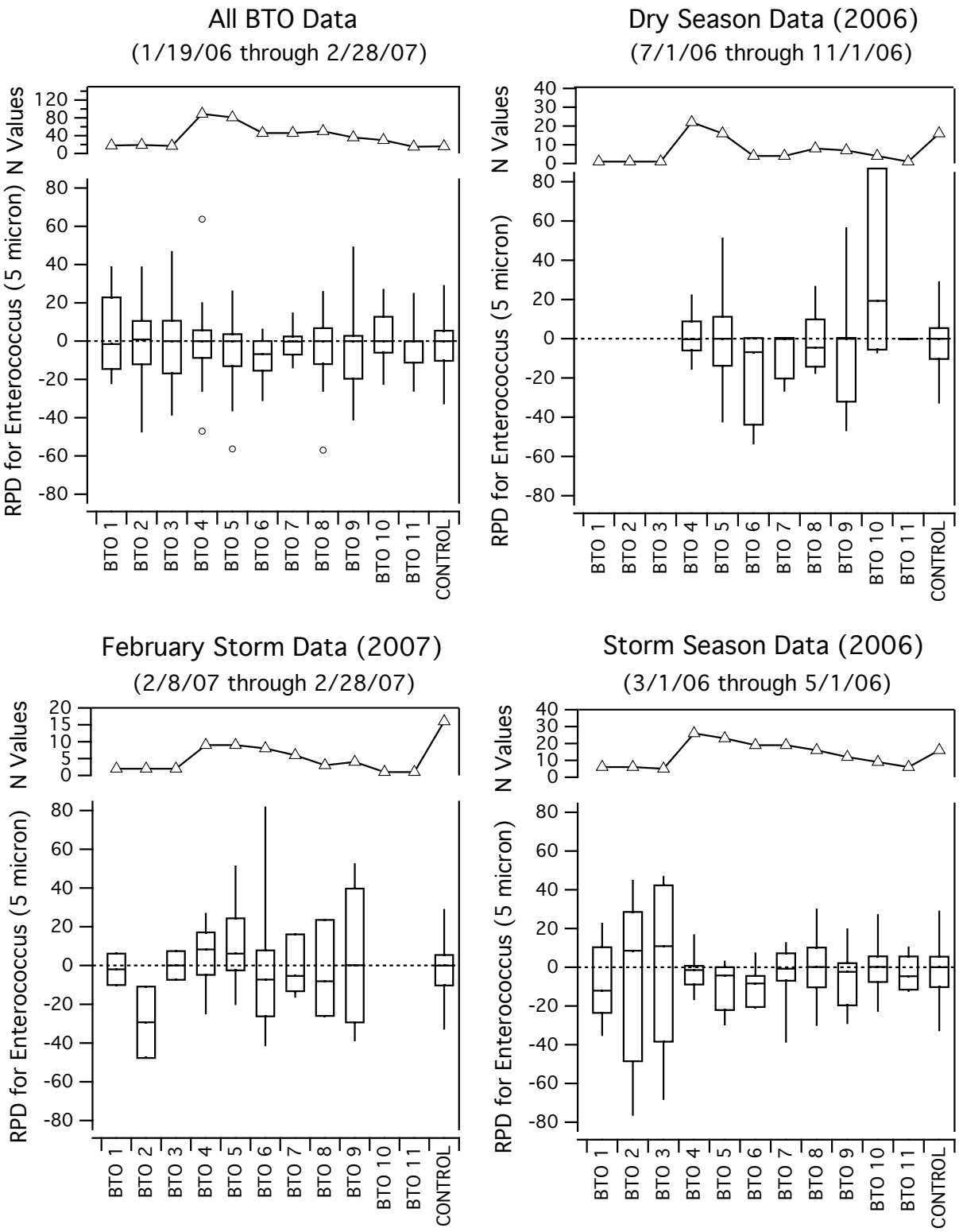

Figure 2-44. Relative percent difference (RPD) calculated from ENT measurements on samples before and after filtration with a 5 micron pore-size filter. Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown are the RPD values for the negative control (see text).

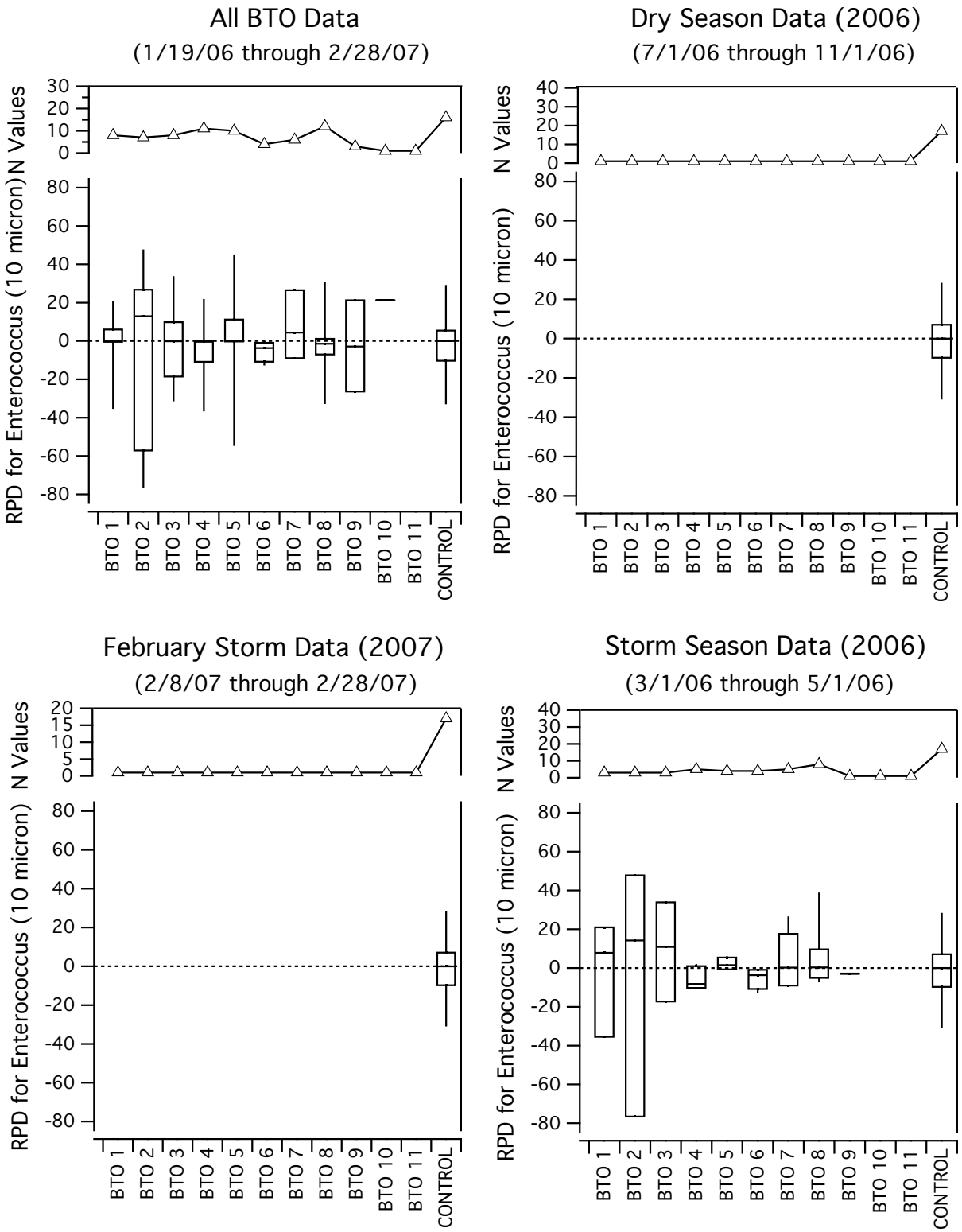

Figure 2-45. Relative percent difference (RPD) calculated from ENT measurements on samples before and after filtration with a 10 micron pore-size filter. Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown are the RPD values for the negative control (see text).

2.3.10.3 Potential Artifacts with the FIB Filtration Assay.

It is important to note that even when the concentration of FIB in a sample is the same before and after filtration, we cannot rule out the possibility that at least some FIB in the sample are associated with large, but relatively rare, particles. Because the concentration of particles in a water sample typically declines like a power-law of particle size[30], small particles are typically orders-of-magnitude more abundant than larger aggregates. If the concentration of free-living cells is much greater than the concentration of FIB-harboring aggregates, then removal of the latter by passage through a 5-micron filter will have a negligible impact on the FIB concentration in a sample.

For example, in a recently published paper, Planchera and Cohen reported that, for water samples collected from a creek in Hawaii, ENT concentration was unchanged before and after filtration through a 5 micron pore-sized filter[31]. However, when particles captured on the 5-micron filter were resuspended and disaggregated, ENT could be cultured off of the retained aggregates. The authors noted that these two apparently contradictory results can be reconciled if particles greater than 5 microns are relatively rare, but these large particles harbor, on average, > 5 ENT cells per particle. A similar situation might very well exist in Newport Bay. In any case, within the resolution of the assay utilized in this study, it appears that, with the exception of EC at within-Bay sites during dry weather, the majority of culturable FIB in Newport Bay are present as free-living cells. It is interesting to note that modeling results presented in Chapter 8 suggest that EC concentrations in Upper Bay during dry weather cannot be accounted for by loading of EC from San Diego Creek and Santa Ana Delhi channel. Based on the filtration results presented here, it is possible that the "extra source" of EC during dry weather periods is resuspension of particle-associated EC from near the sediment bed (see discussion of nephloid layer in the next section).

2.3.11. Seasonal and Down-Bay trends of FIB in Sediments Collected from Creek Tributaries and Within Bay Sites in Newport Bay.

The concentration of FIB in Newport Bay sediments is summarized in Tables 2-7 and 2-8, and displayed graphically in Figs. 2-46 and 2-47. During wet weather, EC concentrations are highest in sediment collected from one or more tributaries (BTO 1 through 3), followed by sediment collected from Upper Bay (BTO 4 through 7), followed by sediment collected from Lower Bay (BTO 8 through 11) (lower right and left panels, Fig. 2-46). During dry weather, EC concentrations are higher in sediments collected from the tributaries (although note the relatively low N values, upper right panel, Fig. 2-46), and very low in most sediment samples collected from within-Bay sediments. This last observation should be considered in light of two results presented earlier in the report: (1) during dry weather, EC concentrations in Upper Bay are often higher at the bottom of the water column and lower at the surface of the water column (Fig. 2-27) and (2) during dry weather, a measureable fraction of EC in the Bay appears to be particle-associated

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[30] Bader, H. (1970) "The hyperbolic distribution of particle sizes" *J.Geophys. Res*. **75** (15): 2822–2830.
[31] Plancherel, Y., and Cowen, J.P. (2007) "Towards measuring particle-associated fecal indicator bacteria in tropical streams" *Water Research* v. **41**: 1501-1515.

(upper right panel, Fig. 2-42). One interpretation consistent with these collective results is that, during dry weather, particle-associated EC settle near the bottom of the water column, in effect forming an EC nepheloid layer. In any case, EC do not appear to reside, to any appreciable degree, in the Bay's bottom sediments during dry weather periods.

The spatial and seasonal patterns for ENT in sediments are displayed in Fig. 2-47. When all of the BTO sediment data are combined (upper left panel, Fig. 2-47), ENT concentrations are highest in sediments collected from Lower Bay (BTO 8 through 10), followed by sediments collected from Upper Bay and the three tributaries (BTO 1 through 7), followed by the site nearest the ocean outlet (BTO 11). Apart from the tributaries, which have relatively low N values (typically N<10), the spatial pattern just described applies to both dry weather (upper right panel, Fig. 2-47) and wet weather (lower right and left panels, Fig. 2-47) periods.

A comparison of FIB measured in the water column and in sediments yields the following assessment (compare Figs. 2-46 and 2-47 with Figs. 2-18 and 2-19):

- All BTO Data: EC concentrations in the water column and sub-tidal sediments both decrease with distance down-Bay. ENT concentrations in the water column and sub-tidal sediments decrease and increase, respectively, with distance down-Bay.
- Dry Season Data (2006): EC concentrations in the water column and sediment are low and relatively constant at most within-Bay sites. ENT concentrations in the water column and sub-tidal sediments decrease and increase, respectively, with distance down-Bay.
- Wet Season Data (2006): EC concentrations in the water column and sub-tidal sediments decrease with distance down-Bay. ENT concentrations in the water column and sub-tidal sediments decrease and stay relatively constant, respectively, with distance down-Bay.
- February Storm Data (2007): The N values are too low (N<10) at individual BTO sites to assess down-Bay trends of EC and ENT in sub-tidal sediments. In the water column, both EC and ENT decrease with distance down-Bay.

2.3.12. Seasonal and Down-Bay trends in Percent Organic Carbon, Percent Water Content, and Average Grain Size of Sediments Collected from Creek Tributaries and Within Bay Sites in Newport Bay.

The percent organic carbon, percent water content, and average grain size of sediments collected from Newport Bay and its tributaries are presented in Figs. 2-48 through 2-50. Among tributaries, sediment samples collected from the Santa Ana Delhi channel (BTO 2) have the highest median organic carbon content (ca., 8%, Fig. 2-48) and the highest median water content (ca., 47%, Fig. 2-49)[32]. Within error, the average grain size is the same at all three tributary sites (ca., 25 microns, Fig. 2-50). Organic carbon and water

[32] The one exception is the Dry Season (2006) results, for which Big Canyon wash has a higher water content.

Table 2-7. ENT measurement statistics for sediment samples collected from tributaries (BTO 1-3), and within-Bay sites (BTO 4-11).

Sampling Site	***Study Period[1]***	***N***	***Log-mean (std)[2]***	***Minimum (MPN/10 g)[5]***	***Maximum (MPN/10 g)[6]***
BTO 1	All Data	35	3.1 (0.8)	16	39000
	2006 Dry Season	9	3.0 (0.7)	200	31000
	2006 Wet Season	7	3.1 (1.2)	16	34000
	2007 Feb. Storm	7	3.9 (0.7)	440	39000
BTO 2	All Data	35	2.7 (1)	1.4	50000
	2006 Dry Season	9	3.2 (0.8)	330	50000
	2006 Wet Season	7	1.8 (1.2)	1.5	900
	2007 Feb. Storm	7	3.7 (0.9)	190	50000
BTO 3	All Data	27	2.1 (1)	1.2	40000
	2006 Dry Season	6	2.0 (1)	1.5	37000
	2006 Wet Season	7	2.7 (1.9)	1.5	40000
	2007 Feb. Storm	2	1.8 (0.9)	15	250
BTO 4	All Data	90	2.4 (1)	2.2	85000
	2006 Dry Season	33	1.4 (1)	2.2	8300
	2006 Wet Season	17	3.3 (0.9)	30	85000
	2007 Feb. Storm	8	3.1 (0.6)	190	17000
BTO 5	All Data	89	2.8 (1)	1.8	120000
	2006 Dry Season	33	3.0 (2)	1.8	67000
	2006 Wet Season	17	2.6 (0.9)	16	120000
	2007 Feb. Storm	8	4.1 (0.3)	5700	45000
BTO 6	All Data	86	2.9 (1)	1.3	42000
	2006 Dry Season	33	3.0 (1)	1.6	42000
	2006 Wet Season	16	3.0 (0.8)	100	13000
	2007 Feb. Storm	8	3.8 (0.9)	53	40000
BTO 7	All Data	73	3.1 (1)	1.3	55000
	2006 Dry Season	33	3.1 (1)	1.3	55000
	2006 Wet Season	11	3.2 (0.6)	68	7000
	2007 Feb. Storm	5	4.1 (0.3)	5000	32000
BTO 8	All Data	79	3.6 (1)	2.9	85000
	2006 Dry Season	33	3.4 (1)	2.9	71000
	2006 Wet Season	14	4.0 (1.0)	260	85000
	2007 Feb. Storm	8	4.0 (0.9)	100	65000
BTO 9	All Data	80	3.6 (0.9)	38	59000
	2006 Dry Season	33	3.8 (0.9)	140	57000
	2006 Wet Season	14	3.1 (0.7)	45	41000
	2007 Feb. Storm	8	4.0 (1)	38	44000
BTO 10	All Data	82	3.4 (0.7)	40	53000
	2006 Dry Season	33	3.5 (0.7)	63	46000
	2006 Wet Season	17	3.4 (0.8)	400	47000
	2007 Feb. Storm	8	3.8 (0.5)	890	37000
BTO 11	All Data	82	1.6 (1)	1.2	34000
	2006 Dry Season	33	1.3 (0.8)	1.3	1300
	2006 Wet Season	17	2.0 (1.5)	1.2	34000
	2007 Feb. Storm	8	1.6 (1)	1.3	920
BTO 12	All Data	0			
	2006 Dry Season	0			
	2006 Wet Season	0			
	2007 Feb. Storm	0			

[1]Denotes samples collected over the following time intervals. All Data: 1/19/06 through 2/28/07; 2006 Dry Season: 7/1/06 through 11/1/06; 2006 Wet Season: 3/1/06 through 5/1/06; 2007 Feb. Storm: 2/8/07 through 2/28/07.
[2]Mean and standard deviation of the log-transformed enterococci bacteria concentration, in units of MPN/10 g dry weight sediment.
[3]Minimum concentration of enterococci bacteria measured in samples collected from a particular site over a particular time.
[4]Maximum concentration of enterococci bacteria measured in samples collected from a particular site over a particular time.

Table 2-8. EC measurement statistics for sediment samples collected from tributaries (BTO 1-3) and within-Bay sites (BTO 4-11) in Newport Bay.

Sampling Site	***Study Period[1]***	***N***	***Log-mean (std)[2]***	***Minimum (MPN/100 mL)[5]***	***Maximum (MPN/100 mL)[6]***
BTO 1	All Data	35	2.5 (1)	1.3	11000
	2006 Dry Season	9	1.3 (1)	1.3	3300
	2006 Wet Season	7	3.1 (0.6)	200	11000
	2007 Feb. Storm	7	2.8 (0.7)	39	7100
BTO 2	All Data	35	1.8 (1)	1.3	23000
	2006 Dry Season	9	1.4 (1)	1.3	1000
	2006 Wet Season	7	2.2 (0.4)	44	490
	2007 Feb. Storm	7	2.4 (1)	1.3	23000
BTO 3	All Data	27	2.6 (1)	1.3	41000
	2006 Dry Season	6	2.9 (1)	80	41000
	2006 Wet Season	7	3.0 (0.9)	15	4500
	2007 Feb. Storm	2	2.5 (0.4)	170	590
BTO 4	All Data	90	1.5 (1)	1.6	8700
	2006 Dry Season	33	0.67 (0.6)	1.6	220
	2006 Wet Season	17	2.9 (0.9)	4.2	8700
	2007 Feb. Storm	8	1.0 (0.8)	2.3	350
BTO 5	All Data	89	1.0 (0.9)	1.5	2400
	2006 Dry Season	33	0.54 (0.5)	1.7	310
	2006 Wet Season	17	2.1 (0.8)	1.5	2400
	2007 Feb. Storm	8	1.1 (1)	1.7	970
BTO 6	All Data	86	0.81 (0.9)	1.3	2200
	2006 Dry Season	33	0.48 (0.7)	1.3	1000
	2006 Wet Season	16	1.9 (1)	1.4	2200
	2007 Feb. Storm	8	0.75 (0.7)	1.3	100
BTO 7	All Data	72	0.76 (0.9)	1.3	4200
	2006 Dry Season	32	0.44 (0.4)	1.3	22
	2006 Wet Season	11	2.3 (1)	1.7	4200
	2007 Feb. Storm	5	0.19 (0.03)	1.4	1.7
BTO 8	All Data	78	0.48 (0.2)	2	33
	2006 Dry Season	33	0.45 (0.2)	2	33
	2006 Wet Season	13	0.54 (0.3)	2.1	30
	2007 Feb. Storm	8	0.47 (0.04)	2.6	3.4
BTO 9	All Data	80	0.90 (0.8)	1.3	1200
	2006 Dry Season	33	0.68 (0.6)	1.4	76
	2006 Wet Season	14	1.5 (1)	1.4	1200
	2007 Feb. Storm	8	0.51 (0.4)	1.7	18
BTO 10	All Data	82	0.69 (0.7)	1.3	940
	2006 Dry Season	33	0.56 (0.6)	1.3	130
	2006 Wet Season	17	1.1 (0.9)	1.6	300
	2007 Feb. Storm	8	0.49 (0.5)	1.5	33
BTO 11	All Data	82	0.47 (0.7)	1.2	9700
	2006 Dry Season	33	0.27 (0.5)	1.3	190
	2006 Wet Season	17	0.61 (0.7)	1.2	123
	2007 Feb. Storm	8	1.2 (1.5)	1.3	9700

[1]Denotes samples collected over the following time intervals. All Data: 1/19/06 through 2/28/07; 2006 Dry Season: 7/1/06 through 11/1/06; 2006 Wet Season: 3/1/06 through 5/1/06; 2007 Feb. Storm: 2/8/07 through 2/28/07.
[2]Mean and standard deviation of the log-transformed *E. coli* concentration, in units of MPN/10 g dry weight sediment. Note that censored data were included in the mean and standard deviation calculations (see definition of censored data in footnotes 5 and 6).
[3]Minimum concentration of *E. coli* bacteria measured in samples collected from a particular site over a particular time.
[4]Maximum concentration of *E. coli* bacteria measured in samples collected from a particular site over a particular time.

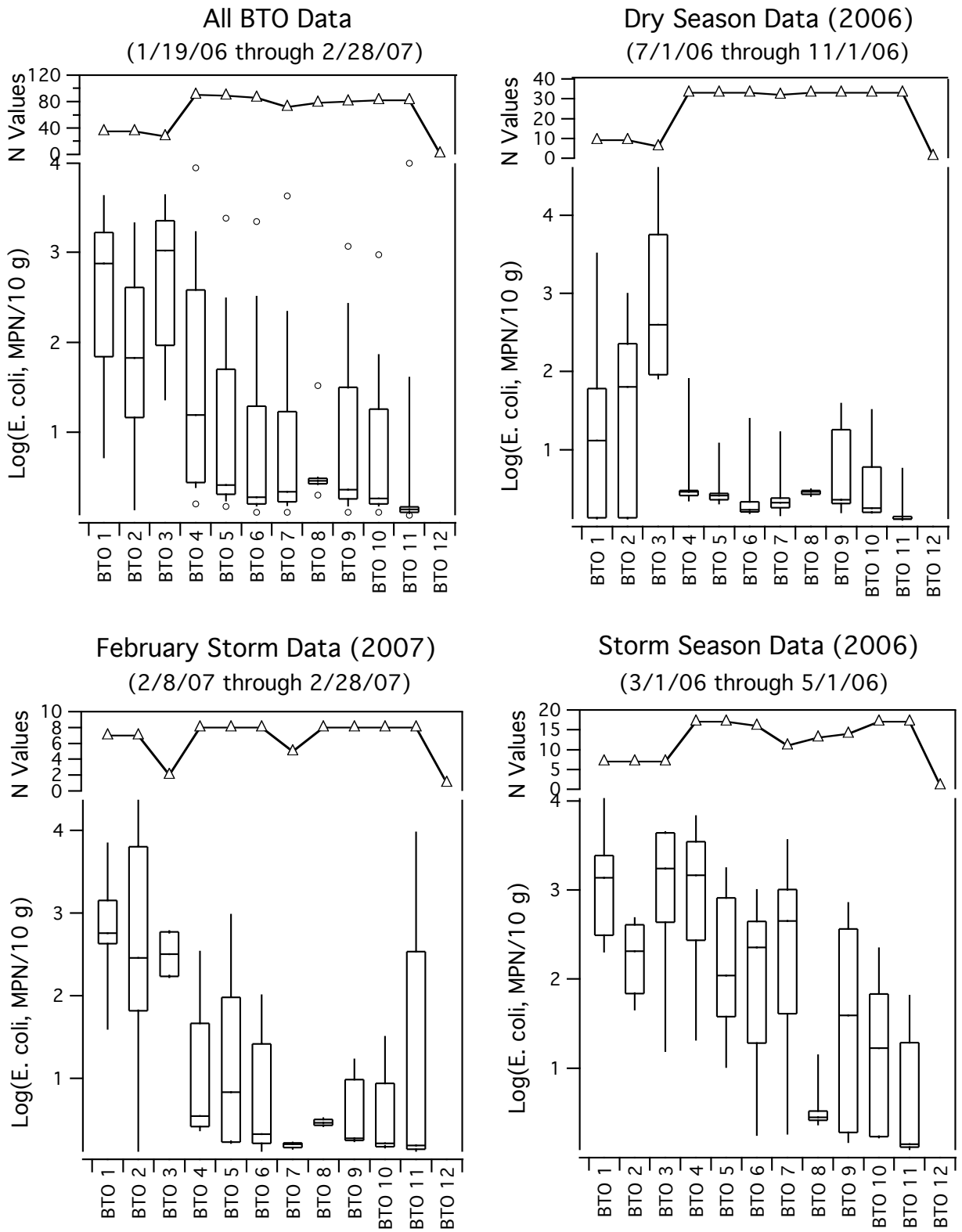

Figure 2-46. EC measured in sediment samples collected from tributary (BTO 1-3), within-Bay sites (BTO sites 4-11), and the offshore control site (BTO 12). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

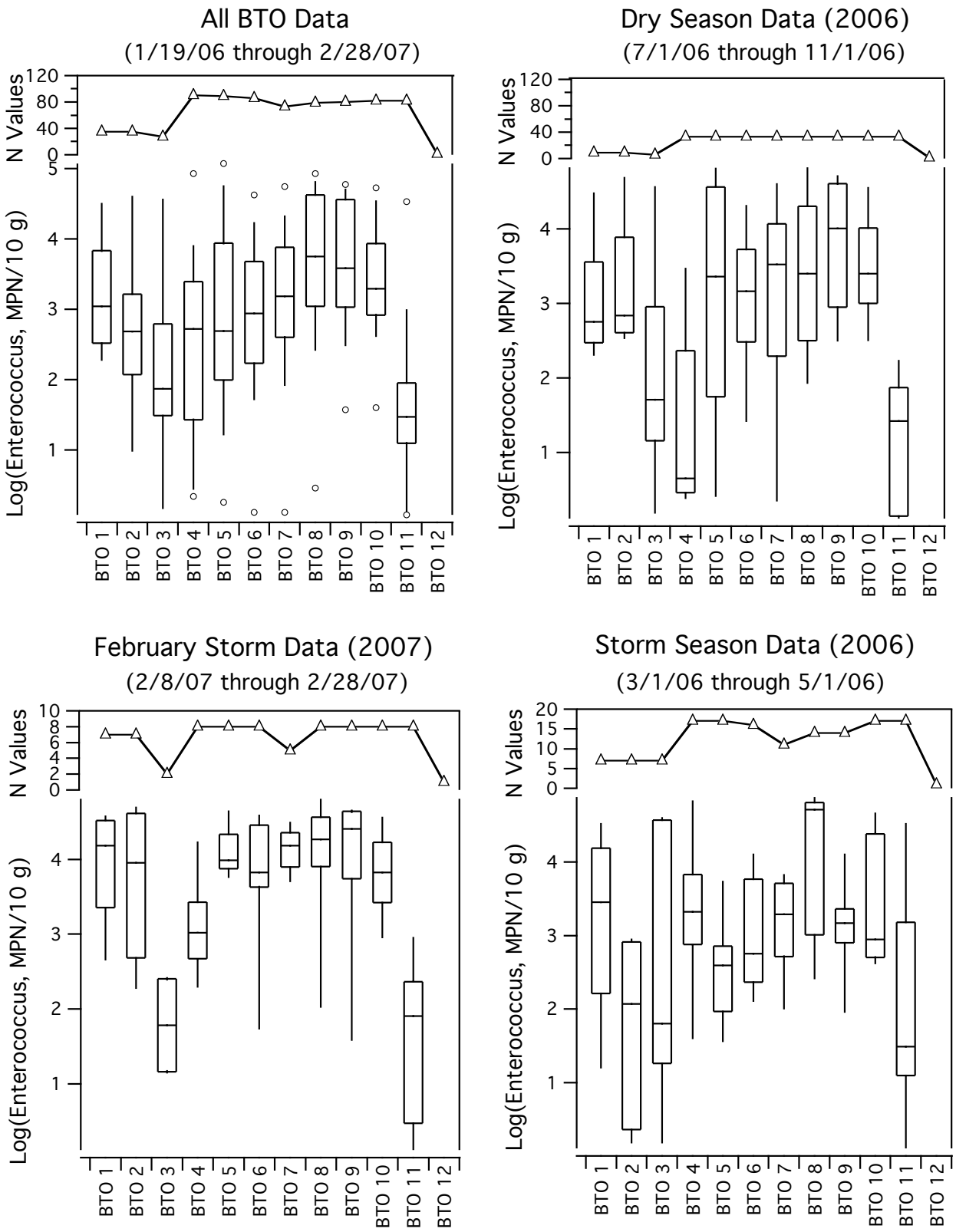

Figure 2-47. ENT measured in sediment samples collected from tributary (BTO 1-3), within-Bay sites (BTO sites 4-11), and the offshore control site (BTO 12). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

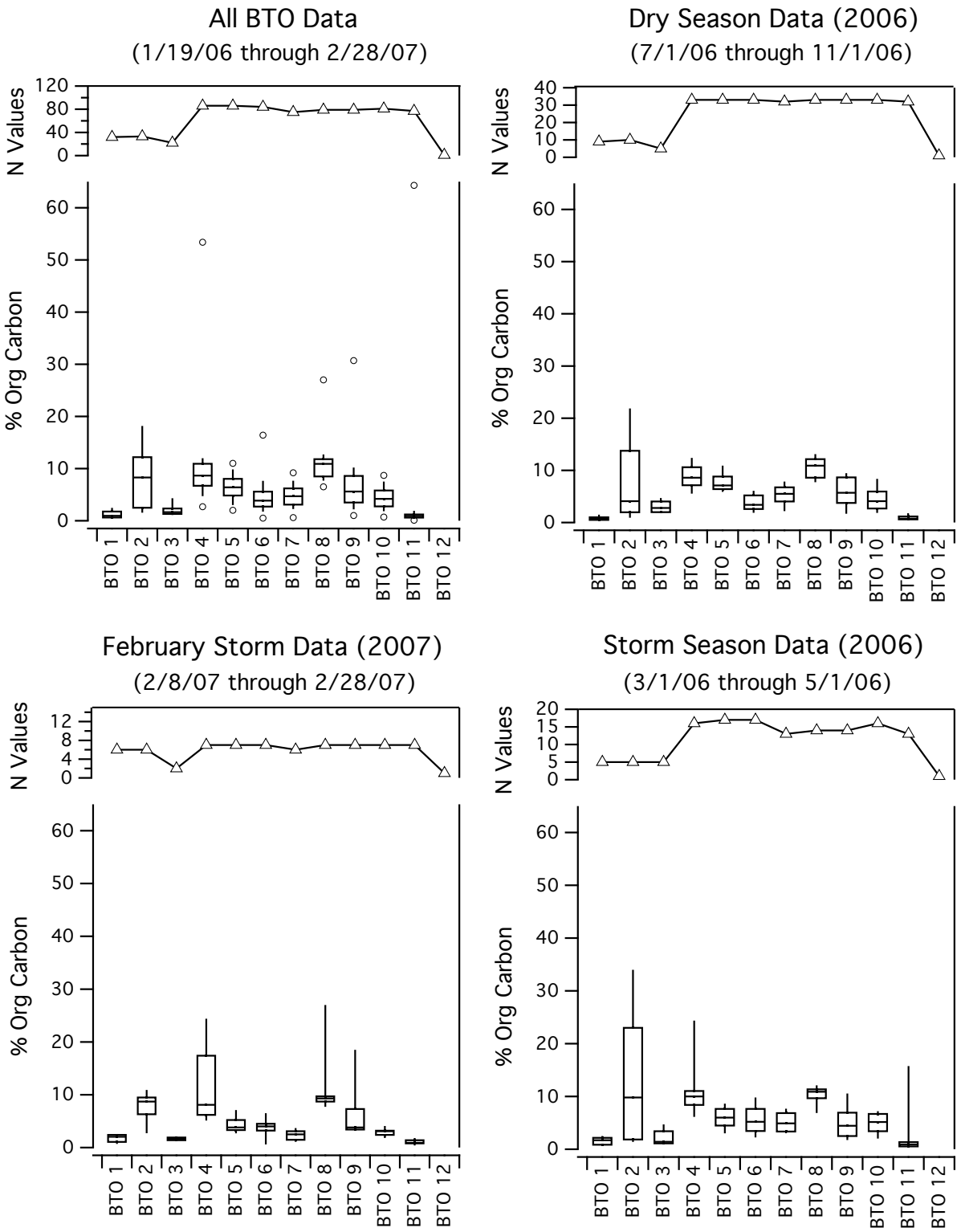

Figure 2-48. Percent organic carbon measured in sediment samples collected from tributary (BTO 1-3), within-Bay sites (BTO sites 4-11), and the offshore control site (BTO 12). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

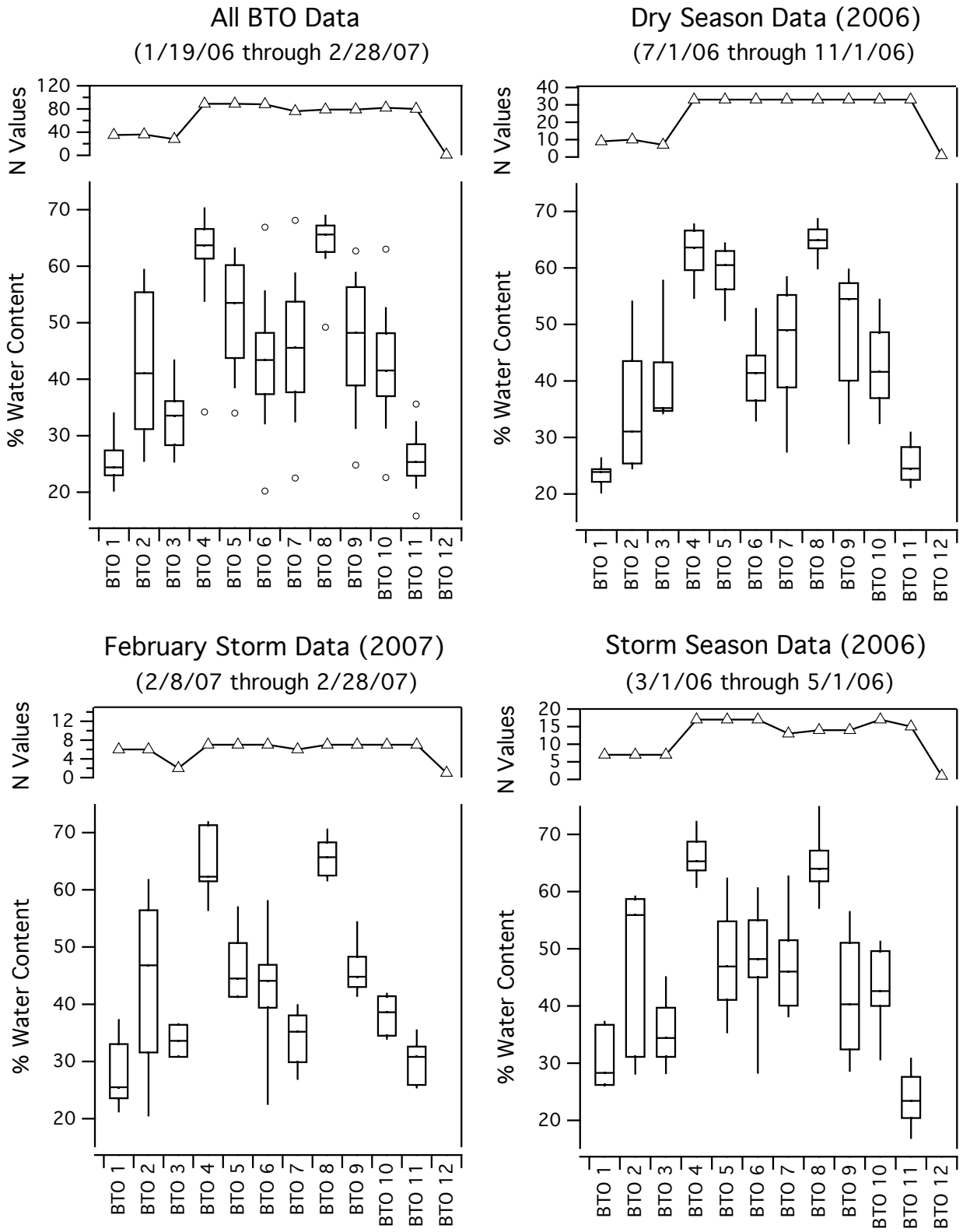

Figure 2-49. Percent water content measured in sediment samples collected from tributary (BTO 1-3), within-Bay sites (BTO sites 4-11), and the offshore control site (BTO 12). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

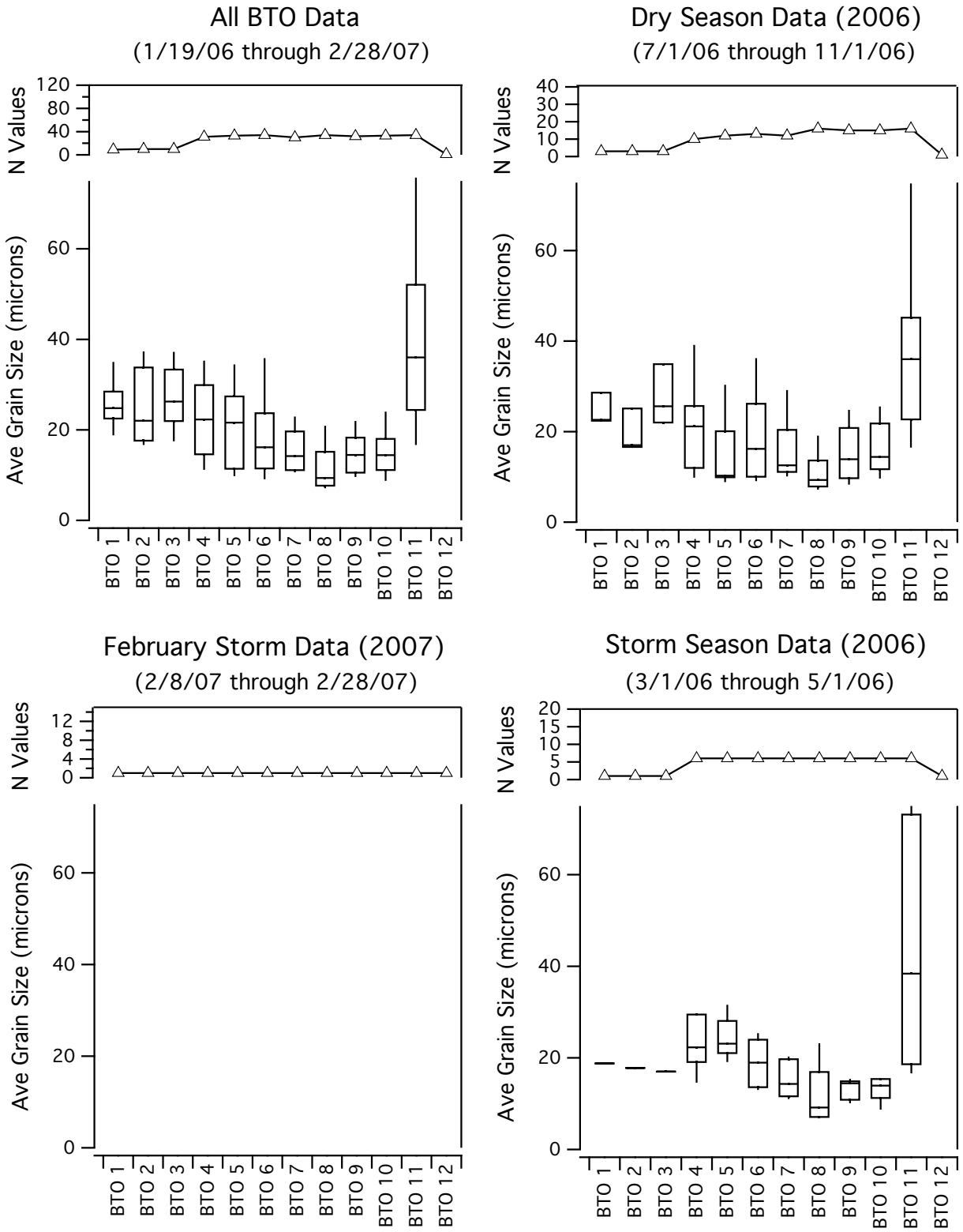

Figure 2-50. Average grain size measured in sediment samples collected from tributary (BTO 1-3), within-Bay sites (BTO sites 4-11), and the offshore control site (BTO 12). Elements of each box represent the median value (middle horizontal line), 25th and 75th percentiles (bottom and top of box), 10th and 90th percentiles (whiskers), and <1 or >99th percentile (circles). Also shown is the number (N values) of measurements collected at each site during the time period indicated.

content are higher (ca., 10% and 65%, respectively) in sediments collected from regions of the Bay that experience less tidal flushing (BTO 4 in Upper Bay and BTO 8 in Western Bay). Median organic carbon and water content are lower (ca., 5% and 45%, respectively) in regions of the Bay that experience better flushing (BTO 6, 7, 9, and 10). Over most of the Bay, average sediment grain size decreases down-Bay, from 25 microns in the tributaries to 17 microns in Lower Bay. The average sediment grain size near the ocean outlet, however, is much higher (ca., 70 microns), perhaps reflecting the tidal input of sand-sized sediments from the open coastline (Fig. 2-50).

2.3.13. Sediment Sample Analyses: Time Series of Cross-Sectional Average and Cross-Sectional Deviations

Each time the boat visited a within-Bay site, sediment samples were collected from three locations in the channel cross section: left bank, right bank, and the deepest point in the tidal channel (the thalweg)[33]. To determine if sediment measurements were consistently high or low at any particular location in the cross section, "deviations" from the cross-sectional average were calculated as follows:

$$\text{Deviation}_i = C_i - (\text{XS Average})$$

$$(\text{XS Average}) = \frac{1}{3}\left[C_L + C_M + C_R\right]$$

where the variables are:

C_L concentration in the left sediment sample
C_m concentration in the middle sediment sample
C_R concentration in the right sediment sample

If the deviation is positive, then the concentration at a particular location is greater than the cross-section average. Conversely, if the deviation is negative, then the concentration at a particular location is lower than the cross-section average.

The results of this analysis are summarized in a series of 32 figures (see Appendix 2F), one each for every combination of the eight within-Bay sites (BTO 4 through 11) and four sediment analytes (EC, ENT, organic carbon content, water content, and average grain size). Here, we present results for a subset of BTO sites representative of the three major geographical regions of the Bay: BTO 4 for Upper Bay, BTO 8 for West Lower Bay, and BTO 10 for East Lower Bay.

2.3.13.1. Sediment Cross-Section: Upper Bay (BTO 4)

[33] The designation "left" and "right" banks assumes that the observer is facing up-Bay (i.e., the direction the flood tide flows).

Sediment EC and ENT in Upper Bay. EC and ENT concentrations in Upper Bay sediment exhibit very strong seasonal variability, with higher concentrations measured during wet weather and lower concentrations measured during dry weather (see cross-sectional averages, Figs. 2-51 and 2-52). No cross-sectional trends are evident for EC, but ENT is consistently higher in sediment collected from the right bank at BTO 4 (see deviations, Figs. 2-51 and 2-52).

Sediment organic carbon, water content, and average grain size in Upper Bay. No cross-sectional trends are evident for organic carbon, water content, or average grain size measured in Upper Bay sediment (see pgs 28, 37, 46, Appendix 2F).

2.3.13.2. Sediment Cross-Section: West Lower Bay (BTO 8)

Sediment EC and ENT in West Lower Bay. EC concentrations measured in West Lower Bay sediment are very low in all but two sediment samples (Fig. 2-53). ENT concentrations in West Lower Bay sediment are high, and exhibit no obvious seasonal pattern (Fig. 2-54). Sediments collected from the left bank at BTO 8 frequently have higher ENT concentrations than sediments collected either the middle or right banks at the same site (Fig. 2-54). Note that the Arches drain discharges to the Bay on the right bank at BTO 8.

Sediment organic carbon, water content, and average grain size in West Lower Bay. No seasonal trends are evident for organic carbon, water content, and average grain size measured on sediment collected in West Lower Bay. While no cross-sectional trends are evident for organic carbon, water content and average grain size tend to be higher in sediments collected from the right bank of the channel at BTO 8 (see pgs 32, 41, 50, Appendix 2F).

2.3.13.3. Sediment Cross-Section: East Lower Bay (BTO 10)

Sediment EC and ENT in East Lower Bay. EC concentrations measured in East Lower Bay sediment are generally higher during storms and lower during dry periods, but no consistent cross-sectional pattern is evident (Fig. 2-55). ENT concentrations, on the other hand, display relatively little seasonal variation, but ENT concentrations are consistently higher in sediments collected from the right bank of the tidal channel (i.e., the Balboa Island side) (Fig. 2-56).

Sediment organic carbon, water content, and average grain size in East Lower Bay. No seasonal trends are evident for organic carbon, water content, and average grain size measured on sediment collected in East Lower Bay. However, organic carbon and water content are frequently higher in sediments collected from the right bank of the channel (i.e., the Balboa Island side of the channel) at BTO 10, and lower in sediments collected from the thalweg or left bank. Average grain size tends to be smaller on the right bank as well (see pgs 34, 43, 52, Appendix 2F).

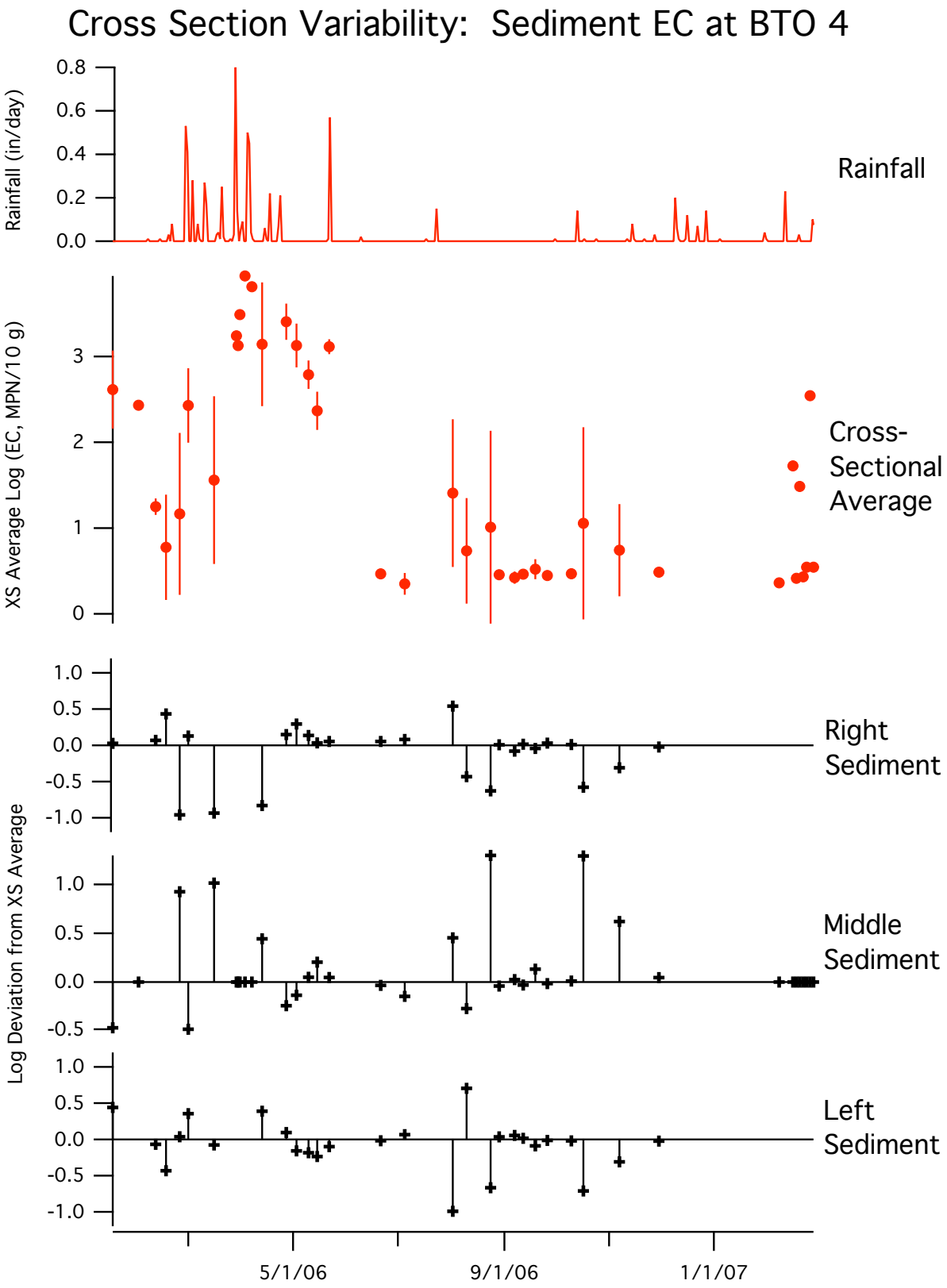

Figure 2-51. Variation of Log(EC) measured in the sediment as a function of time and position in the cross-section at BTO 4. Shown are rainfall history (top panel), cross-sectionally averaged Log(EC) (second panel), and the deviation of Log(EC) from the cross-sectional average at each of three cross-section locations (bottom five panels).

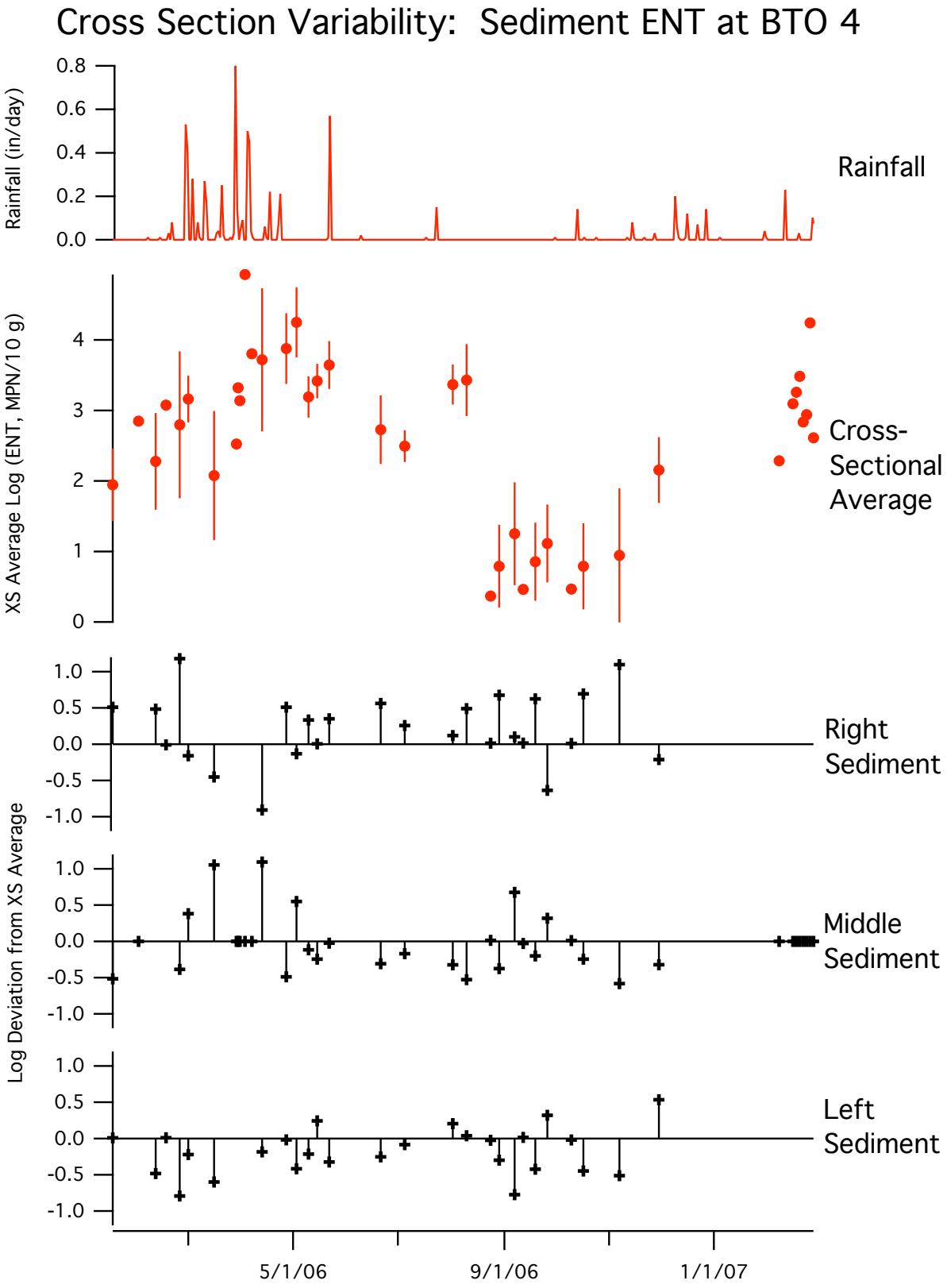

Figure 2-52. Variation of Log(ENT) measured in the sediment as a function of time and position in the cross-section at BTO 4. Shown are rainfall history (top panel), cross-sectionally averaged Log(ENT) (second panel), and the deviation of Log(ENT) from the cross-sectional average at each of three cross-section locations (bottom five panels).

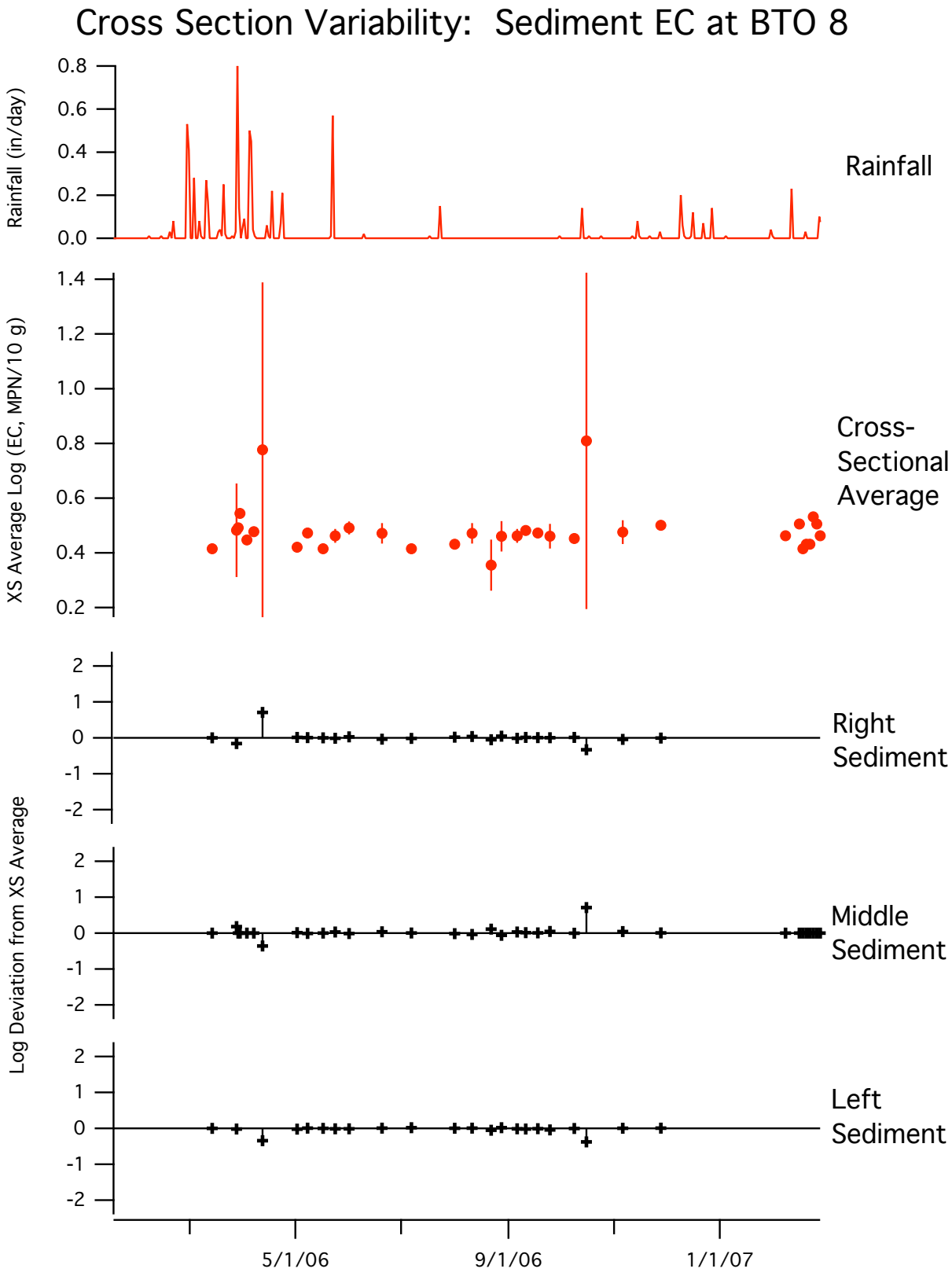

Figure 2-53. Variation of Log(EC) measured in the sediment as a function of time and position in the cross-section at BTO 8. Shown are rainfall history (top panel), cross-sectionally averaged Log(EC) (second panel), and the deviation of Log(EC) from the cross-sectional average at each of three cross-section locations (bottom five panels).

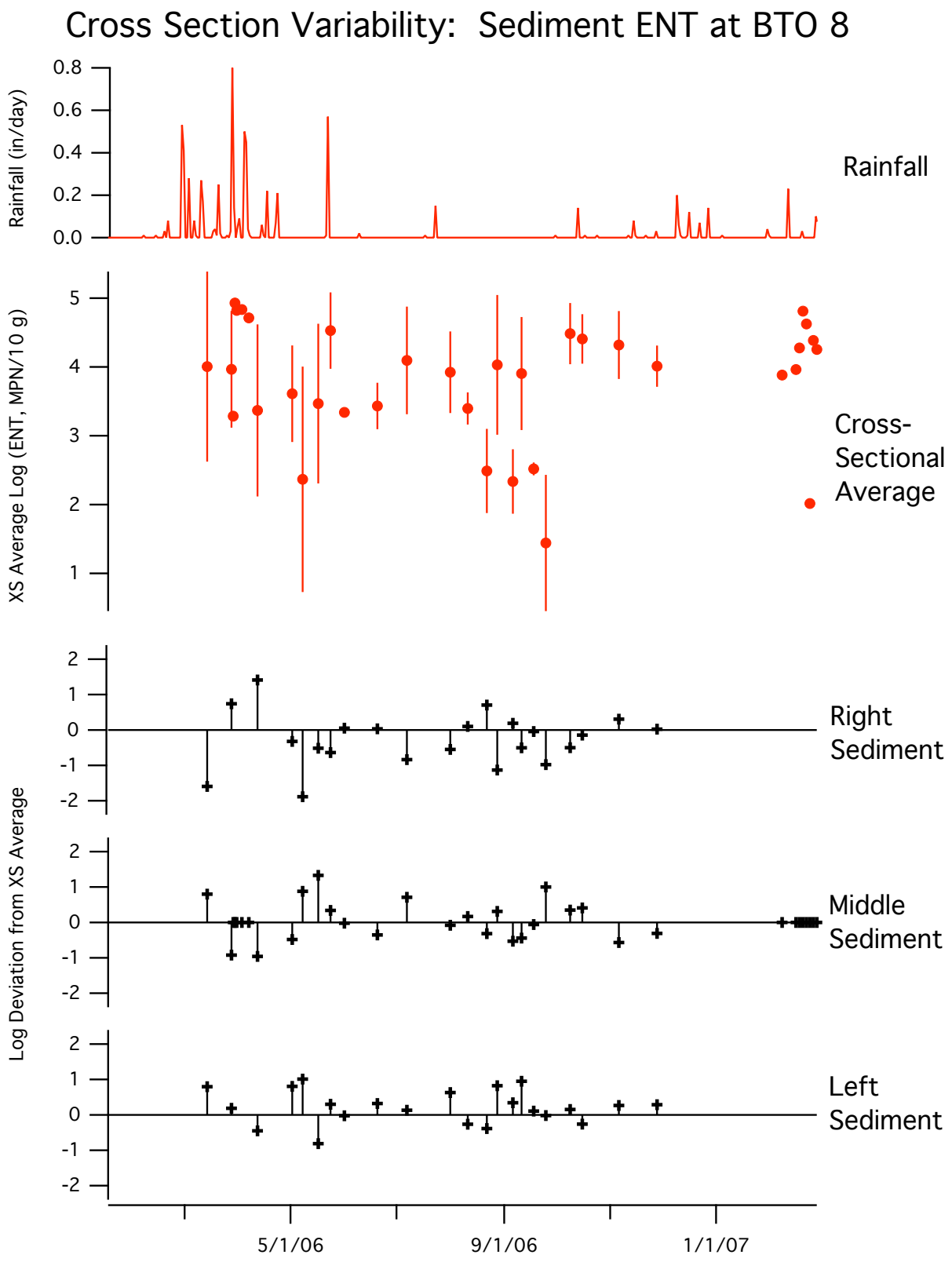

Figure 2-54. Variation of Log(ENT) measured in the sediment as a function of time and position in the cross-section at BTO 8. Shown are rainfall history (top panel), cross-sectionally averaged Log(ENT) (second panel), and the deviation of Log(ENT) from the cross-sectional average at each of three cross-section locations (bottom five panels).

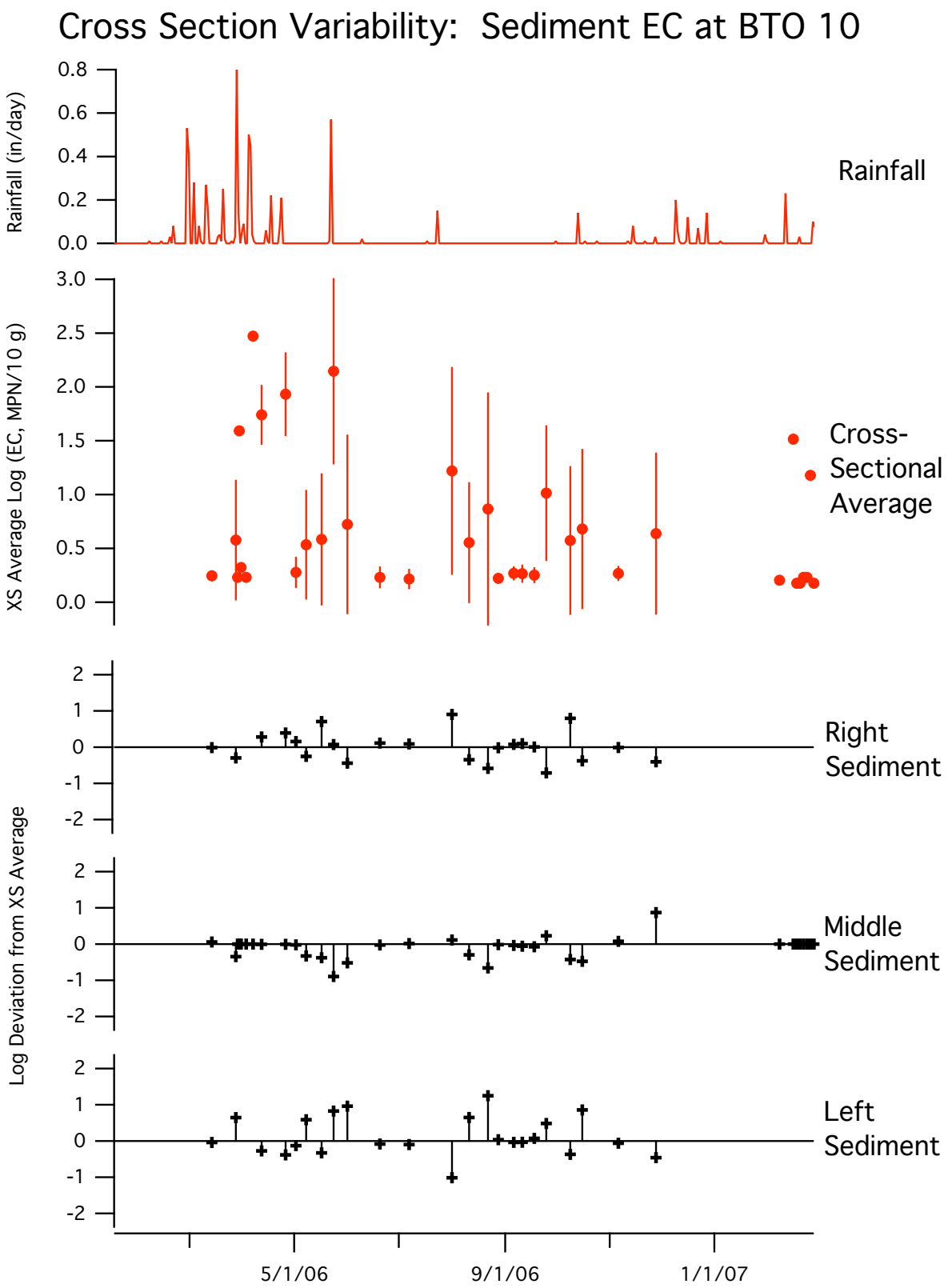

Figure 2-55. Variation of Log(EC) measured in the sediment as a function of time and position in the cross-section at BTO 10. Shown are rainfall history (top panel), cross-sectionally averaged Log(EC) (second panel), and the deviation of Log(EC) from the cross-sectional average at each of three cross-section locations (bottom five panels).

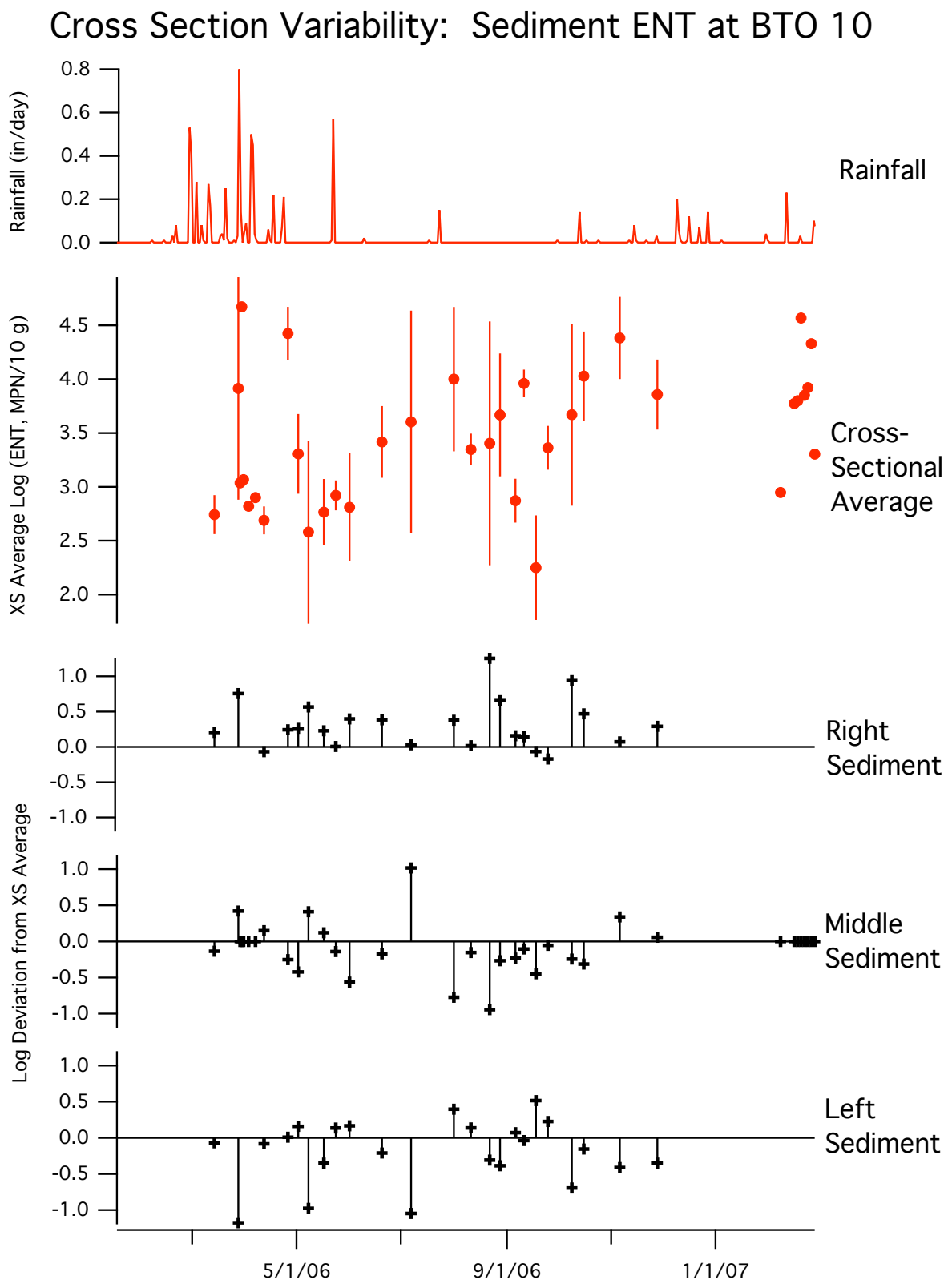

Figure 2-56. Variation of Log(ENT) measured in the sediment as a function of time and position in the cross-section at BTO 10. Shown are rainfall history (top panel), cross-sectionally averaged Log(ENT) (second panel), and the deviation of Log(ENT) from the cross-sectional average at each of three cross-section locations (bottom five panels).

2.3.14. Correlations between FIB concentrations in sediment and bottom water.

Based on the results presented in the last section, it appears that ENT is maintaining viability, and perhaps growing, in the sub-tidal sediments of Newport Bay. This begs the question: *are FIB in the sub-tidal sediments of Newport Bay a significant source of FIB pollution in the overlying water column?* As a first step toward answering this question, Spearman rank correlation coefficients were computed between FIB concentrations measured in the sediments at each BTO transect site, and the simultaneously measured FIB concentration in the overlying water.

The correlation analysis indicates that the concentrations of EC and ENT in the sediment are generally uncorrelated from the concentrations of these bacteria in the overlying water. For the creek data (Table 2-9), only a single correlation is deemed significant at $p<0.05$: at the Santa Ana Delhi channel site ENT measured in the sediment is significantly ($p=0.00$) and positively ($Sp = 0.6$) correlated with ENT measured in the overlying water column. For the within-Bay data (Table 2-10), only a single correlation is deemed significant at $p<0.05$: EC measured in the sediment and water column at the BTO 5 site are significantly ($p=0.04$) and negatively ($Sp = - 0.66$) correlated. If the statistical significance is set at $p<0.01$, FIB concentrations in the sediment and water are not significantly correlated at any within-Bay sampling sites.

The extent to which FIB concentrations in the sub-tidal sediment and overlying water column are uncorrelated is surprising, particularly given that ENT is present at high concentrations in the Bay sediments (see earlier discussion). These *sub-tidal sediment* results stand in contrast to the *intertidal sediment* results presented later in Chapter 6, which indicate that FIB are transported out of intertidal sediments into the water column during rising tides. However, as noted by one of the reviewers on this report, if sediment were an intermittent source of FIB to the overlying water column, for example triggered by tidal resuspension of sediments, FIB concentrations in the sub-tidal sediments might not be significantly correlated with FIB concentrations in the overlying water. Thus, from these results alone sub-tidal sediments cannot be ruled out as a source of FIB in the water column of Newport Bay.

Table 2-9. Spearman Rank Correlation between FIB concentrations in creek water and sediment. Asterisk denotes correlations that are statistically significant ($p<0.05$).

		EC in Creek Water vs EC in Creek Sediment	***ENT in Creek Water vs ENT in Creek Sediment***	***N***
BTO1	Correlation Coefficient	0.27	-0.02	20
	Sig. (2-tailed)	0.25	0.94	
BTO2	Correlation Coefficient	0.04	0.60*	22
	Sig. (2-tailed)	0.85	0.00	
BTO3	Correlation Coefficient	-0.21	-0.04	19
	Sig. (2-tailed)	0.39	0.87	

Table 2-10. Summary of Spearman Rank Correlation tests carried out to assess if sub-tidal sediments affect the concentration of FIB in bottom water (i.e., water collected just above the bed). Entries where the correlation is statistically significant (p<0.05) are denoted with an asterisk.

		EC in Bottom Water vs EC in Sediment	***ENT in Bottom Water vs ENT in Sediment***	***N***
BTO4	Correlation Coefficient	-0.16	0.38	8
	Sig. (2-tailed)	0.71	0.35	
BTO5	Correlation Coefficient	-0.66*	0.31	10
	Sig. (2-tailed)	0.04	0.38	
BTO6	Correlation Coefficient	0.07	0.36	11
	Sig. (2-tailed)	0.85	0.27	
BTO7	Correlation Coefficient	0.23	-0.03	10
	Sig. (2-tailed)	0.53	0.93	
BTO8	Correlation Coefficient	-0.56	0.55	10
	Sig. (2-tailed)	0.09	0.10	
BTO9	Correlation Coefficient	-0.38	0.00	11
	Sig. (2-tailed)	0.25	1.00	
BTO10	Correlation Coefficient	0.00	-0.29	12
	Sig. (2-tailed)	1.00	0.36	
BTO11	Correlation Coefficient	-0.16	0.53	11
	Sig. (2-tailed)	0.63	0.10	

Table 2-11. Spearman Rank Correlations between FIB concentrations, Organic Carbon, Water Content, and Average Grain Size in the sediments. All sediment data collected from the BTO transect study are included.

		Organic C	***Water Content***	***Ave. Size (um)***
EC	Correlation Coefficient	0.485*	0.605*	-0.182*
	Sig. (2-tailed)	0	0	0.003
	N	258	260	260
ENT	Correlation Coefficient	0.317*	0.313*	-0.255*
	Sig. (2-tailed)	0	0	0
	N	258	260	260

Table 2-12. Spearman rank correlation between FIB and nutrients measured in water samples collected from all Upper Bay sites (BTO 4-7). Entries with statistically significant correlations (p<0.05) are denoted with an asterisk.

FIB Group		***Ammonia***	***TKN***	***Nitrate***	***Total P***	***TOC***	***TON***
EC	Correlation Coefficient	0.513*	-0.042	0.517*	0.287*	0.333	0.943*
	Sig. (2-tailed)	0.021	0.823	0.023	0.117	0.067	0.005
	N	20	31	19	31	31	6
ENT	Correlation Coefficient	0.444*	0.344	0.141	0.209	0.464*	0.677
	Sig. (2-tailed)	0.050	0.054	0.565	0.25	0.007	0.140
	N	20	32	19	32	32	6

Table 2-13. Spearman rank correlation between FIB and nutrients measured at all Lower Bay sites (BTO 8-11). Entries with statistically significant correlations (p<0.05) are denoted with an asterisk.

FIB Group		**Ammonia**	**TKN**	**Nitrate**	**Total P**	**TOC**	**TON**
EC	Correlation Coefficient	0.088	-0.155	-0.584	-0.405*	0.040	0.500
	Sig. (2-tailed)	0.835	0.413	0.128	0.026	0.831	0.667
	N	8	30	8	30	31	3
ENT	Correlation Coefficient	0.170	-0.172	-0.701	-0.363*	0.034	0.866
	Sig. (2-tailed)	0.687	0.362	0.053	0.049	0.854	0.333
	N	8	30	8	30	31	3

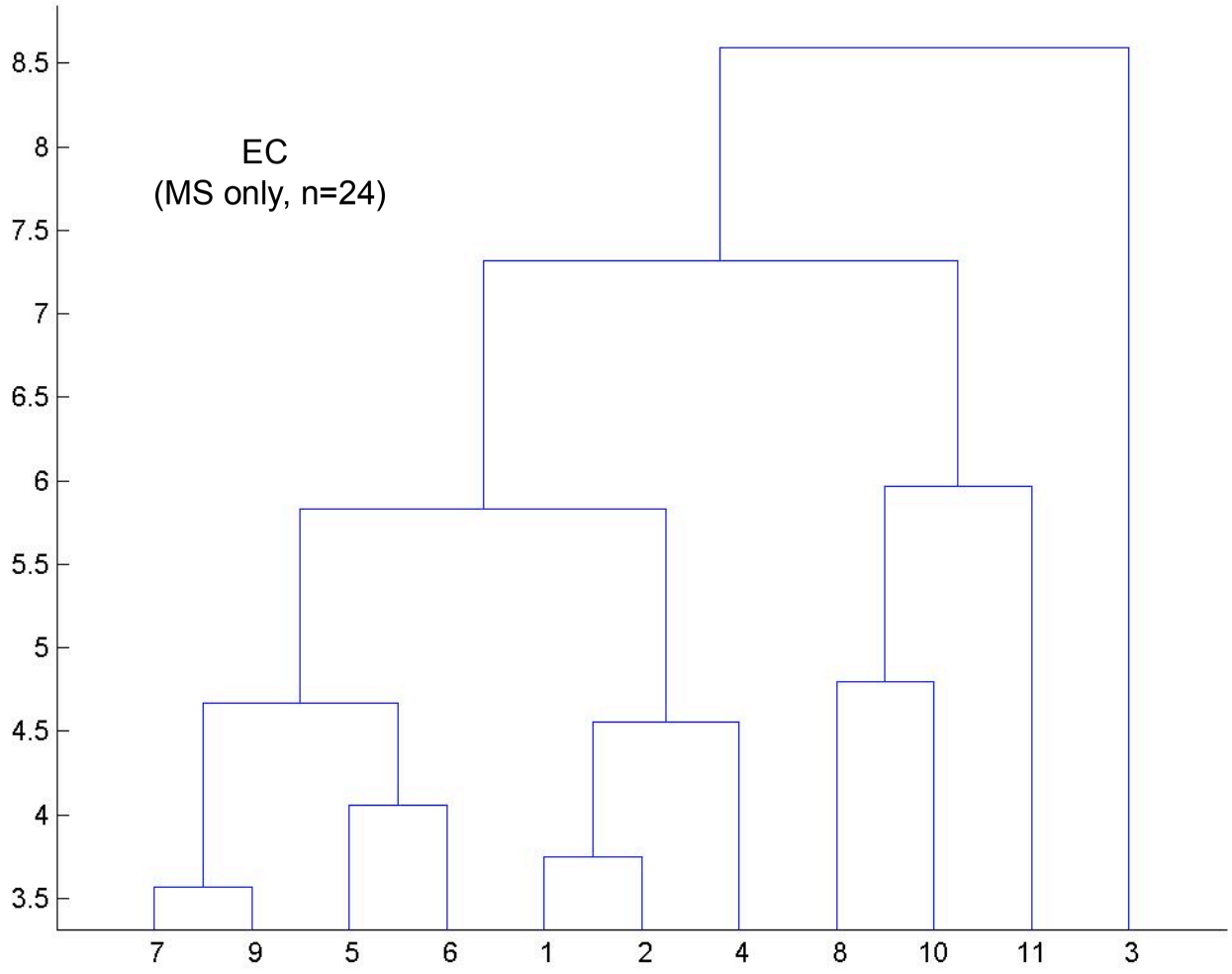

Figure 2-57. Hierarchical clustering of *E. coli* concentrations measured at different BTO sites (indicated on the horizontal axis). The blue ines indicate the degree to which EC concentrations measured at one site is correlated with EC concentrations at another site (the shorter the line distance between two sites, the stronger the correlation). "MS" denotes that the analysis was carried out on measurements from middle surface samples.

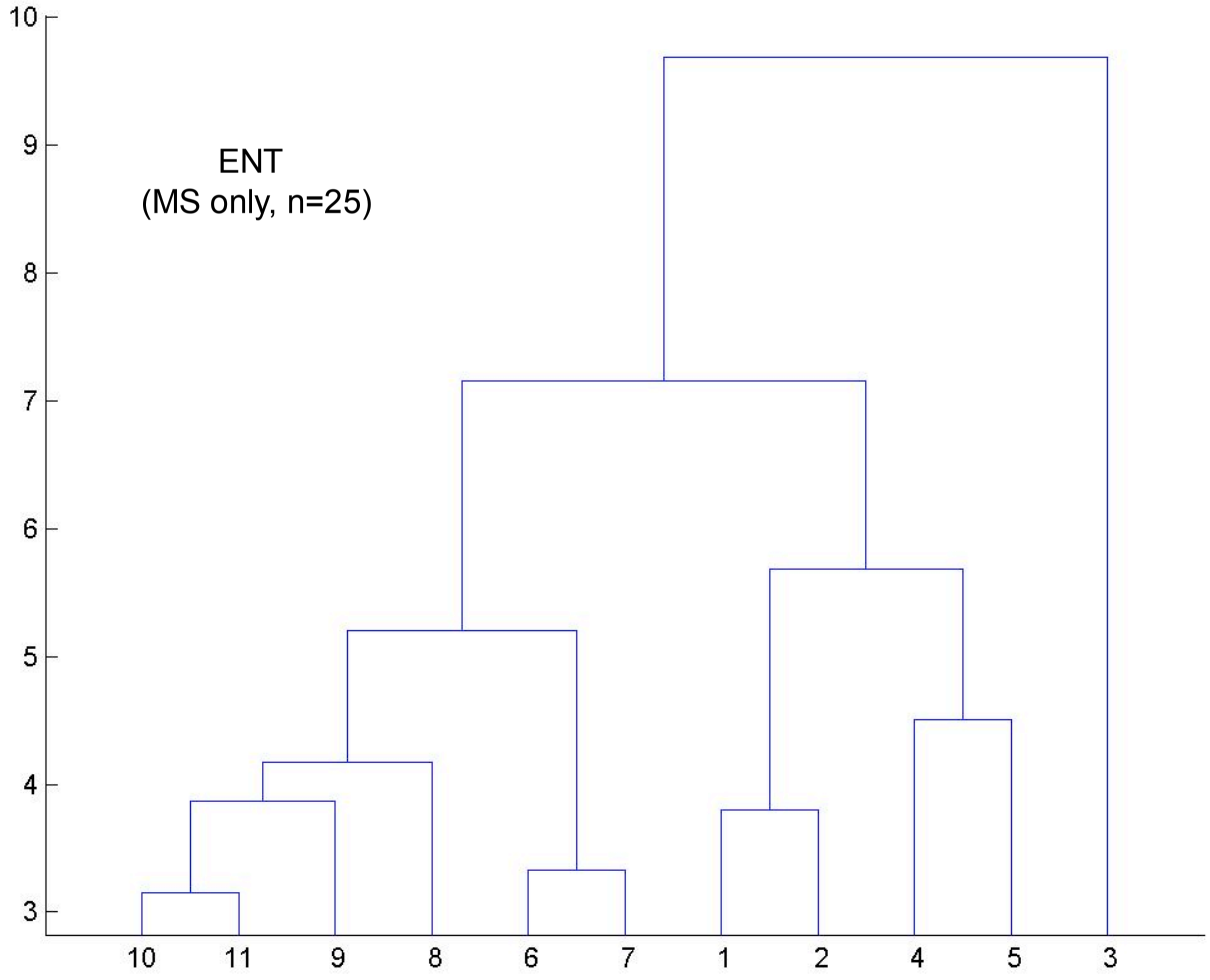

Figure 2-58. Hierarchical clustering of enterococci bacteria concentrations measured at different BTO sites (indicated on the horizontal axis). The blue ines indicate the degree to which ENT concentrations measured at one site is correlated with ENT concentrations at another site (the shorter the line distance between two sites, the stronger the correlation). "MS" denotes that the analysis was carried out on measurements from middle surface samples.

2.3.15. Correlations Between Sediment FIB, Grain Size, Organic Carbon and Moisture Content.

As a next step, we evaluated the degree to which FIB measured in the sediments are correlated with the sediment's organic carbon content, water content, and average grain size. Spearman rank correlation analyses were carried out using all sediment data collected from the BTO transect study (Table 2-11). ENT and EC in the sediments are significantly and positively correlated with the sediment's organic carbon and water content, and negatively correlated with sediment's average grain size. In other words, when all sediment samples collected from Newport Bay are included in the analysis, FIB contamination of the sediment tends to be associated with organic rich fine-grained sediments with elevated water content. The positive correlations with organic carbon and water content are particularly strong in the case of EC (Sp= 0.5 and 0.6, respectively).

2.3.16. Correlations Between Water Column FIB and Nutrients.

To determine if any specific nutrients are associated with high FIB concentrations in the Bay, Spearman rank correlations were calculated for all FIB and nutrient pairs. Because relatively few samples were processed for nutrients, Spearman rank correlations were calculated after first pooling all measurements collected from Upper Bay (Table 2-12) and Lower Bay (Table 2-13) sites.

In Upper Bay, FIB are positively correlated with various nutrients:

1. ENT is significantly ($p<0.05$) and positively correlated (Sp=0.44) with ammonia and TOC (SP=0.5);
2. EC is significantly ($p<0.05$) and positively correlated with ammonia (Sp = 0.51), nitrate (Sp = 0.52), total P (Sp = 0.3), and TON (Sp = 0.94)[34].

In Lower Bay (Table 2-12), the only significant correlations are negative: both EC and ENT are negatively correlated with Total P. It is likely that the positive correlations observed between FIB and nutrients in Upper Bay is, at least in part, a consequence of the fact that both FIB and nutrients are sourced by the tributaries to Upper Bay, particularly during storms.

2.3.17. Cross-correlation of Salinity, Turbidity, and FIB in water and sediments samples.

In this final section we assess how water quality in one part of the Bay co-varies with water quality in a different part of the Bay. To address this issue, dendrograms were prepared from EC and ENT measurements carried out on middle surface samples collected from tributary and within-Bay sites (i.e., BTO sites 1 through 11). To prepare the dendrograms, a hierarchical clustering algorithm was used within the Statistics

[34] Note that the N values for total organic nitrogen (TON) are small (a total of 10 samples across both Upper and Lower Bay) and thus care should be exercised in drawing conclusions regarding the positive correlation reported between FIB and TON in Upper Bay.

Toolbox of Matlab. The results, presented in Figs. 2-57 and 2-58, indicate the degree to which measurements at one site are correlated with measurements at another site. For clustering carried out based on EC measurements, the clustering of BTO sites closely follows their geographical distribution in the Bay (Fig. 2-57):

1. Cluster 1: BTO 1 (San Diego Creek), BTO 2 (Santa Ana Delhi Channel), and BTO 4;
2. Cluster 2: BTO 5, BTO 6, BTO 7, and BTO 9;
3. Cluster 3: BTO 8, BTO 10, and BTO 11.

A similar clustering pattern is evident for ENT (Fig. 2-58). It is particularly noteworthy that sites BTO 1, 2, 4, and 5 tend to cluster together, as this result would to support the idea that FIB impairment in Upper Bay is caused by runoff flowing into the Bay from these two creeks. The fact that within-Bay sites cluster according to their geographical proximity is probably a consequence of the tidal transport of FIB between locations in Newport Bay.

2.4 Summary of BTO Transect Data

2.4.1. Down-Bay Trends in Environmental Parameters

The field studies described in this chapter document a strong ocean-ward gradient in bird populations, wind speed, salinity, temperature, pH, FIB concentration, suspended particle concentration, suspended particle diameter, and nutrients.

2.4.1.1. Down-Bay Trends in Bird Sightings

The average number of bird sightings increased with distance inland, from an average of <5 birds per sampling event at all Lower Bay sites (BTO 8-11) to an average of approximately 300 birds per sampling event at the most inland site (BTO 4). Among the Lower Bay sites, birds are slightly more numerous near the Newport Bay outlet (BTO sites 10 and 11). Birds were most numerous at Upper Bay stations (BTO sites 4-7) during the spring (January through June) and fall (after October) migrations. Bird counts were lower during summer months (June through September), and lower at Lower Bay sites (BTO sites 8-11).

2.4.1.2. Down-Bay Trends in Wind Speed

During the dry season, median wind speeds were generally higher (ca., 2 m/s) and more variable at Upper Bay sites and lower (ca., 1 m/s) and less variable at Lower Bay sites. Wind speeds were most variable during the two storm periods, with 25% of wind measurements exceeding 8 m/s at some sites.

2.4.1.3. Down-Bay Trends in Water Column Salinity

Salinity measurements were carried out on all water samples collected from tributary sites (BTO 1-3), within-Bay sites (BTO 4-11), and the offshore control site (BTO 12).

In addition, depth-averaged salinity measurements were obtained from CTD casts carried out at within-Bay sites (BTO 4-11). Salinities ranged from very low at tributary sites (generally <5), to brackish in Upper Bay (10 to 23), to values typical of the coastal ocean in Lower Bay (ca., 32).

2.4.1.4. Down-Bay Trends in Water Column Temperature

Water temperature decreased with distance down the Bay, from about 16°C (winter season) and 24°C (summer season) in Upper Bay, to 12°C (winter season) and 20°C (summer) near the ocean outlet in Lower Bay. Median depth-averaged temperatures in Newport Bay are approximately 10°C higher during the dry period compared to the storm periods. Runoff from the creeks tends to be a couple of degrees warmer than Bay water. As a result, near freshwater outlets (e.g., San Diego Creek), temperature and salinity stratification of the Newport Bay water column are associated with marked density stratification, and elevated values of the Buoyancy Frequency.

2.4.1.5. Down-Bay Trends in Water Column pH

The pH of water samples collected from Newport Bay is confined to a fairly narrow range of values, between 7 and 9. However, within this narrow range, there is a progressive increase in pH from Upper Bay, to Lower Bay, to the Ocean. These variations in pH appear to originate from the mixing of different water parcels (e.g., creek water with ocean water), generation or consumption of acidity (perhaps associated with microbial metabolism in the marsh sediments), and changes in pH of the coastal ocean.

2.4.1.6. Down-Bay Trends in Water Column FIB

The log-mean concentration of FIB, and percent of samples exceeding marine bathing water single-sample standards, decline with distance down the Bay. Tributary sites had the highest concentrations and single-sample exceedence rates (between 16 and 86% of samples, depending on the tributary and FIB group), followed by sites in the upper reaches of Upper Bay (BTO 4 and 5, exceedence rates between 18 and 38%), followed by sites in the lower reaches of Upper Bay (BTO 6 and 7, exceedence rates between 7 and 17%), followed by sites in Lower Bay (BTO 9-11, exceedence rates between 0 and 8%), followed by the offshore control site (BTO 12, exceedence rate of 0%). At the offshore control site all but one sample had FIB concentrations below the lower limit of detection of 10 MPN/100 mL.

Among Lower Bay sites, samples collected from BTO 8 had relatively high concentrations and exceedence rates for both EC and ENT (7 and 12% for *E. coli* and enterococci bacteria, respectively). This particular site is located in West Newport Bay, in a region with relatively poor tidal mixing and significant sources of dry and wet weather urban runoff (e.g., from Arches Drain).

2.4.1.7. Down-Bay Trends in Suspended Particle Concentration

The *volume-based* particle concentration generally declined with distance down the Bay. Among tributary sites, particle concentrations are highest in San Diego Creek (median 44 ppm), followed by Big Canyon Wash (median 16 ppm), and the Santa Ana Delhi Channel (median 7 ppm). The median particle concentration is approximately the same during dry and wet weather in San Diego Creek (BTO 1), higher during dry weather in the Santa Ana Delhi channel (BTO 2), and higher during wet weather in Big Canyon Wash (BTO 3).

Within the Bay, volume-based particle concentrations are typically higher in Upper Bay (BTO 4 through 7) and lower in Lower Bay (BTO 8 through 12). In Upper Bay, median particle concentrations are approximately 20 ppm during wet and dry seasons. In Lower Bay, median particle concentrations are closer to 10 ppm, with the exception of BTO 9 where median particle concentration reaches 20 ppm during dry weather. Offshore at BTO 12, median particle concentrations are lower (4 ppm) during dry weather and higher (10 ppm) during wet weather. The range of particle concentrations measured at BTO 12 is similar to that measured with the same instrument along the open coastline in Orange County (0 to 10 ppm).

Number-based particle concentrations follow a similar pattern. Among tributary sites, the highest particle numbers are recorded in San Diego Creek (median of approximately 10^9 particles per liter), followed by Big Canyon Wash (median of 10^8 particles per liter), and the Santa Ana Delhi Channel (median ranges between 10^7 and 10^8 particles per liter). Particle numbers are higher during wet weather in San Diego Creek, and higher during dry weather in Santa Ana Delhi Channel. Particle numbers measured at Big Canyon Wash are, within error, the same during dry and wet weather.

Particle number concentrations measured at within-Bay sites exhibit down-Bay and seasonal variability. During dry weather, the median particle number is relatively constant across most of the Bay (BTO 4 through 9, approximately 10^8 particles per liter), dropping off steeply at the ocean outlet (BTO 10 through 12). During storms, the median particle number is highest in Upper Bay, followed by Lower Bay, followed by the ocean.

2.4.1.8. Down-Bay Trends in Suspended Particle Diameter

During dry weather, the median average diameter is similar across the three tributary sites (ca., 6 microns). During wet weather, the median particle diameter in San Diego Creek drops to about 4 microns. The median diameter is relatively constant, at about 5 microns, across most within-Bay sites (BTO 4 through 9). The median particle diameter increases markedly oceanward of BTO 10, particularly during dry weather. Within the Bay, the median particle diameter is slightly larger during dry weather (ca., 6 microns), compared to wet weather (ca., 5 microns).

2.4.1.9. Down-Bay Trends in Water Clarity (Beam c)

Higher beam-c values are associated with more turbid waters. Among the tributary sites, median beam c values decrease in the order San Diego Creek, Big Canyon Wash, and

Santa Ana Delhi Channel. Within the Bay, beam c measurements exhibit down-Bay and seasonal variability. The highest median beam c values were recorded in Upper Bay, followed by Lower Bay, followed by the ocean. This down-Bay trend is especially evident during wet weather, when the median beam c values in Upper Bay are higher and more variable.

2.4.1.10. Down-Bay Trends in Water Column Nutrients

A subset of water samples collected during the BTO studies were analyzed for nutrients, including nitrate, ammonia, Total P, and TOC. Generally speaking, nutrient concentrations were highest in samples collected from one or more tributaries that discharge to Upper Bay, followed by samples collected from within the main channel of Upper Bay, followed by samples collected from Lower Bay, followed by samples collected from the offshore control site. The exception is ammonia, for which the concentrations measured in the upper reaches of Upper Bay are higher than the concentrations measured in the tributary sites.

Among the tributary sites, San Diego Creek has the highest average nitrate concentration (26 mg/L or 420 μM), Santa Ana Delhi Channel has the highest average ammonia concentration (0.13 mg/L or 7.6 μM), and Big Canyon Wash has the highest average total phosphorous (1.1 mg/L or 36 μM) and TOC (9.4 mg/L) concentrations.

Among within-Bay sites, without exception the upper reaches of Upper Bay had the highest nutrient concentrations. Average nutrient concentrations reported here for Upper Bay sites are similar to values published previously for this wetland[35]. The average nitrate (3.1 mg/L or 50 μM) and total phosphorous (0.36 mg/L or 11 μM) concentrations are at the high end of measurements reported for estuaries on both the east[36] and west[37] coasts of the United States.

2.4.1.11. Correlation of FIB and Nutrients in the Water Column

FIB concentrations in Newport Bay are positively and significantly correlated with the concentration of several nutrients. In Upper Bay, FIB are significantly and positively correlated with ammonia (EC, ENT), total organic nitrogen (EC), nitrate (EC), Total P (EC), and TOC (ENT). In Lower Bay, the only significant correlations are negative: both EC and ENT are negatively correlated with Total P. Because FIB are significantly and positively correlated with nutrients only in Upper Bay, which is significantly impacted by stormwater runoff from the San Diego Creek and Santa Ana Delhi Channel, it is likely that the correlations are associative not causal; i.e., FIB are not growth-limited by one or

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[35] Boyle, K.A., Kamer, K., and Fong, P. (2004) "Spatial and temporal patterns in sediment and water column nutrients in a eutrophic southern California estuary" *Estuaries* 27:378-388.
[36] Pruell, R.J., Taplin, B.K., Lake, J.L., Jayaraman, S. (2006) "Nitrogen isotope ratios in estuarine biota collected along a nutrient gradient in Narragansett Bay, Rhode Island, USA" *Marine Pollution Bulletin* 52:612-620.
[37] Sigleo, A.C., Mordy, C.W., Stabeno, P., Frick, W.E. (2005) "Nitrate variability along the Oregon coast: Estuarine-coastal exchange" *Estuarine, Coastal and Shelf Science* 64:211-222.

more of the nutrients tested for. The significant and strongly positive correlations observed between FIB and nutrients in Upper Bay are probably a consequence of the fact that both FIB and nutrients are sourced by the tributaries to Upper Bay, particularly during storms.

2.4.2. Cross-sectional Trends in Water Quality

At all within-Bay sites, water samples were collected from five locations in the tidal channel cross-section: three surface samples (left, middle, and right), a depth-integrated sample, and a bottom sample. Here, the designation "left" and "right" assumes that the observer's back is to the ocean. Hence, for regions of the Bay where the local tidal channel strikes N-S, the "left bank" corresponds to the west side of the channel; in regions of the Bay where the local tidal channel strikes E-W, the "left bank" corresponds to the north side of the channel.

2.4.2.1. Cross-sectional Trends in Water Column Salinity

The water column in the upper reaches of Upper Bay, near the outlet of San Diego Creek (BTO 4), is salinity stratified. Salinity measured at most other sites is well mixed over the vertical, consistent with the classification of Newport Bay as a Type 2a, or well-mixed, estuary (Chapter 1). During storm events, however, salinity stratification can extend into Lower Bay. There is relatively little salinity variation evident in the transverse direction; i.e., the salinity of water collected from the three surface samples is very similar.

2.4.2.2. Cross-sectional Trends in Water Column pH.

As with the salinity measurements described above, the water column at BTO 4 and 5 is relatively well-mixed over the transverse direction, but vertically stratified. Specifically, at the bottom of the water column the pH is generally lower and at the surface of the water column the pH is generally higher. At sites including and ocean-ward of BTO 7, the water column is well-mixed over both the vertical and transverse directions.

2.4.2.3. Cross-sectional Trends in Water Column FIB.

In Upper Bay, the vertical distribution of EC varies with the season. During storms, EC concentrations are higher at the surface of the water column and lower at the bottom of the water column. The opposite pattern prevails during dry weather, with EC concentrations higher at the bottom of the water column and lower at the surface of the water column. At BTO 8, EC concentrations are lower at the bottom of the water column, and higher in samples collected from the left and right banks. At most Lower Bay sites (BTO 9 through 11), EC concentrations are relatively low and well-mixed over the water column. There are no consistent cross-sectional trends in ENT at either Upper Bay or Lower Bay sites.

2.4.2.4. Cross-sectional Trends in Suspended Particle Concentration.

Generally speaking, the concentration of suspended particles is vertically stratified in Upper and Lower Bay, with higher particle concentrations near the bed and lower particle concentrations at the surface of the water column. This vertical pattern is evident for both dry and wet weather periods.

2.4.2.5. Cross-sectional Trends in Water Clarity (Beam c).

Beam c measurements in Upper Bay and at BTO 8 are generally higher at the bottom of the water column and lower at the surface of the water column during both dry and wet weather periods. However, in Lower Bay (BTO 9-11), beam c does not exhibit consistent cross-sectional variability.

2.4.2.6. Cross-sectional Trends in Suspended Particle Diameter.

In Upper Bay and at BTO 8, the average diameter of suspended particles is generally larger at the bottom of the water column and smaller at the surface of the water column. However, in Lower Bay, the average particle diameter exhibits no consistent cross-sectional patterns.

2.4.2.7. Cross-sectional Trends in Nutrients.

Only middle-surface samples were analyzed for nutrients, and hence there is no information available on the cross-sectional variability of nutrients.

2.4.3. Down-Bay Trends in Sediment Data.
2.4.3.1. Down-Bay Trends in Sediment EC.

During wet weather, EC concentrations are highest in sediment collected from one or more tributaries (BTO 1 through 3), followed by sediment collected from Upper Bay (BTO 4 through 7), followed by sediment collected from Lower Bay (BTO 8 through 11). During dry weather, EC concentrations are higher in sediments collected from the tributaries, and very low in most sediment samples collected from within-Bay sediments.

2.4.3.2. Down-Bay Trends in Sediment ENT.

ENT concentrations are highest in sediments collected from Lower Bay (BTO 8 through 10), followed by sediments collected from Upper Bay and the three tributaries (BTO 1 through 7), followed by the site nearest the ocean outlet (BTO 11). Apart from the tributaries, which have relatively low N values (typically N<10), the spatial pattern just described applies to both dry weather and wet weather periods.

2.4.3.3. Down-Bay Trends in Sediment Percent Organic Carbon, Percent Water Content, and Average Grain Size.

Among tributaries, sediment samples collected from the Santa Ana Delhi channel (BTO 2) have the highest median organic carbon content (ca., 8%) and the highest median

water content (ca., 47%). Within error, the average grain size is the same at all three tributary sites (ca., 25 microns). Organic carbon and water content are higher (ca., 10% and 65%, respectively) in sediments collected from regions of the Bay that experience less tidal flushing (BTO 4 in Upper Bay and BTO 8 in Western Bay). Median organic carbon and water content are lower (ca., 5% and 45%, respectively) in regions of the Bay that experience better flushing (BTO 6, 7, 9, and 10). Over most of the Bay, average sediment grain size decreases down-Bay, from 25 microns in the tributaries to 17 microns in Lower Bay. The average sediment grain size near the ocean outlet, however, is much higher (ca., 70 microns), perhaps reflecting the tidal input of sand-sized sediments from the open coastline.

2.4.4. Cross-Sectional Trends in Sediment Data.

At all within-Bay sites, sediment samples were collected from three locations in the tidal channel cross-section: left, middle, and right. As with the water sample notation described above, the designation "left" and "right" assumes that the observer's back is to the ocean.

2.4.4.1. Cross-sectional Trends in Sediment EC.

There are no consistent cross-sectional trends in the concentration of EC measured in the sediments.

2.4.4.2. Cross-sectional Trends in Sediment ENT.

ENT concentrations measured in the sediments are consistently higher along the right bank at Upper Bay sites (BTO 4-7), and higher along the right bank in East Lower Bay (BTO 9-11). Near the outlet of Arches Drain in West Newport Bay (BTO 8), ENT concentrations are consistently elevated in sediments collected from both the middle and right positions in the tidal channel cross-section.

2.4.4.3. Cross-sectional Trends in Sediment Percent Organic Carbon, Percent Water Content, and Average Grain Size.

For sediment samples collected from Upper Bay (BTO 4-7), no cross-sectional trends are evident for organic carbon, water content, or average grain size. For sediment samples collected from West Lower Bay (BTO 8), water content and average grain size tend to be higher in sediments collected from the right bank of the tidal channel. At BTO 10, organic carbon and water content are frequently higher in sediments collected from the right bank of the tidal channel (i.e., the Balboa Island side of the channel), and lower in sediments collected from the thalweg or left bank of the tidal channel. At this site, average grain size tends to be smaller on the right bank as well.

2.4.5. Correlations between Sediment FIB, Organic Carbon, Water Content, and Grain Size.

Spearman rank correlations were carried out to determine if FIB in the sub-tidal sediments of Newport Bay are correlated with other sediment parameters, including organic carbon content, water content, and average grain size. FIB in the sediment tends to be positively correlated with organic-rich, fine-grained sediments with elevated water content. The positive correlations with organic carbon and water content are particularly strong in the case of EC (Spearman Rank Correlations of Sp= 0.5 and 0.6, respectively).

2.4.6. Correlations between FIB in the Sediment and Water Column.

Additional Spearman rank correlations were run to determine if FIB concentrations in the sediment were correlated with FIB concentration in the overlying water column. At Santa Ana Delhi channel site (BTO 2), ENT measured in the sediment is significantly ($p=0.00$) and positively ($Sp = 0.6$) correlated with ENT measured in the overlying water column. For a significance of $p<0.01$, FIB concentrations in the sediment are not significantly correlated with FIB in the overlying water column at any within-Bay sampling sites.

2.4.7. Particle Association of FIB in the Water Column.

To determine if FIB in the water column are associated with suspended particles, a subset of the water samples collected in Newport Bay were assayed for FIB before and after filtration through a 5 or 10 micron filter. In the majority of cases, filtration of water samples through a 5-micron filter did not significantly alter the concentration of EC and ENT. Hence, within the resolution of the measurement method used, most of the FIB in Newport Bay are present as free-living single cells, or are associated with particles smaller than 5 microns in diameter (note that the volume-averaged diameter of EC and ENT is 1.2 microns). This conclusion is consistent with a number of studies suggesting that marine and estuarine waters are typically dominated by free-living (planktonic) cells[38]. While FIB measured in most samples appear to be free-living single-cells, passing samples through a 5-micron pore sized filter did significantly lower the EC concentration in water samples collected from Upper Bay water during the dry season. Also, passage of Santa Ana Delhi samples through the 5 micron pore-sized filter appeared to increase the concentration of FIB, perhaps due to the removal of material that interferes with the EC assay, and/or because the filtration process disrupts EC-laden flocs.

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[38] Palumbo, A.V., Ferbuson, R.L., Rublee, P.A. (1984) "Size of suspended bacterial cells and association of heterotrophic activity with size fraction of particles in estuarine and coastal waters" *Appl. Environ. Microbiol.* 48:157-164; Bidle, K.D. and Fletcher, M. (1995) "Comparison of free-living and particle-associated bacterial communities in the Chesapeake Bay by stable low molecular-weight RNA analysis" *Appl. Environ. Microbiol*. **61**:944-952; Kirchman, D. and R. Mitchell (1982) "Contribution of particle-bound bacteria to total microheterotrophic activity in five ponds and two marshes" *Appl. Environ. Microbiol*. **43**: 200-209; Clarke, K.R. and Joint, I.R. (1986) "Methodology for estimating numbers of free-living and attached bacteria in estuarine water" *Appl. Environ. Microbiol*. 51:1110-1120; Yoon, W.B. and Rosson, R.A. (1990) "Improved method of enumeration of attached bacteria for study of fluctuation in the abundance of attached and free-living bacteria in response to diel variation in seawater turbidity" *Appl. Environ. Microbiol*. **56**:595-600; Murrell, M.C., Hollibaugh, J.T., Silver, M.W., and Wong, P.S. (1999) "Bacterioplankton dynamics in San Francisco Bay: role of particle association and seasonal freshwater flow" *Limnol. Oceanogr*. **44**:295-308.

[1]Chapter 3. Speciation and Molecular Fingerprinting of Newport Bay *Enterococcus* Isolates

3.1. Introduction and Overview

In this chapter we report on the distribution of *Enterococcus* species present in Newport Bay water and sediment samples and type the most common species utilizing Pulsed Field Gel Electrophoresis (PFGE). PFGE is extremely discriminatory and is the "gold standard" for typing bacteria to determine strain relatedness. The procedure is used extensively for tracking disease outbreaks by public health departments and the Centers for Disease Control.

In this study, PFGE was used to examine *Enterococcus* isolates from Newport Bay for inter and intra sample clonal matches with the hope of determining the source and distribution of bacterial pollution in Newport Bay. In this context, bacteria are "clonal" if they are genetically identical at the level of discrimination afforded by the typing method; in this case PFGE. PFGE typing of enterococci isolates could provide information on the possible role that environmental growth plays in the FIB contamination of Newport Bay. Specifically, if all enterococci isolates were found to be clonal, then growth of an environmentally adapted enterococci strain might be inferred.

This chapter satisfies the following task of the Proposition 13 project: the element of sub-task 2.1.4.1 focused on the relatedness of *Enterococcus* isolates from Newport Bay.

3.2. Speciation of Newport Bay *Enterococcus*

The objectives were to speciate enterococci isolates obtained from Newport Bay and to determine species diversity. The hypothesis is that a large species diversity will be indicative of many sources of enterococci while a low species diversity will be indicative of environmental growth.

3.2.1. Speciation of Newport Bay *Enterococcus*: Methods

A subset of the total water and sediment samples collected during the Bay to Ocean (BTO) transect study (see Chapter 2) were analyzed for enterococci using the membrane filtration technique on mEI media (EPA method 1600). Up to seven presumptive *Enterococcus* isolates were picked from each sample and placed on agar slants at the Orange County Sanitation District. The isolates were transferred to the Orange County Public Health Laboratory where they were streaked for purity and speciated utilizing PC12 panels in an

[1] This chapter was prepared by Dr. D. Moore, and edited by Dr. S. Grant, Charles McGee, Donna Ferguson and Joseph Guzman.

Table 3-1. Number of water and sediment samples from which enterococci isolates were collected, indicated by site and month. The first and second numbers in each cell represent the number of water samples and sediment samples analyzed.

	BTO 1	***BTO 2***	***BTO 3***	***BTO 4***	***BTO 5***	***BTO 6***	***BTO 7***	***BTO 8***	***BTO 9***	***BTO 10***	***BTO 11***	***BTO 12***	***Total***
February	1/1	1/1	2/1	1/0	1/0	1/0	0/0	1/0	1/0	1/0	0/0	1/0	11/3
March	1/0	1/0	0/0	11/7	9/6	0/0	0/0	0/0	0/0	0/0	0/0	0/0	22/13
April	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/1
May	1/0	1/0	0/0	1/1	1/0	1/1	1/1	0/0	0/0	0/0	0/0	0/0	6/3
June	1/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0	0/0	1/0	0/0	4/0
July	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
August	3/0	3/1	1/0	1/2	0/0	0/1	0/0	0/0	0/1	0/1	0/0	0/0	8/6
September	3/1	3/0	2/1	2/1	3/0	2/0	1/1	1/0	1/2	0/2	0/0	0/0	18
Total	10/2	10/2	5/2	16/11	14/6	4/2	2/2	2/0	3/3	1/4	1/0	1/0	69/34

Table 3-2. Number of enterococci isolates included in the speciation study, indicated by site and month. The first and second numbers in each cell represent the number of isolates analyzed from water and sediment samples.

	BTO 1	***BTO 2***	***BTO 3***	***BTO 4***	***BTO 5***	***BTO 6***	***BTO 7***	***BTO 8***	***BTO 9***	***BTO 10***	***BTO 11***	***BTO***	***Total***
February	5/5	4/5	10/5	5/0	5/0	2/0	0/0	2/0	5/0	5/0	0/0	1/0	44/15
March	5/0	5/0	0/0	33/20	22/21	0/0	0/0	0/0	0/0	0/0	0/0	0/0	22/13
April	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/3	0/0	0/0	0/3
May	5/0	5/0	0/0	5/5	5/0	5/1	5/1	0/0	0/0	0/0	0/0	0/0	30/7
June	5/0	5/0	0/0	0/0	0/0	0/0	0/0	0/0	5/0	0/0	5/0	0/0	20/0
July	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
August	8/0	10/5	5/0	2/2	0/0	0/1	0/0	0/0	0/1	0/1	0/0	0/0	25/10
September	15/5	15/0	10/5	5/1	9/0	2/0	5/3	3/0	1/5	0/5	0/0	0/0	65/24
Total	43/10	44/10	25/10	50/28	41/21	9/2	10/4	5/0	11/6	5/9	5/0	1/0	249/100

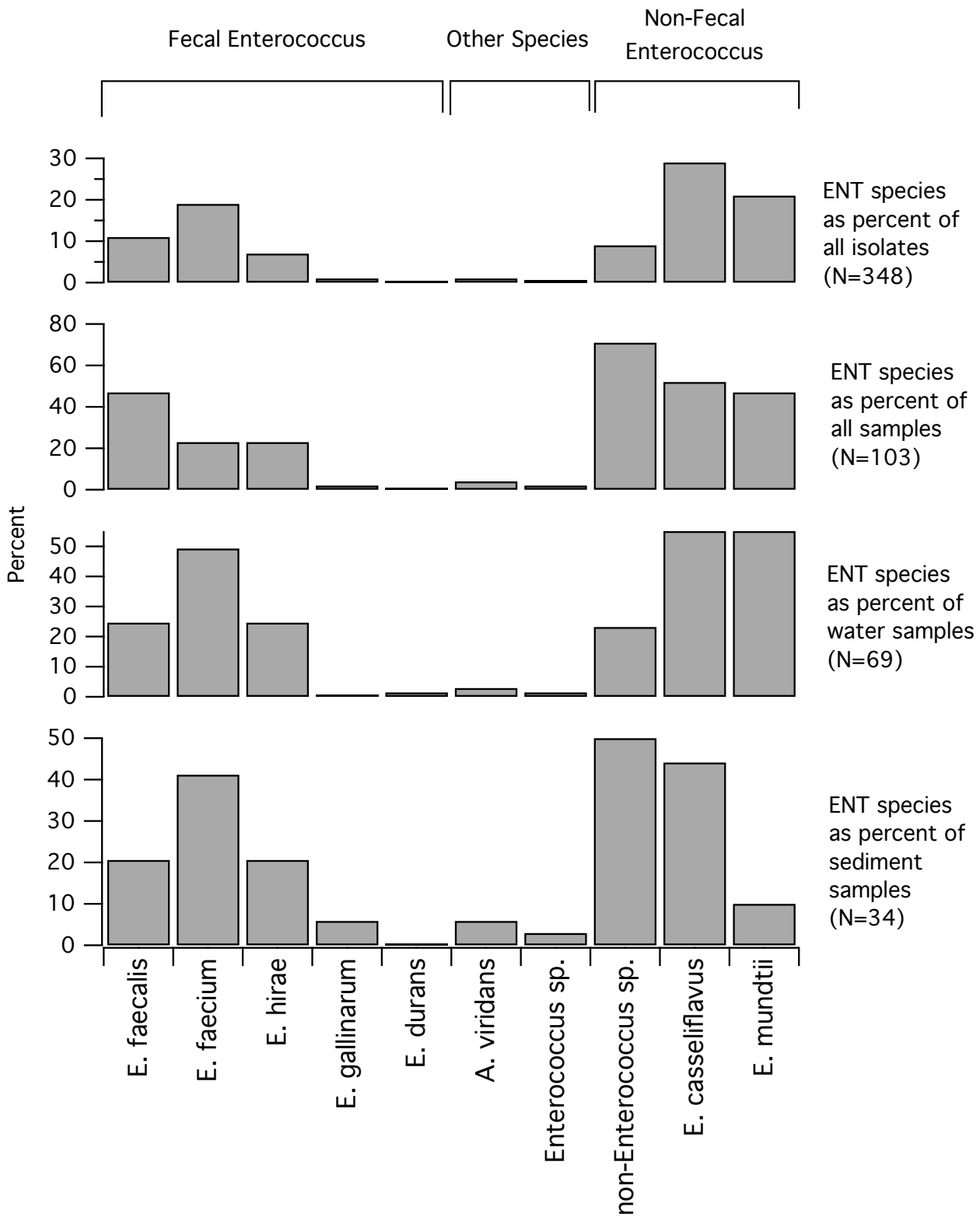

Figure 3-1. *Enterococcus* species distribution across all isolates (top panel), all sediment and water samples (second panel), water samples (third panel), and sediment samples (fourth panels) .

automated Microscan Walk Away instrument with additional biochemical tests as necessary[2].

3.2.2. Speciation of Newport Bay *Enterococcus*: Sample Distribution

The number of samples utilized for the study and the total number of enterococci presumptive isolates that were speciated are indicated by month and sample site in Tables 3-1 and 3-2. Overall, 103 water and sediment samples were included in the study, consisting of 69 water samples and 34 sediment samples. There was an uneven distribution of samples by month collected and site. The total number of water and sediment samples ranged from one sample in one month at sites BTO 11 and 12 to 27 samples over five months at BTO-4 (see Fig. 2-1 for location of BTO sites). The majority of samples were from February to March and August to September. There were only 14 samples taken from April to June and none in July. There was also an uneven distribution of total isolates in the study, due to the uneven sample distribution and the concentration of *Enterococcus* in each sample (Table 3-2).

The original objective was to obtain isolates from weekly samples using a stratified random process. However, since many samples had very low or non-detectable levels of presumptive enterococci bacteria (particularly for water and sediment samples collected in Lower Bay), the temporal component of the study was sacrificed and isolates were obtained from samples collected for other sub-studies. For example, all of the isolates from March and April were obtained from samples collected as part of the diurnal intertidal transect site study (DITS, see Chapter 6 of this report). Also, to obtain additional isolates, extra samples were collected two to three times a week in September.

3.2.3. Overall *Enterococcus* Species Distribution

The distribution of species is presented in Fig. 3-1. Ninety percent of the presumptive isolates were identified as *Enterococcus* species. The three most common species were *E. casseliflavus* (30%), *E. mundtii* (21%) and *E. faecium* (19%). *E. casseliflavus* and *E. mundtii* made up 51% of the total number of isolates, while *E. faecium, E. faecalis, E. hirae, E. gallinarum* and *E. durans* made up 38% of the total number of isolates (top panel, Fig. 3-1).

3.2.4. *Enterococcus* Species Distribution by Sample

The distribution analysis of species by total isolates tested is biased towards samples containing a higher concentration of *Enterococcus* because more isolates were picked from these plates. This results in these samples being over-represented in the overall pool.

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[2] Moore, D.F. et al. (2006) Comparison of 16s rRNA sequencing with conventional and commercial phenotypic techniques for identification of enterococci from the marine environment. *Journal of Applied Microbiology*, 100:1272-1281.

Table 3-3. The number of different samples from which a particular bacterial species was cultured by month[2].

	February [1]***(N=14)***	***March (N=35)***	***April (N=1)***	***May (N=9)***	***June (N=4)***	***July (N=0)***	***August (N=14)***	***Sept. (N=26)***
A. viridans	1	2	0	0	0	0	0	0
E. durans	0	1	0	0	0	0	0	0
E. gallinarum	0	1	0	0	0	0	1	0
E. hirae	4	12	0	0	1	0	4	3
E. mundtii	10	12	1	6	2	0	5	12
E. faecium	4	20	1	6	4	0	1	8
E. faecalis	1	7	0	4	1	0	3	9
E. casseliflavus	8	15	0	4	2	0	8	16

[1]The N value represents the total number of water or sediment samples collected during a particular month.
[2]Because frequently more than one isolate was cultured from the same sample, the sum of numbers in each column will not, in general, equal the total number of samples collected that month.

As a result, further analysis of frequency and distribution of species was done by analyzing presence of an *Enterococcus* species by sample (bottom three panels, Fig. 3-1). Five *Enterococcus* species were present in a significant portion of all samples, including *E. casseliflavus* (51%), *E. mundtii* (47%), *E. faecium* (43%), *E. faecalis* (24%) and *E. hirae* (23%). Sixty-seven percent of samples contained at least one isolate of *E. faecium,E. faecalis, E. hirae, E. gallinarum* and *E. durans.* Seventy one percent of samples contained at least one *E. casseliflavus* or *E. mundtii* isolate (second panel, Fig. 3-1).

3.2.5. *Enterococcus* Species Distribution in Sediment and Water Samples

The distribution of species in water samples and sediment samples was similar, with the possible exception of *E. mundtii* which was present in 52% of water samples and 33% of sediment samples (compare bottom two panels, Fig. 3-1). The frequency of occurrence was similar for all species in Upper Bay and Lower Bay samples except for *E. casseliflavus* that was present in 14% of Lower Bay sediment samples and 38% of Upper Bay sediment samples and *E. mundtii* that was present in 14% of Lower Bay sediment samples and 75% of Upper Bay sediment samples (data not shown). Also, with the number of isolates available to work with, no difference was seen in the frequency of occurrence of species by month. All five of the significant species were present throughout the study period (Table 3-3).

3.2.6. *Enterococcus* Species Distribution: Down-Bay trends

Down-Bay trends in the species distribution of Enterococcus are displayed in Figs. 3-2 and 3-3. To overcome the relatively low number of isolates analyzed at some sites, in these figures the speciation results are pooled for sites BTO 5-7 (in Upper Bay) and BTO

8-12 (in Lower Bay and offshore). By pooling these sites, a similar number of isolates is indicated for each region (see N values noted in the two figures).

The distribution of *Enterococcus* species is remarkably similar across the tributary sites (BTO 1, 2, and 3) and Upper Bay sites (BTO 4 and 5-7). In both tributary and Upper Bay sites, *Enterococcus* is dominated by the non-fecal species *E. casseliflavus* and E. *mundtii,* and to a lesser extent by the fecal species *E. faecalis*, *E. faecium*, and *E. hirae* (Fig. 3-2). With distance down-Bay, the species distribution appears to shift toward a more even distribution of fecal (*E. faecalis, E. faecium, and E. hirae*) and non-fecal (*E. casseliflavus and E. mundtii*) species. This apparent shift in species distribution is especially evident when the fecal (*E. faecalis, E. faecium, and E. hirae, E. gallinarum, E. durans*), non-fecal (*E. casseliflavus, E. mundtii*) and other (other *Enterococcus* or non-*Enterococcus*) species are plotted against distance down-Bay (Fig. 3-3). The non-fecal species dominate over the fecal species at the tributary sites and in Upper Bay (ca., 60% compared to 20%), while the distribution of non-fecal and fecal *Enterococcus* species are nearly even in Lower Bay (Fig. 3-3). It is important to keep in mind that the concentration of *Enterococcus* also declines with distance down-Bay, so the change in species composition could reflect differential die-off of certain (e.g., non-fecal) strains in the Bay, and/or predominance of different sources of enterococci bacteria in Upper and Lower Bay.

3.3. Molecular Fingerprinting of Newport Bay Enterococcus Isolates

PFGE typing of a subset of *Enterococcus* isolates was performed in order to examine isolate relatedness. The hypothesis being tested was that a large population of genetically similar enterococci would be indicative of clonality, which could possibly point to a source of pollution or support the idea that enterococci bacteria are regrowing in Newport Bay.

3.3.1. Molecular Fingerprinting of Newport Bay Enterococcus Isolates: Methods

Four of the most common *Enterococcus sp.* representing all sites and time periods were typed by using PFGE after SmaI restriction endonuclease digestion of the genomic DNA. Four different *Enterococcus* species were selected for molecular typing by PFGE. *E. faecalis* was chosen because it was frequently isolated from water and sediment samples from Newport Bay, and it has been typed in previous environmental studies in Orange County and thus a large database of types exist. *E. casseliflavus, E. mundtii* and *E. faecium* were also frequently isolated from water and sediment samples from Newport

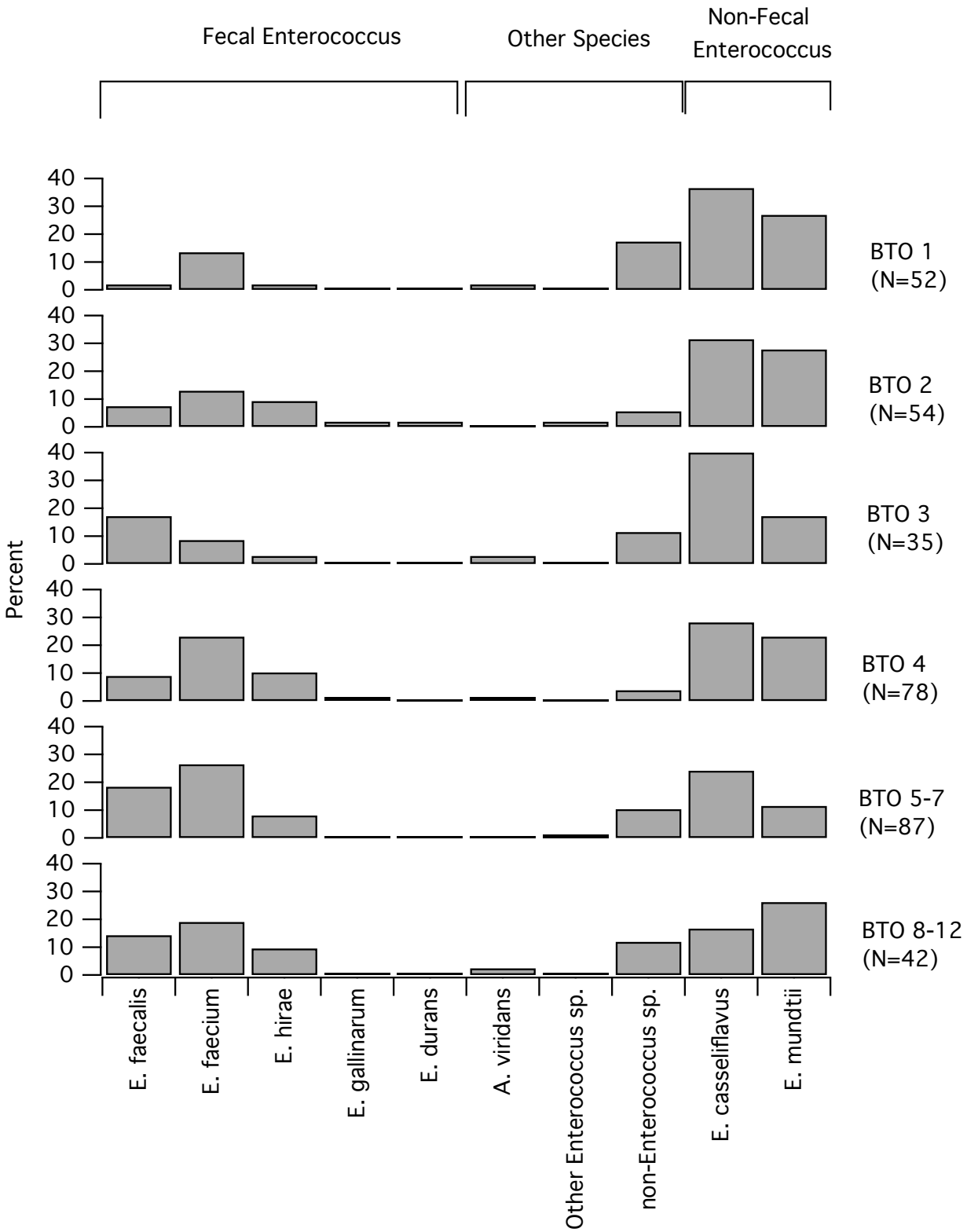

Figure 3-2. *Enterococcus* species distribution at tributary sites (BTO 1, 2, and 3), at Upper Bay sites (BTO 4, 5-7) and Lower Bay and offshore sites (BTO 8-12).

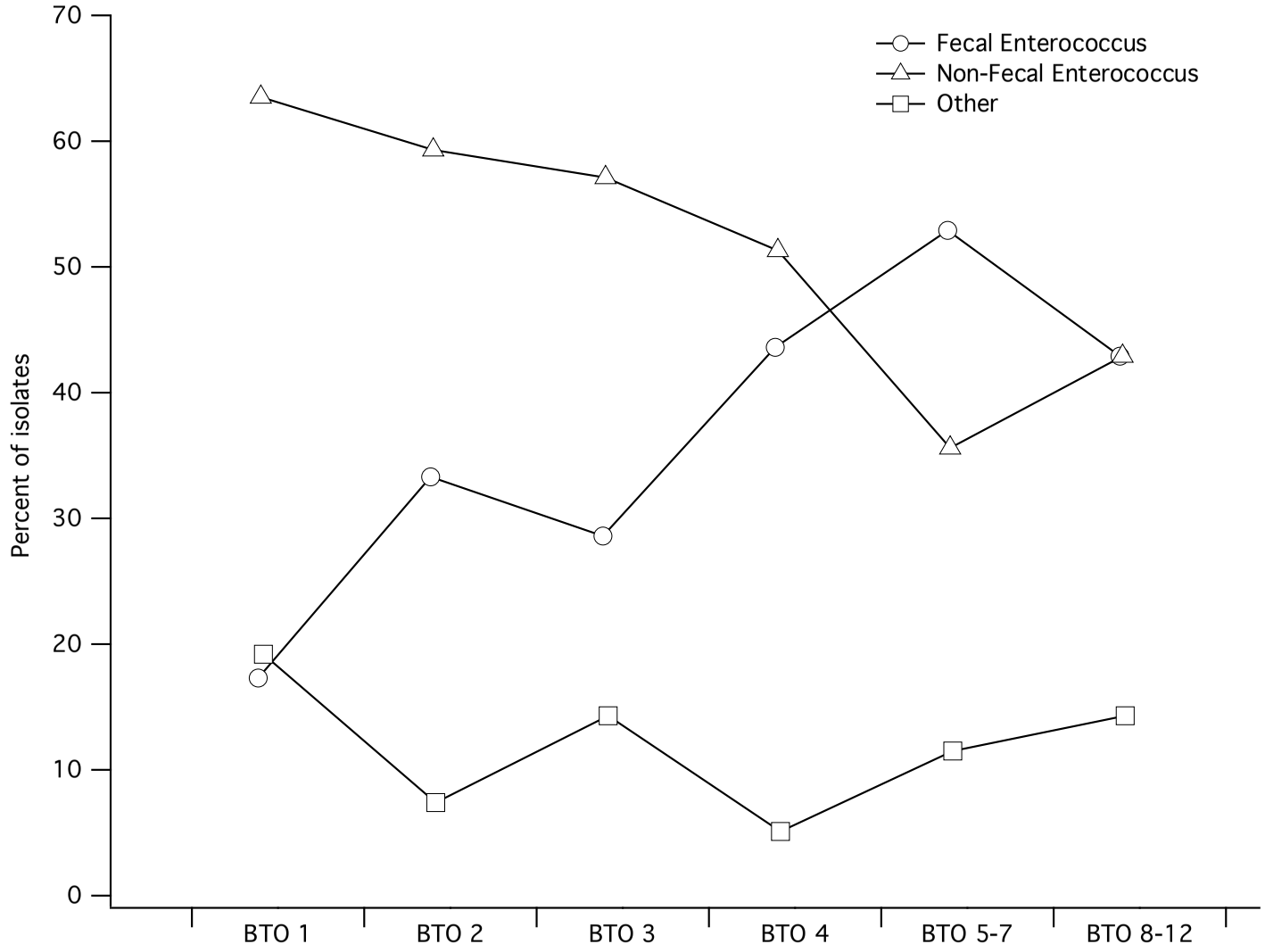

*Figure 3-3. Percent of Enterococcus species at each site classified as -fecal (E. faecalis, E. faecium, and E. hirae, E. gallinarum, E. durans), non-fecal (E. casseliflavus and E. mundtii), or other (*other *Enterococcus* spp. or non-*Enterococcu spp).*

Table 3-4. Number of water and sediment samples included in the PFGE study, indicated by site and month. The first and second numbers in each cell represent the number of isolates from water and sediment samples,

	BTO 1	***BTO 2***	***BTO 3***	***BTO 4***	***BTO 5***	***BTO 6***	***BTO 7***	***BTO 8***	***BTO 9***	***BTO 10***	***BTO 11***	***BTO 12***	***Total***
February	1/1	1/1	2/1	1/0	1/0	1/0	0/0	1/0	1/0	1/0	0/0	1/0	11/3
March	1/0	1/0	0/0	4/3	3/3	0/0	0/0	0/0	0/0	0/0	0/0	0/0	9/6
April	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/1
May	1/0	1/0	0/0	1/1	1/0	1/1	1/0	0/0	0/0	0/0	0/0	0/0	6/2
June	1/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	1/0	0/0	1/0	0/0	4/0
July	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
August	3/0	3/1	1/0	1/1	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	8/3
September	3/0	3/0	2/1	2/1	3/0	2/0	1/1	1/0	1/2	0/1	0/0	0/0	18/6
Total	10/1	10/2	5/2	9/6	8/3	4/1	2/1	2/0	3/2	1/3	1/0	1/0	**56/21**

Table 3-5. Number of isolates included in the PFGE study, by BTO site and enterococcus species. The first and second numbers in each cell indicate the number of isolates from, respectively, water and sediment samples.

	E. faecalis	***E. faecium***	***E. casseliflavus***	***E. mundtii***	***Total***
BTO 1	1/0	7/0	20/0	10/4	38/4
BTO 2	2/2	4/3	15/1	13/2	34/8
BTO 3	6/0	3/0	9/5	4/2	22/7
BTO 4	6/1	6/5	8/6	7/4	27/16
BTO 5	6/2	3/0	6/3	4/0	19/5
BTO 6	1/0	3/0	4/0	1/1	9/1
BTO 7	6/1	2/0	1/0	0/0	9/1
BTO 8	0/0	0/0	1/0	1/0	2/0
BTO 9	2/3	2/3	0/1	3/0	7/7
BTO 10	0/1	0/2	2/0	3/1	5/4
BTO 11	0/0	1/0	3/0	1/0	5/0
BTO 12	0/0	0/0	0/0	1/0	1/0
Total	30/10	31/13	69/16	48/14	178/53

Bay, but these species have not been previously typed in Orange County environmental studies by PFGE fingerprinting.

Altogether, 232 isolates from 77 samples were typed. Included were 85 *E. casseliflavus,* 63 *E. mundtii*, 40 *E. faecalis* and 44 *E. faecium.* Table 3-4 indicates the month and sample site of water and sediment samples included in the PFGE typing. Table 3-5 shows the number of the four most common species by BTO site.

3.3.2. Molecular Fingerprinting of Newport Bay *Enterococcus* Isolates: Results and Discussion

The PFGE patterns, dendogram of strain relatedness, and isolate/sample information is presented in Figures 3-4 through 3-7. Most PFGE types (or fingerprint patterns) were detected only once, consistent with the idea that there are many different strains of each *Enterococcus* species present in the water and sediment of Newport Bay. Not only are there many different types, these types are relatively heterogeneous, in that the dendrograms of the PFGE patterns reveal no meaningful grouping or sub-grouping at any level of relatedness by sampling site, sample type or date taken. Overall the number of pattern matches, indicating either inter-sample or intra-sample clonality, was low for the number of isolates analyzed.

Further analysis of all PFGE type matches or isolate "clonality" was carried out to determine isolate-sample relationships. Inter-sample clonality (two different samples

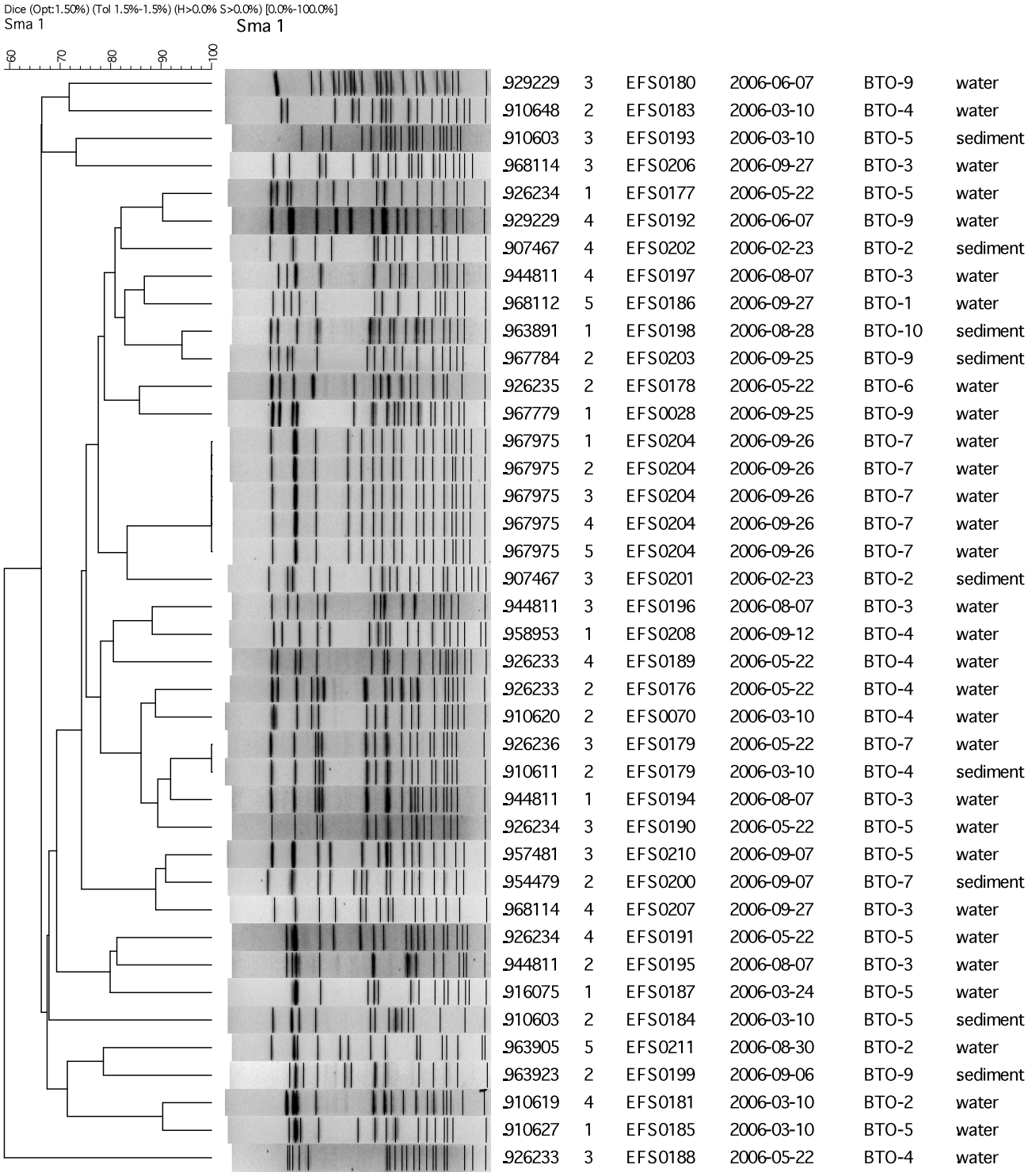

Figure 3-4. PFGE patterns and dendrograms of strain relatedness for *Enterococcus faecalis* isolates cultured from water and sediment samples in Newport Bay.

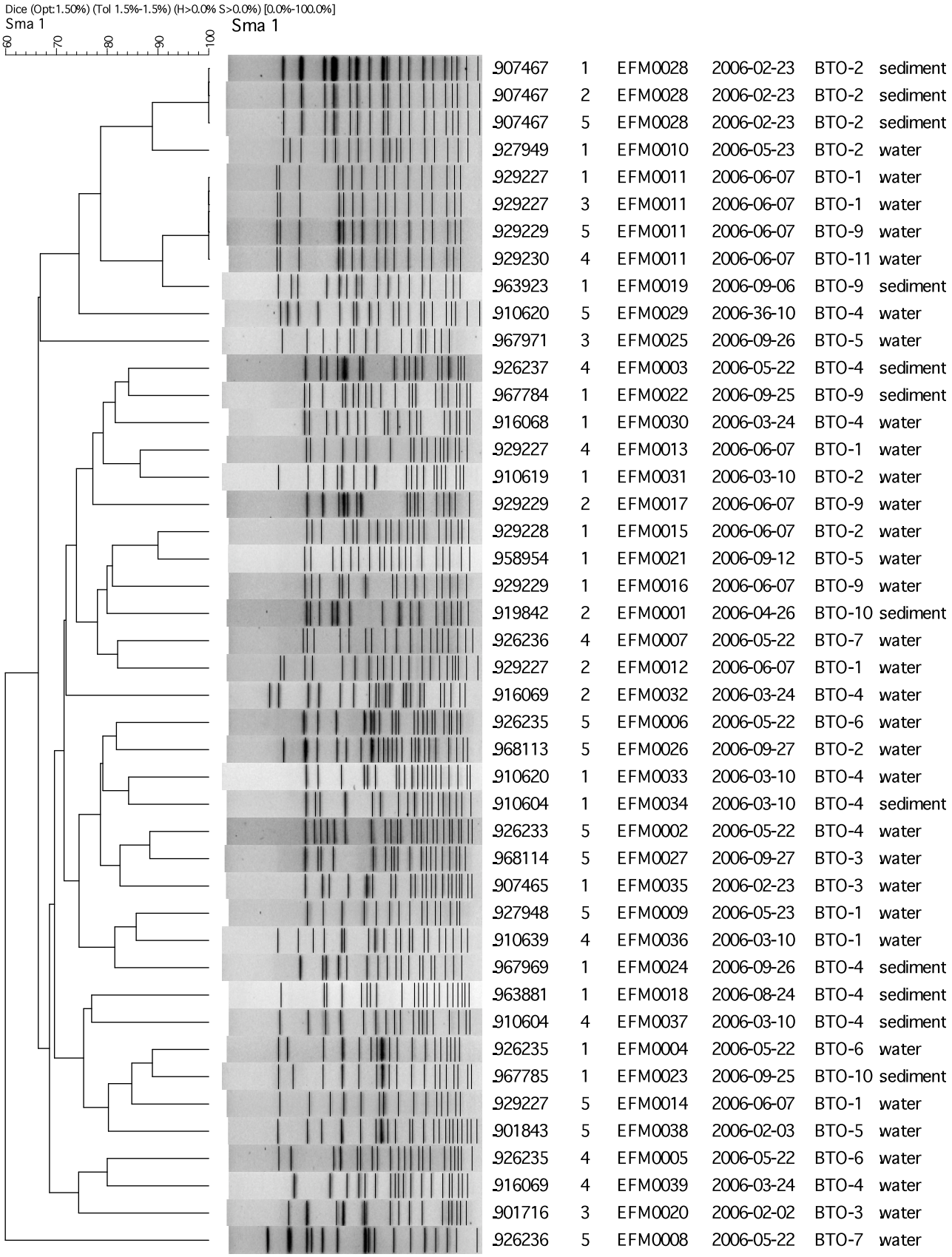

Figure 3-5. PFGE patterns and dendrograms of strain relatedness for *Enterococcus faecium* isolates cultured from water and sediment samples in Newport Bay.

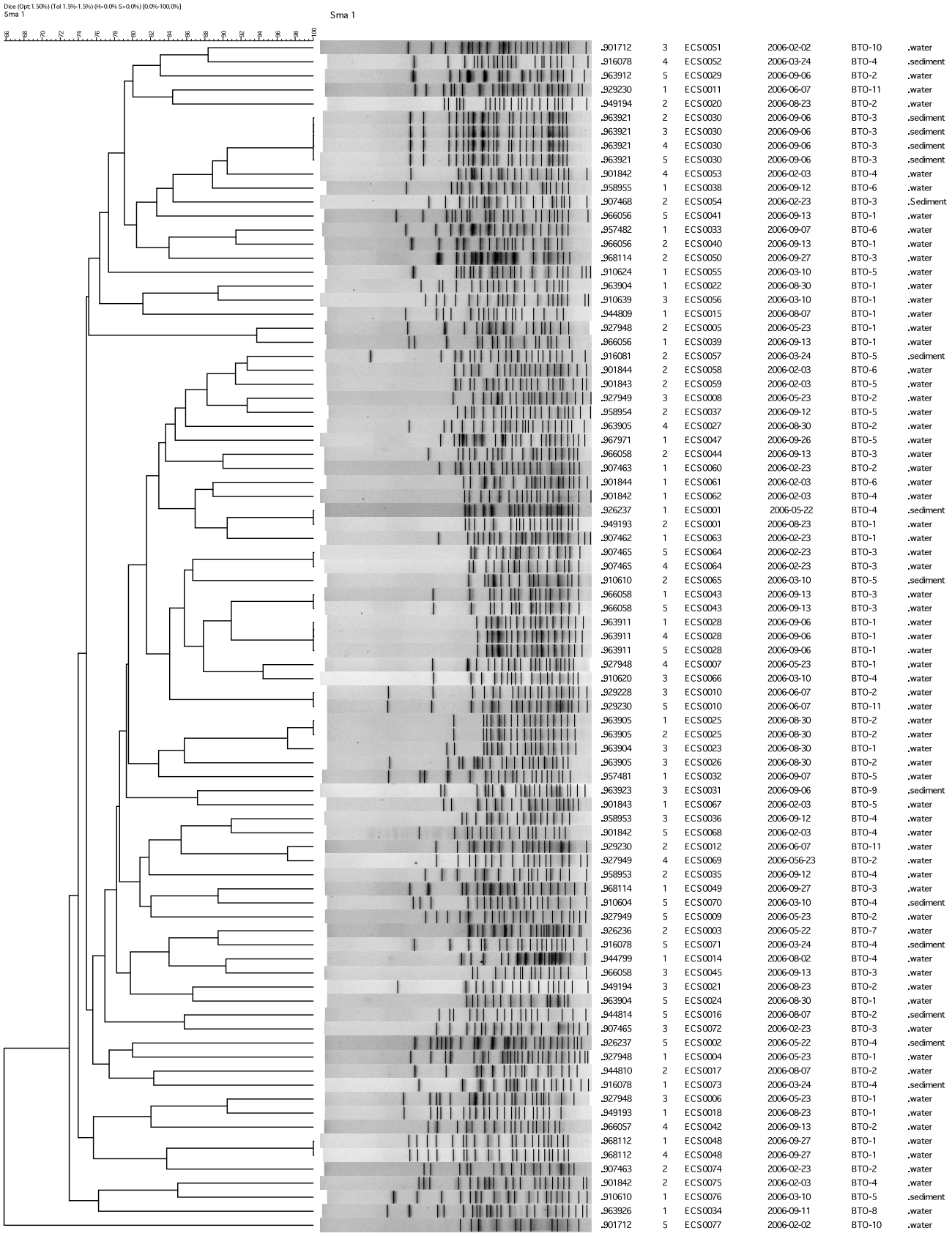

Figure 3-6. PFGE patterns and dendrograms of strain relatedness for *Enterococcus casseliflavus* isolates cultured from water and sediment samples in Newport Bay.

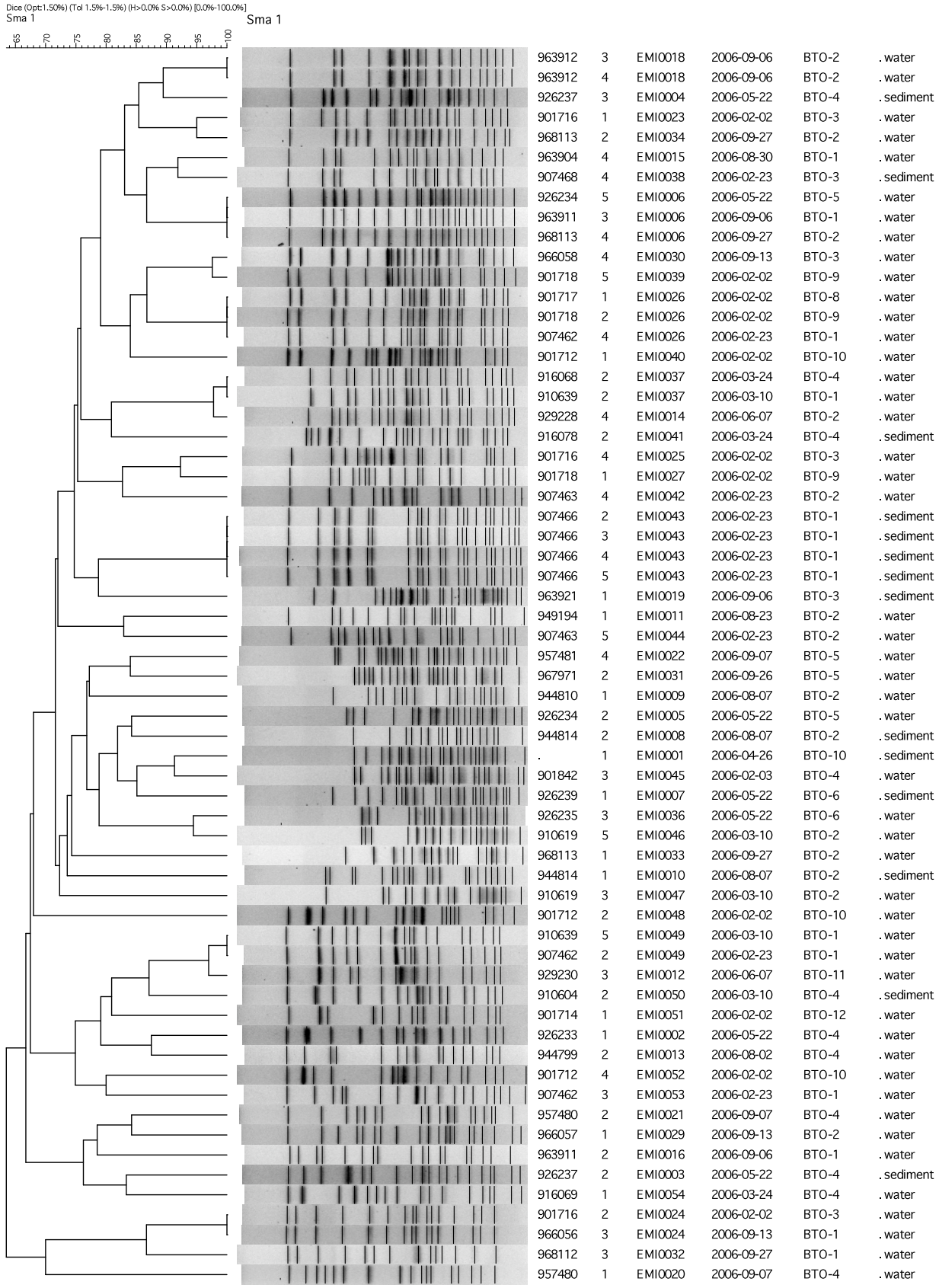

Figure 3-7. PFGE patterns and dendrograms of strain relatedness for *Enterococcus mundtii* isolates cultured from water and sediment samples in Newport Bay.

containing an isolate with the same PFGE pattern) is presented in Figs. 3-8 and 3-9. There were 18 samples that contained 21 clonal isolates seen in other samples. Two of these clonal isolates were from sediment samples and 19 were from water samples. Three samples (907462, 910639, and 929230) had two clonal types present in the same sample. Of the water samples containing clonal isolates, four had an *Enterococcus* concentration ≥104 CFU/ml, thus exceeding the EPA criteria for marine recreational waters. The most clonal matches were seen with *E. mundtii* with five PFGE types matching between two or more samples (Fig. 3-9). The percent of samples containing at least one clonal isolate in two different samples was higher in Lower Bay water samples and lower in Upper Bay water samples. Four of the eight Lower Bay water samples contained three PFGE types that matched types seen in San Diego Creek and Santa Ana Delhi channel (sites BTO-1 and BTO-2). The Lower Bay samples containing the matching types were collected the same day as the creek samples for two PFGE types (ECS0010 and EFM0011) and were collected three weeks earlier for one type (EMI0026).

Twenty-two percent (14/64) of total Upper Bay and creek samples contained isolates with nine different PFGE types matching isolates in other sample (Figs. 3-8 and 3-9). Six were PFGE types seen at San Diego Creek (BTO-1) and another site. One was a PFGE type seen in two different samples collected two weeks apart at BTO-1, one was a PFGE type seen in a BTO-4 sediment sample as well as in a BTO-7 water sample taken two months later, and one was a PFGE type seen in an Upper Bay site (BTO-2) and a Lower Bay site (BTO-11) from water samples taken on the day. The time difference between collection of samples containing matching types ranged from zero days for three PFGE types, 2 to 3 weeks for 3 PFGE types and between 10 week and 32 weeks for four PFGE types. Three matching PFGE types seen in San Diego Creek (BTO-1) and another site were seen in the downstream site three to 16 weeks before being collected at BTO-1. Since BTO-1 is above and upstream of all other sites that are tidally affected, it is possible that San Diego Creek may be the source of these clonal types. However, it is also possible that clonal types found at different sites in the Bay were not transported there from San Diego Creek, but rather originated from a source that releases *Enterococcus* throughout the Bay (i.e., birds, animals, plants or soil).

Intra-sample clonality (two or more isolates from the same sample with the same PFGE pattern) results are presented in Figs. 3-10 and 3-11. Fourteen percent (3 of 21) of sediment samples and 14% (8 of 56) of water samples had multiple isolates with matching PFGE types. One of these types (*E. faecium* EMF0011) also had inter-sample clonality. The majority of intra-sample matches were *E. casseliflavus* PFGE types isolated from Upper Bay (Fig. 3-10). All other species combined had five PFGE types (Fig. 3-11). Six samples contained two matching isolates within the same sample, two samples contained three matching isolates within the same sample, two contained four matching isolates and one sample contained 5 matching isolates within the same sample. The sediment samples that contained clonal isolates had concentrations of *Enterococcus* ranging from 500 to 2980 cfu/10g.

None of the *E. faecalis* types detected in this study matched *E. faecalis* types in a database containing 238 environmental isolates with 131 different PFGE types isolated from studies

Specimen	PFGE Type	Sample Location											
		BTO-1	BTO-2	BTO-3	BTO-4	BTO-5	BTO-6	BTO-7	BTO-8	BTO-9	BTO-10	BTO-11	BTO-12
E. faecalis	EFS0179				03/10/06 sediment 30 910611			5/22/06* water 6000 926236 Exceedence					
E. faecium	EFM0011	06/07/06 water 45 929227								06/07/06 water 38 929229		06/07/06 water 18 929230	
E. casseliflavu	ECS0001	08/23/06 water 20 949193			05/22/06 sediment 1000 926237								
	ECS0010		06/07/06 water 18 929228									06/07/06 water 18 929230	

* Date, Sample type, CFU/ml of sample, Sample number. "Exceedence" indicates sample contains ≥104 CFU/ml

Figure 3-8. Samples containing Enterococci isolates with matching PFGE patterns. Shown are PFGE matches for three of the four species included in the PFGE study (E. faecalis, E. faceium, and E. casseliflavus).

Specimen	PFGE Type	Sample Location											
		BTO-1	BTO-2	BTO-3	BTO-4	BTO-5	BTO-6	BTO-7	BTO-8	BTO-9	BTO-10	BTO-11	BTO-12
E. mundtii	EMI0006	09/06/06 water 66 963911	09/27/06 water 74 968113			05/22/06 water 2100 926234 Exceedence							
	EMI0024	09/13/06 water 54 966056		02/02/06 water 140 901716 Exceedence									
	EMI0026	02/23/06 water 26 907462							02/02/06 water 4 901717	02/02/06 water 240 901718 Exceedence			
	EMI0037	03/10/06 water 100 910639			03/24/06 water 30 916068								
	EMI0049	03/10/06 water 100 910639 02/23/06 water 26 907462											

* Date, Sample type, CFU/ml of sample, Sample number. "Exceedence" indicates sample contains ≥104 CFU/ml

Figure 3-9. Samples containing Enterococci isolates with matching PFGE patterns. Shown are PFGE matches for E. mundtii.

Specimen	PFGE Type	BTO-1	BTO-2	BTO-3	BTO-4	BTO-5	BTO-6	BTO 7	BTO-8	BTO-9	BTO-10	BTO-11	BTO-12
E. casseliflavus	ECS0025		8/30/06* water 10 963905-1,2										
	ECS0028	09/06/06 water 66 963911-1,4,5											
	ECS0030			09/06/06 sediment 600 963921-2,3,4,5									
	ECS0043			09/13/06 water 46 966058-1,5									
	ECS0048	09/27/06 water 14 968112-1, 4											
	ECS0064			02/23/06 water 82 907465-4,5									

* Date, Sample type, CFU/ml of sample, Sample number. "Failure" indicates sample contains ≥104 CFU/ml

Figure 3-10. Samples containing multiple Enterococci isolates with matching PFGE patterns. Shown are PFGE matches for E casseliflavus.

Specimen	PFGE Type	BTO-1	BTO-2	BTO-3	BTO-4	BTO-5	BTO-6	BTO 7	BTO-8	BTO-9	BTO-10	BTO-11	BTO-12
E. faecium	EFM0011	07/06/05 water 45 929227-1,3											
	EFM0028		02/23/06 sediment 500 907467-1,2,5										
E. faecalis	EFS0204							09/26/06 water 24 967975-1,2,3,4,5					
E. mundtii	EMI0018		09/06/06 water 14 963912 -3,4										
	EMI0043	02/23/06 sediment 2980 907466-2,3,4,5											

* Date, Sample type, CFU/ml of sample, Sample number. "Failure" indicates sample contains ≥104 CFU/ml

Figure 3-11. Samples containing multiple Enterococci isolates with matching PFGE patterns. Shown are PFGE matches for E faecium, E. faecalis, and E. mundtii.

conducted in Dana Point Harbor, Lower Santa Ana River and Huntington Beach from 2003 to 2006.

3.4. Summary and Conclusions

1) The study was successful in providing an overview of the species diversity and types of *Enterococcus* present in Newport Bay at the time and locations studied.

2) The majority of *Enterococcus* found in both Upper and Lower Newport Bay are *E. casseliflavus* and *E. mundtii*. *E. faecalis* and *E. faecium* species were seen with less frequency.

2). The vast majority of enterococci clonal matches were between two different samples from San Diego Creek (BTO-1) and Upper and Lower Bay sites. Lower Bay sites with samples containing clonal matches with BTO-1 included BTO-8 (near the outfall of Arches Drain) and BTO-11 (Rocky Point) which is adjacent to the harbor entrance. Since BTO-1 is not affected by tides, these results suggest that some enterococci bacteria seeded into the Bay from San Diego Creek might survive and transport to all regions of Newport Bay. Another interpretation is that the clonal bacteria are not transported from San Diego Creek, but rather from a common source (e.g., birds, plants, animals, soil) that is distributed widely throughout the Bay. The specific source in the creek (birds, plants, animals, etc.) was not identified in this study.

3) None of the *E. faecalis* types detected in this study matched *E. faecalis* types isolated from Dana Point Harbor, Lower Santa Ana River and Huntington Beach from 2003 to 2006. This suggests that the sources of these organisms may be relatively unique to Newport Bay.

4) The large number and heterogeneity of the PFGE types of all four enterococcus species indicates a complex input of sources.

5) The overall effect of regrowth of *Enterococcus* in sediments on the levels of bacterial pollution in Newport Bay could not be determined with the limited data from this study. However, a moderate percentage of sediment and water samples contained clonal isolates representing several species suggest that regrowth may occur, consistent with the observations of high ENT concentrations in the sub-tidal sediments of Newport Bay (see Chapter 2).

6) The high percentages of plant-related enterococci (*E. casseliflavus* and *E. mundtii*) is reported in this study is interesting, and merits additional investigation. Follow-up studies could also more carefully establish down-Bay and temporal variability in enterococci species distribution, and establish clonality to a higher degree of certainty. In this study, only a single enzyme was used to identify clonal matches with PFGE. Two or more enzymes could be employed to differentiate enterococci strains at a finer level of discrimination (e.g., down to a single mutation).

[1]Chapter 4. Phenotyping and Gene Array Analysis of Newport Bay *E. coli* isolates

4.1. Introduction and Overview

This chapter describes a set of three studies aimed at assessing the ecology of *E. coli* in Newport Bay. The three studies include: (1) *E. coli* isolates from Newport Bay were biotyped using commercially available biochemical assays; (2) the shift of *E. coli* biotypes were followed over the course of a microcosm incubation experiment; and (3) a novel gene expression assay was tested with the goal of identifying gene clusters in the *E. coli* genome responsible for environmental adaptation of this organism.

While both Chapters 3 and 4 use microbiological source tracking tools to obtain information about the relative importance of natural vs urban sources of microbial pollution, the target FIB group (*Enterococcus spp*. vs *Escherichia coli*) and methods employed in the two chapters are distinctly different. In Chapter 3, *Enterococcus* isolates were speciated, and some isolates were typed using PFGE. As noted earlier, PFGE was carried out to assess the degree to which *Enterococcus* isolates from Newport Bay are clonal; i.e., genetically identical at the level of discrimination afforded by the PFGE method employed.

In this chapter, putative *E. coli* isolates are assayed using an identification system called API-20E, a commercially available biochemical substrate test kit designed to identify enteric bacteria. Results from the API 20E tests serve to confirm that the presumptive *E. coli* isolates are in fact *E. coli*. Further, different strains of *E. coli* <u>may</u> be distinguished by their API biotypes (a unique numerical code that is assigned the isolate based on the scoring of biochemical reactions). It is important to stress that the API 20E biotypes have considerably less discriminatory power than the PFGE method used to evaluate *Enterococcus* clonality in Chapter 3. The limited discriminatory power of API 20E means that its use to evaluate the diversity of *E. coli* strains in Newport Bay should be viewed as exploratory. Indeed, as will be described shortly, the dominant *E. coli* biotype present in Newport Bay can have many different sources, based on a literature review and API 20E analysis of *E. coli* isolated from fecal samples from different animals. Finally, an experimental method was employed—called a gene expression assay—to assess if *E. coli* strains isolated from Newport Bay have unique gene expression patterns, which may reflect their adaptation to grow in the estuarine environment of Newport Bay.

The work described in this chapter satisfies the following task of the Prop 13 project: (1) the element of sub-task 2.1.4.1 focused on the relatedness of *E. coli* isolates from Newport Bay; (2) the element of sub-task 2.1.4.2 focused on the relatedness of *E. coli* isolates from microcosm studies.

[1] This chapter was prepared by Dr. S. Jiang, and edited by Drs. S. Grant and C. McGee

4.2. Phenotyping of Newport Bay *E. coli* Isolates

The objectives of this study were to examine *E. coli* isolates from the Bay for biotype diversity. The hypotheses tested were that there are specific *E. coli* biotypes better at survival in the environment and/or phenotypic differences could be detected in the organisms that were capable of survival in Newport Bay.

4.2.1. Methods: API 20E Substrate Utilization Tests

A subset of water and sediment sample splits collected in Newport Bay and its tributaries were transported to the Orange County Sanitation District, where they were analyzed for *E. coli* using membrane filtration on mTEC (EPA Method 1603). Presumptive *E. coli* isolates were transferred onto LB agar slants and stored at refrigeration temperature until species and biotype were analyzed using the Analytical Profile Index (API) 20E biochemical test (bioMerieux Vitek, Inc., Hazelwood, MO).

API 20E strips for *Enterobacteriaceae* and other Gram-negative rods were used for phenotyping of isolates. This system uses 23 standardized and miniaturized biochemical tests contained in 23 different wells on a single strip and a database to identify Gram-negative rods. Each isolate was streaked onto LB media plate and incubated at 37 ºC overnight. Sterile saline buffer was used to resuspend an individual bacterial colony that was no more than 24 hours old. The resuspension was then inoculated into each of the wells and stripes were incubated at 37°C. The results were read between 22 to 24 hours after inoculation. A profile number was determined from the sequence of positive and negative test results, then looked up in a codebook having a correlation between numbers and *E. coli* strains. Frozen stock was made for each of the tested bacterial isolates.

4.2.2. Sources and Timing of Newport Bay *E. coli* Isolates

Newport Bay *E. coli* isolates were cultured primarily from water samples (88%); fewer isolates were cultured from sediment samples (12%) (inset pie-chart, Fig. 4-1). Most isolates were cultured from tributary sites (BTO 1-3) and the upper reaches of Upper Bay (BTO 4-5); relatively fewer isolates were cultured from the other transect sites in Newport Bay (BTO 6-11) (upper panel, Fig. 4-1). There was an uneven distribution of isolates by month (bottom panel, Fig. 4-1). For example, 58 presumptive *E. coli* isolates were cultured from water and sediment samples collected from Newport Bay in September 2006. No isolates were cultured from Newport Bay in July, October, and December 2006.

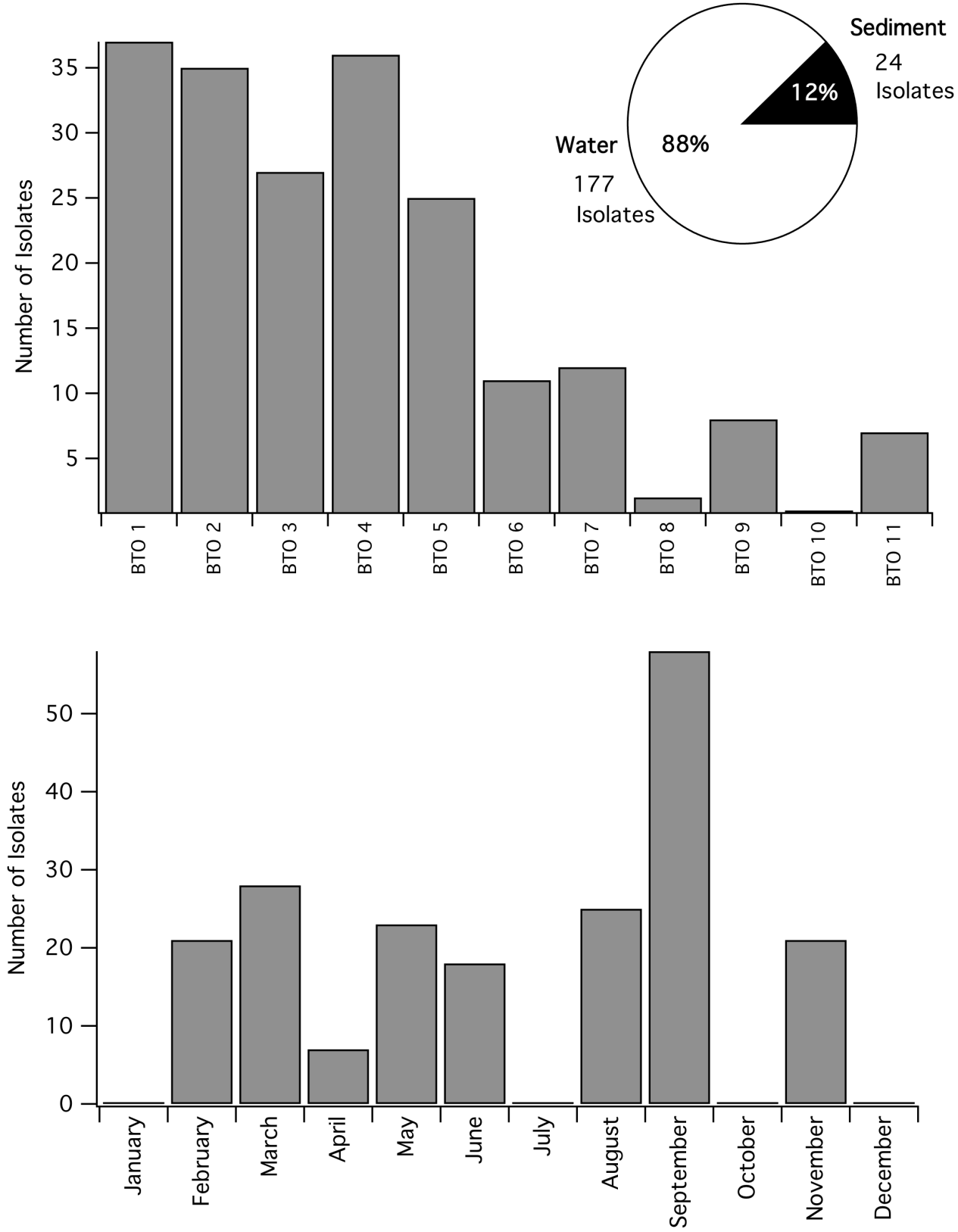

Figure 4-1. Number of *E. coli* isolates included in the API 20E biotyping study, sorted by matrix from which the isolates were obtained (sediment or water, pie chart), location in the Bay (upper panel), and date of collection (bottom panel).

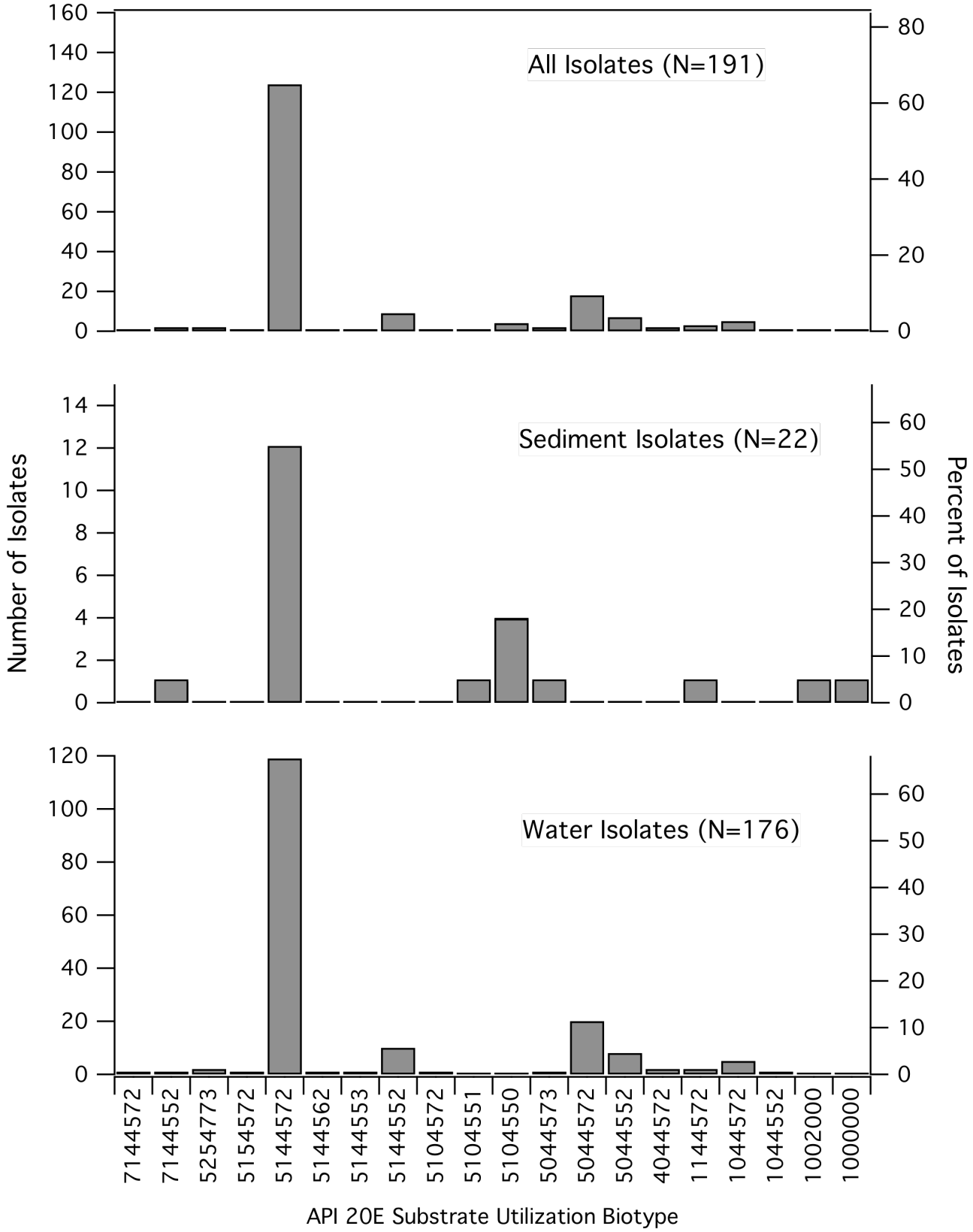

Figure 4-2. Distribution of Newport Bay *E. coli* API biotypes for all isolates (top panel), isolates collected from sediment samples (middle panel), and isolates obtained from water samples (bottom panel).

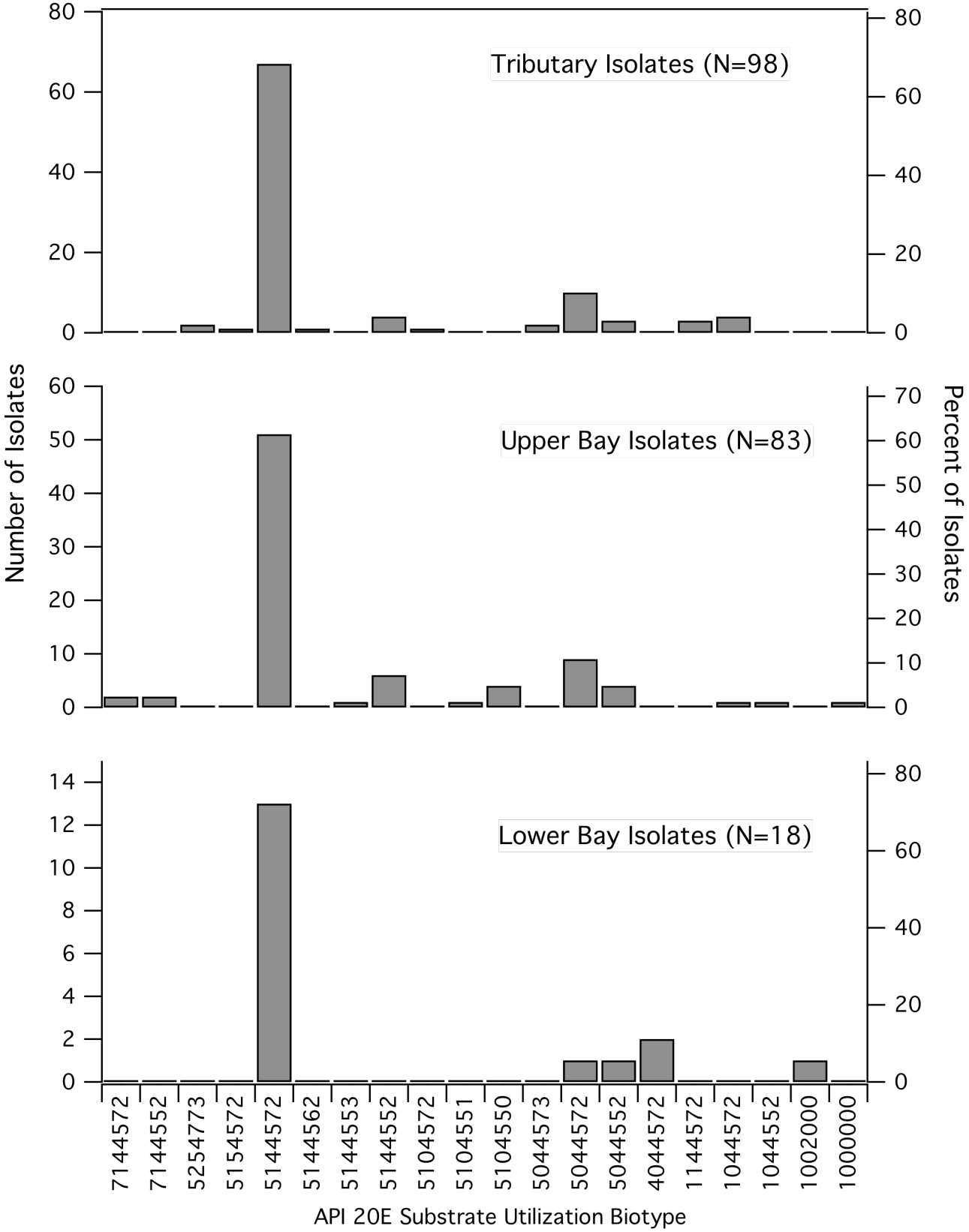

Figure 4-3. Distribution of Newport Bay *E. coli* API biotypes sorted by location in Newport Bay, including tributaries (top panel), Upper Bay (middle panel), and Lower Bay (bottom panel).

4.2.3. Dominant *E. coli* API Biotype

Sixty-five percent of *E. coli* isolates cultured from Newport Bay were identified as a single biotype, API code 5144572 (top panel, Fig. 4-2). This particular biotype is dominant for isolates cultured from sediment (middle panel, Fig. 4-2), from water (bottom panel, Fig. 4-2), and from all three regions of the Bay (tributaries, Upper Bay, and Lower Bay, Fig. 4-3).

4.2.4. Interpretation of API Biotype Data

The API biotyping described in this chapter does not have the discriminatory power of the PFGE method used to analyze enterococci bacteria isolates in Chapter 3. Nevertheless, the results reported here provide some information on the diversity of *E. coli* strains in Newport Bay and its tributaries. In particular, the predominance of a single biotype (API code 5144572) is noteworthy. A review of the literature reveals that this *E. coli* biotype has been isolated from a variety of fecal sources, including a wastewater treatment plant, pig farm manure, and dairy cattle manure in France[2] and rabbit feces in the U.S.[3]. Thus, it cannot be said that this particular biotype always comes from a specific type of fecal pollution. Furthermore, because the dominant biotype in Newport Bay may include multiple sub-strains each with its own environmental and/or fecal source, it would be premature to conclude that tributaries are the primary source of *E. coli* to Newport Bay, as might be surmised by the predominance of *E. coli* 5144572 in both tributary and Bay samples (Fig. 4-3). More discriminating analyses, such as PFGE or rep-PCR, of these isolates might shed light on the environmental significance of this interesting result[4].

API biotyping was also carried out on *E. coli* isolated from several microcosm experiments involving mixtures of Bay and Creek water, as described in Chapter 5 of this report. Unfortunately, *E. coli* concentrations were very low in these microcosms, so very few isolates (3 in one microcosm and 7 in another) were available for API substrate testing. Because of the very low number of isolates available, it is not possible to discern statistically significant trends in *E. coli* biotype diversity (data not shown).

4.3. *E. coli* Gene Expression

The objective of this part of the study is to test the hypothesis that global gene expression patterns of *E. coli* will be different for isolates that have adapted to survive or grow in the natural environment, compared to isolates adapted to grow in human and animal guts. The hypothesis is motivated by the observation that microorganisms respond quickly to selective pressure, because of their short generation time and large population sizes. For

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[2] Vernozy-Rozand, C., Montet, M.P., Lequerrec, F., Serillon, E., Tilly, B., Bavai, C., Ray-Gueniot, S., Bouvet, J., Mazuy-Cruchaudet, C., Richard, Y. (2002) "Prevalence of vertotoxin-producing E. xoli (VTEC) in slurry, farmyard manure, and sewage sludge in France" *J. Applied Microbiol*. 93, 473-478 (see Table 2).
[3] A. Garcia, Fox, J.G. (2003) "The rabbit as a new reservoir host of enterohemorrhagic *Escherichia coli*" *Emerging Infectious Diseases*, 12, 1592-1596.
[4] Santo Domingo, J.W., Banbic, D.G., Edge, T.A., Wuertz, S. (2007) "Quo vadis source tracking? Towards a strategic framework for environmental monitoring of fecal pollution" *Water Research* **41**, 3539-3552.

example, Rehle et al.[5,6] have shown that *E. coli* growing in a warmer environment leads to detectable changes in the bacterial genome after only 2000 generations[7]. Hence, *E. coli* strains adapted to survive or grow in an aquatic environment, such as the Newport Bay, may be identifiable based on their global gene expression patterns.

4.3.1. *E. coli* Gene expression: Methods

E. coli isolated from human sewage, bird and cattle feces, and Newport Bay were used for this study. All except one of the Newport Bay isolates have the dominant biotype as described above, API code 5144572. One each of the bird and sewage isolates and two of the cattle isolates were also identified as the same biotype, consistent with the conclusion above that this biotype cannot be ascribed to any particular source of fecal pollution. Each isolate was cultured under a common condition, 37 ºC in LB medium. This common condition—termed "common garden" in evolutionary terminology—serves as a standardized condition for the microarray hybridization assay.

Total RNA was extracted from cells and used for cDNA synthesis and labeling as described in Riehle et al.[5]. The DNA high-density array was purchased from Affimatrix. The GeneChip® *E. coli* Genome 2.0 Array contains probe sets to detect transcripts from the K12 strain of *E. coli* and three pathogenic strains of *E. coli*. The GeneChip *E. coli* Genome 2.0 Array includes approximately 10,000 probe sets for all 20,366 genes present in four strains of *E. coli*. DNA hybridization and detection were performed by UCI microarray facility following Affirmatrix's standard protocol. The data were analyzed using array analysis software. The expression signals were normalized and all genes on the array were used for generation of similarity profiles (referred to as a dendrogram)

4.3.2. *E. coli* Gene expression: Results

Gene expression patterns obtained for *E. coli* isolated from Newport Bay were indistinguishable from *E. coli* isolated from human or animal feces (data not shown). This result suggests that, based on the global gene expression patterns, *E. coli* isolated from the Newport Bay are similar to *E. coli* isolated from human and animal feces. The use of gene expression for microbial source tracking is new, and thus from these results alone we cannot conclude with certainty that *E. coli* in Newport Bay are derived from human and/or animal feces.

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[5] Riehle, M.M., A. F. Bennett, and A.D. Long (2001) "Genetic architecture of thermal adaptation in *Escherichia coli*" *Proceedings of the National Academy of Sciences*, 98: 525-530.
[6] Riehle, M.M., A.F. Bennett, R.E. Lenski, A.D. Long (2003) "Evolutionary changes in heat-inducible gene expression in *Escherichia coli* lines evolved at high temperature" *Physiol. Genomics* 14:47-58.
[7] Although generation time of *E. coli* in the environment is not known, it could be as short as 20 minutes under ideal conditions.

4.4. Summary

1. *E. coli* in Newport Bay is dominated by a single biotype, API code 5144572.

2. The significance of the low biotype diversity of *E. coli* in Newport Bay cannot be evaluated without further analysis of the isolates belonging to this biotype, perhaps using molecular methods such as PFGE and/or rep-PCR. However, it is likely that *E. coli* belonging to this biotype come from a variety of fecal sources, based on experiments reported here (Section 4.3.2) and a survey of the literature.

3. The experimental design of this study did not detect differences in the global gene expression patterns of *E. coli* isolates from Newport Bay and from human sewage, bird and cattle feces.

[1]Chapter 5. Microcosm studies to characterize the rate and magnitude of natural sources and within-Bay processes that affect the concentration of FIB in Newport Bay

5.1. Introduction and Overview

This chapter summarizes microcosm and field experiments conducted to characterize the input of FIB to Newport Bay from a variety of natural sources (bird droppings, debris mats, sediment), and the influence of environmental variables (sunlight, synergistic interactions between various substrates) on FIB persistence in Newport Bay. Microcosm studies were conducted both in the laboratory (to isolate the influence of specific variables) and in the field (to more realistically simulate conditions in Newport Bay). In several cases, supplemental field investigations were carried out to augment the microcosm studies. Supplemental field investigations included a synoptic study of FIB concentrations in Upper Bay during a macroalgal bloom, and field observations of debris mat formation in Upper and Lower Bay during and following storm events.

This chapter partially satisfies Task 2.1.3.7, by reporting on the following tasks of the Prop 13 contract:

- Task 2.1.3.1: Microcosms seeded with bird droppings
- Task 2.1.3.2: Microcosms seeded with debris mats
- Task 2.1.3.3: Field observations of debris mat abundance
- Task 2.1.3.6: Microcosm studies of environmental factors affecting FIB die-off rates

Table 5-1 summarizes the microcosm experiments presented in this chapter, including the date(s) each microcosm experiment was conducted, the microcosm's unique identifier (needed to access the raw data in the excel spreadsheet where the microcosm data are archived), the source of FIB in the microcosm, the microcosm's experimental design, and the chapter sections where data are presented. In all, 64 separate microcosm experiments are described in this chapter (67 microcosms appear in the list in Table 5-1, but two are control experiments that appear twice in the list). The study team carried out 8 additional field microcosm experiments to evaluate the effect of bird feces and macroalgae on FIB concentrations in Newport Bay and San Diego Creek. These experiments terminated when the dialysis bags ruptured 1 day into the study, thus spurring the development of an alternative, and ultimately successful, approach for conducting microcosm experiments with dialysis bags in the field. The alternative approach involved suspending the dialysis bags in milk crates that, in turn, were suspended in San Diego Creek or Newport Bay. Including these truncated dialysis experiments (for which the archived data are available), a total of 72 separate microcosm experiments were conducted for this study—two more than budgeted in the Prop 13 contract (20 for Task 2.1.3.1, 20 for Task 2.1.3.2, and 30 for Task 2.1.3.6).

[1] Prepared by Drs. K. McLaughlin, S. Grant, and S. Jiang with assistance from Ms. R. Litton and Dr. Weiping Chu

Table 5-1. Summary of microcosm experiments completed for Task 2.1.3.

Study Date	***Identifier***	***Lab/Field***	***FIB source***	***Description***	***Report Sections***
4/10 to 4/17 (2006)	A	Lab (18°C, dark)	San Diego Creek (BTO 1)	Unfiltered Creek water (single-source control)	5.2.1 & 5.2.2
4/10 to 4/17 (2006)	B	Lab (18°C, dark)	Upper Bay (BTO 5)	Unfiltered Bay water (single-source control)	5.2.1 & 5.2.2
4/10 to 4/17 (2006)	C	Lab (18°C, dark)	BTO 1 & BTO 5	1:2 unfiltered Creek:unfiltered Bay	5.2.1 & 5.2.2
4/10 to 4/17 (2006)	D	Lab (18°C, dark)	BTO 1 & BTO 5	1:1 unfiltered Creek:unfiltered Bay	5.2.1 & 5.2.2
4/10 to 4/17 (2006)	E	Lab (18°C, dark)	BTO 1 & BTO 5	2:1 unfiltered Creek:unfiltered Bay	5.2.1 & 5.2.2
4/10 to 4/17 (2006)	F	Lab (18°C, dark)	No-source	Filtered Creek water (negative control)	5.2.1 & 5.2.2
4/10 to 4/17 (2006)	G	Lab (18°C, dark)	BTO 5	1:2 filtered Creek:unfiltered Bay	5.2.1 & 5.2.2
4/10 to 4/17 (2006)	H	Lab (18°C, dark)	BTO 5	1:1 filtered Creek:unfiltered Bay	5.2.1 & 5.2.2
4/10 to 4/17 (2006)	I	Lab (18°C, dark)	BTO 5	2:1 filtered Creek:unfiltered Bay	5.2.1 & 5.2.2
4/10 to 4/17 (2006)	J	Lab (18°C, dark)	No-source	Filtered Bay water (negative control)	5.2.1 & 5.2.2
4/10 to 4/17 (2006)	K	Lab (18°C, dark)	BTO 1	1:2 unfiltered Creek:filtered Bay	5.2.1 & 5.2.2
4/10 to 4/17 (2006)	L	Lab (18°C, dark)	BTO 1	1:1 unfiltered Creek:filtered Bay	5.2.1 & 5.2.2
4/10 to 4/17 (2006)	M	Lab (18°C, dark)	BTO 1	2:1 unfiltered Creek:filtered Bay	5.2.1 & 5.2.2
2/1 to 2/7 (2007)	Light-A	Field (tanks on Kerchoff Dock)	SDC	Unfiltered Creek Water (single-source control); uncovered	5.2.3 & 5.2.4

Table 5-1. Summary of microcosm experiments completed for Task 2.1.3 (cont.).

Study Date	***Identifier***	***Lab/Field***	***FIB source***	***Description***	***Report Sections***
2/1 to 2/7 (2007)	Light-B	Field (tanks on Kerchoff Dock)	BTO 5	Unfiltered Bay Water (single-source control); uncovered	5.2.3 & 5.2.4
2/1 to 2/7 (2007)	Light-C	Field (tanks on Kerchoff Dock)	SDC & BTO 5	1:1 Unfiltered Creek: unfiltered Bay; uncovered	5.2.3 & 5.2.4
2/1 to 2/7 (2007)	Light-D	Field (tanks on Kerchoff Dock)	No source	Filtered Creek water (negative control); uncovered	5.2.3 & 5.2.4
2/1 to 2/7 (2007)	Light-E	Field (tanks on Kerchoff Dock)	BTO 5	1:1 Filtered Creek: unfiltered Bay; uncovered	5.2.3 & 5.2.4
2/1 to 2/7 (2007)	Light-F	Field (tanks on Kerchoff Dock)	No source	Filtered Bay water (negative control) exposed to light	5.2.3 & 5.2.4
2/1 to 2/7 (2007)	Light-G	Field (tanks on Kerchoff Dock)	BTO 1	1:1 Unfiltered Creek: filtered Bay; uncovered	5.2.3 & 5.2.4
2/1 to 2/7 (2007)	Dark-I	Field (tanks on Kerchoff Dock)	BTO 1	Unfiltered Creek (single source control); covered	5.2.3 & 5.2.4
2/1 to 2/7 (2007)	Dark-J	Field (tanks on Kerchoff Dock)	BTO 5	Unfiltered Bay (single source control); covered	5.2.3 & 5.2.4
2/1 to 2/7 (2007)	Dark-K	Field (tanks on Kerchoff Dock)	BTO 1 & BTO 5	1:1 Unfiltered Creek: unfiltered Bay; covered	5.2.3 & 5.2.4
2/1 to 2/7 (2007)	Dark-L	Field (tanks on Kerchoff Dock)	No source	Filtered Creek water (negative control); covered	5.2.3 & 5.2.4
2/1 to 2/7 (2007)	Dark-M	Field (tanks on Kerchoff Dock)	BTO 5	1:1 Filtered Creek: unfiltered Bay; covered	5.2.3 & 5.2.4
2/1 to 2/7 (2007)	Dark-N	Field (tanks on Kerchoff Dock)	BTO 1	1:1 Unfiltered Creek: filtered Bay; covered	5.2.3 & 5.2.4
2/1 to 2/7 (2007)	Dark-O	Field (tanks on Kerchoff Dock)	No source	Filtered Bay water (negative control); covered	5.2.3 & 5.2.4
6/12 to 6/16 (2006)	LC-C	Lab (20°C, dark)	BTO 1	Unfiltered Creek water (single source control)	5.3.1 & 5.3.2

Table 5-1. Summary of microcosm experiments completed for Task 2.1.3 (cont.).

Study Date	***Identifier***	***Lab/Field***	***FIB source***	***Description***	***Report Sections***
6/12 to 6/16 (2006)	LC-MA	Lab (20°C, dark)	BTO 1 & Macroalgae	Unfiltered Creek water & Macroalgae from BTO 5	5.3.1 & 5.3.2
6/12 to 6/16 (2006)	LC-BP	Lab (20°C, dark)	BTO 1 & Bird feces	Unfiltered Creek water & Bird feces homogenate	5.3.1 & 5.3.2
6/12 to 6/16 (2006)	LC-MABP	Lab (20°C, dark)	BTO 1, Bird feces, & Macroalgae	Unfiltered Creek water, Bird feces homogenate, & Macroalgae	5.3.1 & 5.3.2
6/12 to 6/16 (2006)	LB-C	Lab (20°C, dark)	BTO 5	Unfiltered Bay water (single-source control)	5.3.1 & 5.3.2
6/12 to 6/16 (2006)	LB-MA	Lab (20°C, dark)	BTO 5 & Macroalgae	Unfiltered Bay water & Macroalgae from BTO 5	5.3.1 & 5.3.2
6/12 to 6/16 (2006)	LB-BP	Lab (20°C, dark)	BTO 5 & Bird feces	Unfiltered Bay water & Bird feces homogenate	5.3.1 & 5.3.2
6/12 to 6/16 (2006)	LB-MABP	Lab (20°C, dark)	BTO 5, Bird feces, & Macroalgae	Unfiltered Bay water, Bird feces homogenate, & Macroalgae	5.3.1 & 5.3.2
10/2 to 10/6 (2006)	BC	Field (dialysis at Shellmaker)	Upper Bay water (Shellmaker)	Upper Bay water dialyzed against Upper Bay water (single source control)	5.3.3 & 5.3.4
10/2 to 10/6 (2006)	BA	Field (dialysis at Shellmaker)	Upper Bay water (Shellmaker) + Macroalgae	Upper Bay water & Macroalgae dialyzed against Upper Bay water	5.3.3 & 5.3.4
10/2 to 10/6 (2006)	BP	Field (dialysis at Shellmaker)	Upper Bay water (Shellmaker) + Bird Feces	Upper Bay water & Bird Feces dialyzed against Upper Bay water	5.3.3 & 5.3.4
10/2 to 10/6 (2006)	BAP	Field (dialysis at Shellmaker)	Upper Bay water (Shellmaker) + Macroalgae + Bird Feces	Upper Bay water, Macroalgae & Bird Feces dialyzed against Upper Bay water	5.3.3 & 5.3.4
10/2 to 10/6 (2006)	CC	Field (dialysis at BTO 1)	BTO 1 water	BTO 1 water dialyzed against BTO 1 water (single source control)	5.3.3 & 5.3.4
10/2 to 10/6 (2006)	CA	Field (dialysis at BTO 1)	BTO 1 water + Macralgae	BTO 1 water & Macroalgae dialyzed against BTO 1 water	5.3.3 & 5.3.4
10/2 to 10/6 (2006)	CP	Field (dialysis at BTO 1)	BTO 1 water + Bird Feces	BTO 1 water & Bird Feces dialyzed against BTO 1 water	5.3.3 & 5.3.4
10/2 to 10/6 (2006)	CAP	Field (dialysis at BTO 1)	BTO 1 water + Macroalgae + Bird Feces	BTO 1 water, Macroalgae & Bird Feces dialyzed against BTO 1 water	5.3.3 & 5.3.4

Table 5-1. Summary of microcosm experiments completed for Task 2.1.3 (cont.).

Study Date	***Identifier***	***Lab/Field***	***FIB source***	***Description***	***Report Sections***
11/13 to 11/22 (2006)	C-C	Lab (20°C, dark)	BTO 1 water	BTO 1 water (single-source control)	5.4.1 & 5.4.2
11/13 to 11/22 (2006)	C-S	Lab (20°C, dark)	BTO 1 water + BTO 1 sediment	Affect of Creek sediment on Creek FIB survival	5.4.1 & 5.4.2
11/13 to 11/22 (2006)	UB-C	Lab (20°C, dark)	BTO 5 water	BTO 5 water (single-source control)	5.4.1 & 5.4.2
11/13 to 11/22 (2006)	UB-S	Lab (20°C, dark)	BTO 5 water + BTO 5 sediment	Affect of Upper Bay sediment on Bay FIB survival	5.4.1 & 5.4.2
11/13 to 11/22 (2006)	NS-C	Lab (20°C, dark)	BTO 6 water	BTO 6 water (single-source control)	5.4.1 & 5.4.2
11/13 to 11/22 (2006)	NS-S	Lab (20°C, dark)	BTO 6 water + BTO 6 sediment	Affect of North Star sediment on Bay FIB survival	5.4.1 & 5.4.2
11/13 to 11/22 (2006)	LBF-C	Lab (20°C, dark)	BTO 9 water	BTO 9 water (single-source control)	5.4.1 & 5.4.2
11/13 to 11/22 (2006)	LBF-S	Lab (20°C, dark)	BTO 9 water + BTO 9 sediment	Affect of Lower Bay sediment on Bay FIB survival	5.4.1 & 5.4.2
11/13 to 11/22 (2006)	LBS-C	Lab (20°C, dark)	BTO 11 water	BTO 11 water (single-source control)	5.4.1 & 5.4.2
11/13 to 11/22 (2006)	LBS-S	Lab (20°C, dark)	BTO 11 water + BTO 11 sediment	Affect of Lower Bay sediment on Bay FIB survival	5.4.1 & 5.4.2
10/2 to 10/6 (2006)	BC	Field (dialysis at Shellmaker)	Upper Bay water (Shellmaker)	Upper Bay water dialyzed against Upper Bay water (single source control)	5.4.3 & 5.4.4

Table 5-1. Summary of microcosm experiments completed for Task 2.1.3 (cont.).

Study Date	***Identifier***	***Lab/Field***	***FIB source***	***Description***	***Report Sections***
10/2 to 10/6 (2006)	BS	Field (dialysis at Shellmaker)	Upper Bay water (Shellmaker) + Upper Bay Sediment	Upper Bay water & Sediment dialyzed against Upper Bay water	5.4.3 & 5.4.4
10/2 to 10/6 (2006)	BS-a	Field (dialysis at Shellmaker)	Upper Bay water (Shellmaker) + autoclaved Upper Bay Sediment	Upper Bay water & autoclaved Upper Bay Sediment dialyzed against Upper Bay water	5.4.3 & 5.4.4
10/2 to 10/6 (2006)	CC	Field (dialysis at BTO 1)	BTO 1 water	BTO 1 water dialyzed against BTO 1 water (single source control)	5.4.3 & 5.4.4
10/2 to 10/6 (2006)	CS	Field (dialysis at BTO 1)	BTO 1 water + Upper Bay Sediment	BTO 1 water & Upper Bay sediment dialyzed against BTO 1 water	5.4.3 & 5.4.4
10/2 to 10/6 (2006)	CS-a	Field (dialysis at BTO 1)	BTO 1 water + autoclaved Upper Bay Sediment	BTO 1 water & autoclaved Upper Bay Sediment dialyzed against BTO 1 water	5.4.3 & 5.4.4
2/1 to 2/7 (2007)	Light-B	Field (tanks on Kerchoff Dock)	BTO 5 Water	Unfiltered Bay Water (single-source control); uncovered	5.5.1 & 5.5.2
2/1 to 2/7 (2007)	Dark-J	Field (tanks on Kerchoff Dock)	BTO 5 Water	Unfiltered Bay Water (single-source control); covered	5.5.1 & 5.5.2
2/1 to 2/7 (2007)	Light-H	Field (tanks on Kerchoff Dock)	BTO 5 Water + Debris collected from Upper Bay	Affect of Debris on Upper Bay FIB; uncovered	5.5.1 & 5.5.2
2/1 to 2/7 (2007)	Dark-P	Field (tanks on Kerchoff Dock)	BTO 5 Water + Debris collected from Upper Bay	Affect of Debris on Upper Bay FIB; covered	5.5.1 & 5.5.2
2/8 to 2/9 (2007)	A-Control	Field (dialysis at Balboa Yacht Basin)	Lower Bay Water	Unfiltered Lower Bay Water dialyzed against Lower Bay Water (single source control)	5.6.1, 5.6.2, & 5.6.3
2/8 to 2/9 (2007)	B-Polaris	Field (dialysis at Balboa Yacht Basin)	Lower Bay Water + Dry Weather Runoff from Polaris Drain	Unfiltered Lower Bay Water + Unfiltered Polaris Drain Runoff dialyzed against Lower Bay Water	5.6.1, 5.6.2, & 5.6.3
2/8 to 2/9 (2007)	C-Balboa	Field (dialysis at Balboa Yacht Basin)	Lower Bay Water + Dry Weather Runoff from Balboa Curb	Unfiltered Lower Bay Water + Unfiltered Balboa Curb Runoff dialyzed against Lower Bay Water	5.6.1, 5.6.2, & 5.6.3

5.2. Microcosms containing Mixtures of Creek and Bay Water

In this section we describe laboratory and field microcosms consisting of mixtures of Creek and Bay water, with or without filter sterilization. The goals of these experiments were two-fold:

- To evaluate how creek FIB respond to being mixed into Bay water and how Bay FIB respond to being mixed with Creek water (as occurs when San Diego Creek runoff mixes into Newport Bay).
- To evaluate the survival of Creek and Bay FIB upon exposure to environmental variables (e.g., solar intensity, nutrients, salinity, temperature) typical of Newport Bay.

5.2.1. Methods: Laboratory Microcosms Containing Mixtures of Creek and Bay Water

Water samples from Upper Newport Bay (Station BTO 5) and San Diego Creek (BTO 1) were collected in sterile 20 L carboys (see Fig. 2.1 in Chapter 2 for BTO sampling sites). Carboys were rinsed three times with sample water before filling. These samples were transferred to the laboratory at UCI within 2 hours of collection. Five liters of Bay and creek water were filter sterilized through a 0.2 μm TFF cartridge to remove all FIB. Unfiltered water was kept in the dark at 4°C until all of the filter-sterilized samples were prepared.

Microcosms consisted of 2 L sterile Erlenmeyer flasks, which were kept in a temperature-controlled room held at 18°C in the dark. There were 4 control microcosms (Bay water alone, creek water alone, filter-sterilized Bay water alone, and filter-sterilized creek water alone) and 9 microcosms with different mixtures of source waters. The mixed microcosms included 1:2, 1:1, and 2:1 mixtures of creek water and Bay water, with or without filter sterilization. The total volume in each microcosm was 1500 mL. Microcosms were swirled in the morning, during sampling, and in the evening of each day to minimize hypoxia (low dissolved oxygen). The following microcosms were prepared:

- 1500 ml Creek Water
- 1500 ml Bay Water
- 500 ml Creek Water + 1000 ml Bay Water (1:2)
- 750 ml Creek Water + 750 ml Bay Water (1:1)
- 1000 ml Creek Water + 500 ml Bay Water (2:1)
- 1500 ml 0.2 um filtered Creek Water
- 500 ml filtered Creek Water + 1000 ml Bay Water (1:2)
- 750 ml filtered Creek Water + 750 ml Bay Water (1:1)
- 1000 ml filtered Creek Water + 500 ml Bay Water (2:1)
- 1500 ml 0.2 um filtered Bay Water
- 500 ml Creek Water + 1000 ml filtered Bay Water (1:2)
- 750 ml Creek Water + 750 ml filtered Bay Water (1:1)
- 1000 ml Creek Water + 500 ml filtered Bay Water (2:1)

Sub-samples were withdrawn from each microcosm using sterile serological pipettes. Sub-samples were collected immediately following microcosm construction (time 0), 3 hours, 6 hours, 12 hours, 24 hours (1 day), 48 hours (2 days), 72 hours (3 days), 96 hours (4 days), and

120 hours (5 days). Water samples were analyzed for FIB using IDEXX Colilert-18 and Enterolert tests, implemented in a 96 well quantitray format. For the 1:10 dilution employed, the dynamic range of the Colilert-18 and Enterolert assays is 10 to 24,192 MPN/100 mL. Samples that had concentrations below the detection limit of 10 MPN/100 mL were assigned a left censored value of 1 MPN/100 mL Samples that had concentrations in excess of the detection limit of 24192 MPN/100 mL were assigned a right censored value equal to the upper limit value.

5.2.2. Results and Discussion: Laboratory Microcosms Containing Creek and Bay Water

Microcosm Salinity Variability.

Salinity of the microcosms varied from 1 to 22 depending on the mixing ratio of creek and Bay waters. Table 5-2 indicates salinity measurements recorded on the final day of the experiment. Salinity was not recorded in each microcosm until the final day to prevent contamination by the salinity probe.

Table 5-2. Salinity measured in laboratory microcosms containing different ratios of Creek and Bay waters.

Microcosm:	**Bay**	**Creek**	**1:2 Creek: Bay**	**1:1 Creek: Bay**	**2:1 Creek: Bay**
Salinity:	22	1	17	12	6

Concentrations and Die-off of Escherichia coli and Enterococci Bacteria

The results of the microcosm studies are shown as time series plots in Figs. 5-1 (*E. coli*) and 5-2 (enterococci bacteria). The different panels in each figure correspond to different sources of FIB. The top panel shows the results obtained when both creek and Upper Bay bacteria are present; the second panel shows the results when only Upper Bay bacteria are present; the third panel shows the results when only San Diego Creek bacteria are present.

The results presented in Figs. 5-1 and 5-2 indicate that: (1) FIB are present at higher concentrations in the creek water than in the Bay water; (2) FIB in the creek water die-off relatively slowly when mixed with filter sterilized Bay water; (3) FIB in Bay water die-off relatively rapidly when mixed with filter sterilized Creek water. At the start of the microcosm experiments, FIB concentrations were 10 to 1000 fold higher in the creek water than in the Bay water (top panels, Figs. 5-1, 5-2). In microcosms containing only bacteria from the Bay (i.e., creek water was filter sterilized), *E. coli* and enterococci bacteria concentrations declined rapidly with time, usually dropping below the lower-limit of detection within 12 hours (middle panel, Figs. 5-1, 5-2). The die-off of Bay bacteria occurred rapidly for all dilution ratios of unfiltered Bay to filter sterilized Creek waters. In microcosms containing only bacteria from the creek (i.e., Bay water was filter sterilized), *E. coli* and enterococci bacteria concentrations declined less rapidly with time, usually dropping no more than a factor of 10 to 100 (depending on the experiment and the Bay:creek ratio) over the course of the 5 day experiment (bottom panel, Figs. 5-1, 5-2). These results suggest that San Diego Creek could be a source of long-lived FIB in the Bay.

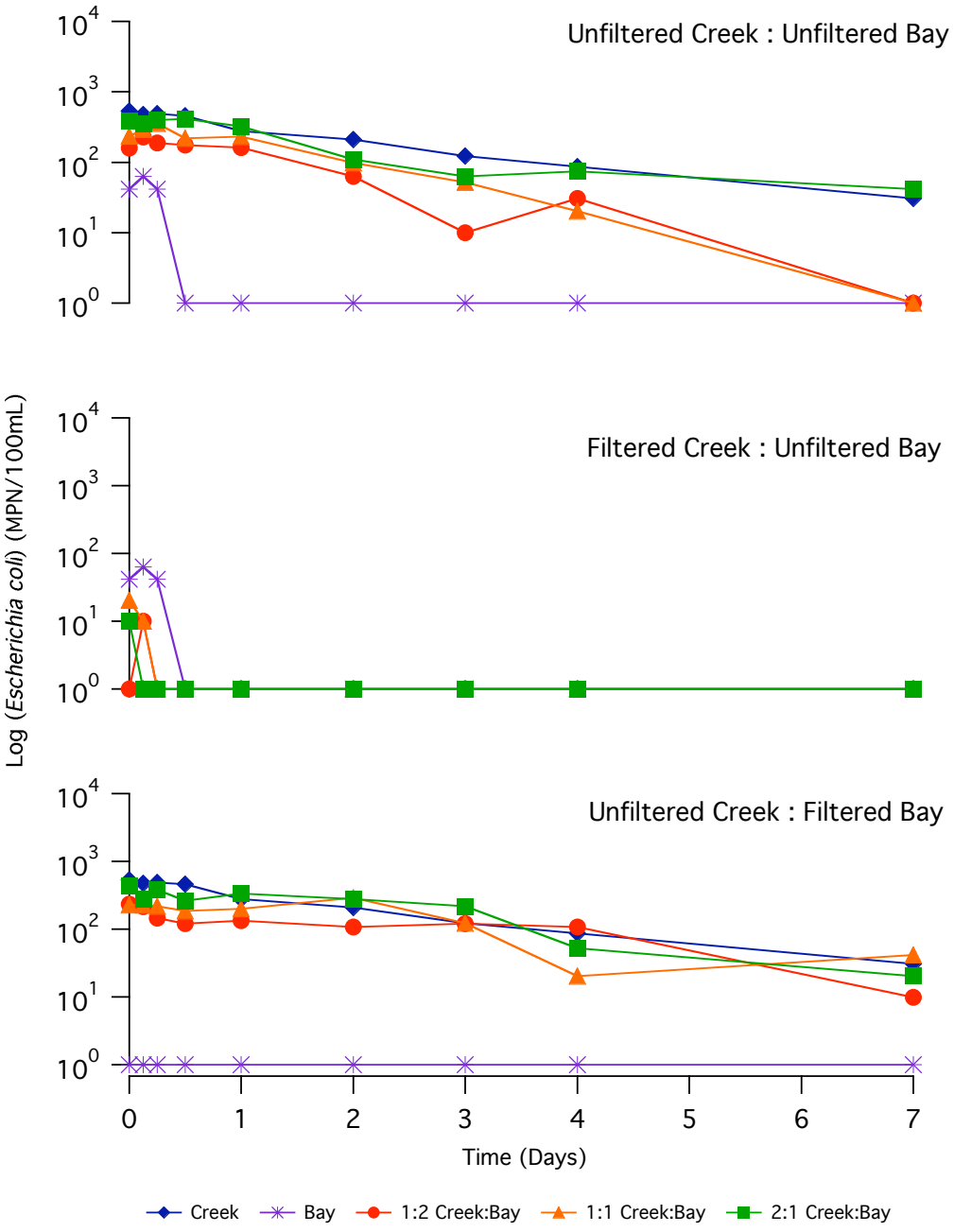

Figure 5-1. Time series measurements of *Escherichia coli* in microcosms containing mixtures of unfiltered creek water with unfiltered bay water (top panel), filter sterilized creek water with unfiltered bay water (middle panel), and unfiltered creek water with filter sterilized bay water (bottom panel).

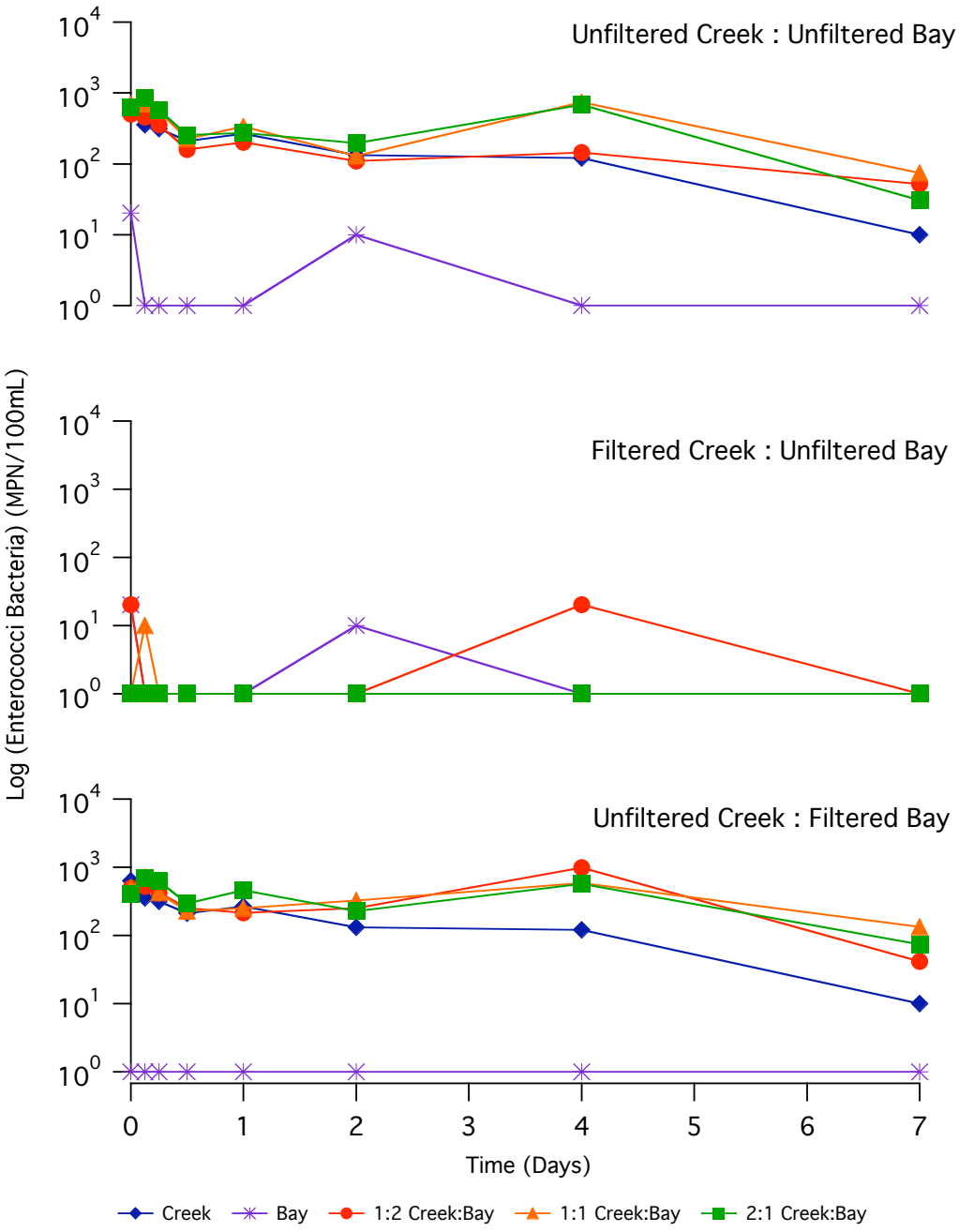

Figure 5-2. Time series measurements of enterococci bacteria in microcosms containing mixtures of unfiltered creek water with unfiltered bay water (top panel), filter sterilized creek water with unfiltered bay water (middle panel), and unfiltered creek water with filter sterilized bay water (bottom panel).

5.2.3. Methods: Field Microcosms Containing Creek and Bay Water

Water samples from Upper Bay (Station BTO 5) and San Diego Creek (BTO 1) were collected in sterile 20 L carboys. Carboys were rinsed three times with sample water before filling. These samples were transferred to the lab within 2 hours of collection. Ten liters of both Bay water and Creek water were filter sterilized through a 0.2 μm TFF cartridge. Filtration served to remove FIB from the water samples. Unfiltered water was kept in the dark at 4°C until all of the filtered samples were prepared.

Field microcosm experiments were conducted on the dock at the Kerchoff Lab in Lower Bay. Each microcosm was prepared in a sterilized 2.5 gallon glass aquarium. Once prepared, the aquariums were placed in one of two child-paddling pools. Bay water was continuously pumped into these pools with a bilge pump that was placed over the side of the dock into the Bay. Bay water was allowed to flow over the edge of the pools such that the temperature of water in the aquariums was close to the Bay water temperature (approximately 19°C). Half of the microcosms were uncovered, and thus exposed to changes in solar irradiance and atmospheric deposition. The other half were wrapped in aluminum foil, and therefore excluded from light and atmospheric deposition. There were 8 control microcosms and 9 mixed microcosms. Because there the mixing ratio did not have much effect on FIB survival in the laboratory microcosms (see last section), only of 1:1 mixtures of Bay and creek water were tested in the field microcosm studies. The total volume in each microcosm was 5 liters. The following microcosms were prepared:

- 5 L Creek Water exposed to light
- 5 L Bay Water exposed to light
- 2.5 L Creek Water + 2.5 L Bay Water (1:1) exposed to light
- 5 L 0.2 um filtered Creek Water exposed to light
- 2.5 L filtered Creek Water + 2.5 L Bay Water (1:1) exposed to light
- 5 L 0.2 um filtered Bay Water exposed to light
- 2.5 L Creek Water + 2.5 L filtered Bay Water (1:1) exposed to light
- 5 L Creek Water in the dark
- 5 L Bay Water in the dark
- 2.5 L Creek Water + 2.5 L Bay Water (1:1) in the dark
- 5 L 0.2 um filtered Creek Water in the dark
- 2.5 L filtered Creek Water + 2.5 L Bay Water (1:1) in the dark
- 5 L 0.2 um filtered Bay Water in the dark
- 2.5 L Creek Water + 2.5 L filtered Bay Water (1:1) in the dark

Sub-samples were withdrawn from each microcosm using sterile syringes. Sub-samples were collected immediately following microcosm construction (time 0), 3 hours, 6 hours, 12 hours, 24 hours (1 day), 48 hours (2 days), 72 hours (3 days), 96 hours (4 days), 120 hours (5 days), and 172 hours (6 days). Samples were analyzed for FIB using IDEXX Colilert-18 and Enterolert.

Table 5-3. Salinity measured in field microcosms containing mixtures of Bay and Creek water.

Microcosm:	**Bay**	**Creek**	**1:1 Creek: Bay**
Initial Salinity:	30	1	15
Final Salinity (Uncovered):	32	1	17
Final Salinity (Covered):	30	1	16

5.2.4. Results and Discussion: Field Microcosms Containing Creek and Bay Water

Microcosm Salinity Variability

Salinity in the microcosms varied from 1 to 30, depending on the mixing ratio of each microcosm. Table 5-3 indicates salinity measurements recorded on the first and last days of the experiment. A slight (1 to 2 unit) increase in salinity is evident for the uncovered microcosm, presumably due to evaporation that occurred over the 6-day experiment.

Concentrations and Die-off of Escherichia coli and Enterococci Bacteria

FIB died-off more rapidly in open microcosms than in covered microcosms. Bay populations of *E. coli* and enterococci bacteria decayed below the detection limit (10 MPN/100 mL) in under 6 hours in the open microcosms, and in under 60 hours in the covered microcosms (middle panels, Figs. 5-3 and 5-4). Freshening of the Bay water by addition of filter sterilized creek water did not affect the die-off rates of FIB in either the open or covered microcosms (middle panels, Figs. 5-3 and 5-4). Creek populations of *E. coli* and enterococci bacteria follow a similar trend, although in general the creek bacteria die-off more slowly than the Bay bacteria. Populations of FIB in the creek declined approximately 100 fold over approximately 120 hours in the covered microcosms, and approximately 1000 fold over approximately 60 hours in the open microcosms (compare blue lines in light and dark microcosms, bottom panels, Figs. 5-3 and 5-4). Addition of filter sterilized Bay water to the creek water did not obviously change the die-off rate of creek FIB, in either the open or covered microcosms.

5.2.5. Conclusions: Laboratory and Field Microcosms Containing Creek and Bay Water

The field and laboratory microcosm studies support the following conclusions: (1) During dry weather periods (when these studies were conduced), FIB concentrations in San Diego Creek are generally higher than in Upper Newport Bay; and (2) Creek-derived FIB die-off less rapidly than Bay-derived FIB, when seeded into mixtures of Creek and Bay waters. The field microcosms also highlight the impact of sunlight on die-off rates, thus confirming for this site what has been documented in the literature at other locales[2]. While die-off occurs more rapidly in open microcosms compared to closed microcosms, it is important to note that populations of FIB from the creek still survive for a relatively long period of time in the uncovered microcosms (at least 40 hours). Natural predators in the Bay water (e.g., protozoans) may remove FIB in

[2] Sinton, L.W., Finlay, R.K., Lynch, P.A. *Applied and Environ. Microbiol.*, 1999, v. 65: 3605-3613.

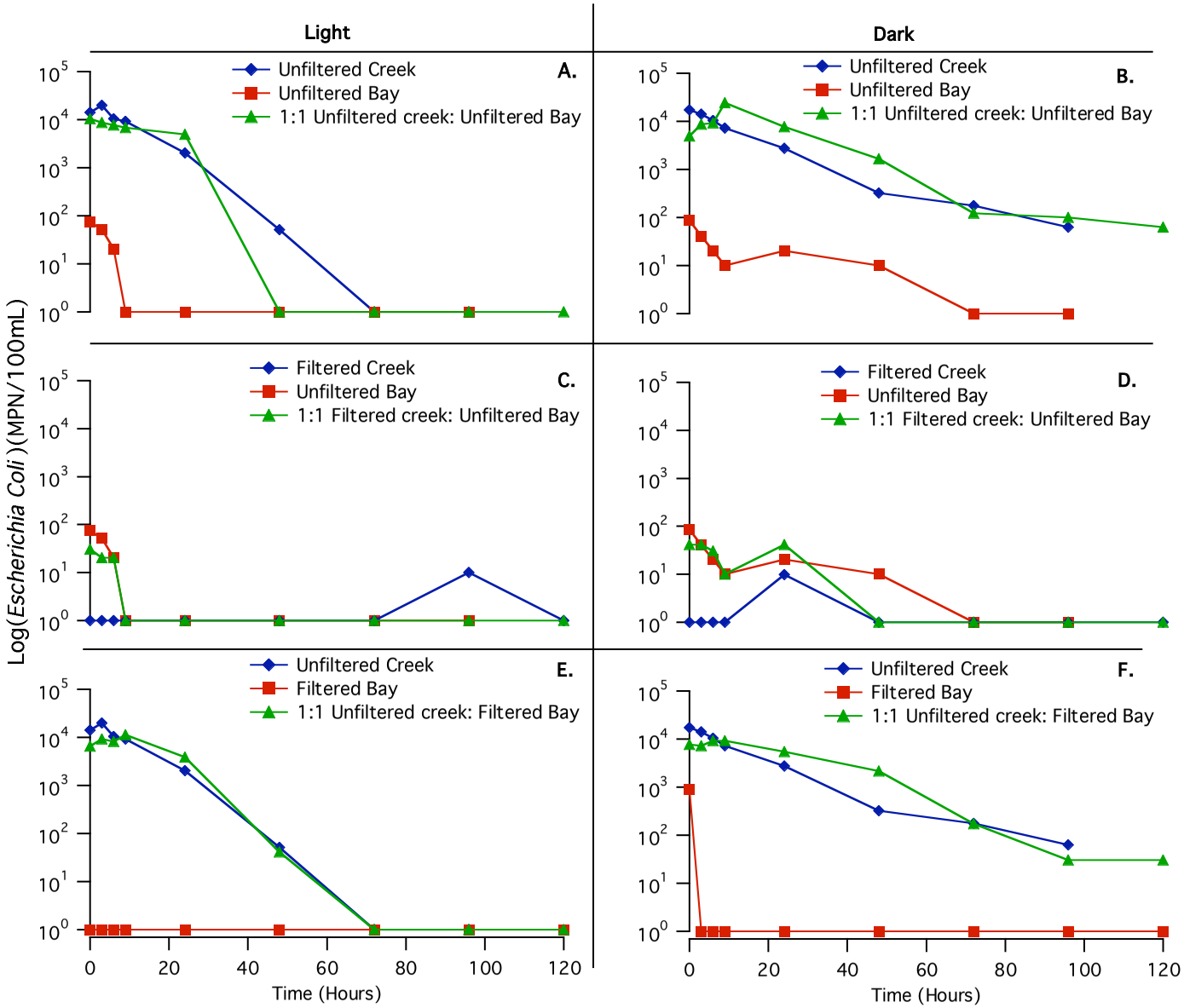

Figure 5-3. Time series measurements of *Escherichia coli* in field microcosms open (left column of panels) or not open (right column of panels) to light and atmospheric deposition. The rows correspond to mixtures of unfiltered creek and Bay water (panels A,B), filter sterilized creek water and unfiltered Bay water (panels C,D), and unfiltered creek water and filtered Bay water (panels E,F).

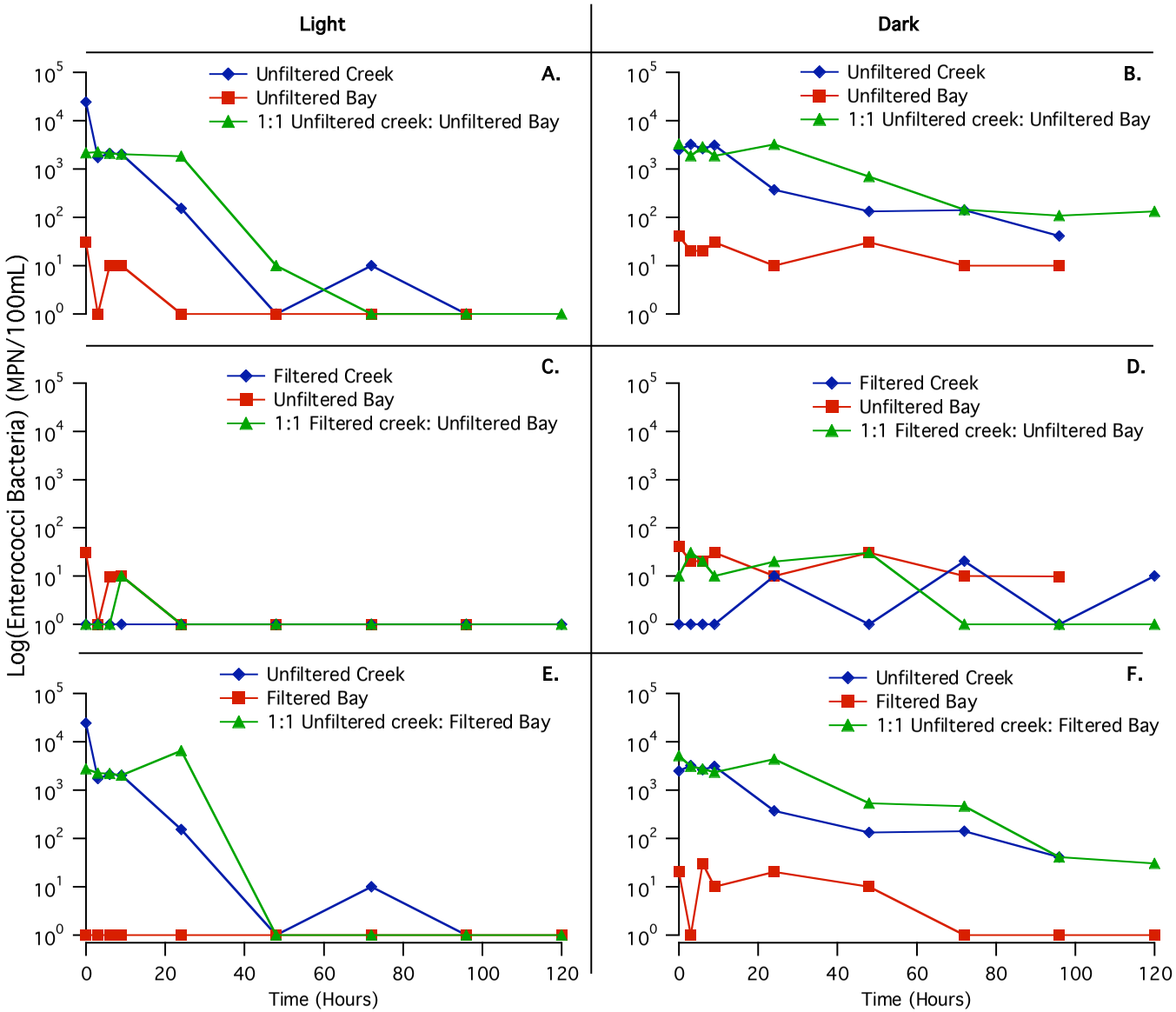

Figure 5-4. Time series measurements of enterococci bacdteria in field microcosms open (left column of panels) or not open (right column of panels) to light and atmospheric deposition. The rows correspond to mixtures of unfiltered creek and Bay water (panels A,B), filter sterilized creek water and unfiltered Bay water (panels C,D), and unfiltered creek water and filtered Bay water (panels E,F).

microcosms that contain unfiltered Bay water[3,4,5]. However, this hypothesis would not explain why *E. coli* and enterococci bacteria are long-lived in microcosms containing both unfiltered creek and unfiltered Bay water, especially those microcosms with a high percentage of FIB from the creek; i.e., the microcosm with a 2:1 ratio of Creek to Bay water (green lines, top panels, Figs. 5-1 and 5-2). Taken as a whole, both laboratory and field microcosm experiments support the hypothesis that FIB flowing into the Bay from San Diego Creek constitute a significant, and potentially long-lived, source of FIB pollution in Newport Bay.

5.3. Microcosms Containing Macroalgae and Bird Feces

In this section we describe microcosm experiments carried out with water from San Diego Creek or Newport Bay, with or without addition of macroalgae and bird feces collected from Newport Bay. These laboratory and field microcosm studies were carried out to:

- Characterize the regrowth and/or decay of FIB from bird droppings and the effects of changing nutrient and salinity conditions.
- Characterize the regrowth and/or decay of FIB from macroalgae and the effects of changing nutrient and salinity conditions.
- Characterize synergistic effects of adding both bird droppings and macroalgae on FIB regrowth in Bay and Creek waters.

5.3.1. Methods: Laboratory Microcosms Containing Bird Feces and Macroalgae

Feces from a number of different birds (including gulls, ducks, pelicans, and geese) were collected from the Upper Newport Bay Ecological Reserve, the boat ramp at the Newport Dunes Resort, and the Jetties at the outlet of Lower Newport Bay. Feces were collected using a sterile spatula into a sterile 100 mL bottle. Only moist (recently deposited) bird feces were collected. Bird feces were transported to the laboratory on ice within 6 hours of collection, pooled, and then homogenized by stirring and sonication (for 30 seconds) in the laboratory. Five grams of pooled and homogenized bird feces were placed into a sterile saline buffer solution to create a "bird feces stock solution". The bird feces stock solution was vigorously shaken, sonicated for 30 seconds to disburse FIB, serially diluted (1:10, 1:100, 1:1000, 1:10,000, 1:100,000, and 1:1,000,000), and assayed for FIB using IDEXX Colilert-18 and Enterolert tests in a 96 well quantitray format. Bird feces stock solution was stored at 4°C in the dark over night before seeding the laboratory microcosms.

Waters from Upper Newport Bay (Station BTO 5) and San Diego Creek (BTO 1) were collected in sterile 20 L carboys. Carboys were rinsed three times with sample water before filling. Macroalgae (from *Enteromorpha spp*. and *Ulva spp*. growing along the margins of Upper Bay)

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[3] Davies, C.M., J.A.H. Long, M. Donald, N.J. Ashbolt (1995) "Survival of Fecal Microorganisms in Marine and Freshwater Sediments" *Appl. Environ. Microbiol*. 61:1888-1896.

[4] Enzinger, E.M., R.C. Cooper (1976) "Role of bacteria and protozoa in the removal of Escherichia coli from estuarine waters. *Appl. Environ. Microbiol*. 31:758-763.

[5] Hartke, A., S. Lemarinier, V. Pichereau, Y. Auffray (2002) "Survival of Enterococcus faecalis in seawater microcosms is limited in the presence of bacterivorous zooflagellates" *Current Microbiology*. 44:329-335.

was also collected from the surface waters at BTO 5 and transferred into a sterile 500 mL bottle that had been rinsed 3 times with bay water. These samples were transported to the lab within 6 hours of collection on the day the microcosms were to be prepared. Water samples were not filtered before the experiments.

Laboratory microcosms consisted of sterile 4 L Erlenmeyer flasks held in a temperature-controlled room held at 20°C in the dark. There were 2 control microcosms (Bay water alone and creek water alone) and 6 microcosms with addition of bird feces solution and/or macroalgae. The three different amendments consisted of: 30 grams wet weight macroalgae, 50 mL of bird feces solution, and 30 grams wet weight macroalgae plus 50 mL of bird feces solution. The total volume in each microcosm was 3 L. Microcosms were swirled in the morning, during sampling, and in the evening of each day to minimize hypoxia. The following microcosms were prepared:

- San Diego Creek Water (unfiltered)
- San Diego Creek Water + Macroalgae
- San Diego Creek Water + Bird Feces Solution
- San Diego Creek Water + Macroalgae + Bird Feces Solution
- Upper Newport Bay Water (unfiltered)
- Upper Newport Bay Water + Macroalgae
- Upper Newport Bay + Bird Feces Solution
- Upper Newport Bay + Macroalgae + Bird Feces Solution

Sub-samples were withdrawn from each microcosm using sterile serological pipettes. Sub-samples were collected immediately following microcosm construction (time 0), 3 hours, 6 hours, 12 hours, 24 hours (1 day), 48 hours (2 days), 72 hours (3 days), 96 hours (4 days), and 120 hours (5 days). Water samples were analyzed for FIB using IDEXX Colilert-18 and Enterolert tests, implemented in a 96 well quantitray format. For the 1:10 dilution employed, the dynamic range of the Colilert and Enterolert assays is 10 to 24,192 MPN/100 mL. Censored values were handled as described earlier.

5.3.2. Results and Discussion: Laboratory Microcosms Containing Bird Feces and Macroalgae

Salinity was consistent for all Bay water microcosms (29.2) and all creek water microcosms (0.8). The addition of macroalgae and bird feces stock solution was the only variable that changed among the different microcosms. Figs. 5-5 and 5-6 show results for *E. coli*, and enterococci bacteria respectively. The top panel in each figure shows results for the microcosms with Bay water and the bottom panel shows results for microcosms with creek water. Results for *Escherichia coli* and enterococci were similar for each of the microcosm treatments and will be discussed together. Note that no laboratory microcosms were carried out with filter-sterilized Bay or Creek water.

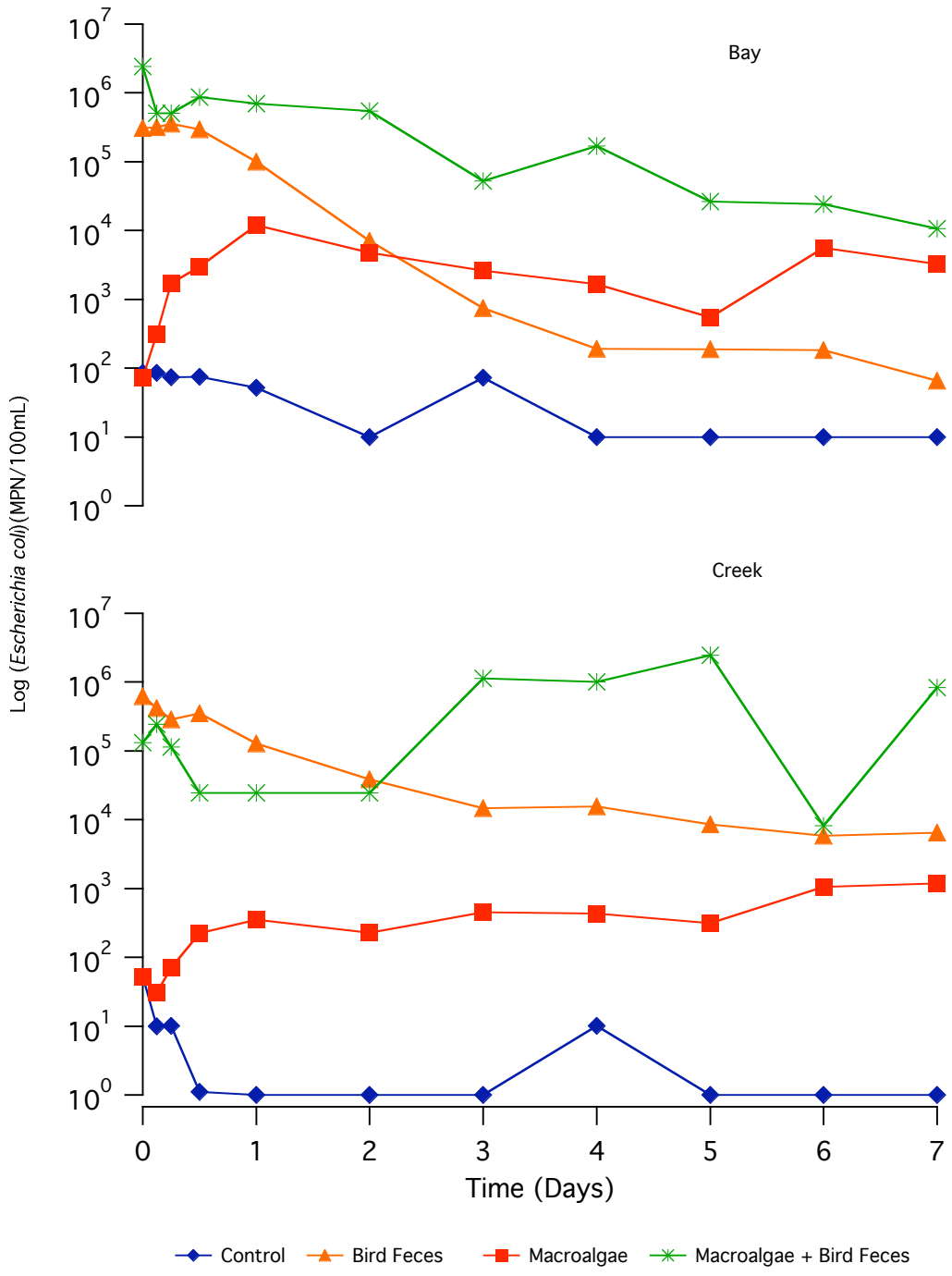

Figure 5-5. Time series measurements of *Escherichia coli* in laboratory microcosms amended with macroalgae and/or bird feces. Results shown for Bay water (top panel) and creek water (bottom panel).

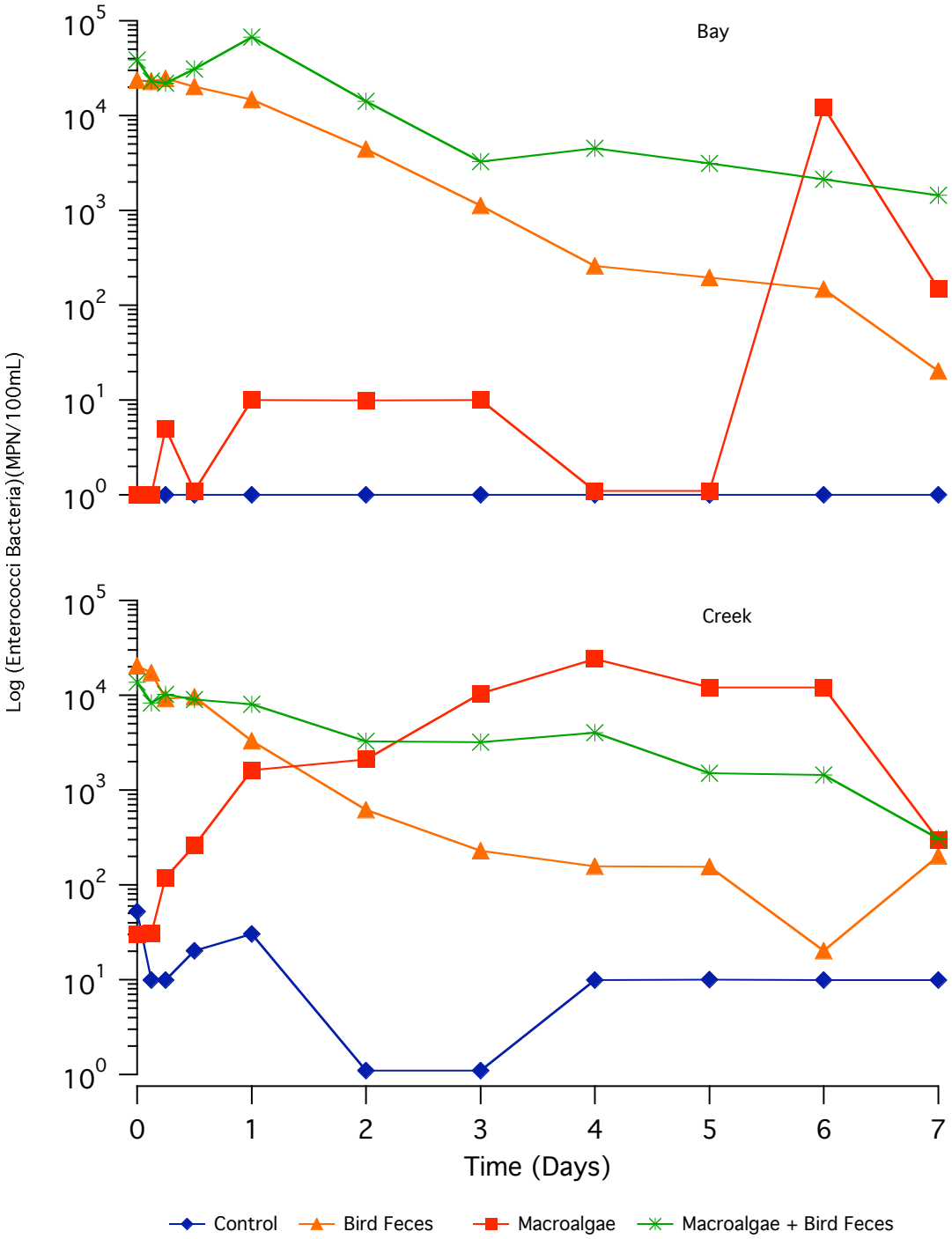

Figure 5-6. Time series measurements of enterococci bacteria in laboratory microcosms amended with macroalgae and/or bird feces. Results shown for Bay water (top panel) and creek water (bottom panel).

Bird Feces as a Source of FIB in Bay and Creek Water.

The concentrations of *E. coli* and enterococci were relatively low in the unamended creek and unamended Bay waters (blue diamonds, top and bottom panels, Figs. 5-5 and 5-6). The bird feces stock solution had very high concentrations of FIB[6], so when microcosms were amended with the bird feces stock solution, the initial FIB concentrations were very high (*ca*., 10^5 to 10^6 MPN/100 mL for *E. coli*, and 10^4 MPN/100 mL for enterococci bacteria, orange triangles in Figures 5-5 and 5-6). Populations of *E. coli* and enterococci bacteria from the bird feces decayed steadily over the 5-day microcosm experiments. The die-off of *E. coli* populations was more rapid when bird feces stock solution was mixed into Bay water, and less rapid when the stock solution was mixed into creek water (compare orange curves in top and bottom panels, Fig. 5-5). The die-off of enterococci bacteria populations from the bird feces, on the other hand, was similar in Creek and Bay waters (yellow curves, top and bottom panels, Fig. 5-6). Although there is some variability in the die-off rate of FIB in the different microcosms, it is important to note that these bacteria are still relatively long-lived. In almost all cases, the time to 90% die-off (or T90) is at least 1 day.

Macroalgae aids FIB regrowth/persistence:

Generally speaking, the addition of macroalgae improved the persistence of *E. coli* and enterococci bacteria. In microcosms amended with macroalgae, *E. coli* increased by a factor of between 10 (creek water) to 100 (Bay water) within the first 24 hours (orange curves, top and bottom panels, Fig. 5-5). In the microcosm containing macroalgae and Creek water, enterococci bacteria increase by nearly 1000 fold over the first four days of incubation. In the microcosm containing macroalgae and Bay water, enterococci concentrations were highly variable, with concentrations near the detection limit (10 MPN/100 mL) for the first five days of incubation, increasing >1000 fold on the 6th day, and then dropping 100 fold on the 7th day (red squares, top panel, Fig. 5-6). With few exceptions, the highest FIB concentrations, and the slowest die-off, were observed in microcosms that were amended with both macroalgae and bird feces (green crosses in Figs. 5-5 and 5-6).

5.3.3. Methods: Field Microcosms Containing Macroalgae and Bird Feces

Field microcosms consisted of dialysis bags (MWCO 12000-14000 Daltons, Spectra/Por Dialysis Membrane, Rancho Dominguez, CA) suspended in a milk crate that was, in turn, suspended in either Newport Bay (at the US Fish and Game Dock on Shellmaker Island), or in San Diego Creek (at the Campus Dr. crossing, BTO 1). The MWCO of the dialysis bag allows dissolved nutrients and organic carbon to pass through the bag walls, while separating bacterial populations inside and outside the bag. Dialysis bags were cut into 2.5 ft long segments (for a total volume of approximately 3 L), clamped on one end, filled with sample water +/- amendments, and clamped at the other end. The time 0 sub-sample was collected immediately using sterile serological pipettes. Bags were then re-clamped and gently placed into plastic milk crates that were lowered into either the Creek or the Bay.

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[6] FIB in bird feces ranged from 10^8 to 10^{10} *E. coli* and enterococci bacteria per gram of feces, wet weight

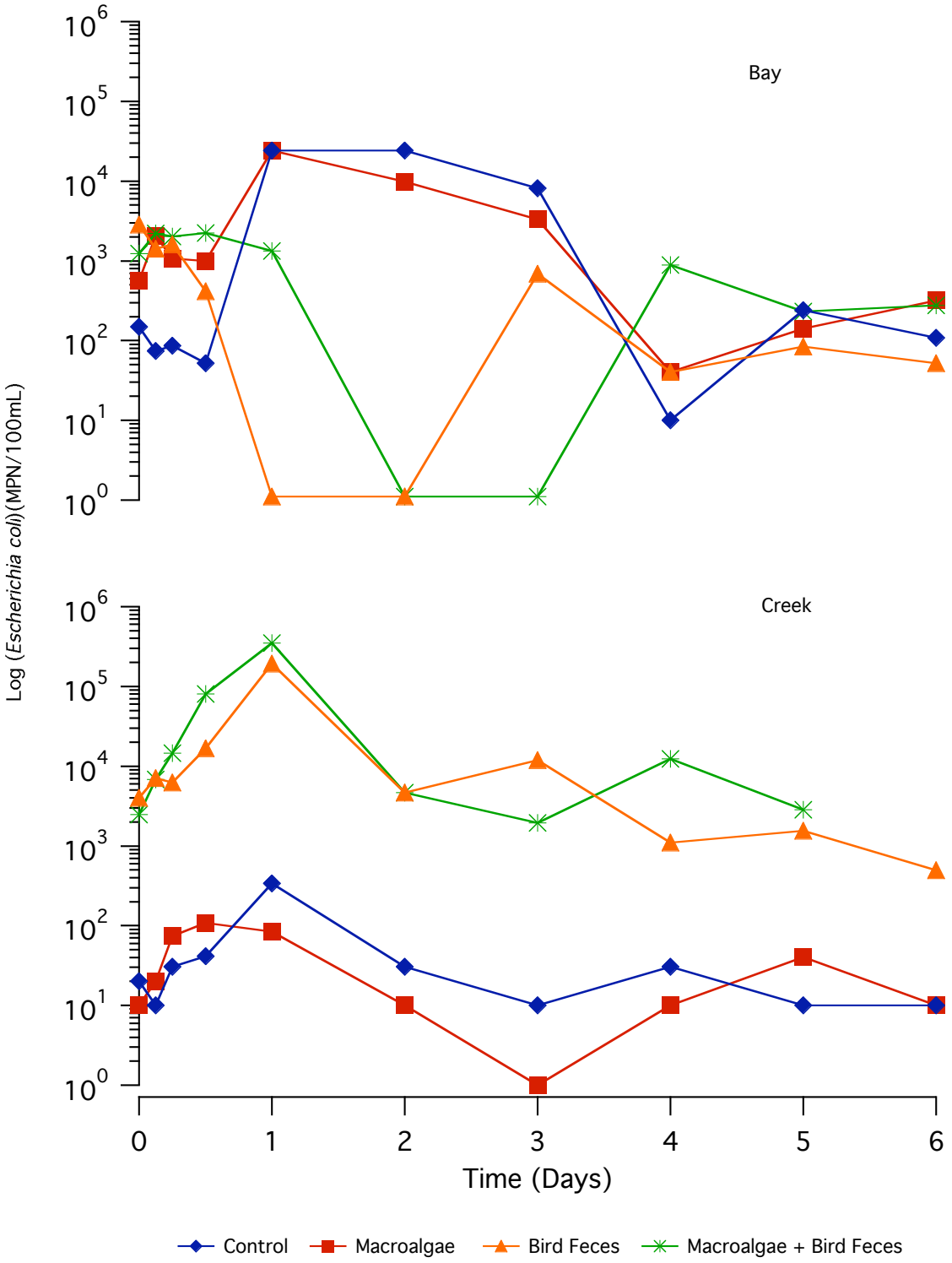

Figure 5-7. Time series measurements of *Escherichia coli* in field microcosms amended with macroalgae and/or bird feces. Results shown for Bay water (top panel) and creek water (bottom panel).

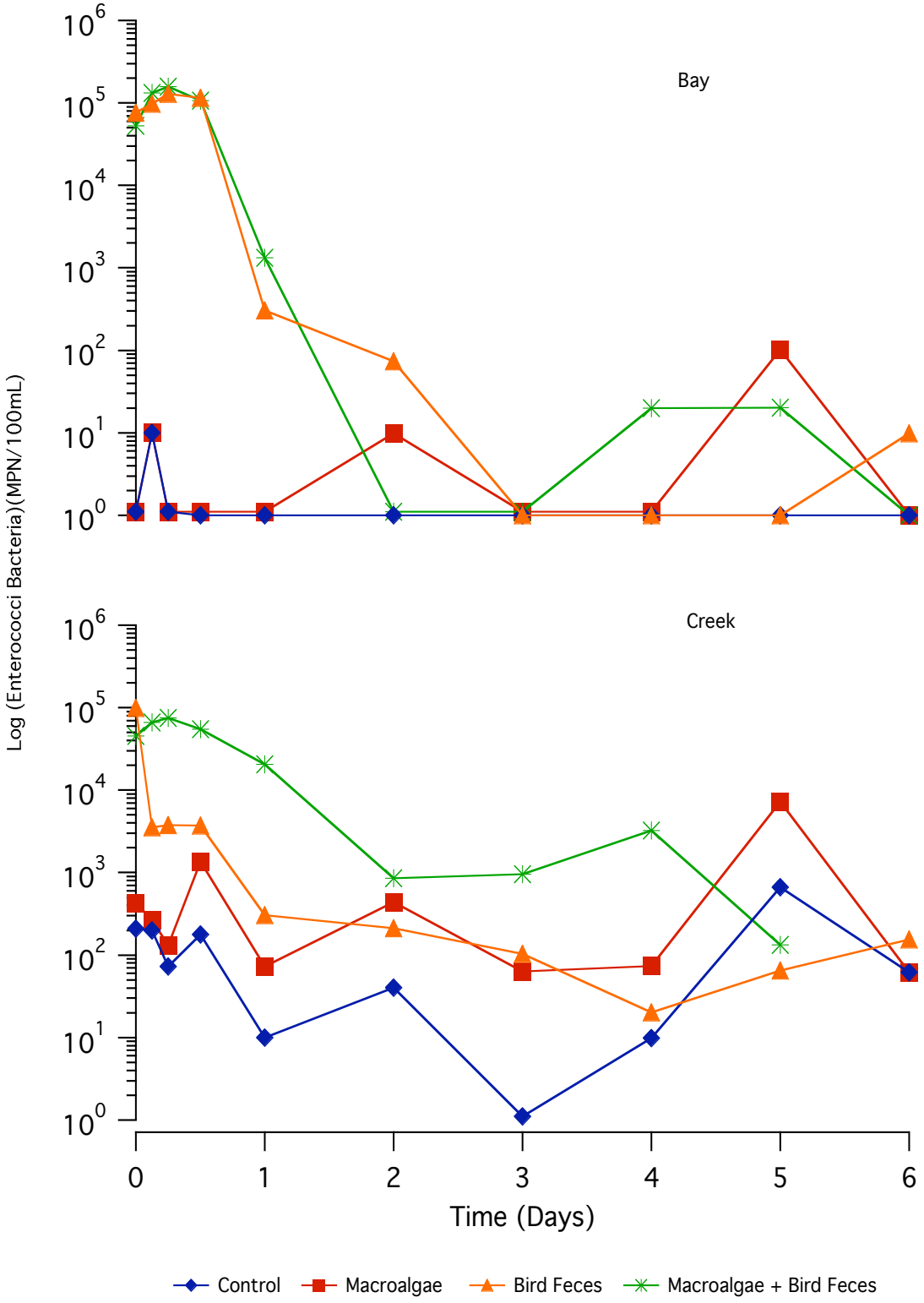

Figure 5-8. Time series measurements of enterococci bacteria in field microcosms amended with macroalgae and/or bird feces. Results shown for Bay water (top panel) and creek water (bottom panel).

At the San Diego Creek site, milk crates were placed in the center of the stream such that the microcosms were completely covered by creek water. The milk crate was then roped to 2 cinder blocks that kept the crate from being washed down stream. At the Shellmaker Island site, the crates were tied to a cleat on the dock, and lowered to a depth such that the tops of the microcosms were covered (but not too deep, to prevent the microcosms from floating out of the crate). The milk crates were necessary because, during preliminary field tests, the end clips came off while retrieving the dialysis bags and the microcosms were lost.

Because the laboratory and field experiments were separated in time by 3.5 months (see Table 5-1), fresh bird feces stock solution and macroalgae were prepared for the field experiments using the procedure described earlier (Section 5.3.1). There were two control microcosms (dialysis bags filled with Bay or Creek source water without amendment), and 6 microcosms with addition of bird feces solution and/or macroalgae. The three different amendments consisted of: 30 grams wet weight macroalgae, 50 mL of bird feces solution, and 30 grams wet weight macroalgae plus 50 mL of bird feces solution. The following microcosms were prepared:

- Upper Newport Bay Water; dialysis bag deployed at Shellmaker Island Dock (BC)
- Upper Newport Bay Water + Macroalgae; dialysis bag deployed at Shellmaker Island Dock (BA)
- Upper Newport Bay Water + Bird Feces Homogenate; dialysis bag deployed at Shellmaker Island Dock (BP)
- Upper Newport Bay Water + Macroalgae + Bird Feces Homogenate; dialysis bag deployed at Shellmaker Island Dock (BAP)
- San Diego Creek Water; dialysis bag deployed in San Diego Creek (Campus crossing) (CC)
- San Diego Creek Water + Macroalgae; dialysis bag deployed in San Diego Creek (Campus crossing) (CA)
- San Diego Creek Water + Bird Feces Homogenate; dialysis bag deployed in San Diego Creek (Campus crossing) (CP)
- San Diego Creek Water + Macroalgae + Bird Feces; dialysis bag deployed in San Diego Creek (Campus crossing) (CAP)

5.3.4. Results and Discussion: Field Microcosms Containing Macroalgae and Bird Feces

Results for the field experiment are shown in Figs. 5.7 (*E. coli*) and 5.8 (enterococci bacteria). The top panel in each figure shows the results from microcosms suspended in Upper Bay and the lower panel shows results from microcosms suspended in San Diego Creek.

With one exception (*E. coli* suspended in Bay water, top panel, Fig. 5-7), the addition of bird feces resulted in high initial FIB concentrations. In some cases, these high initial concentrations were sustained for a long period of time (*E. coli* in San Diego Creek, bottom panel, Fig. 5-7), while in other cases FIB concentrations decayed within several days to near the lower limit of detection (enterococci bacteria in Bay Water, top panel, Fig. 5-8). In only one case did the addition of macroalgae appear to act synergistically with the bird feces to sustain high FIB concentrations (enterococci bacteria suspended in San Diego Creek, bottom panel, Fig. 5-8). In all other cases, the addition of macroalgae either alone, or in combination with addition of bird

feces, had little effect on FIB concentrations, relative to the unamended control (compare red and blue lines, Figs. 5-7 and 5-8).

5.3.5. Conclusions: Laboratory and Field Microcosms containing Macroalgae and Bird Feces

The results from the laboratory and field microcosms generally support the idea that bird feces are a potential source of FIB in Bay and Creek waters. However, the length of time over which high FIB concentrations are sustained is highly variable (ranging from days to weeks), depending on the nature of the microcosm (lab or field), source water (creek or Bay) and FIB group (*E. coli* or enterococci bacteria). One of the most striking findings is that FIB concentrations responded very differently to macroalgae, depending on whether the microcosm experiments were conducted in the laboratory or field. In laboratory settings, addition of macroalgae to Bay or Creek Waters was associated with the apparent regrowth of *E. coli* and enterococci bacteria. In field settings, the addition of macroalgae had little or no effect on FIB concentrations, compared to the unamended control. One possible explanation for these different outcomes is that, despite our effort to prevent hypoxia by gentle mixing (see methods section), laboratory microcosms containing macroalgae became hypoxic (based on their sulfidic smell) as the macroalgae decayed. By contrast, field microcosms remained well oxygenated throughout the study. Thus, hypoxic conditions brought on by the decay macroalgae may be conducive to sustaining, and even growing, FIB.

5.3.6. Spatial Distribution of FIB and Nutrients During a Macroalgal Bloom in Upper Bay

The laboratory microcosm experiments presented earlier indicate that macroalgae may promote the survival, and perhaps even growth, of FIB in the Bay and creek waters, particularly in the case where bird feces are also present. However, survival patterns observed in the field microcosms were more complex, calling into question whether bird feces and macroalgae (either alone or in combination) significantly influence FIB concentrations in the Bay. To explore this issue further, a special study was carried out (with supplemental funds from UCI fellowships awarded to graduate and undergraduate students in the Grant lab) to examine the spatial distribution of FIB concentrations in Upper Bay during a macroalgae bloom. The macroalgae bloom, which initiated in early May (2006) and persisted through the fall, consisted predominantly of *Ulva spp.* and *Enteromorpha spp*. The field study was carried out near the height of the bloom event, during which macroalgae completely covered the mud-flats, and were also present as floating mats throughout the Upper Bay. Macroalgae mats were most dense around the margins of Unit 1 Basin, near sampling site BTO 4 (upper panel, Fig. 5-9).

5.3.6.1. Methods: Special Study on Spatial Distribution of FIB and Nutrients During a Macroalgal Bloom in Upper Bay

To assess the impact of macroalgae bloom events on FIB concentrations within Upper Bay, we carried out a spatially intensive synoptic survey of *E. coli,* enterococci bacteria, nutrients, dissolved organic carbon, and chlorophyll in the upper reaches of Upper Bay, in Unit Basin 1 where site BTO 4 was located (compare top panel Fig. 5-9 with Fig. 2-1, Chapter 2).

The study was conducted at the height of a macroalgae bloom event on June 6, 2006. Thirty sites were sampled over an evenly spaced grid between 0830 and 1100 hours (bottom panel, Figure 5-9). The sampling grid was laid out so that water samples were taken at the edge of the water line, within the mudflat areas, and in the center of the channel. At every site, water samples were collected from the surface of the water column, and immediately capped and placed on ice in the dark. Samples were then transferred back to UCI within a holding time of 6 hours where they were analyzed for FIB using the IDEXX Colilert-18 and Enterolert defined substrate tests, implemented in a 96 well quantitray format. These samples were also measured for pH, salinity and chlorophyll. At every other site, additional water samples were collected from the surface of the water column and analyzed for nutrients (nitrate, ammonium, TKN, total phosphorous), chlorophyll fluorescence, and total organic carbon. Bottles used for collecting nutrient samples were acidified with 1 mL of concentrated hydrochloric acid. Bottles used for collecting water for total organic carbon analysis were acidified with 1mL of concentrated sulfuric acid. Nutrient and total organic carbon samples were stored at $4^{o}C$ and transported to Orange County Public Works (OCPW), which sent them to Associated Laboratories for analysis.

5.3.6.2. Results and Discussion: Special Study on Spatial Distribution of FIB During a Macroalgal Bloom in Upper Bay

Macroalgal mats were most dense on the left bank of the basin where extensive mud flats are present at low tide (field observations, data not shown). Salinity measurements (lower right panel in Figure 5-10) indicate a strong east-west salinity gradient. EC concentrations were also elevated along the left bank.

Spearman rank coefficients were calculated to determine correlations between the three FIB groups and salinity, chlorophyll, pH, ammonia, total Kjeldahl nitrogen (TKN), nitrate, total phosphorus, and total organic carbon (TOC). Based on the correlation results reported in Table 5-4, *E. coli* concentrations are significantly ($p<0.05$) and negatively correlated with salinity (Sp=-0.7), and significantly ($p<0.05$) and positively correlated with ammonia (Sp=0.8), TKN (Sp=0.8), nitrate (Sp=0.6), and total organic carbon (Sp=0.6). *E. coli* concentrations are not significantly ($p>0.05$) correlated with chlorophyll, pH, or total phosphorous. *Enterococcus* concentrations are not significantly correlated ($p>0.05$) with salinity, chlorophyll, pH, ammonia, TKN or nitrate, but are very significantly ($p=0.001$) and highly positively (Sp=0.8) correlated with total phosphorous.

The log-transformed concentrations of *E. coli* and *Enterococcus* are plotted against nutrients measured during the synoptic study in Figs. 5-11 through 5-16. Note that, while the Spearman rank correlation between *E. coli* concentration and total phosphorous is low and not significant, the cross plot (bottom panel, Fig. 5-12) reveals that there may be two separate sources of these bacteria with very different total phosphorous correlations: one source associated with water high in total phosphorous, and another source associated with water low in total phosphorous. It is interesting to compare the range of nutrient concentrations measured during the synoptic study, with measurements of the same nutrients in San Diego Creek during the creek sampling phase of the Prop 13 study (see Chapter 2). Based on the nutrient data presented in Table 5-5, with the exception of nitrate, nutrient concentrations measured in San Diego Creek and Unit 1 Basin are

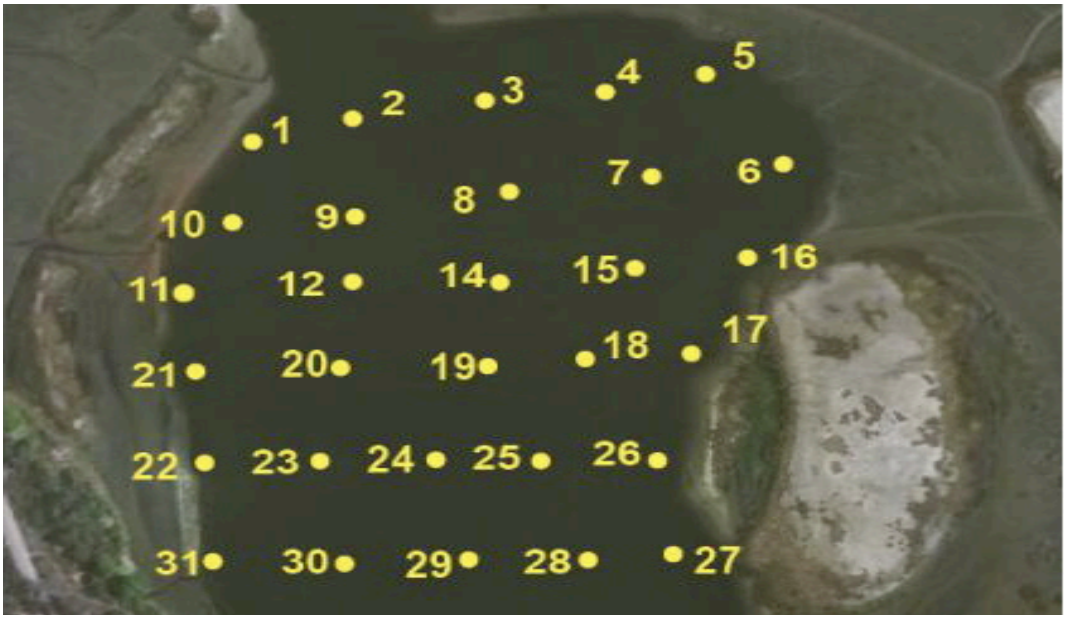

Figure 5-9. Location of sites sampled on June 6 (2006) during a macroalgae bloom in the Unit 1 Basin of Upper Newport Bay. Location of Unit 1 Basin indicated (by the white rectangle) in top panel.

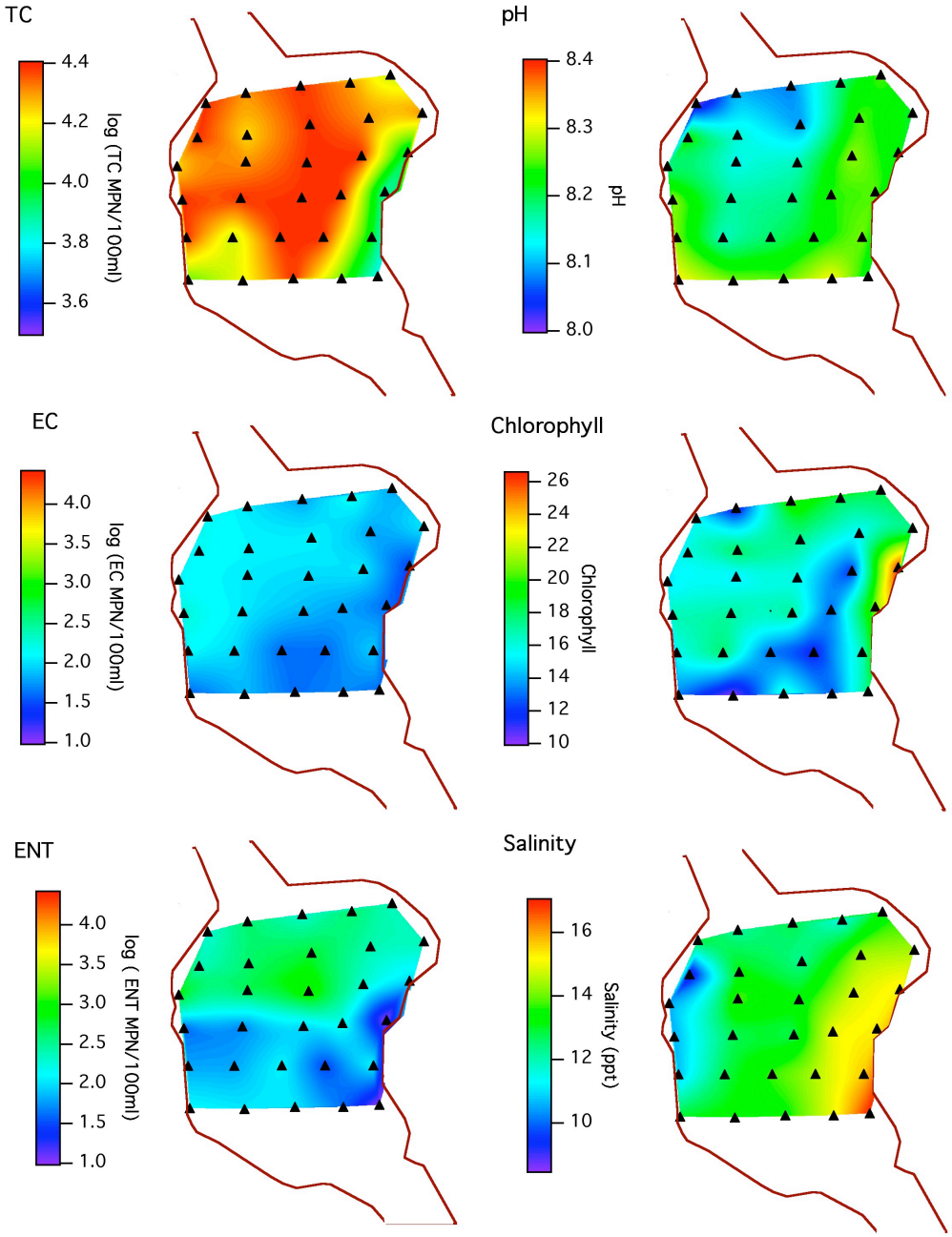

Figure 5-10. Spatial distribution of total coliform (TC), *Escherichia coli* (EC), enterococci bacteria (ENT), pH, chlorophyll, and salinity during the synoptic study on June 6 (2006). Mudflat areas are along both sides of the plots extending out to the second row of points on both the right and left sides. For the color plots of FIB, the transition in color from light blue to green roughly corresponds to exceedence of the AB411 single-sample thresholds for TC (10,000 MPN/100 mL), EC (400 MPN/100 mL), and ENT (104 MPN/100 mL)."

Table 5-4: Spearman Rank Coefficient Results for Macroalgal Upper Basin Study

		Sal.	Chloro.	pH	Ammonia	TKN	Nitrate	Total P	TOC
Log (TC)	Correlation Coefficient	-0.45*	0.26	-0.27	0.76**	0.51	0.54*	0.17	0.51
	Sig. (2-tailed)	0.013	0.17	0.16	0.002	0.06	0.05	0.57	0.06
	N	30	30	30	14	14	14	14	14
Log EC	Correlation Coefficient	-0.70**	0.0	-0.31	0.78**	0.76**	0.58*	0.24	0.61*
	Sig. (2-tailed)	0	0.99	0.09	0.001	0.002	0.03	0.41	0.02
	N	30	30	30	14	14	14	14	14
Log ENT	Correlation Coefficient	-0.33	0.077	-0.45*	0.1	0.28	0.17	0.80**	0.04
	Sig. (2-tailed)	0.39	0.69	0.01	0.8	0.33	0.6	0.001	0.9
	N	30	30	30	14	14	14	14	14

*Correlation is significant at $p<0.05$ (2-tailed).
**Correlation is significant at $p<0.01$ (2-tailed).

Table 5-5. Comparison of nutrient measurements in San Diego Creek and Unit 1 Basin

	Ammonia (mg/L)	TKN (mg/L)	Nitrate (mg/L)	Total P (mg/L)	TOC
Upper Bay Unit Basin 1 (this chapter)	0.3 +/-0.05 (n=14)	1.0 +/-0.08 (n=14)	3.0 +/-0.9 (n=14)	0.4 +/- 0.2 (n=14)	7.0 +/- 0.5 (n=14)
San Diego Creek (Chapter 2)	0.2 (n=1)	1.1 +/- 0.5 (n=4)	26 +/- 15 (n=4)	0.52 +/- 0.13 (n=4)	7.4 +/-2 (n=4)

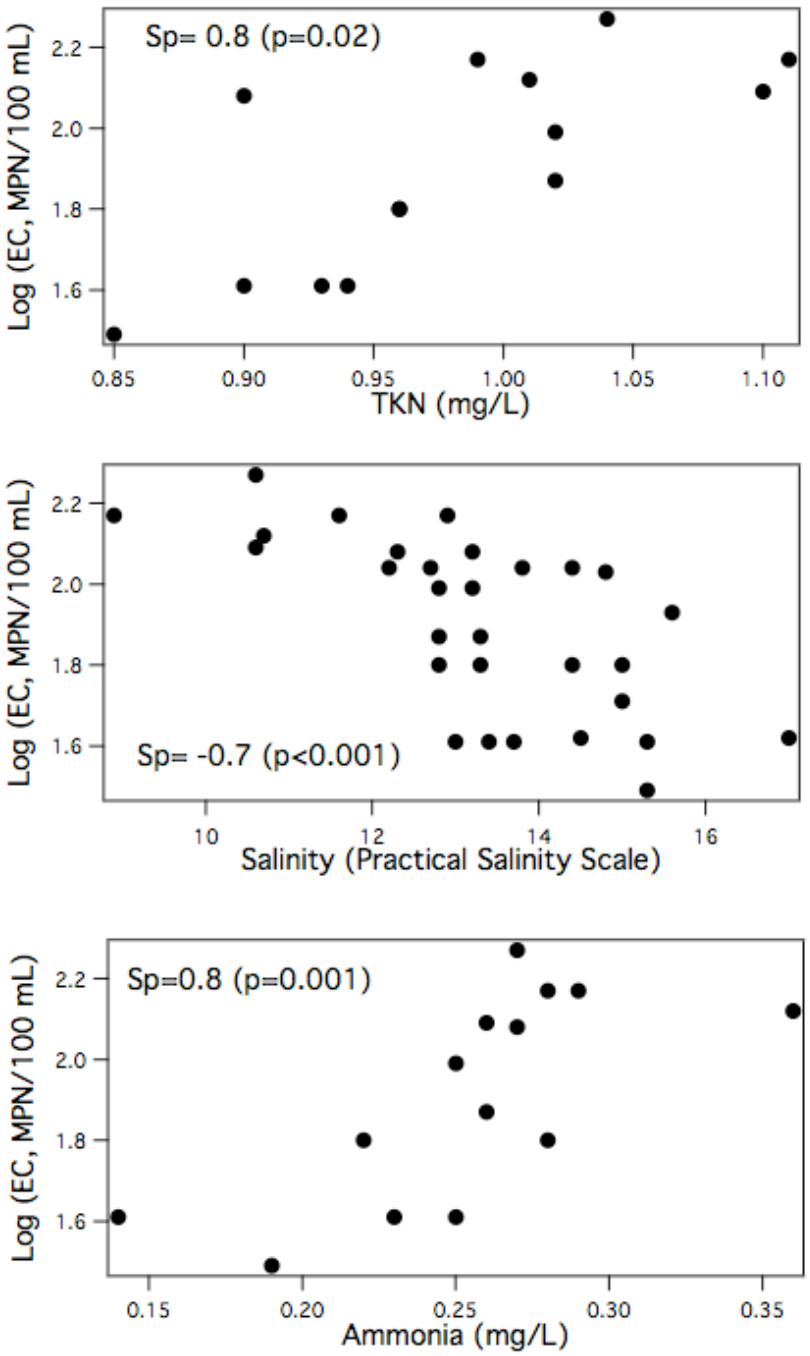

Figure 5-11. Cross plots of log-transformed *E. coli* concentration against nutrient concentrations measured during the synoptic study in Upper Bay (Unit 1 Basin) on June 6 (2006). By way of comparison, the log-transformed AB411 single-sample standard for EC is Log(400 MPN/100 mL)=2.6.

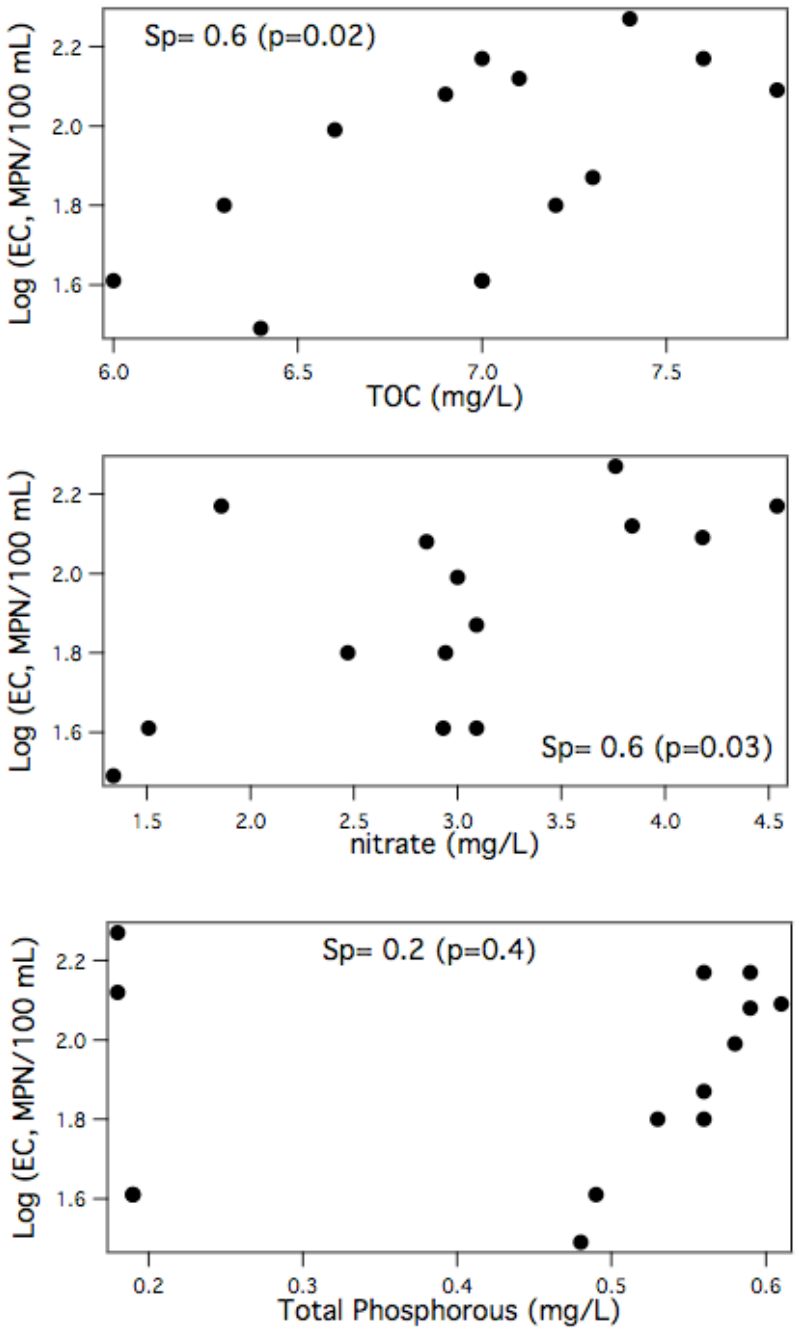

Figure 5-12. Cross-plots of log-transformed *E. coli* concentration against nutrient concentrations measured during the synoptic study in Upper Bay (Unit 1 Basin) on June 6 (2006). By way of comparison, the log-transformed AB411 single-sample standard for EC is Log(400 MPN/100 mL)=2.6.

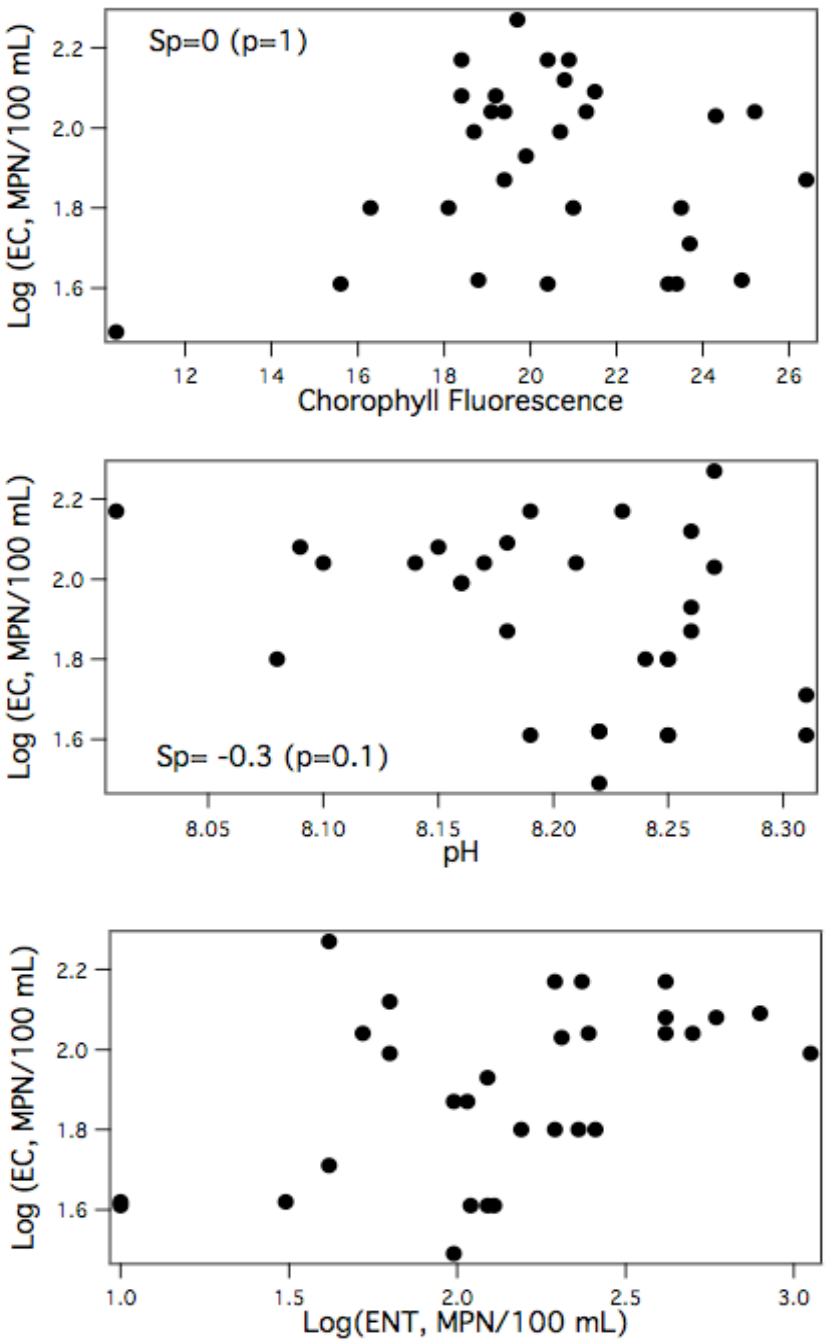

Figure 5-13. Cross-plots of log-transformed *E. coli* concentration against nutrient concentrations and enterococci bacteria concentration measured during the synoptic study in Upper Bay (Unit 1 Basin) on June 6 (2006). By way of comparison, the log-transformed AB411 single-sample standard for EC is Log(400 MPN/100 mL)=2.6.

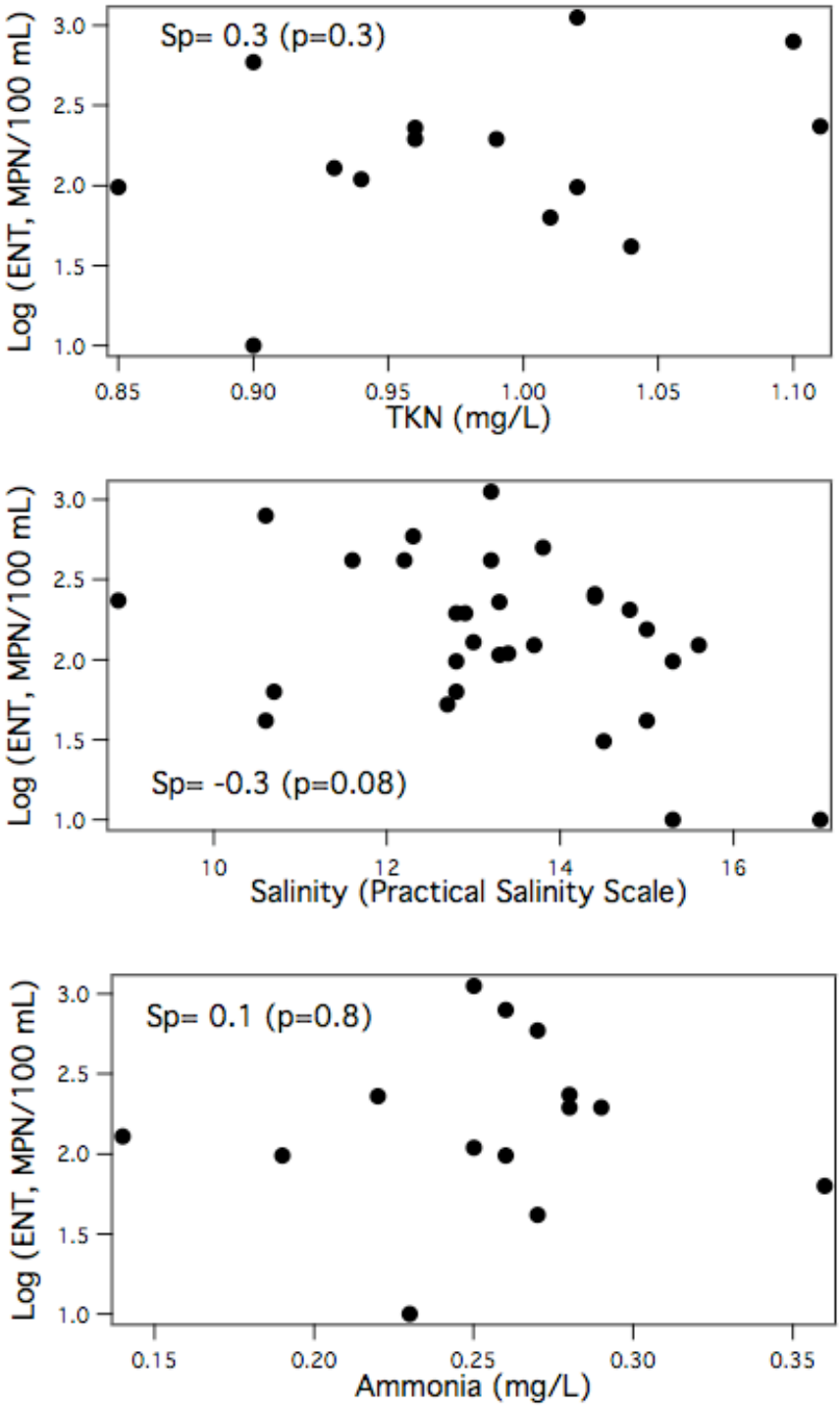

Figure 5-14. Cross-plots of log-transformed *Enterococcus* concentration against nutrient concentrations measured during the synoptic study in Upper Bay (Unit 1 Basin) on June 6 (2006). By way of comparison, the log-transformed AB411 single-sample standard for ENT is Log(104 MPN/100 mL)=2.0.

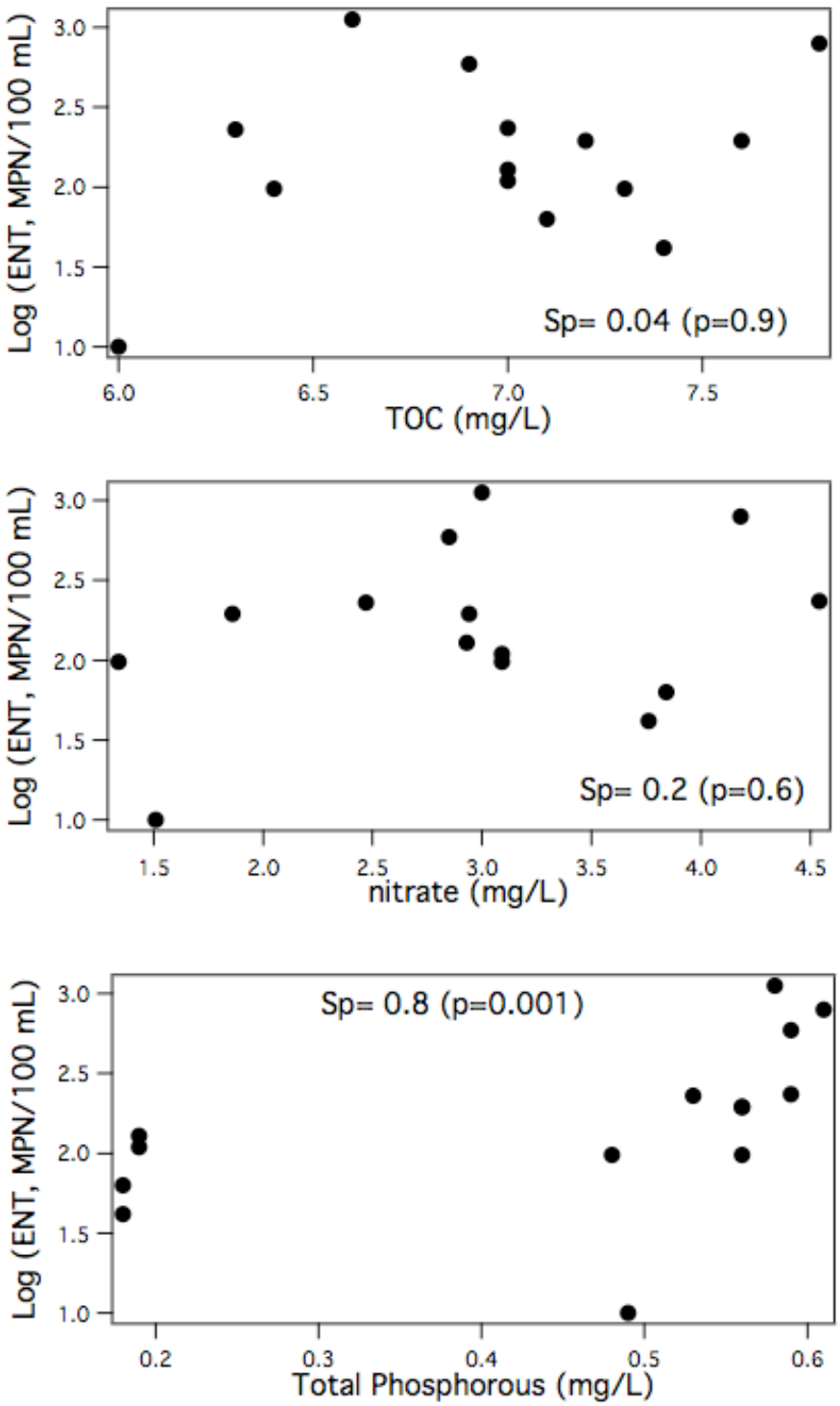

Figure 5-15. Cross-plots of log-transformed *Enterococcus* concentration against nutrient concentrations measured during the synoptic study in Upper Bay (Unit 1 Basin) on June 6 (2006). By way of comparison, the log-transformed AB411 single-sample standard for ENT is Log(104 MPN/100 mL)=2.0.

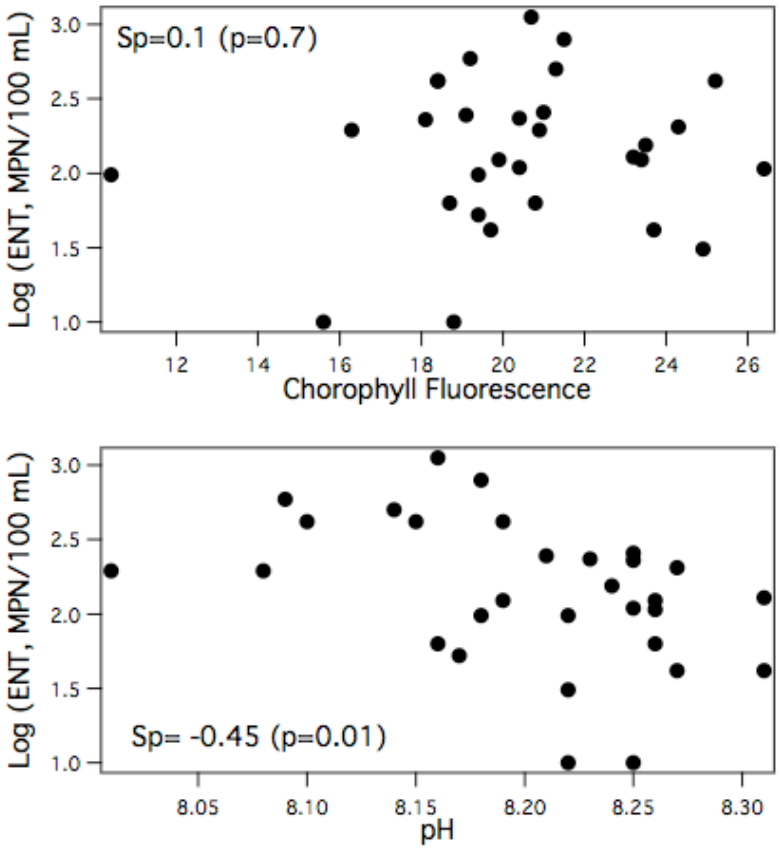

Figure 5-16. Cross plots of log-transformed *Enterococcus* concentration against nutrient concentrations measured during the synoptic study in Upper Bay (Unit 1 Basin) on June 6 (2006). By way of comparison, the log-transformed AB411 single-sample standard for ENT is Log(104 MPN/100 mL)=2.0.

similar. In the case of nitrate, however, the concentration measured in San Diego Creek is >8 times higher than the concentration measured in the upper reaches of Upper Bay.

5.3.6.3. Conclusions: Special Study on Spatial Distribution of FIB During a Macroalgal Bloom in Upper Bay

The significant and strongly negative correlation between *E. coli* and salinity is consistent with the idea that at least a portion of this FIB group originates from the discharge of freshwater into Upper Bay from San Diego Creek. Significant positive correlations between *E. coli* and other nutrients (TKN, ammonia, TOC, and nitrate) could be due to the discharge of these nutrients from San Diego Creek water, or due to secondary but unidentified sources of *E. coli* in Upper Bay. The very poor and non-significant correlation between *Enterococcus* and salinity, on the other hand, suggests that San Diego Creek is not the only source of this FIB group, or that *Enterococcus* from the creek die-off rapidly in the Bay (although this last hypothesis is not supported by the results of the Bay/Creek Microcosms studies, see Section 5.2).

The strong positive correlation between *Enterococcus* and total phosphorous is intriguing. A potential source of phosphorus in Upper Bay is fecal matter deposited by birds congregating along the shoreline. Bird droppings contain high concentrations of phosphorous, and are a significant contribution to the cycling of phosphorous in urban systems[7]. In this interpretation, the macroalgae mats in Upper Bay may play a role in promoting high FIB concentrations along the shoreline in Unit 1 Basin, by providing a habitat for birds congregating along the shoreline (e.g., by increasing the abundance and/or diversity of benthic organisms that serve as food for shoreline birds), creating hypoxic microniches were FIB can regrow (compare laboratory and field microcosm results with macroalgae, Sections 5.3), and perhaps lowering cross-shore mixing rates and thus trapping FIB close to the shore. Alternatively, if environmentally adapted *Enterococcus* species are growing in the Bay, it is possible that they do so only under specific nutrient and environmental conditions, such as when nitrogen is limiting (and phosphorous is in excess). These possibilities will be investigated further as the data described here is packaged into journal articles.

5.4. Laboratory and Field Microcosms Containing Bay and Creek Sediment

In this section we explore the influence of sediments on the concentration and survival of FIB in Newport Bay and San Diego Creek. These laboratory experiments were carried out to:

- Determine if sediments provide an environment conducive to the survival and/or regrowth of FIB in Bay and creek environments.
- Determine if regrowth and/or decay of FIB associated with sediments is affected by sediment quality (e.g. grain size, organic carbon content, presence of benthic infauna)
- Determine if regrowth and/or decay of FIB associated with sediments varies with the nutrient and salinity regime of overlying waters.

[7] Fujita, M., Koike, F. (2007) "Birds transport nutrients to fragmented forests in an urban landscape", *Ecological Applications* **17**: 648-654.

5.4.1. Methods: Laboratory Microcosms Containing Sub-tidal Bay Sediment and Creek Sediment

Samples of sediment and bottom water were collected from a variety of sites in Newport Bay with a range of organic carbon content and grain size:

- San Diego Creek (BTO 1), creek sediments
- Upper Newport Bay (BTO 5), fine-grained organic-rich sub-tidal sediments
- North Star Beach (BTO 6), fine-grained sub -tidal sediments with shells
- Harbor Island (BTO 9), fine-grained sub-tidal sediments
- Rocky Point (BTO 11), coarse-grained sandy sub-tidal sediments

Sub-tidal sediment samples from within-Bay sites were retrieved from the top 1 to 10 cm using a Wildco Petite Ponar sediment grab sampler deployed from the boat, and stored on ice in sterile 500 mL bottles in the dark. Bottom water from within-Bay sites was collected at each site using a Van Doren Bottle. Water samples from each site were poured into 3 two-liter sterile bottles (6 liters per site) and stored on ice in the dark. Water and sediment from creek (BTO 1) site were collected as grab samples. Sediment and water samples were delivered to the laboratory within the 6-hour holding time on the day the microcosms were prepared. Water samples were not filtered before the experiments.

Laboratory microcosms consisted of 4 L sterile Erlenmeyer flasks held in a temperature-controlled room held at 20°C in the dark.. There were 5 control microcosms (bottom water only—no sediment) and 5 microcosms with water and sediment. Three-hundred grams of wet sediment were weighed into 3 L of bottom water for each sediment type. The total volume in each microcosm was 3 L. Microcosms were swirled in the morning, during sampling, and in the evening of each day to reduce hypoxia. The following microcosms were prepared (note bold letters refer to microcosm preparation in Table 5-6 below):

- **A**: San Diego Creek Water (BTO 1)
- **B**: BTO 1 Water with BTO 1 Creek Sediment
- **C**: Upper Newport Bay Water (BTO 5)
- **D**: BTO 5 Water with BTO 5 Sediment
- **E:** North Star Beach Water (BTO 6)
- **F**: BTO 6 Water with BTO 6 Sediment
- **G**: Lower Newport Bay Water (BTO 9)
- **H**: BTO 9 Water with BTO 9 Sediment
- **I**: Lower Newport Bay Water (BTO 11)
- **J**: BTO 11 Water with BTO 11 Sediments

Sub-samples were withdrawn from each microcosm using sterile serological pipettes. Sub-samples were collected immediately following microcosm construction (time 0), 3 hours, 6 hours, 12 hours, 24 hours (1 day), 48 hours (2 days), 72 hours (3 days), 96 hours (4 days), and 120 hours (5 days). Water samples were analyzed for FIB using IDEXX Colilert-18 and Enterolert, implemented in a 96 well quantitray format. For the 1:10 dilution, the dynamic range of the Colilert and Enterolert assays is 10 to 24,192 MPN/100 mL. Samples that had

Table 5-6. Salinity measured in laboratory mixtures of water and sediment (see microcosm list above for letter designation) .

Microcosm	BTO 1 (A & B)	BTO 5 (C & D)	BTO 6 (E & F)	BTO 9 (G & H)	BTO 11 (I & J)
Salinity	0.9	28.5	31.1	31.8	32.1

concentrations below the detection limit of 10 MPN/100 mL were assigned a value of 1 MPN/100 mL Samples that had concentrations in excess of the detection limit of 24192 MPN/100 mL were assigned the upper limit value.

5.4.2. Results and Discussion: Laboratory Microcosms Containing Bay and Creek Sediment

Salinity in each of the microcosms reflected the source water's relative positions in the Bay. The lowest salinity was measured in the microcosms containing Creek water, and the highest salinity was measured in microcosms containing water from Lower Bay (Table 5-6). Salinities measured in the microcosms with Bay water (Microcosms C through J) varied only by 4 units (practical salinity scale).

Figures 5-17 and 5-18 display time series measurements of *E. coli* and enterococci bacteria in the microcosms with or without sediment amendments. Each panel displays results for a particular combination of sediment and source water; the blue diamonds represent the control (water only) and the red squares indicate water plus sediment. The addition of sediment increased the initial concentration of *E. coli* in the water phase in only one case, for sediment collected from site BTO 5 (Figure 5-18). In the four remaining cases, addition of the sediment did not significantly increase the starting concentration of *E. coli*. In four out of five microcosms, the addition of sediment appeared to prolong the survival of *E. coli* in the water phase. The exception was the microcosm containing water and sediment collected from North Star Beach (BTO 6), for which the addition of sediment did not prolong *E. coli* survival, relative to the no-sediment control. The bulk of BTO 6 sediment was comprised of broken shells instead of organic rich sediment, which may account for the no-effect on *E. coli* survival in this case.

The addition of Newport Bay sediment increased the initial concentration of enterococci bacteria in all cases (BTO 5,6, 9, and 11) (Fig. 5-18). The addition of San Diego Creek sediment, on the other hand, did not increase the initial concentration of enterococci bacteria (BTO 1) (Fig. 5-18). In general, enterococci bacteria appeared to survive better in microcosms amended with sediment (Fig. 5-18).

5.4.3. Methods: Field Microcosms Containing Bay and Creek Sediment

Field microcosms consisted of dialysis bags (MWCO 12000-14000 Daltons, Spectra/Por Dialysis Membrane, Rancho Dominguez, CA) suspended in a milk crate that was, in turn, suspended in either Newport Bay (at the US Fish and Game Dock on Shellmaker Island), or in San Diego Creek (at the Campus Dr. crossing, BTO 1). The MWCO of the dialysis bag allows dissolved nutrients and organic carbon to pass through the bag walls, while separating bacterial populations inside and outside the bag. Dialysis bags were cut into 2.5 ft long segments (for a

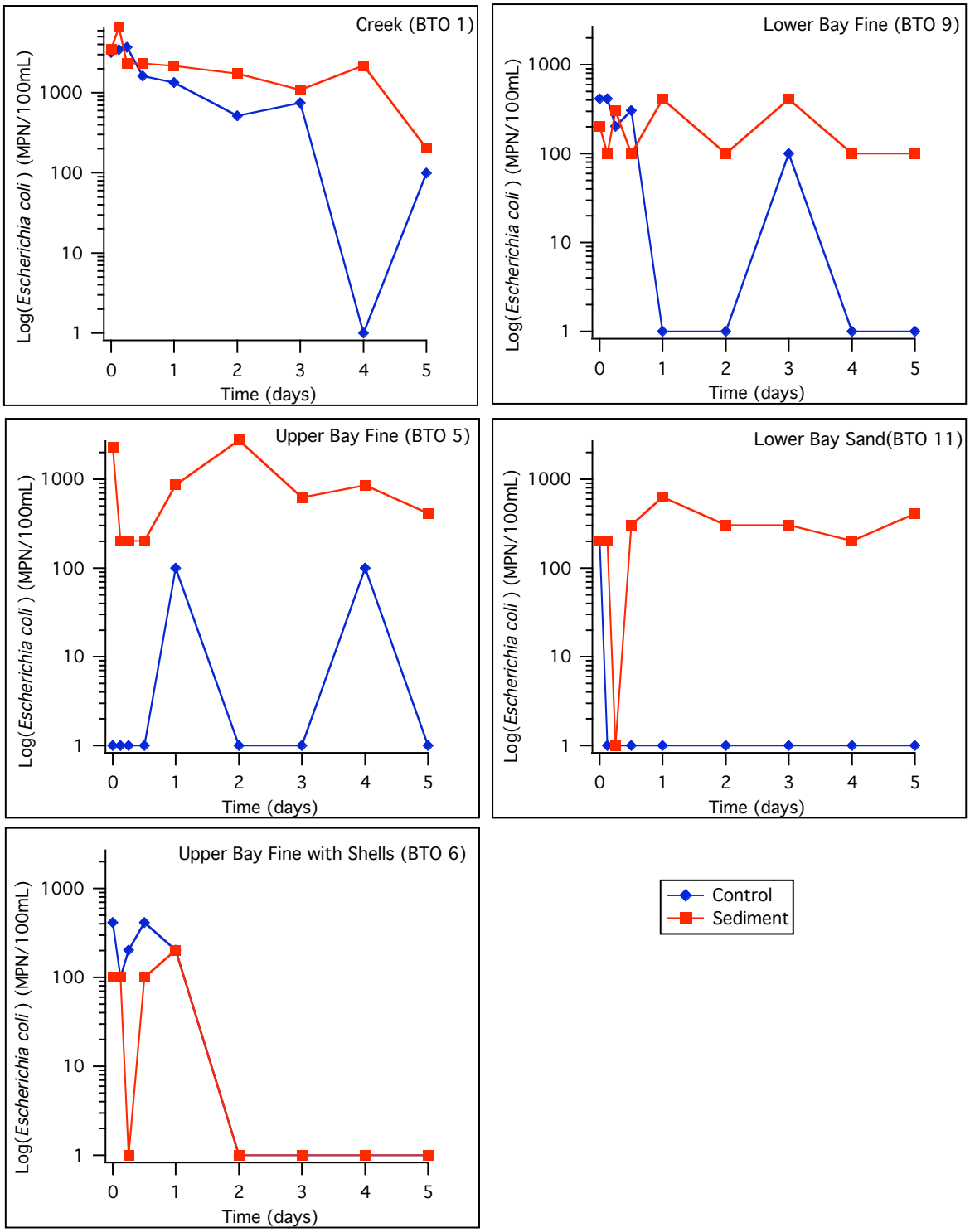

Figure 5-17. The effect of adding sediment to *Escherichia coli* survival in laboratory microcosms.

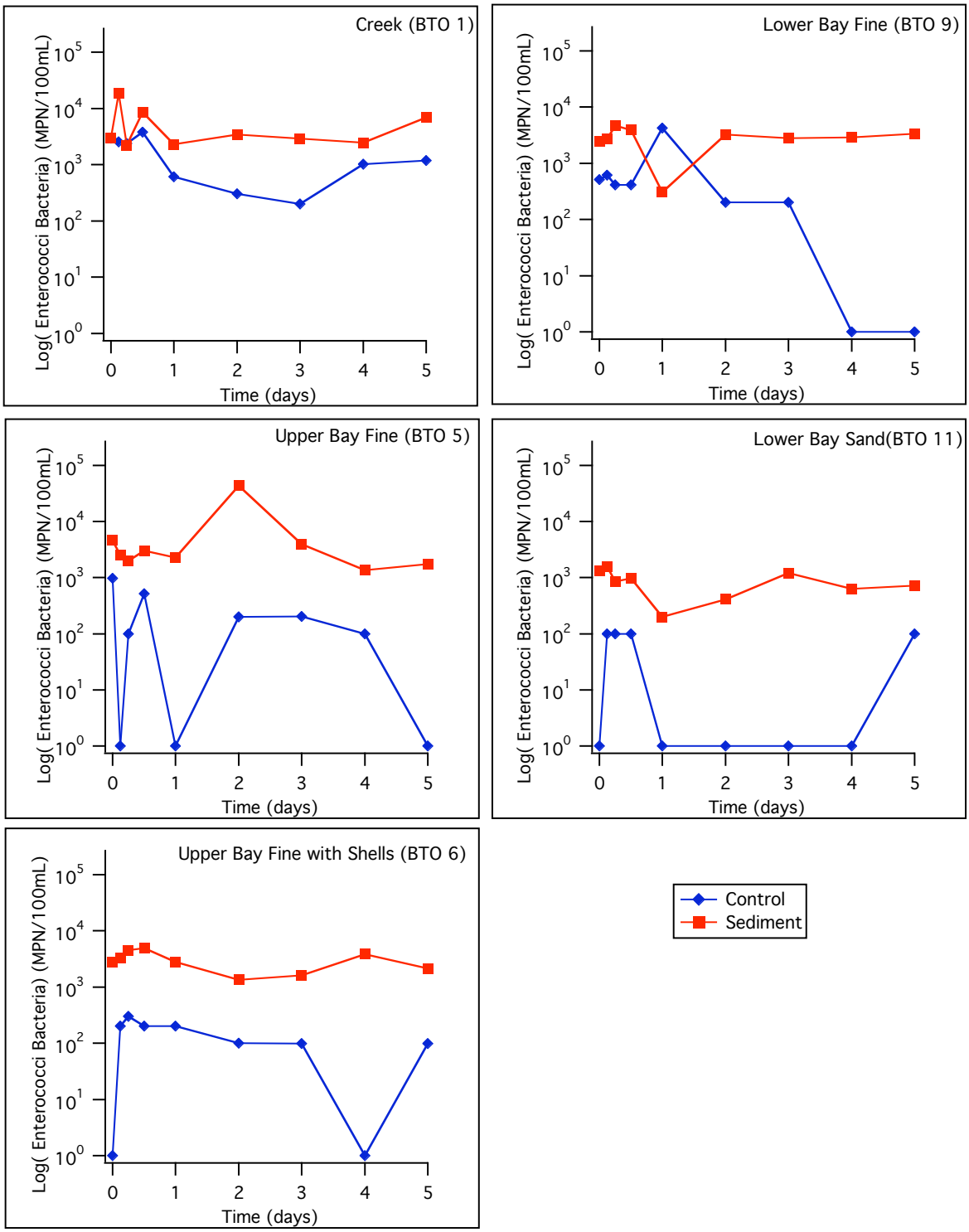

Figure 5-18. The effect of adding sediment on enterococci bacteria survival in laboratory microcosms.

total volume of approximately 3 L), clamped on one end, filled with sample water +/- sediment, and clamped at the other end. The time 0 sub-sample was collected immediately using sterile serological pipettes. Bags were then re-clamped and gently placed into plastic milk crates that were lowered into either the Creek or the Bay.

At the San Diego Creek site, milk crates were placed in the center of the stream such that the microcosms were completely covered by creek water. The milk crate was then roped to 2 cinder blocks that kept the crate from being washed down stream. At the Shellmaker Island site, the crates were tied to a cleat on the dock, and lowered to a depth such that the tops of the microcosms were covered (but not too deep, to prevent the microcosms from floating out of the crate). The milk crates were necessary because, during preliminary field tests, the end clips came off while retrieving the dialysis bags and the microcosms were lost.

Sub-tidal sediment was collected from Upper Bay (BTO 5) in the morning of the experiment with a Wildco Petite Ponar. Half of this sediment was autoclaved for 45 minutes to eliminate FIB present in the sediment. The autoclaved and non-autoclaved sediment samples were then transported to the field site (either Shellmaker Island or San Diego creek) within 6 hours of their collection from the Bay. Microcosms were prepared on site by filling the dialysis bags with source water, with or without addition of one hundred and fifty grams of sediment (either autoclaved or non-autoclaved), wet weight. Once constructed the microcosms were clamped on both ends and shaken. There were 2 control microcosms (dialysis bags filled with Bay or Creek source water without amendment), and 4 microcosms with added sediment. The following microcosms were prepared:

- Bay water control
- Bay water with non-autoclaved Upper Bay sediment
- Bay water with autoclaved Upper Bay sediment
- Creek water control
- Creek water with non-autoclaved Upper Bay sediment
- Creek water with autoclaved Upper Bay sediment

5.4.4. Results and Discussion: Field Microcosms Containing Bay and Creek Sediment

For field microcosms suspended in Upper Bay water:

1. Addition of Upper Bay sediment did not appear to increase the concentration of *E. coli*, relative to the no-sediment control (middle panel, Fig. 5-19).
2. Addition of Upper Bay sediment appears to increase the concentration of enterococci bacteria, relative to the no-sediment control (bottom panel, Fig. 5-19). This increase occurred within hours when non-autoclaved sediments were added (red curve), and within days when autoclaved sediments were added (green curve).

For field microcosms suspended in San Diego Creek water:

1. Addition of Upper Bay sediment appears to substantially increase (>1000 fold) the concentration of *E. coli*, relative to the no-sediment control (middle panel, Fig. 5-20).

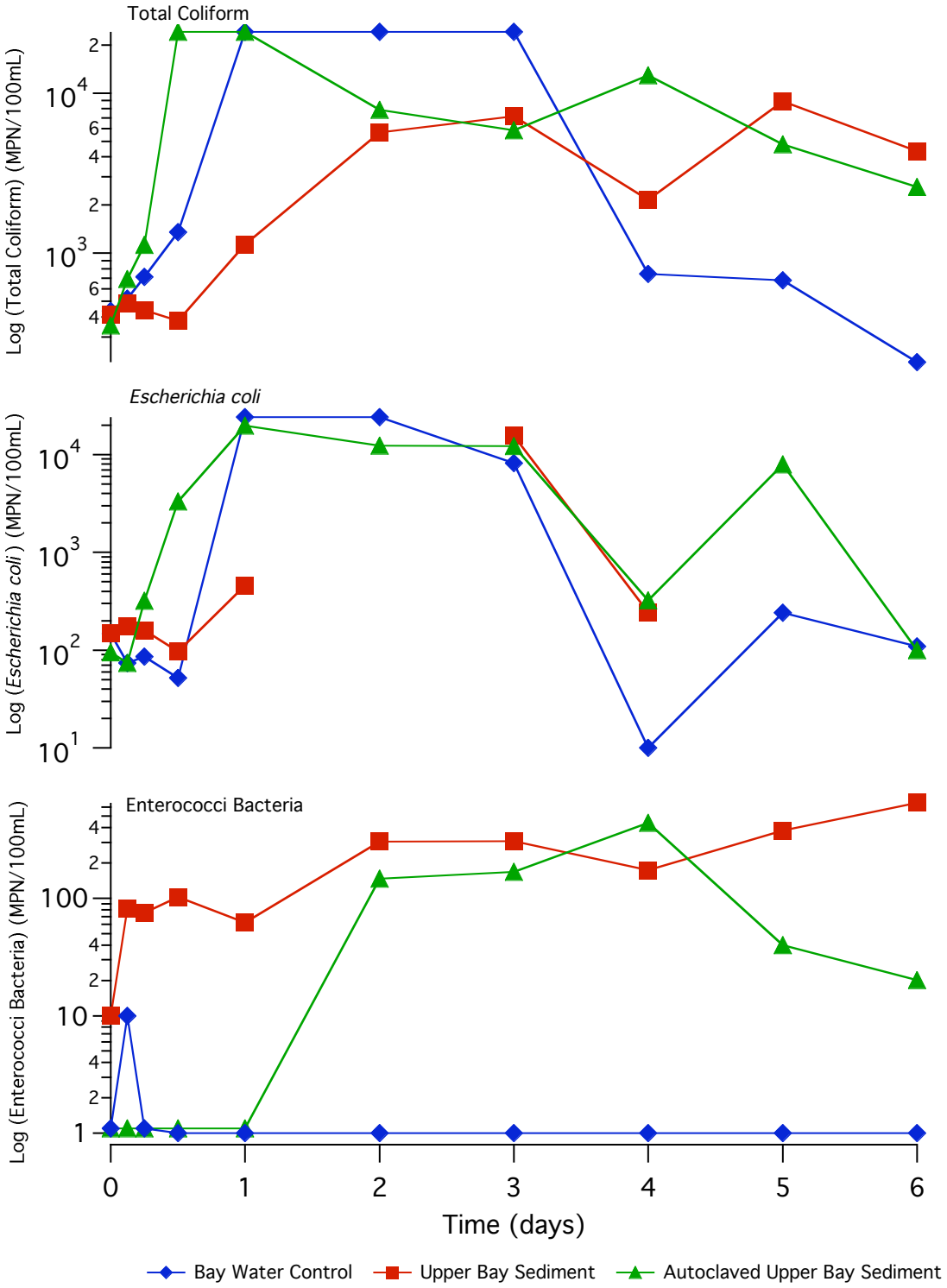

Figure 5-19. Microcosms consisting of water from Upper Bay mixed with sediment from Upper Bay with or without autoclaving.

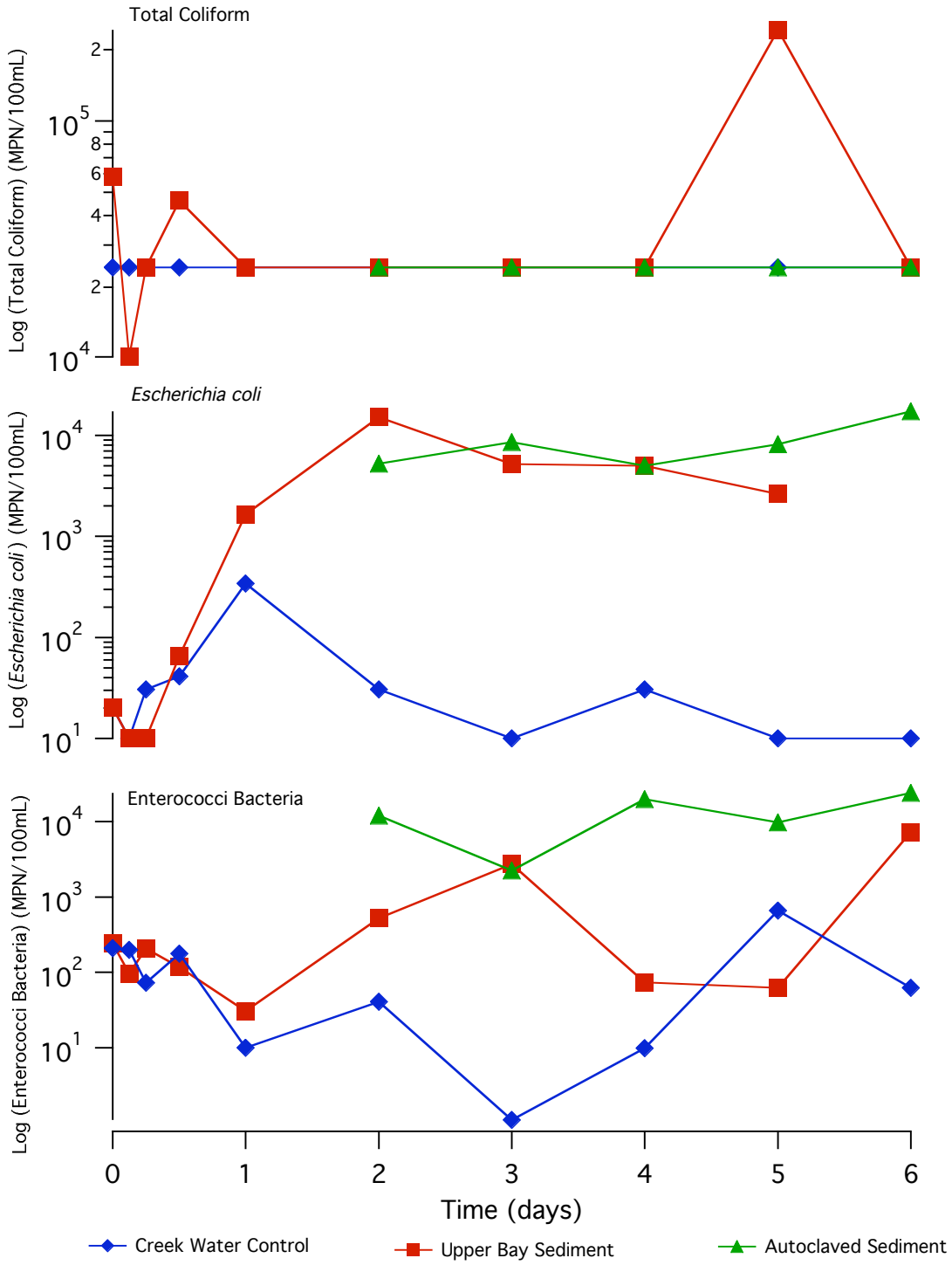

Figure 5-20. Microcosms consisting of water from San Diego Creek mixed with sediment from Upper Bay with or without autoclaving.

1. This increase was observed when both autoclaved and non-autoclaved sediments were added.
2. Addition of Upper Bay sub-tidal sediment also appeared to increase the concentration of enterococci bacteria, although, compared to *E. coli*, the effect is less dramatic (bottom panel, Fig. 5-20). The concentration of enterococci bacteria increases to a greater extent when the microcosm is amended with autoclaved sediment (green curve).

5.4.5. Conclusions: Microcosms Containing Bay Sub-tidal Sediment and Creek Sediment

In the laboratory, sediment addition increased the starting concentration of enterococci bacteria in the water phase, and enhanced the survival of both enterococci bacteria and *E. coli* in the water phase over time. In the field, the sediment addition to microcosms suspended in Upper Bay resulted in rapid and significant (in some cases >10,000 fold) increases in the concentration of enterococci bacteria, but not *E. coli*. Addition of Upper Bay sediment to San Diego Creek water, on the other hand, led to substantial increases in *E. coli* concentration, but had little affect on the concentration of enterococci bacteria. Together, these results support the hypothesis that, in Newport Bay, sub-tidal sediment is a potential source of enterococci bacteria and can prolong the survival of both enterococci bacteria and *E. coli*.

5.5. Field Microcosms Containing Debris Mat Material

Debris mats often appear in Newport Bay during and following storm events. These mats consist of loosely bound plant stalks and sticks mixed together with trash and macroalgae. This material can be transported in runoff to the Bay from the surrounding watershed, or it can be mobilized from within the Bay by the turbulence associated with large storm flows. Debris mats range in size from a half-meter diameter to 5-6 meters across. Because they are typically present in the Bay when FIB concentrations are highest (after storm events), a microcosm study was carried out to determine how debris mats affect FIB concentrations in Newport Bay. The debris mat microcosms were designed to determine if debris mats: (1) are a potential source of FIB in Newport Bay; (2) prolong FIB survival by shielding from solar radiation.

5.5.1. Methods: Field Microcosms Containing Debris Mat Material

Debris mat experiments were conducted simultaneously with the creek water/ Bay water microcosms (Section 5.2.5). Water from Upper Bay (Station BTO 5) was collected in sterile 20 L carboys, and then transported to the lab in under 2 hours from collection. Debris mat material was collected from the Bay as grab samples, placed in Ziploc bags, and stored on ice. Because we were specifically interested in the role the debris mats might play in shielding FIB from solar die-off, these experiments were conducted exclusively in the field.

Field microcosm experiments were conducted on the dock at the Kerchoff Lab in Lower Bay. Each microcosm was prepared in a sterilized 2.5 gallon glass aquarium. Microcosms containing debris were prepared by adding 5L of source water to a 2.5 gallon sterile glass aquarium. Debris was then layered into the aquarium until the debris formed a contiguous layer, approximately 3 to 5 cm thick, across the water surface. Once prepared, the aquariums were placed in one of two

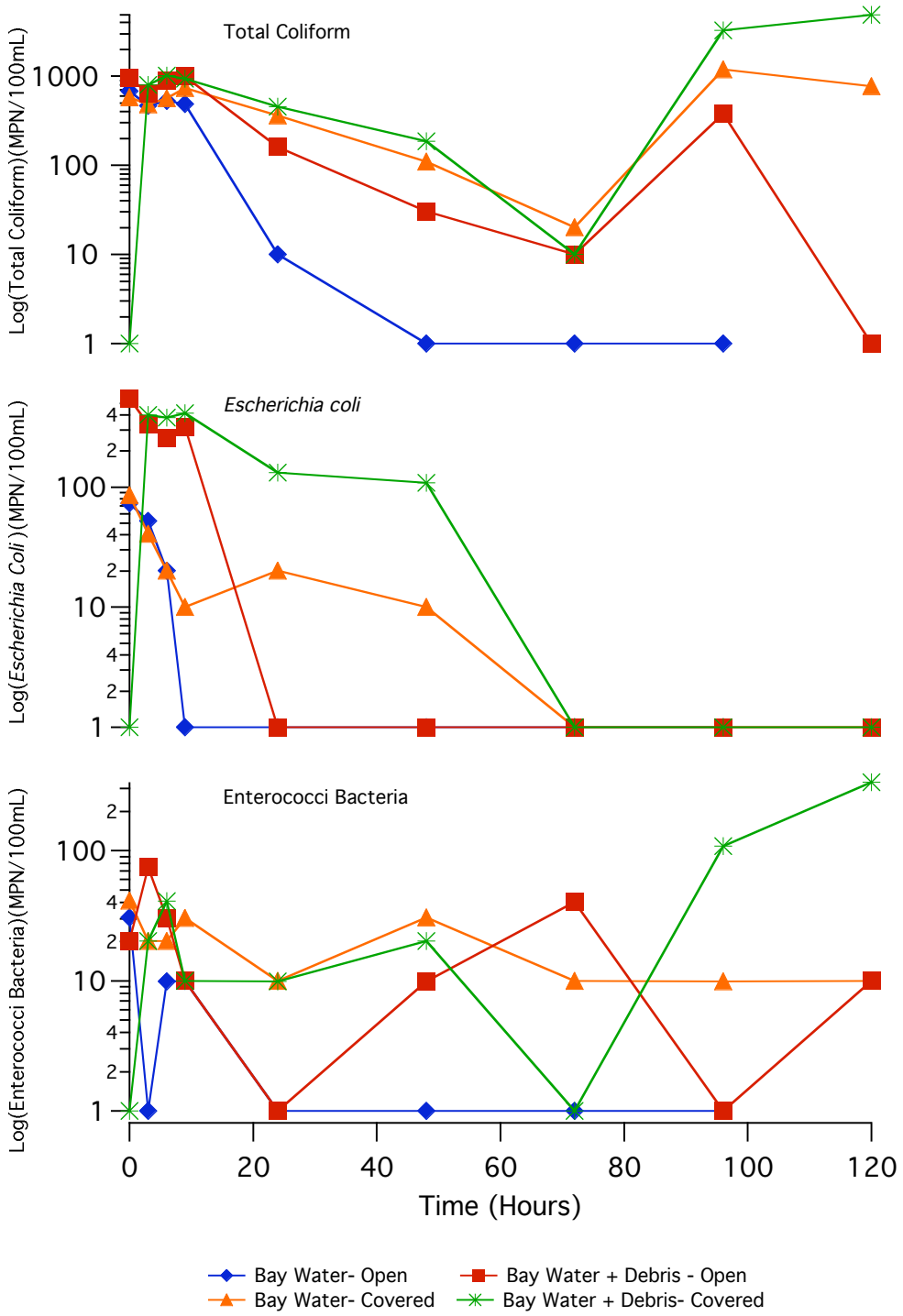

Figure 5.21. Field microcosms consisting of mixtures of Bay water and debris mat materials, either exposed to UV radiation ("Open") or not ("Covered").

child-paddling pools. Bay water was continuously pumped into these pools with a bilge pump that was placed over the side of the dock into the Bay. Bay water was allowed to flow over the edge of the pools such that the temperature of water in the aquariums was close to the Bay water temperature (approximately 19°C). Half of the microcosms were uncovered, and thus exposed to changes in solar irradiance and atmospheric deposition. The other half were wrapped in aluminum foil, and therefore excluded from light and atmospheric deposition. There were 2 control microcosms (Bay water in both light and dark) and 2 microcosms containing Bay water and debris mat material in both light and dark. In total four field microcosms were prepared:

- 5 L Upper Newport Bay (BTO 5) Water – Uncovered
- 5 L Upper Newport Bay (BTO 5) Water – Covered
- 5 L Upper Newport Bay (BTO 5) Water + Debris –Uncovered
- 5 L Upper Newport Bay (BTO 5) Water + Debris –Covered

5.5.2. Results and Discussion: Field Microcosms Containing Debris Mat Material

The concentration of *E. coli* measured decreased over time, but the rate of decrease varied, depending on the source water, the presence (or absence) of debris, and whether or not the microcosm was covered (middle panel, Fig. 5-21). The concentration of enterococci bacteria was low in this set of microcosms (<100 MPN/100 mL), and hence it is difficult to assess what affect, if any, debris had on enterococci survival (although it can be said that they do not appear to be a significant source of these bacteria). In general, enterococci bacteria survived longest in the covered microcosms, and in open microcosms containing debris (bottom panel, Fig. 5-21).

5.5.3. Conclusions: Field Microcosms Containing Debris Mat Material

In summary, debris mat material may shield FIB from the harmful effects of solar radiation, and thus prolong the survival of these bacteria in Bay waters. However, the microcosm data presented here provide little support for the idea that debris mats are significant sources of FIB in the Bay. This result contradicts a preliminary study of debris mats carried out in advance of the Prop 13 project, which found that debris mats harbor very high concentrations of FIB. The debris mat sampled during the preliminary study appeared to consist of plant material washed into the Bay from San Diego Creek watershed during a storm event. During the Prop 13 study, on the other hand, debris mats consisted primarily of plant material generated from within the Bay. Perhaps the different origin of the debris (watershed in the earlier sampling event, and within the marsh during the Prop 13 study) accounts for the different results obtained.

5.5.3. Observations of Algal Mats and Debris Mats in Upper and Lower Newport Bay

Algal mats were observed, particularly at Upper Bay sites, nearly constantly from May through November 2006. Debris mats, on the other hand, were observed rarely. In almost all cases, debris mat sightings occurred in the first few days following a major storm. Debris mats, which we defined as coalesced mats of reeds, sticks, leaves, algae, and/or foam, quickly rafted up onto

the mudflats on the high tide following a storm. Materials from these debris mats were collected from the sampling boat and used as amendments in the microcosm studies described above.

Photographs taken from the sampling boat were not finely resolved enough to permit a quantitative analysis of debris mat size and frequency, as originally proposed in the Prop 13 project. Qualitatively, most of the debris mats were small in size, typically <1m on each side. The largest debris mat (approximately 2m by 2.5m in size) was observed near BTO 5, while the sampling boat was transiting from BTO 5 to BTO 4. When the sampling boat returned to BTO 5 approximately 1 hour later, the debris mat had disappeared, either by breaking into smaller fragments, or by rafting up onto the mudflat. In addition to debris mats and algal mats, large pieces of trash were occasionally observed. For example, a large plastic traffic median (1 m tall, 1.2m wide, and 10 cm thick) was observed floating in Upper Bay after a storm. Large pieces of trash did not appear to "seed" the formation of debris mats; i.e., they were not typically associated with other trash or organic debris.

5.6. Field Microcosms Containing Mixtures of Dry Weather Runoff and Bay Water

A final set of microcosm studies were conducted to assess the die-off rate of FIB in dry weather runoff after mixing into the brackish waters of Newport Bay. The goal of these experiments was to:

- Determine if FIB in dry weather runoff obeys mass balance when mixed with Bay water (i.e., their concentration can be predicted from simple dilution calculations).
- Determine if the survival of runoff-derived FIB in Bay water can be modeled with a single-hit model for the solar die-off of bacteria.

Because we were specifically interested in the synergistic affects of sunlight and Bay water on FIB die-off rates, these microcosm studies were conducted in the field only (i.e., there were no laboratory studies). Further, to assess the influence of sunlight on FIB die-off rates, coincident measurements of solar irradiation were converted into solar insolation (a measure of the integrated exposure of bacteria to solar radiation), which was used to characterize solar-enhanced die-off rates of FIB in the microcosms.

5.6.1. Methods: Field Microcosm of Dry Weather Runoff and Bay Water.

On 2/9/2007 07:00 (PST) approximately 200 mL of dry weather runoff was collected from 2 sites in Lower Newport Bay (from curb flow on Balboa Island and from within Polaris drain). At the Balboa site, runoff was sampled from water flowing along a street curb; at the Polaris site, runoff was sampled from water flowing down the center of the open and concrete-lined channel. These two runoff samples were separately diluted (1 part runoff into 9 parts Bay water) into brackish water collected from Lower Bay for a final volume of 2 L. Each bay/runoff mixture was immediately transferred into two dialysis bags (MWCO 12000-14000 Daltons, Spectra/Por Dialysis Membrane, Rancho Dominguez, CA) and suspended in the Lower Bay over the side of a dock located in the Balboa Yacht Basin. 20 mL aliquots were collected from the dialysis bags using a sterilized syringe, once every half hour for the first two hours, once every hour for the

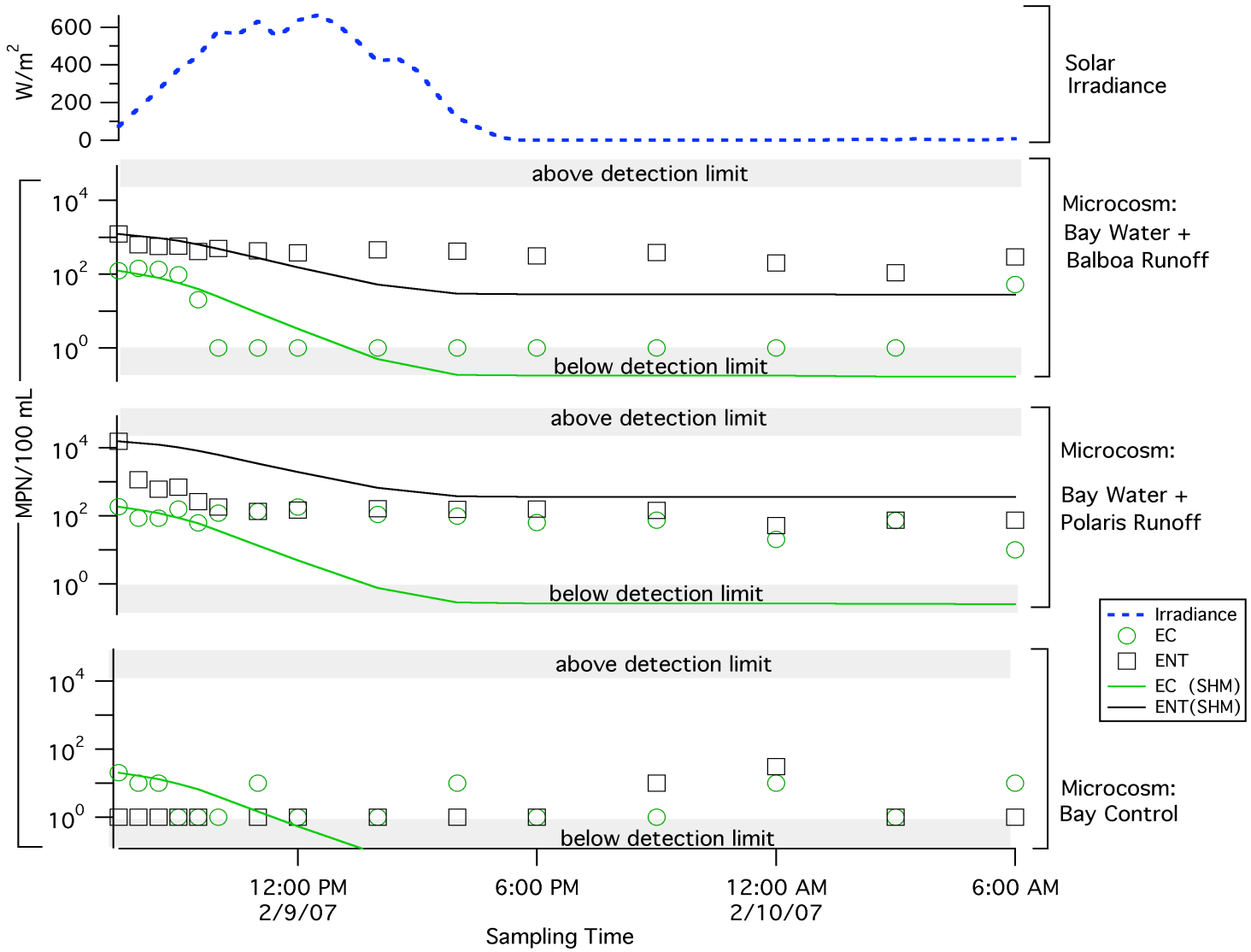

Figure 5-22. Patterns of FIB survival in microcosm mixtures of dry weather runoff and Bay water. Three microcosm results are shown, including a mixture of Bay water and Balboa Island runoff (top panel), a mixture of Bay water and runoff from Polaris (middle panel), and Bay water alone (bottom panel). EC= *E. coli*; ENT= enterococci bacteria. SHM= single hit model for FIB die-off (see text).

Table 5-7. FIB concentrations measured in runoff samples, Bay water, and mixtures of runoff and Bay water. Numbers in parentheses indicate predicted concentrations using mass balance.

Microcosm Preparation	**Initial FIB concentration (MPN/100mL)**	
	Escherichia coli	*Enterococcus spp.*
Bay water (control)	20	<10
Polaris Runoff	880	1500
Balboa Runoff	1100	24000
Bay water + Polaris Runoff	190 (88)	16000 (150)
Bay water + Balboa Runoff	122 (110)	1200 (2400)

next two hours, once every two hours for the next four hours and once every three hours for the rest of the study. Aliquots were analyzed for FIB using IDEXX Colilert-18 and Enterolert, implemented in a 96 well quantitray format. In total three field microcosms were deployed:

- Lower Newport Bay Water
- Lower Newport Bay Water + Dry Weather Runoff from Balboa Island curb
- Lower Newport Bay Water + Dry Weather Runoff from Polaris Drain

Solar insolation $S(t)$ as a function of time t was calculated from coincident hourly measurements of total incoming solar radiation $I(t)$ at the nearby San Joaquin marsh (33.66º, -117.85º) by a CM3 Pyranometer (Kipp and Zonen, Delft, The Netherlands). Solar radiation measurements were integrated to obtain insolation as a function of time t from the start of the microcosm experiments:

$$S(t) = \int_0^t I(t)dt \qquad \text{Eq. 5-1}$$

5.6.2. Results and Discussion: Initial Dilution Results.

E. coli and enterococci bacteria concentrations generally obey mass balance as runoff mixes into Bay water; i.e., the osmotic shock experienced by bacterial populations during the dilution event did not cause an instantaneous die-off of the bacteria. *E. coli* and enterococci concentrations initially present in the three microcosms, and in samples of the runoff alone, are shown in Table 5-7. FIB concentrations in undiluted runoff were significantly higher (from 40 to > 1000 times) than FIB concentrations in the unamended Bay water. With one exception, the initial FIB concentrations measured in microcosms containing 1:10 mixtures of runoff and Bay water are within a factor of three (or better) of the concentration predicted based on a 1:10 dilution factor (predicted values shown in parentheses in Table 5-7). The one exception was enterococci bacteria in the microcosm containing a mixture of Polaris runoff and Bay water, for which the

concentration measured after dilution (16000 MPN/100 mL) is 100 times higher than the concentration predicted from a 1:10 dilution (150 MPN/100 mL).

5.6.3. Results and Discussion: FIB Survival in Microcosms of Dry Weather Runoff and Bay Water

E. coli and enterococci in dry weather runoff exhibit variable survival patterns after dilution into the brackish waters of Newport Bay (Fig. 5-22). In some cases, bacteria concentrations in the microcosms remained relatively constant over the 24-hour monitoring period (enterococci bacteria in Bay Water + Balboa Runoff; EC in Bay Water + Polaris Runoff). In other cases, bacteria concentrations decrease 100 to 1000 fold within the first couple of hours, and then remain relatively constant for the rest of the experiment (enterococci bacteria in Bay Water + Polaris Runoff) or drop below the lower-limit of detection (*E. coli* in Bay Water + Balboa Runoff).

5.6.4. Results and Discussion: Evaluation of Single-Hit Model for the Die-off of Runoff FIB in Bay Water

As a consequence of the variable FIB survival patterns just noted, the single-hit model does a relatively poor job of capturing the die-off of runoff-derived FIB in the brackish waters of Newport Bay. The single-hit die-off model predicts that FIB concentration in the microcosms (C_{micro}) should decline with time (t) according to the following equation[8,9]:

$$C_{micro} = C_i e^{-k'S(t)} \qquad \text{Eq. 5-2}$$

where C_i is the initial concentration of FIB in the microcosm at time $t = 0$ (right after the runoff and Bay water are mixed), $S(t)$ is solar insolation, and k' is a solar modulated die-off rate constant specific to each fecal indicator group. For an initial evaluation of the single-hit model, we used rate constants reported by Sinton et al for the die-off of sewage-derived FIB in ocean water (ref. 5). With this choice of k' values, eq. 5-2 predicts that FIB concentrations in the microcosms decrease rapidly from the start of the experiment (7:30 am) until early evening (5:00 pm) and remain constant throughout the night (solid lines in Fig. 5-22). Although there are a few cases where the predicted and measured trends are similar (e.g., *E. coli* in the Bay Water + Balboa Runoff microcosm), in general the single-hit model with Sinton's rate constants over-estimates (often by multiple log-cycles) the die-off of FIB from dry weather runoff after mixing into the Bay. It should be noted that eq. 5-2 does not account for shoulder effects associated with the initial resistance of bacteria to solar exposure, which can be significant for the survival of sewage-derived ENT in ocean water (ref. 8).

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[8]Sinton, L. W., R. K. Finlay, and P. A. Lynch. Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater, *Appl. Environ.Microbiol.*, **1999**, 65(8), 3605– 3613.

[9]Sinton, L.W., Davies-Colley, R. J., Bell, R. J. Inactivation of enterococci and fecal coliforms from sewage and meatworks effluents in sea water chambers. **1994**. *Applied and Environmental Microbiology*. 2040-2048.

5.7. Summary of Chapter

1. Creek FIB are present at higher concentrations, longer-lived, and more resilient to changes in salinity, than Bay FIB.
2. Consistent with the literature, bird feces harbor very high concentration of FIB, but after entering the Bay bird-derived FIB die-off at a rate that depends on solar irradiation levels (faster die-off in the presence of sunlight, slower die-off in the dark). In all cases, FIB from bird feces survived for relatively long periods of time (i.e., the T90 was generally greater than 1 day).
3. In laboratory microcosms, the addition of macroalgae significantly increases the survival of FIB from bird feces. However, the protective effects of macroalgae on FIB survival and/or regrowth were not replicated in field experiments, perhaps due to different redox conditions (oxic in the case of field microcosms, and hypoxic in the case of laboratory microcosms).
4. A synoptic study of FIB in Upper Bay during a macroalgae bloom found significant positive correlations between *E. coli* and other nutrients (TKN, ammonia, TOC, and nitrate), perhaps signaling the presence of contaminated runoff from San Diego Creek. By contrast, *Enterococcus* was not correlated (either positively or negatively) with salinity, which could be interpreted in at least two ways: (1) San Diego Creek is not the only source of Enterococcus in the Unit 1 Basin; or (2) San Diego Creek is a significant source of *Enterococcus* in the Unit 1 Basin, but these organisms die off rapidly once they enter the Bay. However, the second hypothesis is not supported by the results of the Bay/Creek Microcosms studies (see item 1 above), which indicate that Creek *Enterococcus* are relatively stable when diluted into Bay waters. A strong positive correlation was noted between *Enterococcus* concentration and total phosphorous, which might indicate that bird droppings contributed to enterococci bacteria concentrations along the shore in the Unit 1 Basin.
5. There is some evidence that debris mat material may aid in survival of FIB in creek and Bay water. In principle, both debris mat material and macroalgae could provide nutrients and organic carbon to the water column as they decay, which FIB can potentially use as a substrate for maintenance or regrowth. Macroalgae and debris mat material can also provide shading from the germicidal action of UV radiation.
6. Bay sediments contribute *Enterococcus* to the water phase of the microcosms, and the presence of sediments in the microcosms appears to reduce the die-off of these organisms. This result was observed in both laboratory and field experiments.
7. Bay sediments appear to contribute relatively little *E. coli* to the water phase, but may aid in their survival. It is also possible that turbidity contributed by sediments provide UV shading, thus reducing sunlight-induced die-off of FIB, as recently suggested by Kay et al[10]. Sediments can also contribute nutrients and organic carbon to the water-column, particularly if the sediment is fine grained, which, in turn, can be used by FIB to sustain their populations.

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[10] Kay, D., C.M. Stapleton, M.D. Wyer et al (2005) "Decay of intestinal enterococci concentrations in high-energy estuarine and coastal waters: towards real-time T-90 values for modeling faecal indicators in recreational waters. *Water Research*, 2005, **39**, pgs. 655-667.

8. FIB in dry weather runoff behave relatively conservatively when diluted into high salinity water from Lower Bay. Thus runoff-derived FIB do not precipitously die-off when mixed into high salinity Newport Bay waters.
9. The Single-Hit-Model for UV mediated die-off of FIB generally underestimates the survival of runoff-derived FIB in the high salinity waters of Newport Bay.

[1]Chapter 6. Diurnal Intertidal Sediment (DITS) Studies

6.1. Introduction and Overview

A number of recent studies have implicated beaches—either the beach itself, or shallow groundwater discharge through the beach face—as the source of FIB impairment in ankle depth waters along the shore[2,3,4,5,6,7]. Based on the microcosm results presented in the last chapter, FIB might originate from the surface of the beach in at least two ways: (1) deposition of bird feces on the surface of the beach, which mobilize into the water column during rising tides; (2) growth of environmentally adapted strains of FIB in the beach sand and/or in decaying vegetation (e.g., macroalgae or wracklines) concentrated along the shore.

As a first step toward evaluating the beach as a potential source of FIB, in this chapter we describe a field sampling effort in which water and sediment samples were collected along two cross-shore transects in Upper Bay, near sites BTO 4 and 5. Sampling campaigns were carried out a total of four times (twice during wet weather, and twice during dry weather). During each sampling campaign, water and sediment samples were collected from a set of cross-shore sites every 3 hours for 12 or 24 hours (depending on the study). Sediment samples were analyzed for FIB, organic carbon, water content, and grain size distribution. Water samples were analyzed for FIB, and a subset of water samples were analyzed for nutrients.

The studies described in this report satisfy sub-task 2.1.3.4 in its entirety. This chapter satisfies the reporting element of sub-task 2.1.3.7.

6.2. Methods

The locations of DITS sampling sites are presented in Fig. 6-1. The timing of the four DITS studies is presented in Figs. 6-2 and 6-3, relative to measurements of solar irradiance, rainfall, and flow from the San Diego Creek and Santa Ana Delhi channel.

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[1] Prepared by S. Grant, with assistance from Drs. K. McLaughlin, J. Ahn and R. Litton.

[2] Yamahara, K.M., Layton, B.A., Santoro, A.E., Boehm A.B. (2007) "Beach sands along the California Coast are diffuse sources of fecal bacteria to coastal waters" *Environ. Sci. Technol*. **41**:4515-4521.

[3] Oshiro, R., Fujioka, R. (1995) "Sand, soil, and pigeon droppings—Sources of indicator bacteria in the waters of Hanauma Bay, Oahu, Hawaii" *Water Sci. Technol*. **31**:251-254.

[4] Desmarais, T.R.; Solo-Gabriele, H.M., Palmer, C.J. (2002) "Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment" *Appl. Environ. Microbiol*. **68**:1165-1172.

[5] Whitman, R.L.; Nevers, M.B. (2003) "Foreshore sand as a source of *Escherichia coli* in nearshore water of a Lake Michigan beach" *Appl. Environ. Microbiol*. **69**:5555-5562.

[6] Ferguson, D.M., Moore, D.F., Getrich, M.A., Zhowandai, M.H. (2005) "Enumeration and speciation of enterococci found in marine and intertidal sediments and coastal water in southern California" *J. Appl. Microbiol*. **99**:598-608.

[7] Lee, C.M., Lin, T., Lin, C.C., Kohbodi, G.A., Bhatt, A., Lee, R., Jay, J.A. (2006) "Persistence of fecal indicator bacteria in Santa Monica Bay beach sediments" *Water Res*. **40**:2593-2602.

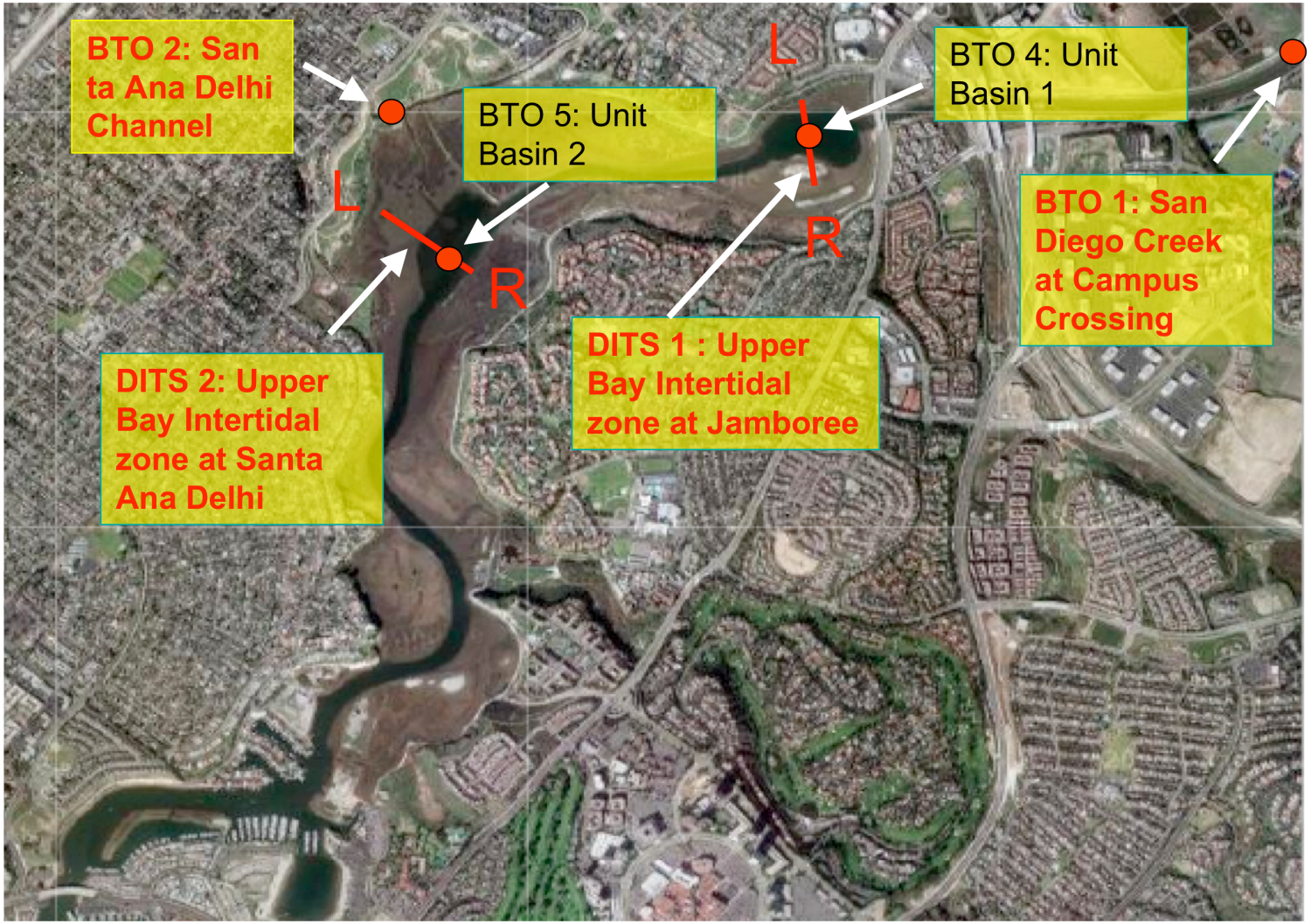

Figure 6-1. Map of Diurnal Intertidal Sediment (DITS) transect sites and tributary sampling locations (labels in red lettering on yellow background). DITS 1 is co-located with BTO 4, and DITS 2 is co-located with BTO5. "L" and "R" refer to the left and right side of the DITS transects (assuming the observer is facing inland).

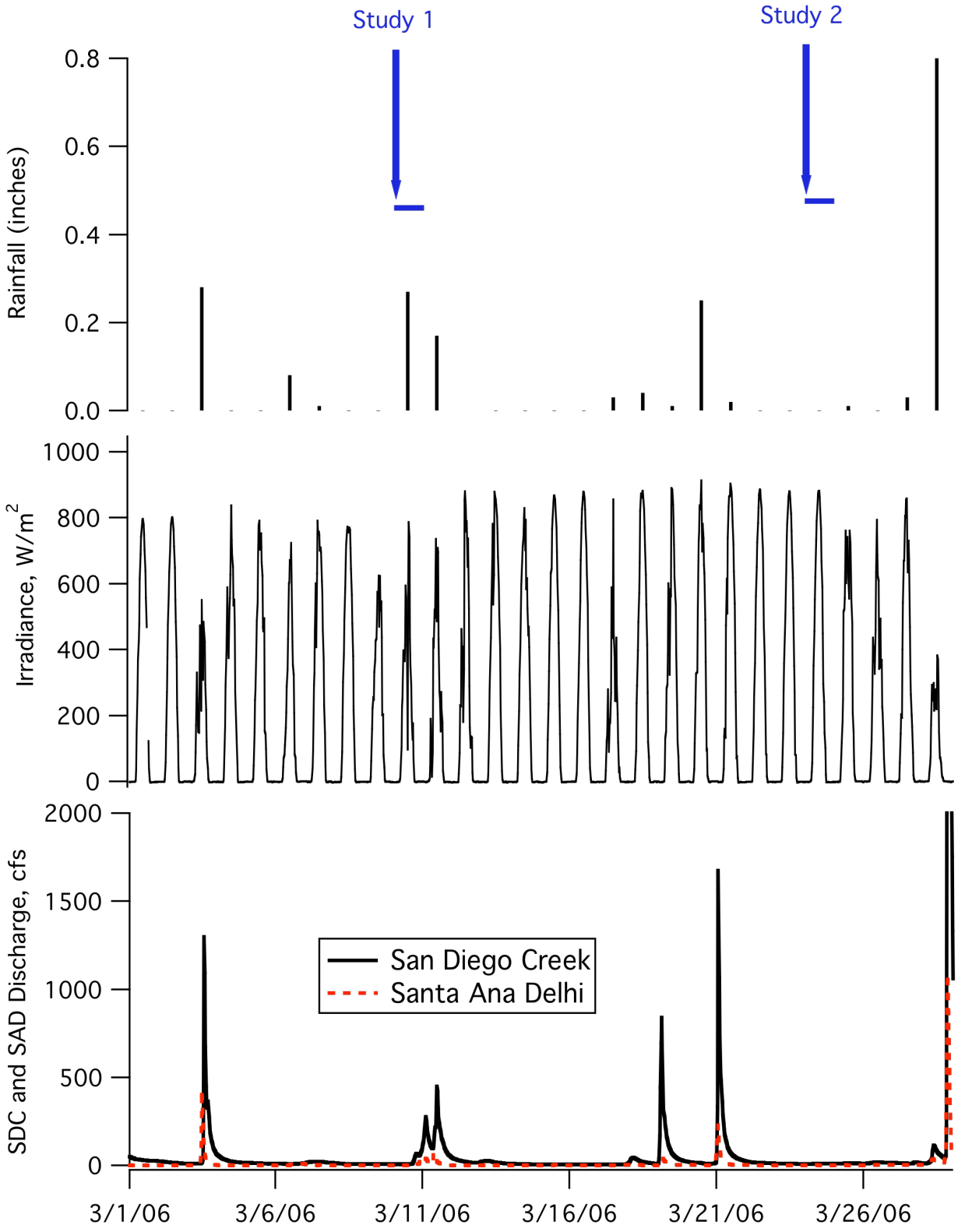

Figure 6-2. Timing of the two wet weather DITS studies relative to records of rainfall, solar irradiance, and volumetric discharge from the San Diego Creek and Santa Ana Delhi channel. Study 1: March 10, 2006; Study 2: March 24, 2006.

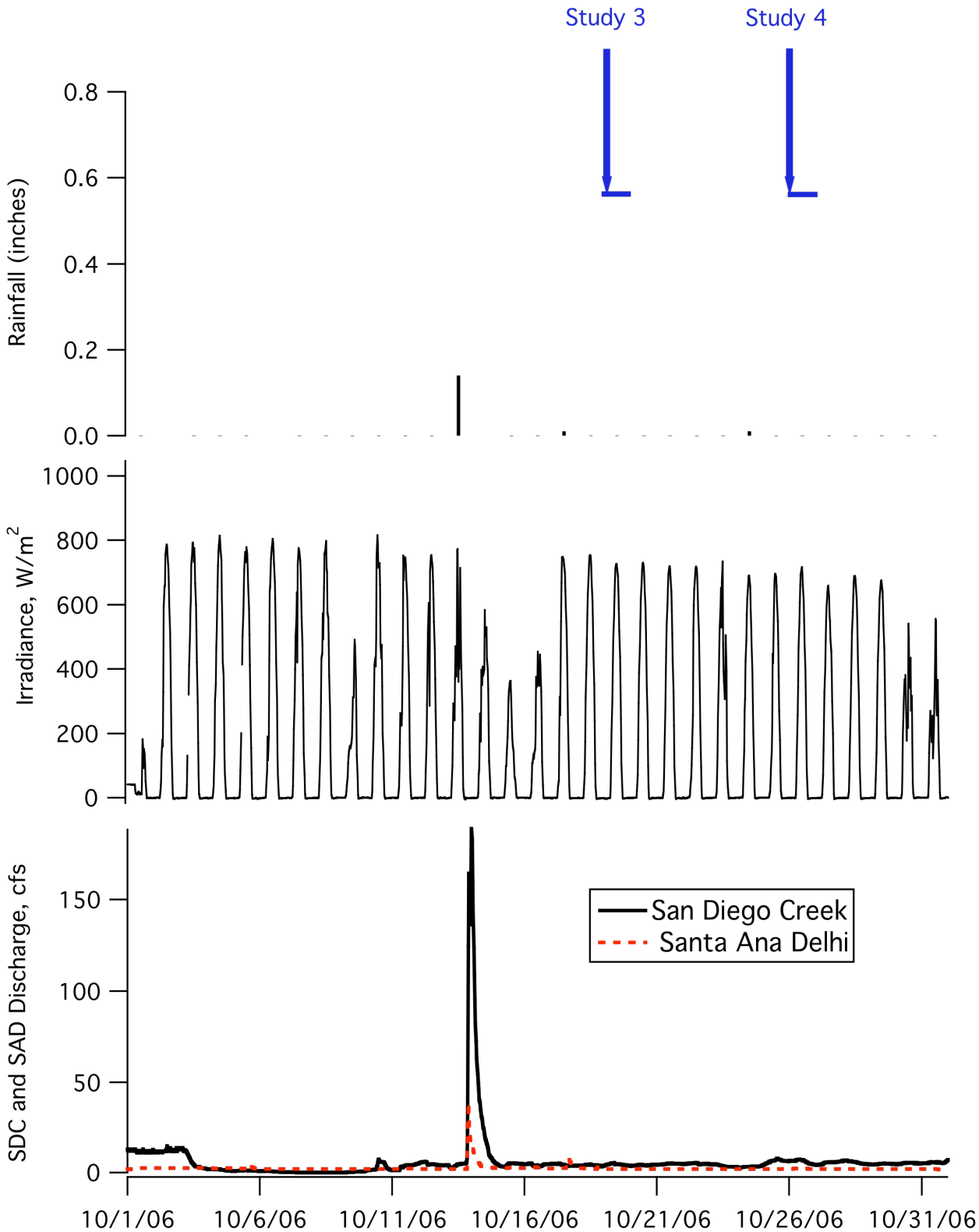

Figure 6-3. Timing of the two dry weather DITS studies relative to measurements of rainfall, solar irradiance, and volumetric discharge from the San Diego Creek and Santa Ana Delhi channel. Study 3: October 19, 2006; Study 4: October 26, 2006.

During each of the four DITS studies, water and sediment samples were collected every three hours at two DITS transect sites in Upper Bay, referred to as DITS 1 and DITS 2 (Fig. 6-1). DITS 1 and 2 are co-located with the Bay-To-Ocean (BTO) transect sites BTO 4 and BTO 5 (see Fig. 2-1, Chapter 2). Surface water samples were collected every three hours from the two creek sites (BTO 1 and BTO 2), and from five locations at the two DITS transects: (1) the channel center (thalweg); (2) halfway from the thalweg to the right bank (where "right" and "left" assume the observer is facing inland); (3) halfway from the thalweg to the left bank; (4) the right bank; and (5) the left bank. A bottom water sample was collected from the channel thalweg using a Van Doren Bottle.

Coincident with the water sampling, sediment samples were collected from above and below the waterline at the left and right banks, and from the channel thalweg. Sediment samples at the banks were collected by hand over the side of a kayak by pushing a sterile conical tube into the surface of the sediment. Sediment samples were collected at the thalweg from the research boat using the Petite Ponar sediment sampler; sediment from the Ponar were sub-sampled on the boat. After collection, all water and sediment samples were immediately placed in an iced cooler, and transported to UCI for analysis with a holding time of less than 6 hours.

At the lab, water samples were analyzed for FIB (total coliform, *Escherichia coli*, and enterococci bacteria), particle size distributions using the LISST-100, and salinity (salinity data included in Appendix 6A). Surface water samples from the channel thalweg were also analyzed for a suite of nutrients, included ammonia, total Kjeldahl nitrogen (TKN), nitrate, total phosphorous, and total organic carbon (TOC). Nutrient analyses were only carried out on thalweg surface water samples collected during the March 10, March 24, and October 26 sampling dates. Sediment samples were analyzed for FIB, grain size distribution, and organic carbon content. CTD casts were carried out at the channel thalwag, as well as measurements wind speed and direction, air temperature, relative humidity and solar irradiance. Digital photos were taken of the mudflats during sampling (only during daylight hours). All methods employed are described in Chapter 2.

In the BTO study described in Chapter 2, the right and left bank water and sediment samples were collected from relatively deep (>1 m) water, out of concern that sampling at shallower depths might result in the grounding of the boat. During the DITS studies, on the other hand, left and right bank sampling was carried out using kayaks. Thus left and right bank water and sediment samples were collected in <10 cm of water, right where the waterline meets the shoreline. This distinction—between the very shallow bank sampling conducted during the DITS study, and the relatively deep bank sampling conducted during the BTO study—should be kept in mind when comparing the results reported here, and the longer-term lower frequency sampling BTO studies described in Chapter 2. The sampling protocol described above was repeated a total of four times, twice during wet weather (March 10, 2006 and March 24, 2006) and twice during dry weather (October 19, 2006 and October 26, 2006). Wet weather studies were conducted approximately concurrent with a sequence of storm events (Studies 1 and 2 in Fig. 6-2).

Table 6-1. Weather conditions during the first wet weather study on March 10, 2006.

Date and Time	***DITS Transect***	***Wind Speed (m/s)***	***Wind Direction***	***Air Temp. (C)***	***Humidity (%)***
3/10/06 7:20	1 (BTO 4)	5.3	60	11.1	62
3/10/06 9:30	1 (BTO 4)	4.8	8	11.5	61
3/10/06 13:12	1 (BTO 4)	5	196	11.0	69
3/10/06 16:00	1 (BTO 4)	3.3	163	11.4	64
3/10/06 19:45	1 (BTO 4)	1.4	101	9.6	76
3/10/06 8:20	2 (BTO 5)	5.3	289	11.6	57
3/10/06 10:30	2 (BTO 5)	5.2	283	11.6	61
3/10/06 13:55	2 (BTO 5)	5.2	160	11.3	67
3/10/06 16:45	2 (BTO 5)	4.1	180	11.4	45
3/10/06 19:00	2 (BTO 5)	0.4	143	9.8	79

Table 6-2. Weather conditions during the second wet weather study on March 24, 2006.

Date and Time	***DITS Transect***	***Wind Speed (m/s)***	***Wind Direction***	***Air Temp. (C)***	***Humidity (%)***
3/24/06 7:00	1 (BTO 4)	1.1	312	10.2	89
3/24/06 9:30	1 (BTO 4)	2.7	160	15.2	70
3/24/06 12:30	1 (BTO 4)	2.4	220	19.8	50
3/24/06 15:15	1 (BTO 4)	3.2	240	16.6	69
3/24/06 19:30	1 (BTO 4)	1.2	92	13.7	79
3/24/06 21:45	1 (BTO 4)	0.4	92	12	92
3/24/06 23:45	1 (BTO 4)	1.5	299	12.3	89
3/24/06 7:50	2 (BTO 5)	0.9	123	13.6	73
3/24/06 10:06	2 (BTO 5)	2.8	113	15.4	70
3/24/06 13:15	2 (BTO 5)	3.2	200	18.3	58
3/24/06 16:00	2 (BTO 5)	2.7	210	16.6	56
3/24/06 20:10	2 (BTO 5)	1	75	13	83
3/24/06 22:20	2 (BTO 5)	1	326.2	11.1	93
3/25/06 00:00	2 (BTO 5)	2	329	11.6	90

Dry weather studies were conducted in the fall after the peak macroalgal bloom (although macroalgae were still present in the Upper Bay at the time the dry weather studies were conducted) (Studies 3 and 4 in Fig. 6-3).

6.3. Results

6.3.1. Wet Weather DITS Studies (March 10 and March 24, 2006)

Study Timing. A rainfall event (0.3 inches of rain) occurred during Study 1 (around noon on March 10) (Fig. 6-2). Flow from the San Diego Creek and Santa Ana Delhi channel increased, respectively, from base flows of 9 and 2 cubic feet per second (cfs) before the rain event, to peak flows of 250 and 38 cfs in the evening on March 10. Rain did not fall during Study 2 (March 24), although a storm passed through the area several days earlier on March 21, generating 0.25 inches of rainfall, and peak flows from San Diego Creek and Santa Ana Delhi Channel of 1,600 and 230 cfs, respectively. At least eight separate rain events were recorded in the three weeks leading up to, and including, the wet weather sampling on March 10 and 24, 2006 (Fig. 6-2).

Meteorology. Tables 6-1 and 6-2 summarize meteorological measurements during the two wet-weather studies. During the March 10 study, wind speed varied between 1 and 5 m/s, with higher winds coming primarily out of the south or west. During the March 24 study, wind speed varied between 1 and 3 m/s, with higher winds coming primarily out of the southwest. Air temperature was fairly constant on March 10 (*ca*., 11°C), and varied over the day (from 10°C in the early morning to 20°C at noon) on March 24. During both the March 10 and 24 studies, humidity varied diurnally, with lower humidity recorded during daylight, and higher humidity recorded at night.

Water Measurements. Time series and probability distribution plots of the raw data collected at DITS 1 and 2 are included in Appendix 6A. FIB statistics for the two wet-weather DITS studies are presented in Table 6-3; because of the small number of samples collected at each site, data from the two wet weather studies were combined to generate the statistics presented. At DITS 1, single-sample exceedences for EC and ENT are highest in water samples collected from the right and left banks, and lower in water samples collected at the other transect sites (cross-hatched bars in Fig. 6-4). At DITS 2, single-sample exceedences for EC and ENT occurred only in samples collected from the left bank, where up to 25% of all samples exceeded the single sample standard for ENT (solid bars in Fig. 6-4).

Sediment Measurements Time series and distribution plots of the raw sediment data collected at DITS 1 and 2 are included in Appendix 6A. Table 6-4 and Fig. 6-5 present statistical data on sediment measurements at DITS 1 and 2 during wet weather sampling. Because a small number of sediment samples were collected during the individual wet weather studies, data from the two wet weather studies were combined to generate the data presented in Table 6-4 and Fig. 6-5. The concentration of EC and ENT is the same, within error, at all DITS 1 sites (ca., $10^{2.5}$ MPN/10 g for both ENT and EC). At DITS 2, on the other hand, EC and ENT concentrations are highest in sediment collected from just above the waterline on the left bank (solid bars, Fig. 6-5); sediment samples collected above the waterline on the right bank also had higher EC concentrations, compared to sediment samples collected from the thalweg.

Table 6-3. FIB measurement statistics for water samples collected from tributaries (BTO 1,2), DITS 1, and DITS 2 sites during the wet weather studies on March 10 and March 24, 2006.

Sampling Site	***Enterococcus spp.***			***E. coli***		
	N	Log-mean[a]	% above standard[a]	N	Log-mean[a]	% above standard[a]
BTO 1	12	1.0 +/- 0.6	0	12	1.8 +/- 0.6	8
BTO 2	12	1.3 +/- 0.5	17	12	1.9 +/- 0.6	17
DITS 1: Left	12	1.2 +/- 0.6	17	12	1.8 +/- 0.7	17
DITS 1: Middle/Left	11	0.9 +/- 0.4	0	11	1.4 +/- 0.4	0
DITS 1: Middle	12	0.8 +/- 0.5	0	12	1.4 +/- 0.4	0
DITS 1: Middle/Bottom	12	1.5 +/- 0.5	8	12	1.4 +/- 0.5	0
DITS 1: Middle/Right	11	1.2 +/- 0.6	9	11	1.3 +/- 0.5	0
DITS 1: Right	12	1.2 +/- 0.7	17	12	1.8 +/- 0.5	8
DITS 2: Left	12	1.3 +/- 1.0	25	12	2.3 +/- 0.7	25
DITS 2: Middle/Left	11	0.9 +/- 0.50	0	11	1.7 +/- 0.6	0
DITS 2: Middle	12	0.5 +/- 0.52	0	12	1.5 +/- 0.5	0
DITS 2: Middle/Bottom	12	0.8 +/- 0.5	0	12	1.3 +/- 0.7	0
DITS 2: Middle/Right	10	0.8 +/- 0.4	0	10	1.4 +/- 0.6	0
DITS 2: Right	12	0.8 +/- 0.7	0	10	1.6 +/- 0.7	0

[a]Single-sample standards for marine bathing waters in California of ENT=104 MPN/100 mL and Fecal Coliform (FC)=400 MPN/100 mL, where it is assumed that FC and EC are equivalent.

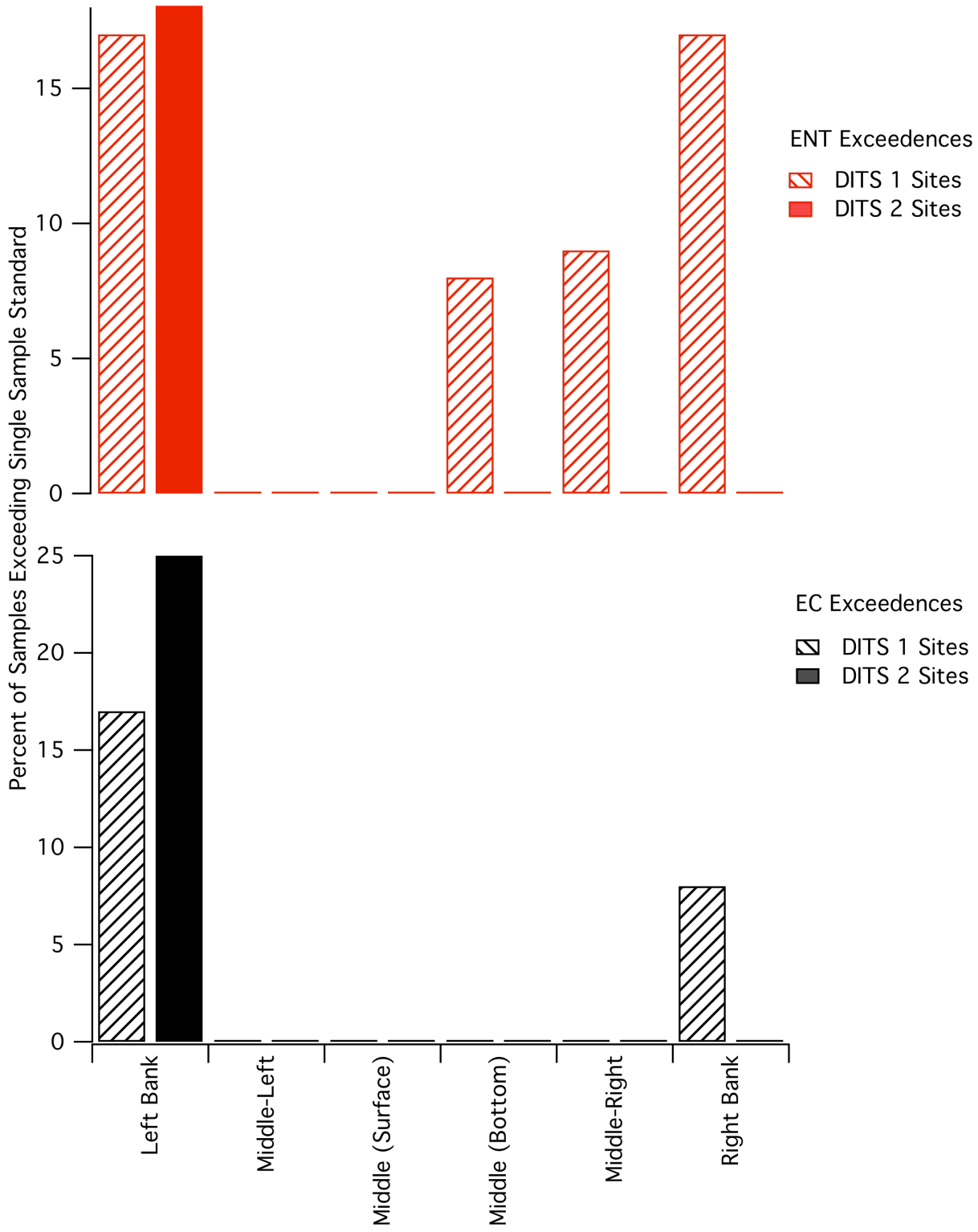

Figure 6-4. Percentage of samples collected from the six locations in the tidal cross-section at DITS 1 or 2 that exceeded the single-sample standards for EC (400 MPN/100 mL) or ENT (104 MPN/100 mL during the two wet-weather studies (March 10 and March 24, 2006).

Table 6-4. FIB measurement statistics for sediment samples collected from the DITS 1, and DITS 2 sites during the wet weather studies on March 10 and March 24, 2006.

Sampling Site	***Enterococcus spp. (MPN/10g)***		***E. coli (MPN/10g)***	
	N	Log-mean	N	Log-mean
DITS 1: Left (dry)	12	2.9 +/- 0.9	12	2.5 +/- 0.8
DITS 1: Left (wet)	12	2.4 +/- 0.8	11	2.3 +/- 0.6
DITS 1: Middle	12	2.2 +/- 1.0	12	2.2 +/- 0.9
DITS 1: Right (wet)	12	2.7 +/- 1.0	12	2.6 +/- 0.8
DITS 1: Right (dry)	12	2.8 +/- 0.8	12	2.4 +/- 0.8
DITS 2: Left (dry)	11	3.1 +/- 1.1	11	2.6 +/- 0.8
DITS 2: Left (wet)	12	1.9 +/- 0.7	12	2.0 +/- 07
DITS 2: Middle	12	2.0 +/- 1.3	12	0.9 +/- 0.7
DITS 2: Right (wet)	12	1.4 +/- 0.6	12	1.4 +/- 0.6
DITS 2: Right (dry)	11	1.8 +/- 0.90	11	1.9 +/- 0.6

The fact that FIB concentrations are highest in sediments collected from above the water line on the left bank of the DITS 2 transect is noteworthy. First, water samples collected from the left bank of the DITS 2 transect also had high concentrations of both EC and ENT (solid bars, Fig. 6-4). Indeed, at the DITS 2 transect, only water samples collected from the left bank exceeded California marine bathing water standards for EC and ENT (Fig. 6-4). There appears to be a significant source of FIB along the left bank of the DITS 2 transect that contaminates both the sediments and water column there. Second, the fact that sediments above the water line have the highest concentrations of FIB suggests that these bacteria originate either within the sediments themselves (e.g., by re-growth) or on top of the sediments (e.g., by deposition of bird droppings).

Photographs of the Mudflats. Large numbers of birds congregate on the mudflats to feed and loaf, as is evident in the photographs taken at DITS 1 and 2 on March 10 (Fig. 6-6). In the close-up photograph of the mudflat at DITS 1 (top panel, Fig. 6-6) the birds appear

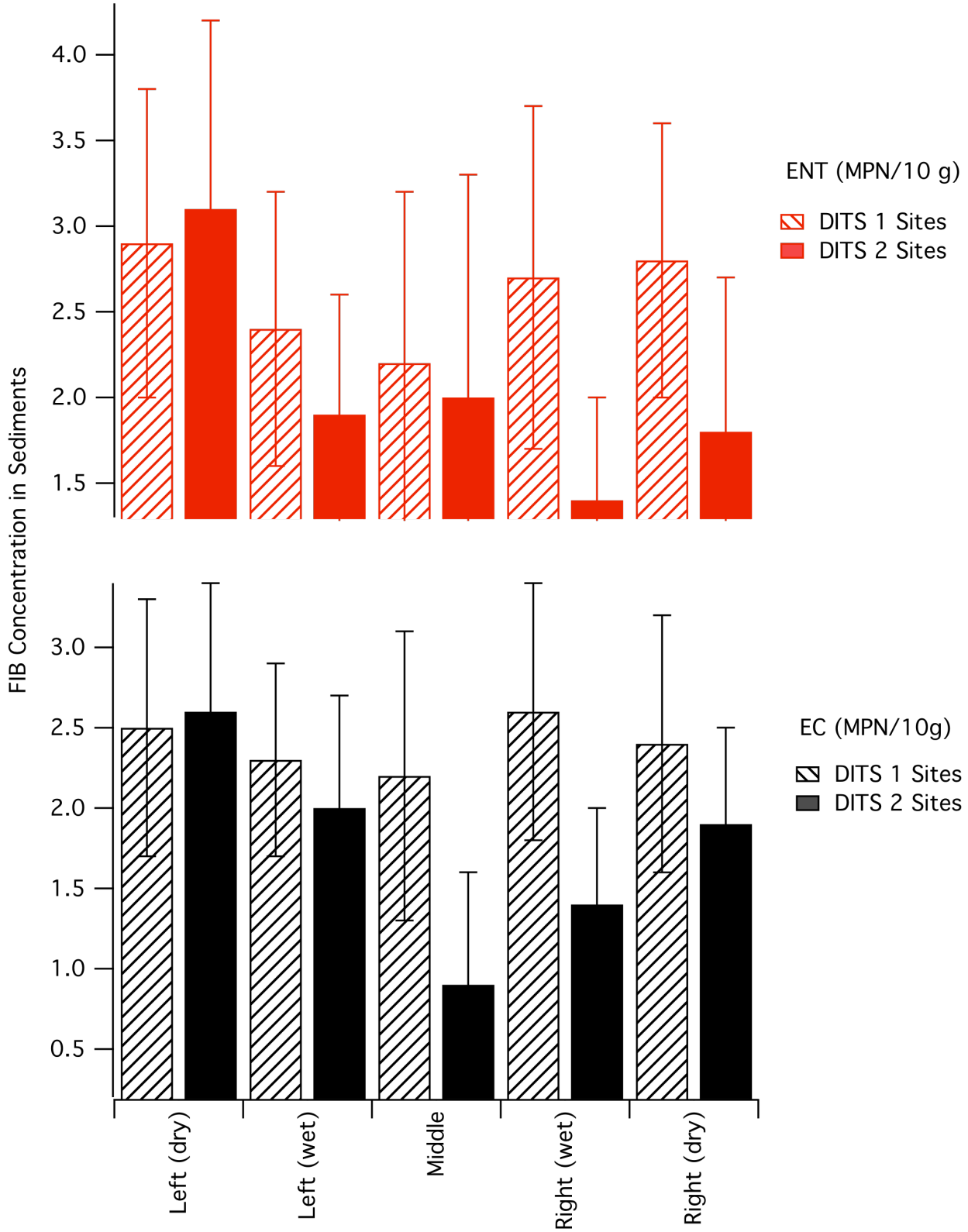

Figure 6-5. Log-mean concentration of EC or ENT measured in sediments collected from the five locations in the tidal cross-section at DITS 1 or 2 during the two wet-weather studies (March 10 and March 24, 2006).

to congregate close to the water's edge, perhaps providing a fresh source of FIB at the waterline. As will be documented later in this chapter when the bird census data are presented, the number of birds present on the mudflats is variable in both space and time, which might account for the highly variable nature of FIB concentrations in sediment collected from the banks of the tidal channel at DITS 1 and 2, and the fact that tidal cycling of FIB concentrations in the sediment is not consistent across sampling sites and study dates (see time series plots in Appendix 6A).

Interpretation of Results from the Wet Weather DITS Study. Collectively, data acquired during the wet weather DITS studies appear consistent with a tidal washing process in which FIB deposited on, or residing in, the mudflats (e.g., from bird droppings, regrowth on macroalgae and/or sediment) are transferred into the water column as the water's edge moves up the mudflat during rising tides. Evidence in support of the tidal washing hypothesis includes:

1. EC and ENT exceedence rates are higher in water samples collected from the right and/or left banks, and lower in water samples collected from the center of the tidal channel (Fig. 6-4), consistent with the idea that at least some FIB are entering the Bay from the mudflats.
2. With the exception of EC measured at DITS 1, EC and ENT concentrations are higher in intertidal sediment samples collected above the waterline from the right and/or left banks, and lower in sub-tidal sediment collected at the thalweg, consistent with the idea that the mudflats are a source of FIB.
3. FIB concentrations measured in water samples collected at the right and/or left banks are generally higher than FIB concentrations measured in samples collected from BTO 1 and 2 (Table 6-3), consistent with the idea that FIB along the shoreline in Upper Bay are not coming exclusively from the creeks.

6.3.2. Dry Weather Studies (October 19 and October 26, 2006)

Study Timing. Fig. 6-3 displays the timing of the two dry weather DITS studies relative to measurements of rainfall (top panel), solar irradiance (second panel), and flow from the San Diego Creek and Santa Ana Delhi channel (third panel). A rain event in the afternoon on October 15—approximately 5 days prior to the start of Study 3—triggered storm water runoff pulses from the San Diego Creek (peak flow of 189 cfs) and the Santa Ana Delhi Channel (peak flow of 39 cfs). Within 24 hours of the rain event, flow in the two creeks returned to pre-storm levels (*ca*., 5 and 2 cfs, respectively). Following the rain event, but prior to the start of Study 3, there were several days when the peak solar irradiance appears to be lower than normal, presumably due to cloud cover. However, during the two study periods the peak solar irradiance (*ca*., 800 W/m^2) is typical of clear summer days, and no rainfall or elevated flow from the San Diego Creek and Santa Ana channel were recorded. From these data it is not unreasonable to refer to these two studies in October as "dry weather studies".

Figure 6-6. Photographs of birds on the mudflats at DITS 1 and DITS 2 during the wet weather study on 3/10/06.

Table 6-5. Meteorology during the first dry weather study on October 19, 2006.

Date and Time	***DITS Transect***	***Wind Speed (m/s)***	***Wind Direction***	***Air Temp. (C)***	***Humidity (%)***
10/19/06 6:44	1 (BTO 4)	0.9	46	10.7	73
10/19/06 9:30	1 (BTO 4)	0.5	7	21.2	33
10/19/06 12:40	1 (BTO 4)	1.7	196	23	29
10/19/06 15:20	1 (BTO 4)	4.1	299	25.2	21
10/19/06 18:30	1 (BTO 4)	1	16	17	50
10/19/06 20:10	1 (BTO 4)	0.6	281	12	76
10/19/06 7:30	2 (BTO 5)	1.8	67	11.1	73
10/19/06 10:20	2 (BTO 5)	0.3	283	24.9	28
10/19/06 13:15	2 (BTO 5)	3	58	23.6	22
10/19/06 16:10	2 (BTO 5)	3.4	194	23.9	31
10/19/06 18:59	2 (BTO 5)	0.5	221	16.2	67
10/19/06 21:20	2 (BTO 5)	0.3	180	12.9	78

Table 6-6. Meteorology during the second dry weather study on October 26, 2006.

Date and Time	***DITS Transect***	***Wind Speed (m/s)***	***Wind Direction***	***Air Temp. (C)***	***Humidity (%)***
10/26/06 7:20	1 (BTO 4)	0.2	57	10.9	99
10/26/06 9:45	1 (BTO 4)	1.4	214	19.1	74
10/26/06 12:30	1 (BTO 4)	3.5	282	25.2	21
10/26/06 15:45	1 (BTO 4)	2.7	102	25.2	20
10/26/06 18:20	1 (BTO 4)	0.3	261	20	58
10/26/06 20:50	1 (BTO 4)	0.6	151	16.5	66
10/26/06 7:40	2 (BTO 5)	0.3	245	10.7	99
10/26/06 10:25	2 (BTO 5)	2.4	4	18.7	62
10/26/06 13:15	2 (BTO 5)	3.1	279	25.2	28
10/26/06 16:20	2 (BTO 5)	2	154	25.2	15
10/26/06 19:00	2 (BTO 5)	0.5	10	18.3	58
10/26/06 21:20	2 (BTO 5)	0.2	134	15.5	72

<u>*Meteorology*</u>. Tables 6-5 and 6-6 summarize meteorological measurements carried out during the two dry-weather studies. During the October 19 study, wind speed varied between 0.3 and 4.1 m/s, with higher winds coming primarily out of the south or west. Winds were weaker during the October 26 study (0.2 to 3.5 m/s) and, again, the stronger winds were predominantly out of the south or west. During both sampling dates, air

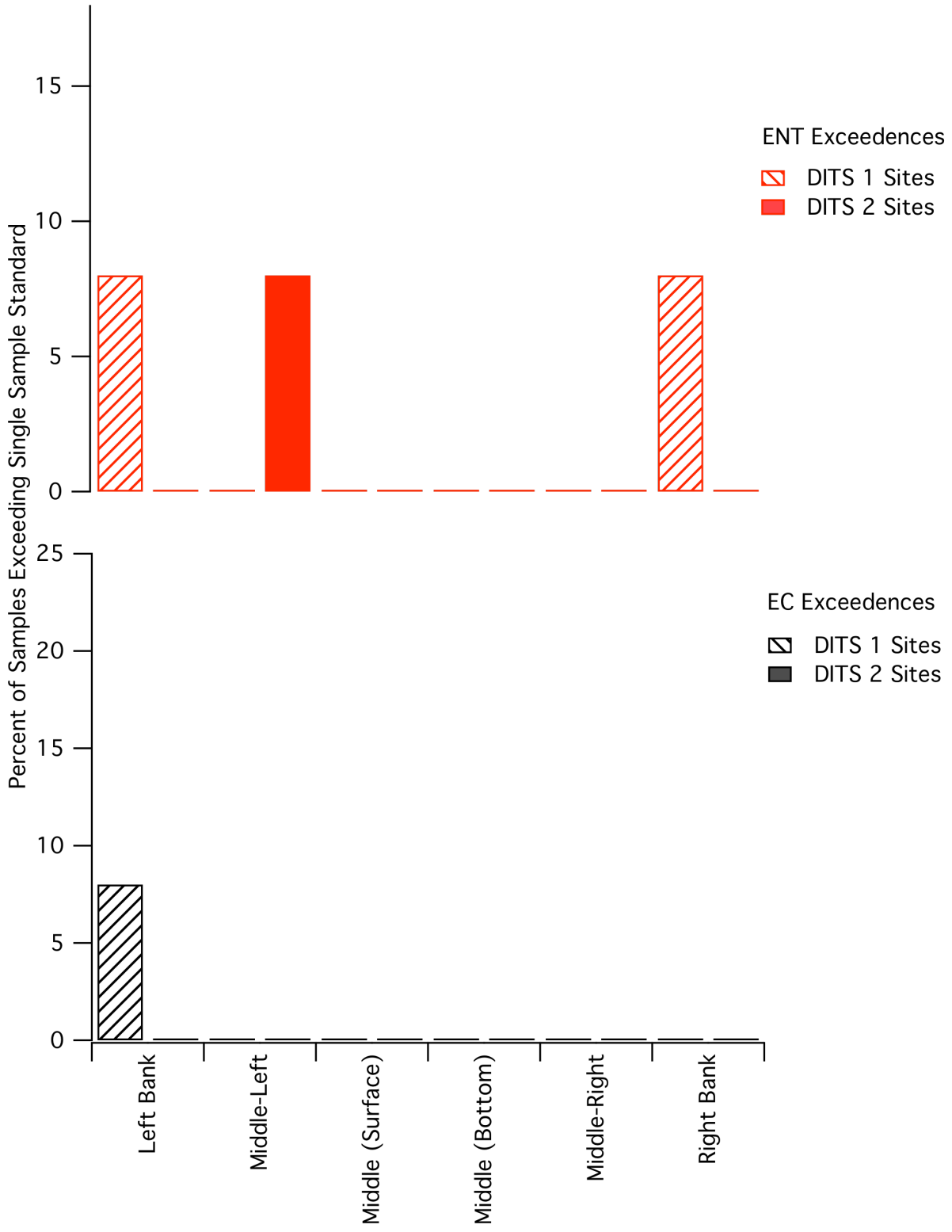

Figure 6-7. Percentage of samples collected from the six locations in the tidal cross-section at DITS 1 or 2 that exceeded the single-sample standards for EC (400 MPN/100 mL) or ENT (104 MPN/100 mL during the two dry-weather studies (October 19 and October 26, 2006).

Table 6-7. FIB measurement statistics for water samples collected from tributaries (BTO 1,2), DITS 1, and DITS 2 sites during the dry weather studies on October 19 and October 26, 2006.

Sampling Site	***Enterococcus spp. (MPN/100 mL)***			***E. coli (MPN/100 mL)***		
	N	Log-mean[a]	% above standard[a]	N	Log-mean[a]	% above standard[a]
BTO 1	12	1.4 +/- 0.5	0	12	2.7 +/- 0.4	58
BTO 2	12	2.0 +/- 0.6	42	12	2.7 +/- 0.4	67
DITS 1: Left	12	0.9 +/- 0.9	8	12	2.9 +/- 0.6	8
DITS 1: Middle/Left	11	0.4 +/- 0.6	0	12	1.5 +/- 0.7	0
DITS 1: Middle	12	0.2 +/- 0.4	0	12	1.5 +/- 0.6	0
DITS 1: Middle/Bottom	12	1.5 +/- 0.5	0	12	0.92 +/- 0.7	0
DITS 1: Middle/Right	12	0.7 +/- 0.7	0	12	1.6 +/- 0.6	0
DITS 1: Right	12	1.2 +/- 0.8	8	12	1.8 +/- 0.7	0
DITS 2: Left	12	1.0 +/- 0.8	0	12	1.3 +/- 0.8	0
DITS 2: Middle/Left	11	0.7 +/- 0.8	8	12	1.6 +/- 0.5	0
DITS 2: Middle	12	0.3 +/- 0.7	0	12	0.9 +/- 0.9	0
DITS 2: Middle/Bottom	12	0.6 +/- 0.6	0	12	0.7 +/- 0.5	0
DITS 2: Middle/Right	12	0.8 +/- 0.6	0	12	1.4 +/- 0.8	0
DITS 2: Right	12	0.8 +/- 0.7	0	12	1.3 +/- 0.8	0

[a]Single-sample standards for marine bathing waters in California of ENT=104 MPN/100 mL and Fecal Coliform (FC)=400 MPN/100 mL, where it is assumed that FC and EC are equivalent.

temperature varied diurnally from about 11°C in the morning and evening, to 25°C at noon. Humidity followed the opposite diurnal pattern, with higher humidity during the morning and evening, and lower humidity at noon.

Water Measurements. Time series and probability distribution plots of the raw data collected at DITS 1 and 2 are included in Appendix 6A. FIB statistics for the two dry-weather DITS studies are presented in Table 6-7 and Fig. 6-7. Because of the small number of samples collected at each site, data from the two dry weather studies were combined to generate the statistics presented in the table and figure. Compared to the wet weather measurements, EC and ENT exceedence rates were generally lower at DITS 1 and 2 during the two dry weather studies (compare single-sample exceedences rates plotted in Figs. 6-4 and 6-7). At DITS 1, only water samples collected from the left and/or right banks exceeded single-sample standards for EC and ENT (hatched bars, Fig. 6-7). At DITS 2 only middle-left samples exceeded the single sample standard for ENT, and no samples exceeded the single-sample standard for EC (solid bars, Fig. 6-7). It is also interesting to note that, in contrast with the wet weather studies described above, during the two dry weather studies FIB concentrations were generally lower in samples collected from Upper Bay at DITS 1 and 2 and higher in samples collected from BTO 1 (EC only) and BTO 2 (both EC and ENT) (Table 6-7).

Sediment Measurements. Time series and distribution plots of the raw sediment data collected at DITS 1 and 2 are included in Appendix 6A. Table 6-8 and Fig. 6-8 present statistical data on sediment measurements at DITS 1 and 2 during dry weather sampling. Because a small number of sediment samples were collected during the individual dry weather studies, data from the two dry weather studies were combined to generate the results presented in Table 6-8 and Fig. 6-8. FIB concentrations measured in the sediments during the dry weather DITS studies are comparable to, or higher than, concentrations measured in the sediments during the wet-weather DITS studies (e.g., compare Figs 6-5 and 6-8). This result is significant given that, compared to the wet weather studies, water column FIB concentrations were significantly lower during the dry weather DITS studies (see discussion above). Apparently, the concentration of FIB in sediments is not, by itself, a good predictor of the concentration of FIB in the water column. This last observation is consistent with lack of significant correlation between FIB in subtidal sediments and FIB concentrations in the overlying water column reported in Chapter 2.

At the DITS 1 transect, ENT concentrations are highest in sediment collected from above the waterline at the left and right banks, lower in sediment below the waterline at the left and right banks, and lowest in sediment collected from the channel thalweg (hatched bars, top panel, Fig. 6-8). A similar pattern is evident for EC, although the concentration of this bacterial group is similar, within error, in sediment collected above and below the waterline at the right and left banks (hatched bars, bottom panel, Fig. 6-8). At the DITS 2 transect, EC concentrations are also higher in sediment collected above the waterline and lower in sediment collected from below the waterline and at the thalweg (solid bars, bottom panel, Fig. 6-8). Within error, ENT concentrations are approximately the same in sediment samples collected from the cross-section at DITS 2 (solid bars, top panel, Fig. 6-8).

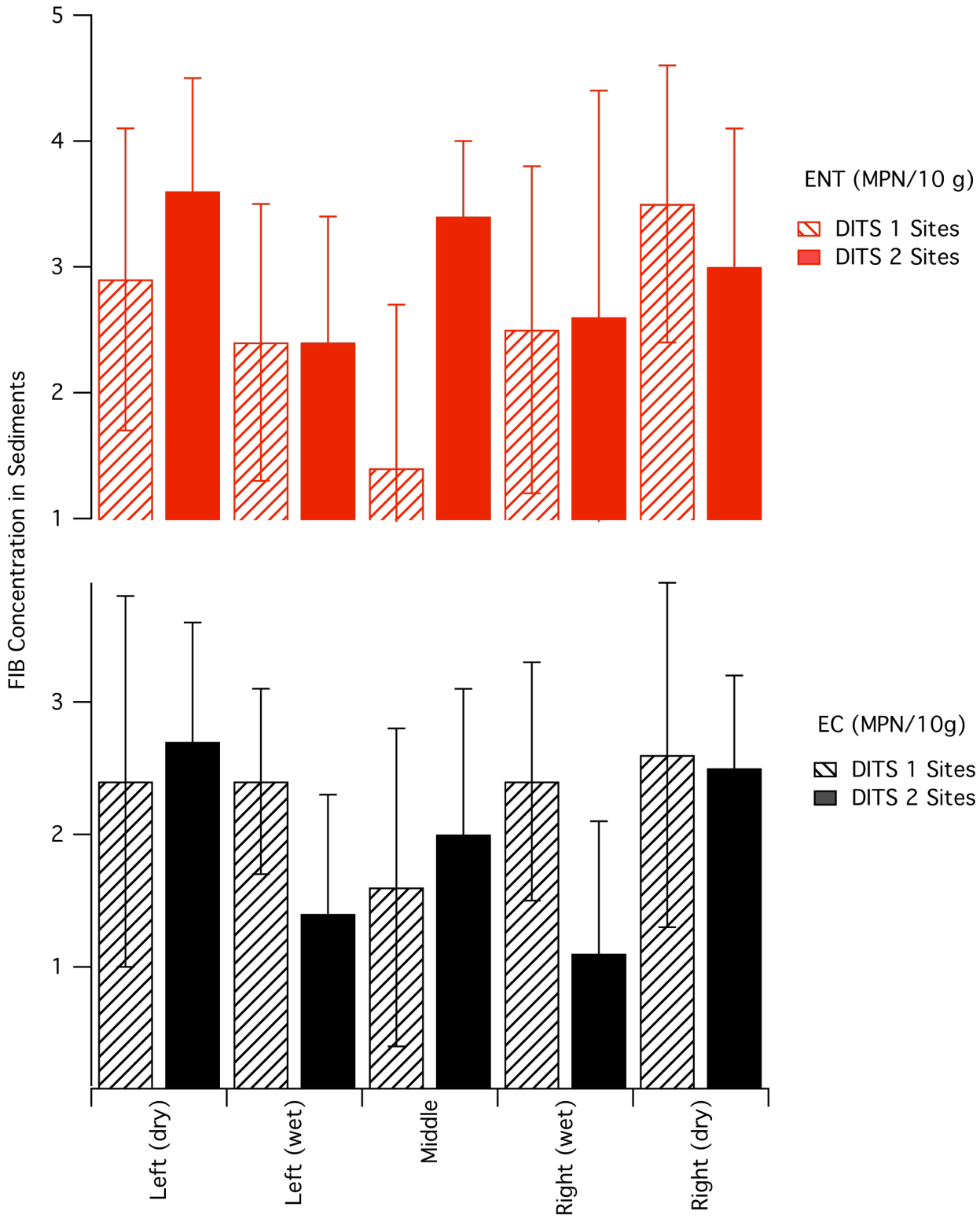

Figure 6-8. Log-mean concentration of EC or ENT measured in sediments collected from the five locations in the tidal cross-section at DITS 1 or 2 during the two dry-weather studies (October 19 and October 26, 2006).

Table 6-8. FIB measurement statistics for sediment samples collected from the DITS 1 and 2 sites during the dry weather studies (Oct. 19 and 26, 2006).

Sampling Site	***Enterococcus spp. (MPN/10g)***		***E. coli (MPN/10g)***	
	N	Log-mean	N	Log-mean
DITS 1: Left (dry)	12	2.9 +/- 1.2	11	2.4 +/- 1.4
DITS 1: Left (wet)	12	2.4 +/- 1.1	12	2.4 +/- 0.7
DITS 1: Middle	12	1.4 +/- 1.3	12	1.6 +/- 1.2
DITS 1: Right (wet)	12	2.5 +/- 1.3	12	2.4 +/- 0.9
DITS 1: Right (dry)	12	3.5 +/- 1.1	12	2.6 +/- 1.3
DITS 2: Left (dry)	5	3.6 +/- 0.9	5	2.7 +/- 0.9
DITS 2: Left (wet)	12	2.4 +/- 1.0	12	1.4 +/- 0.9
DITS 2: Middle	12	3.4 +/- 0.6	12	2.0 +/- 1.1
DITS 2: Right (wet)	12	2.6 +/- 1.8	12	1.1 +/- 1.0
DITS 2: Right (dry)	6	3.0 +/- 1.1	6	2.5 +/- 0.7

Interpretation of the Dry Weather DITS Study. As with the wet weather studies described above, FIB data collected during the dry weather studies are consistent with the hypothesis that the mudflats are a source of FIB, at least in the very shallow waters along the shoreline:

1. For cases where FIB concentrations were elevated (i.e., DITS 1), FIB concentrations in the water were higher at the banks of the tidal channel, and lower in the center of the tidal channel.
2. With a couple of exceptions, FIB concentrations are higher in sediment collected from the left and right banks, and lower in sediment collected from the thalweg.
3. Furthermore, in sediment samples collected from the left and right banks, FIB concentrations are frequently higher above the waterline, and lower below the waterline, consistent with the idea that FIB accumulate in or on the exposed mudflats.

6.3.3. Sediment Average Grain Size

Temporal and spatial trends in the average grain size of sediment collected from the DITS 1 and 2 transect sites are displayed in Fig. 6-9. All sediment samples had an average grain size within the silt size range (4 to 63 microns). At both transect sites (DITS 1 and 2) and during both dry and wet weather, the average grain size is finer in sediment collected from the channel thalweg (middle station) and coarser in sediment collected from the left and right banks[8]. Sediments are coarser across all transect sites during the dry weather study, and finer across all transect sites during the wet weather study. During the dry weather study, sediments collected from the DITS 1 transect are finer than sediments collected from the DITS 2 transect.

Spatial and temporal trends in the average grain size of sediments in Upper Bay are likely a dynamic balance between the (allochthonous) supply of suspended particles from the San Diego Creek and Santa Ana Delhi channel, within-Bay (autochthonous) tidal resuspension of particles, and physicochemical processing and transport of suspended particles in the Bay. At our field site, this dynamic balance results in two dominant trends: (1) a fining of sediments toward the center of the tidal channel, and coarsening of sediments toward the edges of the channel; and (2) a coarsening of sediments during dry weather, and a fining of sediments during wet weather.

6.3.4. Sediment Organic Carbon and Moisture Content

Tables 6-9 and 6-10 present means (and standard deviations) of the percent organic carbon and percent water content measured in sediment collected from the DITS 1 and 2 transect sites. These data are plotted in Figs. 6-10 and 6-11.

Organic Carbon Content in Sediment Samples. Mean percent organic carbon measured in sediment samples ranged from 3.5 to 16.2 % during the wet weather studies (Table 6-9) and from 4.8 to 11.4% during the dry weather studies (Table 6-10). Unlike the average grain size data presented above, the mean percent organic carbon data do not exhibit consistent cross-sectional trends. In some cases, the percent organic carbon is higher in sediments collected from the thalweg (e.g., DITS 1 during dry weather, Fig. 6-10), and in other cases the opposite pattern prevails (e.g., DITS 2 during both dry and wet weather, Fig. 6-10). Sediment organic content was lowest during the dry weather study at DITS 2, intermediate during the wet weather study at DITS 1, and highest during the wet weather study at DITS 2 and the dry weather study at DITS 1 (Fig. 6-10).

[8] The bank-ward coarsening of grain size does not appear to be an artifact of the different methods used to collect sediment from the channel thalwag (Petite Ponar) and from the left and right banks (hand collection of surficial sediments with a conical tube). Sediment collected from the middle-right and middle-left stations (which were both collected with the Ponar) are, in seven out of eight cases, more coarse than sediments collected from the channel thalwag (which was also collected with the Ponar)

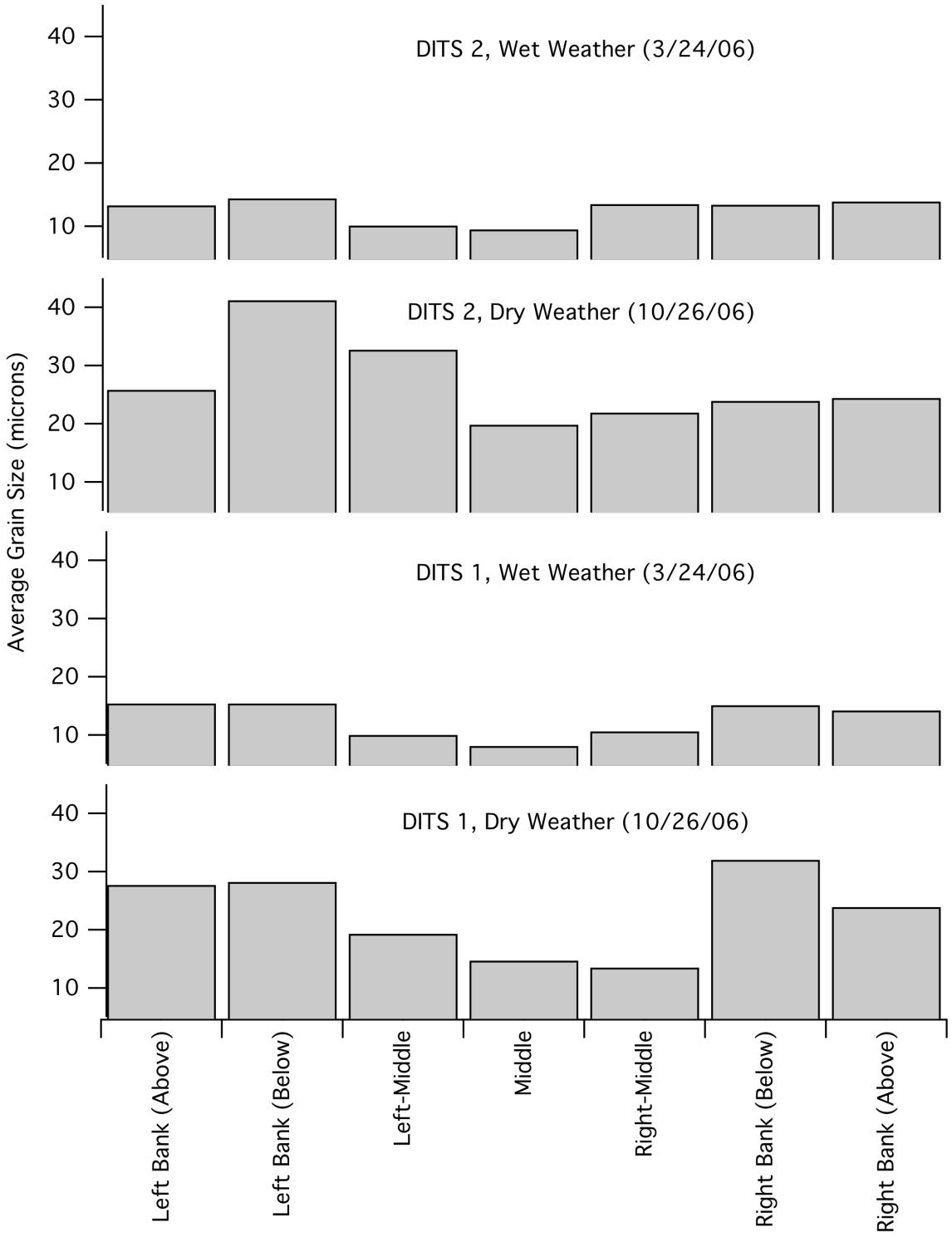

Figure 6-9. Average grain size of sediment collected from the DITS 1 and 2 transect sites during wet and dry weather. "Above" and "Below" refer to sediment samples collected from above and below the waterline at the right or left banks. These average grain size distributions were calculated from sediment samples collected during a high-high tide on the dates indicated.

Table 6-9. Measurement statistics for organic carbon and water content in sediment samples collected from the DITS 1 and DITS 2 sites during the wet weather studies on March 10 and 24, 2006.

Sampling Site	***% Organic Carbon***			***% Water Content***		
	N	mean	s.d.	N	mean	s.d.
DITS 1: Left (dry)	10	6.12	4.3	12	58.0	9.6
DITS 1: Left (wet)	10	10.9	11.9	12	56.0	8.1
DITS 1: Middle	9	8.0	2.3	12	44.0	5.7
DITS 1: Right (wet)	10	4.4	1.5	12	56.3	7.7
DITS 1: Right (dry)	9	3.5	1.3	12	62.8	9.6
DITS 2: Left (dry)	9	16.2	20.0	5	47.2	15.1
DITS 2: Left (wet)	12	8.2	6.9	12	50.2	5.4
DITS 2: Middle	9	9.2	5.6	12	50.9	7.6
DITS 2: Right (wet)	11	6.4	2.3	12	52.0	6.9
DITS 2: Right (dry)	9	12.3	10.7	6	52.8	9.1

Water Content in Sediments. The mean percent water content of sediment ranged from 44 to 62.8% during the wet weather studies (Table 6-10) and from 38.6 to 55.8 % during dry weather studies (Table 6-10). As with the organic carbon data just described, there are no trends in the mean percent water content that are consistent across all sites and both dry and wet weather (Fig. 6-11). At the DITS 1 site, water content is lower in sediment collected from the center of the channel, and higher in sediment collected from the edges (bottom two panels, Fig. 6-11). At the DITS 2 transect site, average percent water content is either highest in the center of the channel (DITS 2 during dry weather, second panel, Fig. 6-11) or progressively increases going from the left bank to the right bank (DITS 2 during wet weather, top panel, 6-11).

6.3.5. Bird Census Data

Coincident with the water and sediment sampling described above, digital photographs were taken of the mudflats at the DITS 1 and 2 transect sites. Altogether, 250

Table 6-10. Measurement statistics for organic carbon and water content in sediment samples collected from the DITS 1 and DITS 2 sites during the dry weather studies on October 19 and 26, 2006.

Sampling Site	***% Organic Carbon***			***% Water Content***		
	N	mean	s.d.	N	mean	s.d.
DITS 1: Left (dry)	11	5.77	4.3	11	55.8	18.7
DITS 1: Left (wet)	12	10.1	7.9	12	48.2	16.8
DITS 1: Middle	12	11.4	15.8	12	38.6	15.0
DITS 1: Right (wet)	12	4.8	3.0	12	54.3	11.0
DITS 1: Right (dry)	12	5.8	8.7	12	60.4	13.4
DITS 2: Left (dry)	5	6.9	1.1	5	39.2	4.0
DITS 2: Left (wet)	12	7.3	3.0	12	45.6	6.0
DITS 2: Middle	12	5.6	1.1	12	48.9	5.3
DITS 2: Right (wet)	12	6.6	3.7	12	45.4	7.4
DITS 2: Right (dry)	6	8.9	4.1	6	40.0	9.5

photographs were electronically archived, capturing images from the two wet weather studies (March 10 and 24, 2006) and one of the dry weather studies (October 19, 2006). Birds visible on these photos were counted to yield a time series of bird counts on the left and right banks of the DITS 1 and DITS 2 transect sites.

When these bird counts are averaged by transect site (DITS 1 or 2) and sampling date (March 10, March 24, October 19) several patterns emerge (Fig. 6A-33, Appendix 6A). During all three sampling events at DITS 1, there are consistently more birds visible on the right bank, and fewer birds visible on the left bank. Comparing the two transect sites, there are more birds present on the mudflats at the DITS 1 transect, and fewer birds present on the mudflats at the DITS 2 transect. As can be seen from the map in Fig. 6-1, the intertidal mudflat at both sites is extensive. Standard deviations associated with the average bird counts are quite large; in many cases the coefficient of variation (i.e., standard deviation divided by mean) exceeds unity.

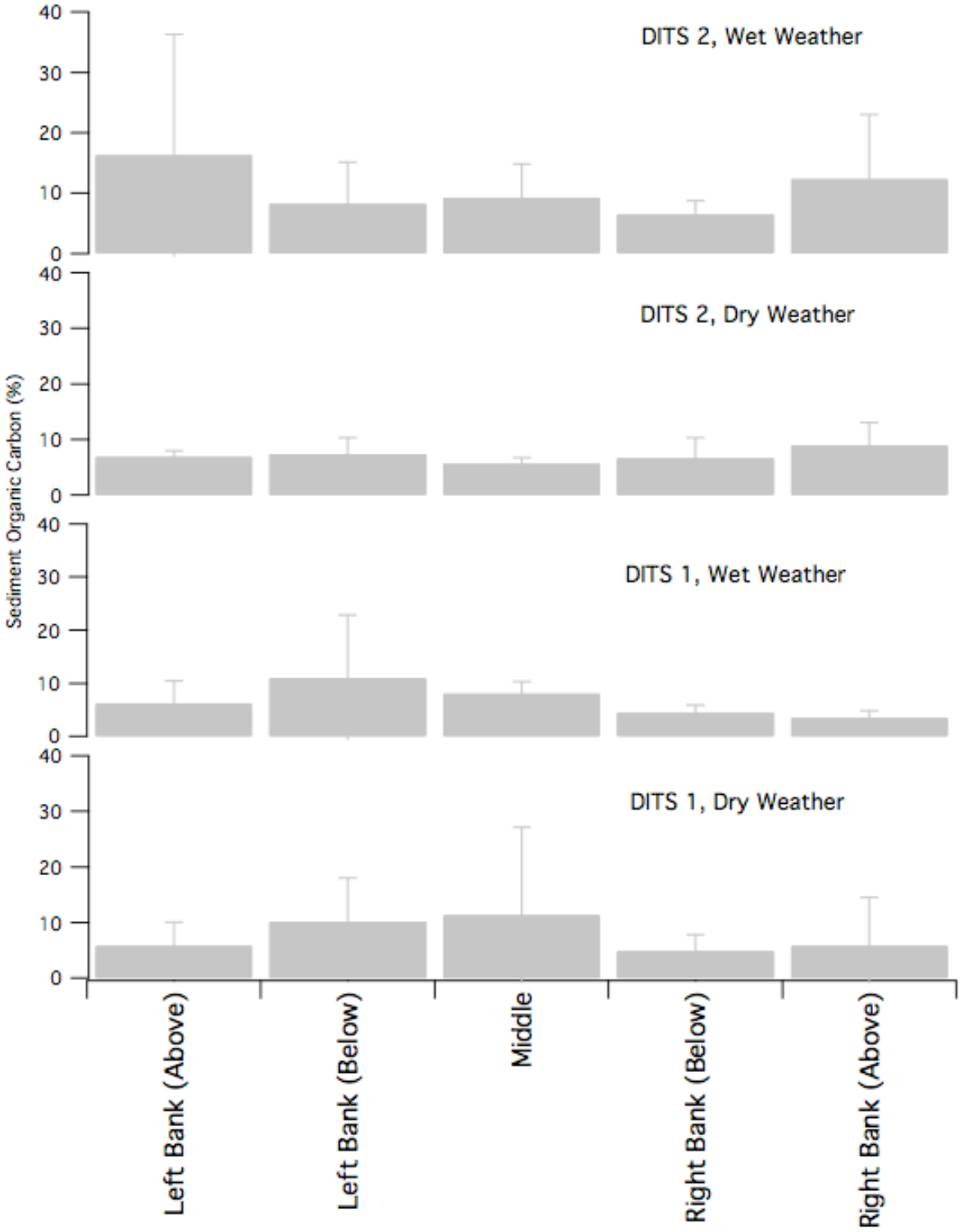

Figure 6-10. Organic carbon content of sediment samples collected from the DITS 1 and 2 transects during the dry and wet weather studies. These graphs are generated from the data included in Table 6-9 and 6-10.

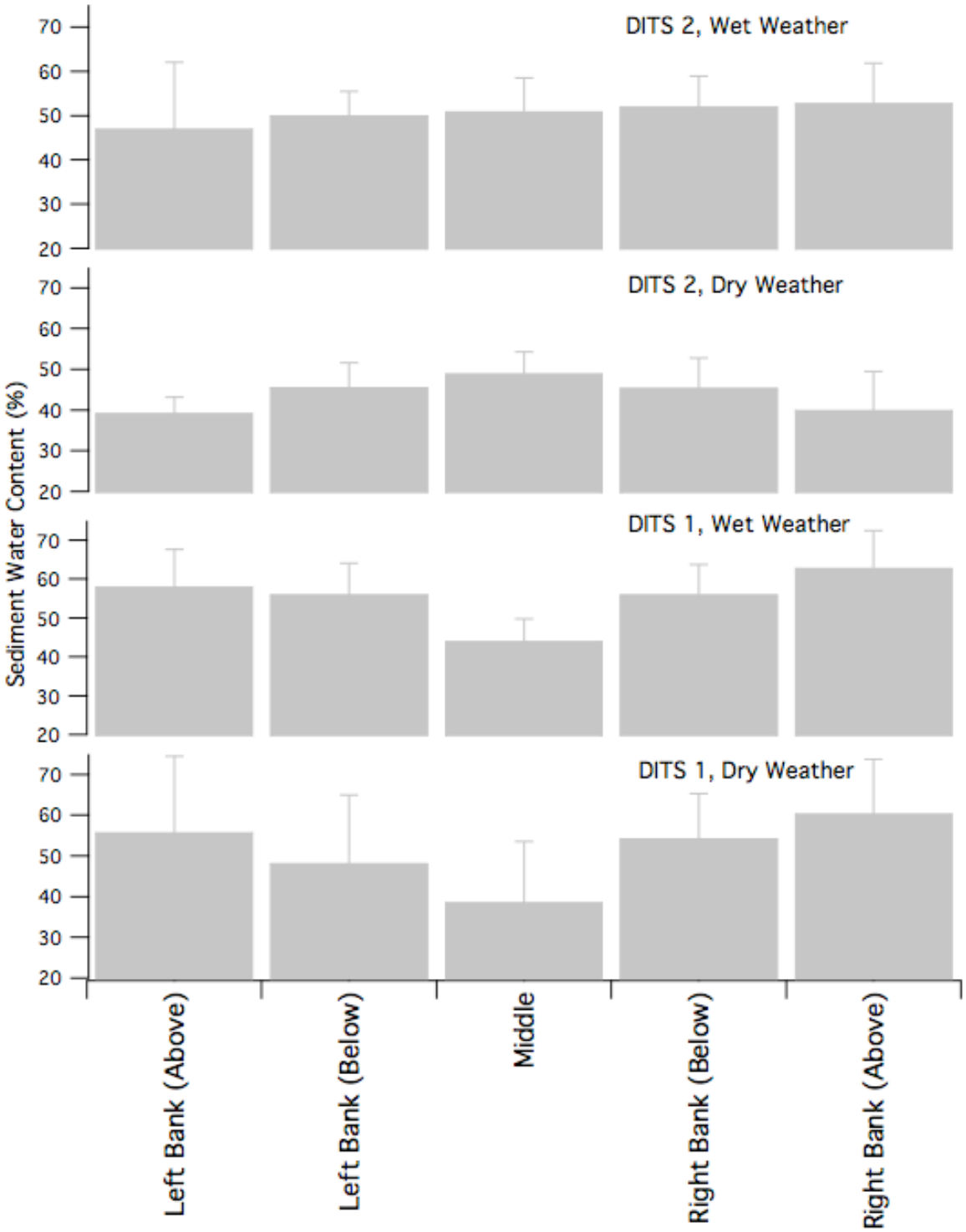

Figure 6-11. Water content of sediment samples collected from the DITS 1 and 2 transects during the dry and wet weather studies. These graphs are generated from the data included in Table 6-9 and 6-10.

To better understand the origin of the variability just noted, bird count data were plotted as time series (Figs. 6A-34 through 6A-36, Appendix 6A). During the March 10 study, there was considerable temporal variability in the number of birds counted on the mudflats (Fig. 6A-34). With one exception, the highest bird counts occurred in the mid-morning during a falling tide. Later in the day, bird counts were generally low. The exception occurred at the left bank of BTO 4, where bird counts were uniformly low (<200) throughout the day. The drop-off in bird counts after noon at most sites may be related to the change in tide (from a falling to rising tide) and/or a small storm that passed through the area.

Bird counts were lower during the March 24th DITS study, and relatively constant over the approximately 8 hours during which pictures were taken (Fig. 6A-35). The sequence of photos taken during the March 24th study coincide with the morning ebb tide, and hence it is not possible to assess the possible influence of changing tides on bird loafing and feeding behavior.

During the October 19 study, bird counts were higher on the mudflats at the DITS 1 transect, and lower on the mudflats at the DITS 2 transect (Fig. 6A-36). Toward the end of the afternoon ebb tide, a large flock of birds congregated on the left bank of the DITS 1 transect (approximately 500 birds counted in the photos). At other sampling times, relatively few birds (<20) were visible on the left bank of the DITS 1 transect. The number of birds loafing or feeding on the right bank of the DITS 1 transect were more stable throughout the period of the study, fluctuating between 200 and 500 birds. A modest number (<200) of birds was observed on the right bank of the DITS 2 transect, and virtually no birds (<10) were observed on the left bank of the DITS 2 transect.

6.3.6. Nutrient Data

A subset of samples collected at DITS 1 and 2 from the surface of the water column at the thalweg were analyzed for a suite of nutrients, including nitrate, ammonia, total Kjeldahl nitrogen (TKN), total phosphorous, and total organic carbon (TOC). Mean and standard deviations computed from these data break out into two groups (Table 6-11): (1) nitrate and total phosphorous vary both spatially (higher at DITS 1, lower at DITS 2) and by study (higher during wet weather, lower during dry weather); (2) ammonia, TKN, and TOC vary spatially (higher at DITS 1, lower at DITS 2) but not by study (wet and dry weather concentrations are not significantly different). These results are consistent with the results presented in Chapter 2, where it was reported that San Diego Creek is a significant source of nitrate and total phosphorous, while ammonia may be generated within Upper Bay, for example by dissimilative reduction of nitrate.

Table 6-11. Nutrient measurements on water samples collected from the surface of the water column at the middle transect site during the DITS 1 and 2 studies. Maximum acceptable reporting detection limits for the nutrients were as follows: 0.4 mg/L (nitrate), 0.1 mg/L (ammonia), 0.2 mg/L (TKN, Phosphate, TOC).

Site	***Date***	***N***	***NO_3^{-1} (mg/L)***	***NH_3 (mg/L)***	***TKN (mg/L)***	***Total P (mg/L)***	***TOC (mg/L)***
DITS 1	3/10/06	5	8 +/- 0.7	0.25 +/- 0.17	0.88 +/- 0.05	0.54 +/- 0.02	5.1 +/- 0.22
DITS 2	3/10/06	5	2.8 +/- 0.9	0.16 +/- 0.09	0.82 +/- 0.15	0.48 +/- 0.03	3.4 +/- 0.43
DITS 1	3/24/06	7	6.8 +/- 0.6	0.26 +/- 0.05	0.61 +/- 0.16	0.73 +/- 0.09	7.3 +/- 0.44
DITS 2	3/24/06	7	4.7 +/- 1.1	0.21 +/- 0.04	0.34 +/- 0.2	0.6 +/- 0.07	6.4 +/- 0.46
DITS 1	10/26/06	6	N.D.	0.24 +/- 0.03	0.77 +/- 0.1	0.12 +/- 0.01	4.7 +/- 0.69
DITS 2	10/26/06	6	N.D.	0.22 +/- 0.03	0.57 +/- 0.2	0.10 +/- 0.01	3.5 +/- 0.52

[a]Average and standard deviation of measurements on water samples collected from the surface of the channel thalwag
[b]N.D., not detected.

6.4. Summary of DITS Study Results

Despite the very different FIB exceedence rates measured in water samples collected during the wet weather studies, on the one hand, and dry weather studies, on the other hand, in both cases the mudflats appear to be a source of FIB in the very shallow waters along the shoreline in Upper Bay. While the study design adopted here cannot definitively determine the source of FIB on the mudflats, several observations point to the deposition of bird feces:

1. Birds are present at high numbers along the mudflats at DITS 1 and 2 during both wet and dry weather studies (see bird census results below). The physical location of the birds (near the water's edge) coincides with the location where FIB concentrations in both water and sediment are highest.
2. Along the banks of the tidal channel, in many cases FIB concentrations are higher in sediment collected above the waterline, and lower in sediment collected below the waterline, even during periods of time when the tide is falling (see time series in Appendix 6A). This suggests that there is fast replenishment of FIB on the exposed mudflat as the tide recedes, consistent with our photographic observations that birds congregate, and presumably defecate, right at the water's edge (top panel, Fig. 6-6). If regrowth in the sediment was the dominant source

of FIB on the mudflat, it would presumably take time to regenerate FIB in the sediments after they were washed by the tide.

3. Water samples collected during the dry weather studies had lower FIB exceedence rates compared to water samples collected during the wet weather studies. The microcosm studies described in Chapter 2 found that bird feces harbor very high concentration of both EC and ENT; however, FIB from bird feces die-off at a rate that depends on solar irradiation levels (faster die-off in the presence of sunlight, slower die-off in the dark). The maximum solar irradiance was relatively similar (700 to 800 W/m^2) for three of the DITS studies (March 24, October 19, and October 26) (compare Figs. 10-3 and 10-21). The peak solar irradiance is lower during the first study on March 10 (ca., 400 W/m^2) because of a rain event that day. Further, during the two dry weather studies the concentration of suspended particles was high (ca., 100 ppm) across a range of particle sizes (see particle size data in Appendix 6A). During the two wet weather studies, on the other hand, the concentration of suspended particles was lower (ca., 50 ppm) and confined to large particle size classes (70 to 80 microns). As noted in a recent article[9], suspended particles can affect the influence of sunlight on FIB survival in estuarine environments. These two factors—lower solar irradiance on March 10, and lower concentrations and larger sizes of suspended particle on March 10 and 24—may have resulted in slower FIB die-off rates, and higher overall exceedence rates of FIB from bird feces in the water column during the wet weather studies.

Measurements on Inter-tidal and Sub-tidal Sediments. Sediments collected from the DITS 1 and 2 transect sites exhibited spatial and seasonal trends relative to average grain size, organic content, and water content. The average grain size of sediment samples showed two primary spatial and seasonal trends:

1. A fining of sediments toward the center of the tidal channel, and coarsening of sediments toward the edges of the channel; and
2. A coarsening of sediments during dry weather periods, and a fining of sediments during wet weather.

Sediment organic content was lowest during the dry weather study at DITS 2, intermediate during the wet weather study at DITS 1, and highest during the wet weather study at DITS 2 and the dry weather study at DITS 1. Water content of the sediment tended to follow the same spatial patterns just noted for average grain size; i.e., all else being equal, water content increased with increasing average grain size.

Bird Census. Estimates of the number of birds present on the mudflats during the DITS 1 and 2 studies suggest that bird populations in the Bay are highly variable in space and

[9]Kay, D.; Stapleton, C.M.; Wyer, M.D.; Mcdonald, A.T.; Crowther, J.; Paul, N.; Jones, K.; Francis, C.; Watkins, J.; Wilkinson, J.; Humphrey, N.; Lin, B.; Yang, L.; Falconer, R.A.; Gardner, S. Decay of intestinal enterococci concentrations in high-energy estuarine and coastal waters: towards real-time T-90 values for modeling faecal indicators in recreational waters. *Water Research.* **2005**, *39*, 655-667.

time. Despite this variability, it can be said that large numbers of birds congregate on the mudflats in Upper Bay (frequently >300 birds could be counted in the images), and more birds tend to loaf on the right bank, compared to the left bank, of the tidal channel at DITS 1 and 2. Some diurnal variability was also noted; e.g., in several cases, birds seemed to prefer to congregate on the mudflats during falling tides in the early morning or late evening.

Nutrient Concentrations in the Water Column. Water samples from the thalweg of the tidal channel were analyzed for a suite of nutrients. From these data, it appears that San Diego Creek is a significant source of nitrate and total phosphorous. San Diego Creek may also be an important source of ammonia, TKN, and TOC, but within-Bay processes may be a source of ammonia from the microbially mediated conversion of nitrate to ammonia under anaerobic conditions[10], and the fixation of organic carbon by macro- and micro-algae present in the Bay[11].

[10] Cole, J. (1996) "Nitrate reduction to ammonia by enteric bacteria: redundancy, or a strategy for survival during oxygen starvation?" *FEMS Micro. Lett*. **136**:1-11.

[11] Boyle, K.A., Kamer, K., Fong, P. (2004) "Spatial and temporal patterns in sediment and water column nutrients in a eutrophic southern California estuary" *Estuaries* **27**:378-388.

[1]Chapter 7. Urban Sources of FIB in Lower Newport Bay

7.1 Introduction and Overview

This chapter summarizes efforts to characterize the input of FIB into Newport Bay from urban sources, including storm drains, sewer lines, and shallow groundwater. This chapter satisfies sub-task 2.1.2.5 of the Prop 13 project, by reporting on:

1) Areas of the Bay where subsurface sewer collection line leaks may contribute to near shore FIB contamination (Task 2.1.2.1.);
2) FIB concentrations in shallow groundwater seeps in areas where subsurface sewage leaks may contribute to near shore FIB contamination (Task 2.1.2.2.)
3) Ongoing efforts by the City of Newport Beach, conduct dye tests of pump-out stations, visually inspect wharves and docks for wash-off activities that may contaminate the Bay (Task 2.1.2.3).
4) Concentration of FIB in storm drain runoff flowing into Lower Bay during dry and wet weather periods (Task 2.1.2.4).

7.2. FIB in Shallow Groundwater

7.2.1. Methods

Pits were dug by hand in the beach sand, approximately 3 to 5 m from the water's edge, at five sites along the shoreline in Lower Bay. The depth of each pit, which varied from 0.15 to 0.30 m depending on the pit, was chosen so that shallow groundwater covered the bottom of the pit to a depth of at least 5 cm. The pits were dug and sampled between 6 and 8 AM on November 16, 2006, coincident with the dry weather storm drain survey (described later). Water samples were collected from the bottom of the pits using sterile 50 mL syringes, which were then emptied into sterile 100 mL bottles, capped and placed on ice in the dark. Water samples were transported back to UCI within a holding time of 6 hours, and analyzed for FIB, pH, and salinity using methods described in Chapter 2.

7.2.2. Results

Sewer Line Survey and Shallow Groundwater Sites. Sites for shallow groundwater testing were selected with several factors in mind: (1) they should be sited in a variety of geographical locations in Lower Bay with various land-use types; (2) they should be proximal (< 30 m) to sewer mains (diameters ≥ 8 inches) of various ages and material composition; (3) they should be located in sandy stretches of beach that are readily accessible on foot, so that the groundwater pits could be easily dug and sampled; and (4) they should be located reasonably close to recreational areas where impacts on surface water quality might affect bather health.

[1] This chapter was prepared by S. Grant with data collection and assistance provided by K. McLaughlin, J. H. Ahn, R. Stein, R. Litton, V. Thulsiraj, A. Pednekar, and B.F. Sanders.

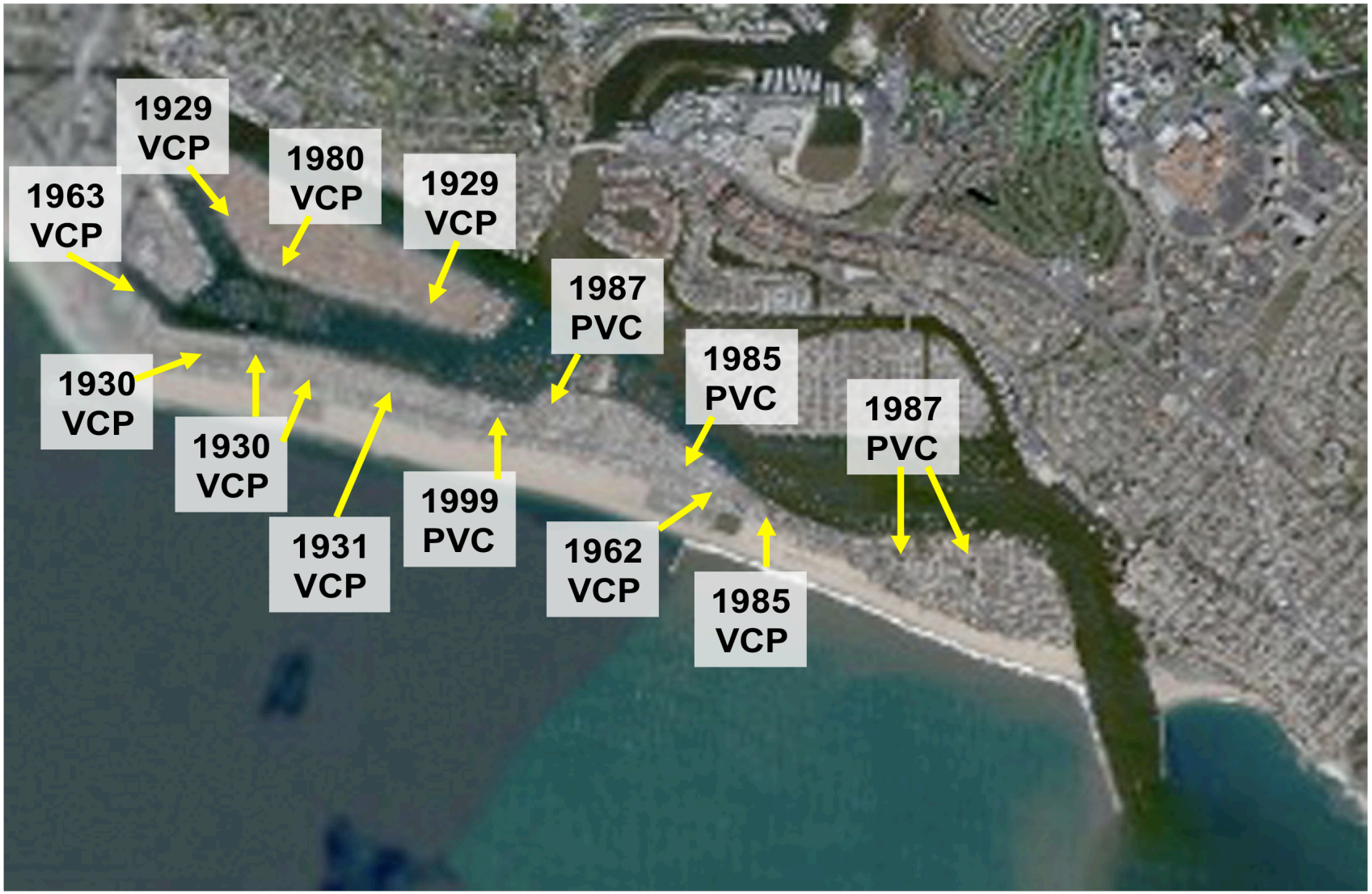

Figure 7-1. Ages and composition of sanitary sewer lines in Lido Island and Balboa Peninsula. VCP = vitrified clay pipe; PVC= polyvinyl chloride pipe.

Figure 7-2. Location of shallow groundwater testing, and water quality measurements.

Table 7-1. Coordinates for the 5 locations in Lower Bay where shallow groundwater was sampled and tested for FIB, salinity, and pH.

Station ID	***Latitude***	***Longitude***
G1	33° 36.006	117° 53.651
G2	33° 36.319	117° 54.192
G3	33° 36.369	117° 54.693
G4	33° 36.491	117° 55.226
G5	33° 36.760	117° 55.326

Based on the above criteria, Mr. Robert Stein (Public Works Department, City of Newport Beach) prepared a map of candidate sampling sites (Fig. 7-1). This figure indicates the age and composition of sewer mains located proximal to the shoreline in Lower Bay. Sewer mains in the Lower Bay are composed of either vitrified clay pipe (VCP) or polyvinyl chloride (PVC). Older VCP lines have cement joints, which are relatively leak free[2]. In the 1960's, rubber-gasket wedge-lock joints were used for connecting VCP sewer main segments. Since 1970, the City of Newport Beach has been installing PVC sewer lines as an alternate to VCP. Both materials are used today for new installations. In 1988, the City implemented a program that requires upgrades of sewer laterals to VCP with wedge-lock joints or PVC for new construction and major remodel projects.

The 15 candidate sites were further evaluated for ease of access, proximity to a sandy beach suitable for digging a shallow pit, and proximity to recreational beaches. Based on these criteria, five testing sites for shallow groundwater testing were selected. The latitude and longitude of these sites are included in Table 7-1; site locations are shown on the map in Fig. 7-2. The 5 shallow groundwater testing sites include 4 locations on the Balboa Peninsula with sewer lines made of either VCP or PVC and ranging in age from 1929 to 1999, and one location on Lido Isle where the VCP sewer main was laid in 1929[3].

<u>*Shallow Groundwater Testing*</u>. Measurements of salinity, pH, and FIB concentrations are shown in Fig. 7-2. Water collected from the shallow groundwater pits had near oceanic salinities (31 to 32). TC and EC concentrations were typically low, with no groundwater pit samples exceeding the California marine bathing water single-sample standards for these two organisms. ENT concentrations in the shallow groundwater exceeded the marine bathing water single-sample standard of 104 MPN/100 mL at two locations on the Balboa Peninsula. One exceedence (at the 9th street site, ENT = 292 MPN/100 mL) is near a sewer main constructed of VCP and installed in 1931. The other exceedence (at Alvarado Place, ENT= 1118 MPN/100 mL) is located near a sewer main constructed of PVC and installed in 1985. Based on these limited data it would be premature to draw a

[2]"Status Report on the Development of a Reporting Methodology for Subsurface Discharges of Sewage," Prepared for Orange County Sanitation District September 30, 2005 by Brown and Caldwell.

[3] According to a City of Newport Beach timeline, the first sanitary sewers were laid in 1922 (http://www.city.newport-beach.ca.us/nbpl/AboutNBPL/newport_beach_time_line.htm), and thus the sanitary sewer lines currently buried on Lido Isle must be among the first laid in the area.

link between the sewage collection system and water quality in the shallow groundwater in Lower Bay. However, given that ENT concentrations were somewhat elevated at one site (9th Street) and significantly elevated at another site (Alvarado Place), and given that 2 of 5 shallow groundwater sites had elevated ENT concentrations, further testing of the shallow groundwater in Lower Bay may be warranted.

7.3. Pump-Out Stations, Runoff from Wash-off Activities

There are 10 public and 13 private pump-out stations in Newport Beach. The City of Newport Beach conducts dye tests on these pump-out stations on a weekly basis. If leaks are discovered, the City and the Orange County Health Care Agency test the surrounding area for FIB contamination. Typically, one to two postings occur on an annual basis due to leaking pump-out stations. Given that all pump-out stations in Lower Bay are monitored on a routine basis by the City of Newport Beach, no additional dye studies were conducted as part of the Prop 13 project.

Observations from the research boat identified several practices in Lower Bay that may contribute to FIB pollution in Lower Bay, including wash-off from commercial fishing boats as they transit through the Bay, and wash-off from dock facilities in and around the Pavilion on the Balboa Peninsula. Efforts to collect water from these wash-down activities were not successful, primarily because the individuals involved did not consent to us sampling the wash off water. Consequently, a synoptic study of water quality along the shoreline in Lower Bay was carried out—with partial funding from the UCI Minority Biomedical Research Support (MBRS) program under the direction of program fellow, Vanessa Thulsiraj—to obtain a snapshot of water quality along the shoreline of Lower Newport Bay, during high and low tides.

7.4. Synoptic Survey of Water Quality in Lower Bay

The goal of the synoptic survey was to measure water quality at 80 to 90 locations along the shoreline in Lower Bay during a dry weather period in the summer, and to determine how water quality there is modulated by: (1) tide conditions (high tide vs. low tide), and (2) cross-shore location (water's edge, 100 feet bay-ward). All samples were analyzed for FIB (TC, EC, and ENT), salinity, and pH, so that relationships among and between these variables could be explored.

7.4.1. Methods

Over 320 water samples were collected from 86 locations along the shoreline in Lower Bay, on August 3 and 4 (2006) and again on August 8 and 9 (2006). Sampling was

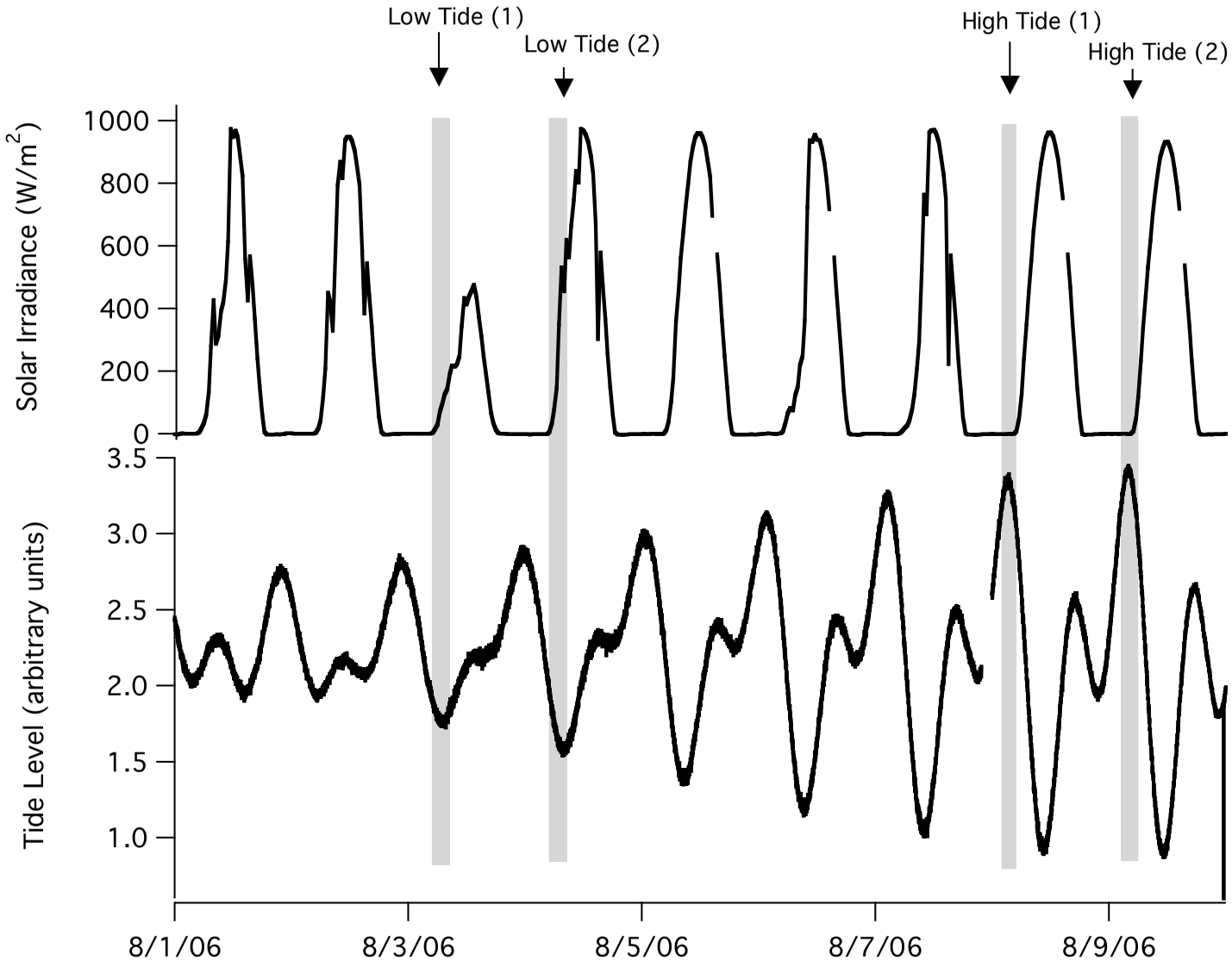

Figure 7-3. Timing of the four synoptic sampling events in Lower Bay relative to solar irradiance (top panel) and tide level (bottom panel). Tide level is pressure measured at the SCCOOS shoreline sensor located at the end of Newport Pier (www.sccoos.org). Solar irradiance recorded at the San Joaquin Marsh.

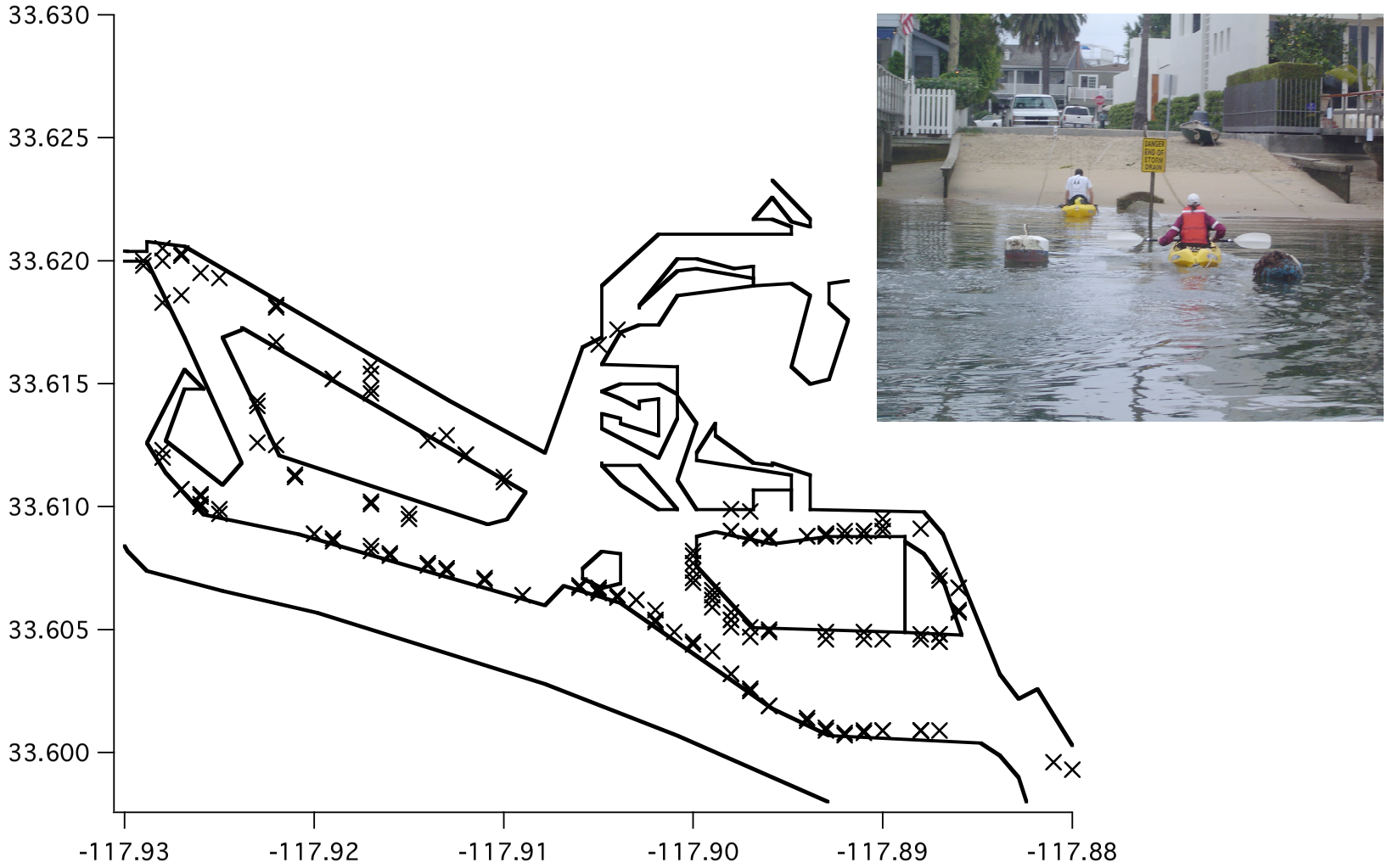

Figure 7-4. Map of Lower Bay showing sites where surface water samples were collected from two locations along the shoreline: (1) water's edge, and (2) 100 feet bay-ward. Inset is a photograph of samples being collected from the water's edge in kayaks, near the outlet of a storm drain.

carried out at low tide during the August 3-4 sampling, and at high tide during the August 8-9 sampling (see Fig. 7-3 for sample timing relative to solar irradiance and tide level). For each tide condition, sampling was conducted over two consecutive nights because, with the resources available, it was not possible to sample the entire Lower Bay shoreline in one night and keep sample holding times under 6 hours. For both low- and high-tide studies, the east side of Lower Bay was sampled the first night, and the west side of the Bay was sampled the second night. To minimize the impact of changing solar irradiance on FIB concentrations in the Bay, sampling was conducted during the midnight or early morning hours (between 4:30 AM and 8:30 AM on August 3 and 4; between 1:30 AM and 5:00 AM on August 8; and between 2:30 AM and 6:00 AM on August 9). The synoptic was carried out during dry and warm conditions, typical of summers in southern California. The only recorded rain at Newport Beach was 0.15 inches that fell 1.5 weeks (on July 23) prior to the start of the Lower Bay synoptic study.

Sampling locations were chosen to provide relatively uniform coverage of the Lower Bay shoreline, and to be proximal to outlets of storm drains discharging to the Bay. Fig. 7-4 is a map of Lower Bay showing the location of all sites sampled during the synoptic study; the inset is a photograph of a typical sampling site in Lower Bay. The latitudes and longitudes of sampling sites are included in Appendix 7A.

Two samples were collected at each sampling site, one at the water's edge (typically directly in front of a storm drain, see inset photograph in Fig. 7-4) and one 100 feet bay-ward of the storm drain. The water's edge samples were collected from a kayak; bay-ward samples were collected from the research boat. All water samples were collected from the surface of the water column in 500 mL sterilized polypropylene bottles. After sampling, bottles were immediately capped and placed on ice, and then transported back to the laboratory at UCI where they were analyzed within a holding time of 6 hours for TC, EC, ENT, salinity, and pH, using methods described in Chapter 2.

7.4.2. Results

FIB, salinity, and pH measurements collected during the synoptic studies are presented in tabular and graphical forms in Appendix 7A (Tables 7A-3 and 7A-4 and Figs. 7A-3 through 7A-6). For a fixed tide condition (i.e., low or high tide), water samples collected closer to shore have lower salinity, lower pH, and higher EC and ENT concentrations. The exception is salinity measured at high tide, for which there is not a significant difference between samples collected at water's edge and 100 feet bay-ward. For a fixed cross-shore location (i.e., water's edge or 100 feet bay-ward), samples collected at low tide have lower salinity, lower pH, and higher EC and ENT concentrations. Median (i.e., 50th percentile) FIB concentrations decreased in the order: water's edge at low tide > 100 feet bay-ward at low tide > water's edge at high tide > 100 feet bay-ward at high tide.

The statistical trends noted above are reflected in the log-mean concentrations of ENT and EC, and in the percentage of samples with EC and/or ENT concentrations above the California single-sample marine bathing water standards (Table 7-2). A relatively high

Table 7-2. FIB measurement statistics for the Lower Bay synoptic study.

		Enterococcus spp. (MPN/100 mL)			***E. coli (MPN/100 mL)***		
Sample Location	Tide	N	Log-mean[a]	% > s.s.s.[b]	N	Log-mean[a]	% > s.s.s.[b]
Water's Edge	High	86	0.8 +/- 0.9	8	87	1.6 +/- 0.8	7
Water's Edge	Low	87	1.3 +/- 1.0	20	87	1.9 +/- 0.7	17
100 ft Bay-ward	High	87	0.5 +/- 0.6	2	87	1.3 +/- 0.5	0
100 ft Bay-ward	Low	87	1.0 +/- 0.9	8	87	1.7 +/- 0.6	7

[a]Average and standard deviation of log-transformed FIB concentrations
[b]Percent of samples with FIB concentrations over California marine bathing single-sample standards (s.s.s.).

percentage of samples collected at the water's edge at low tide had EC and ENT concentrations in excess of single-sample standards (17 and 20% respectively). In contrast, very few samples collected 100 feet bay-ward during high tide exceeded single-sample standards for EC and ENT (0 and 2%, respectively).

The next two maps of Lower Bay show sites where water samples failed one or both single-sample standards for EC and ENT (Figs. 7-5 and 7-6). During low tide, single-sample standards for EC and ENT were exceeded at many sites along the shoreline in Lower Bay (green squares, red triangles, and light blue circles denote sites where ENT, EC, or both ENT and EC exceeded standards). Based on these data, regions of the Bay prone to water quality exceedences include: (1) Turning Basin in West Newport; (2) north-east shore of Lido Isle; (3) north-east shore of Balboa Peninsula; (4) east shore of Balboa Island (grey boxes in Fig. 7-5).

During high tide, on the other hand, only two samples exceeded single-sample standards, including one sample collected on the north-east shore of Lido Isle that exceeded the single-sample standard for ENT, and one sample collected in the Turning Basin that exceeded the single-sample standards for both EC and ENT (Fig. 7-6). The influence of tides on water quality has been documented at open coastal beaches in southern California[4,5]. Compared to previous published studies, however, the extent to which water quality along the shoreline in Lower Bay is affected by tide stage is remarkable.

[4] Boehm, A.B., S.B. Grant, J.H. Kim, S.L. Mowbray, C.D. McGee, C.D. Clark, D.M. Foley, D.E. Wellman (2002) "Decadal and shorter period variability of surf zone water quality at Huntington Beach, California" *Environmental Science and Technology*, v. **36**: 3885-3892.

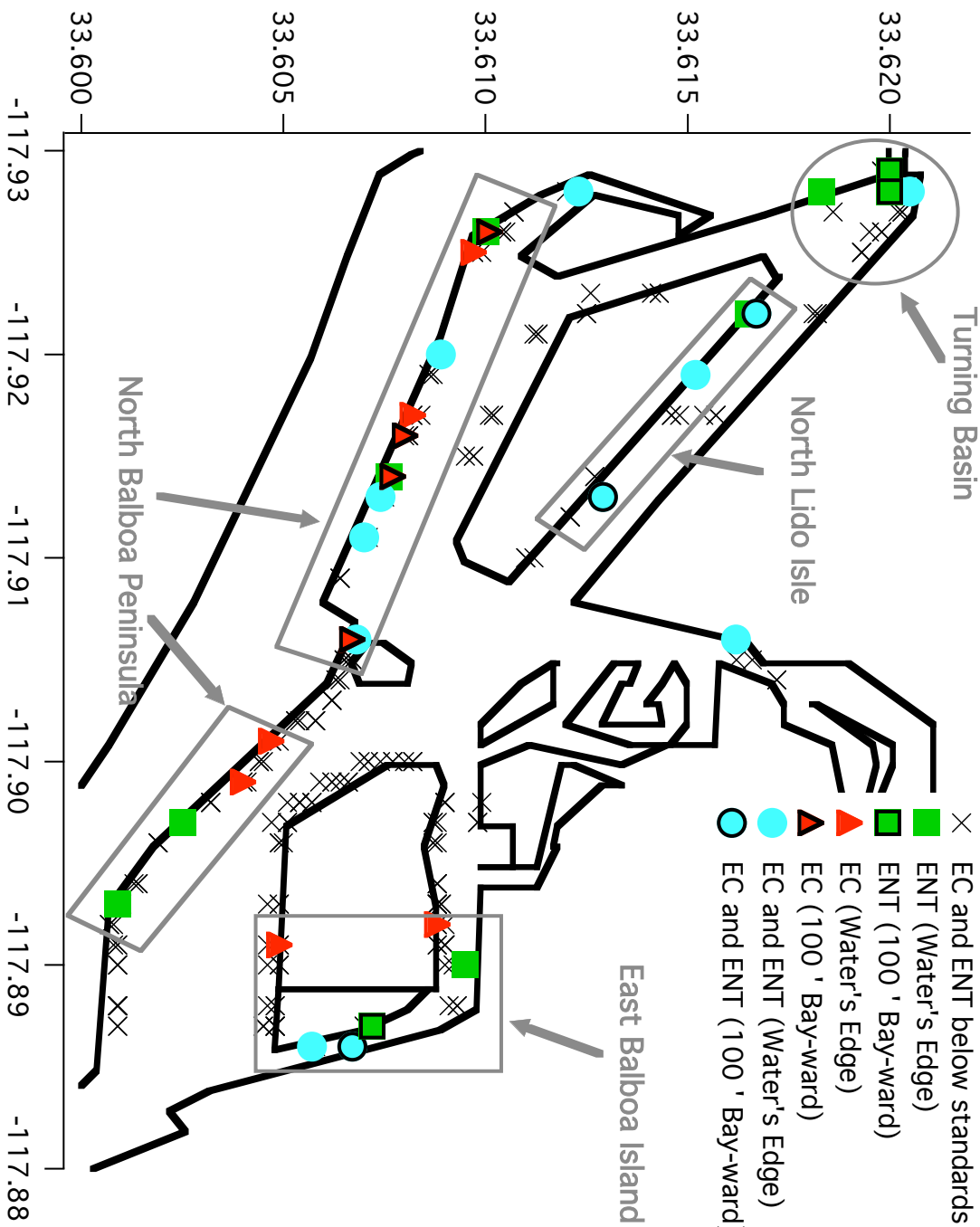

Figure 7-5. Map of Lower Bay showing where water samples exceeded single-sample standards for EC (red triangles), ENT (green squares), or both EC and ENT (light blue circles) during the low-tide synoptic sampling on August 3rd and 4th (2006). Crosses denote the location of samples that did not exceed either EC or ENT standards.

[5] Grant, S.B., J.H. Kim, B.H. Jones, S.A. Jenkins, J. Wasyl, C. Cudaback (2005) "Surf zone entrainment, along-shore transport, and human health implications of pollution from tidal outlets" *J. Geophys. Res*. v. **110**, C10025.

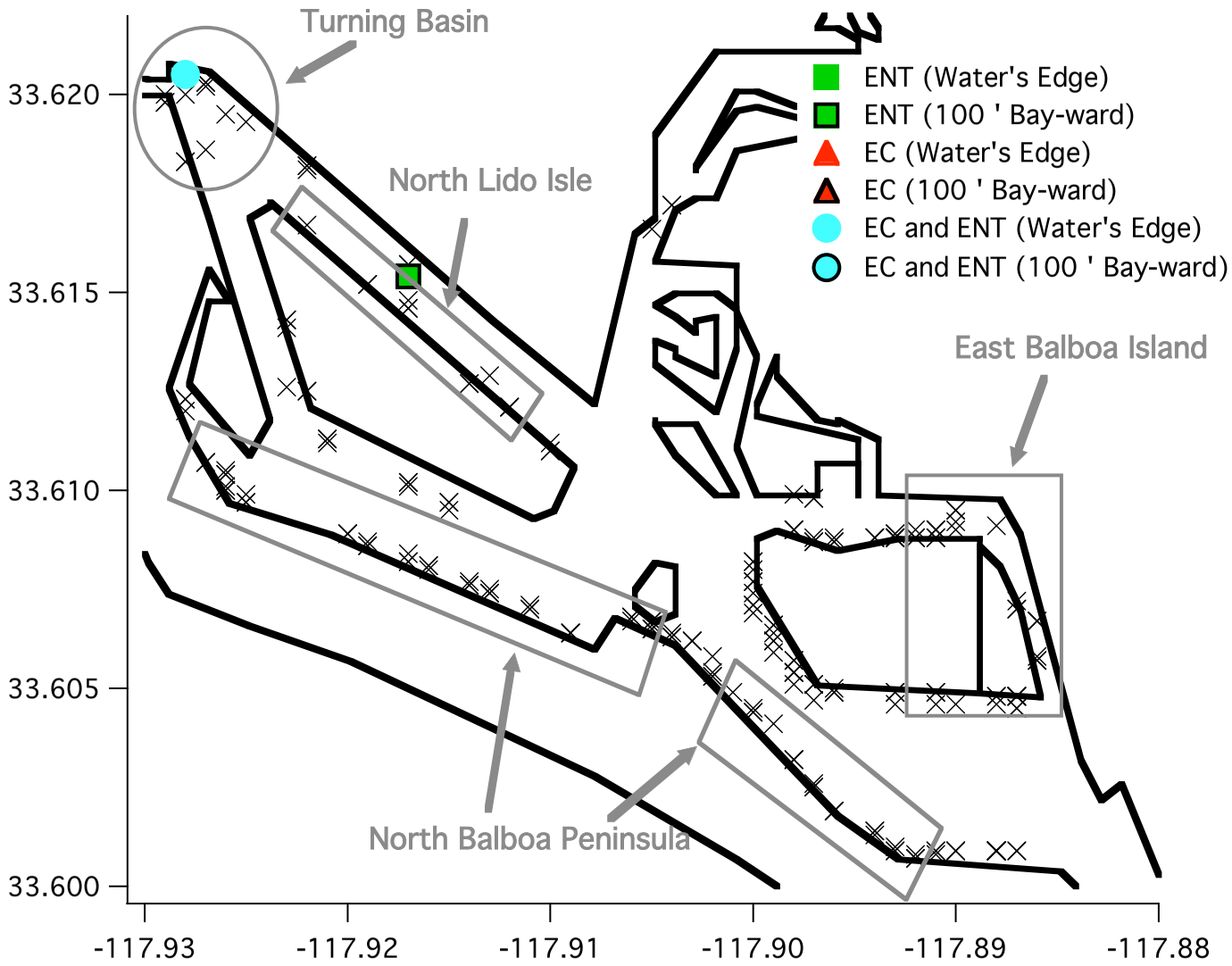

Figure 7-6. Map of Lower Bay showing where water samples exceeded single-sample standards for EC (red triangles), ENT (green squares), or both EC and ENT (light blue circles) during the high-tide synoptic sampling on August 8th and 9th (2006). Crosses denote the location of samples that did not exceed either EC or ENT standards.

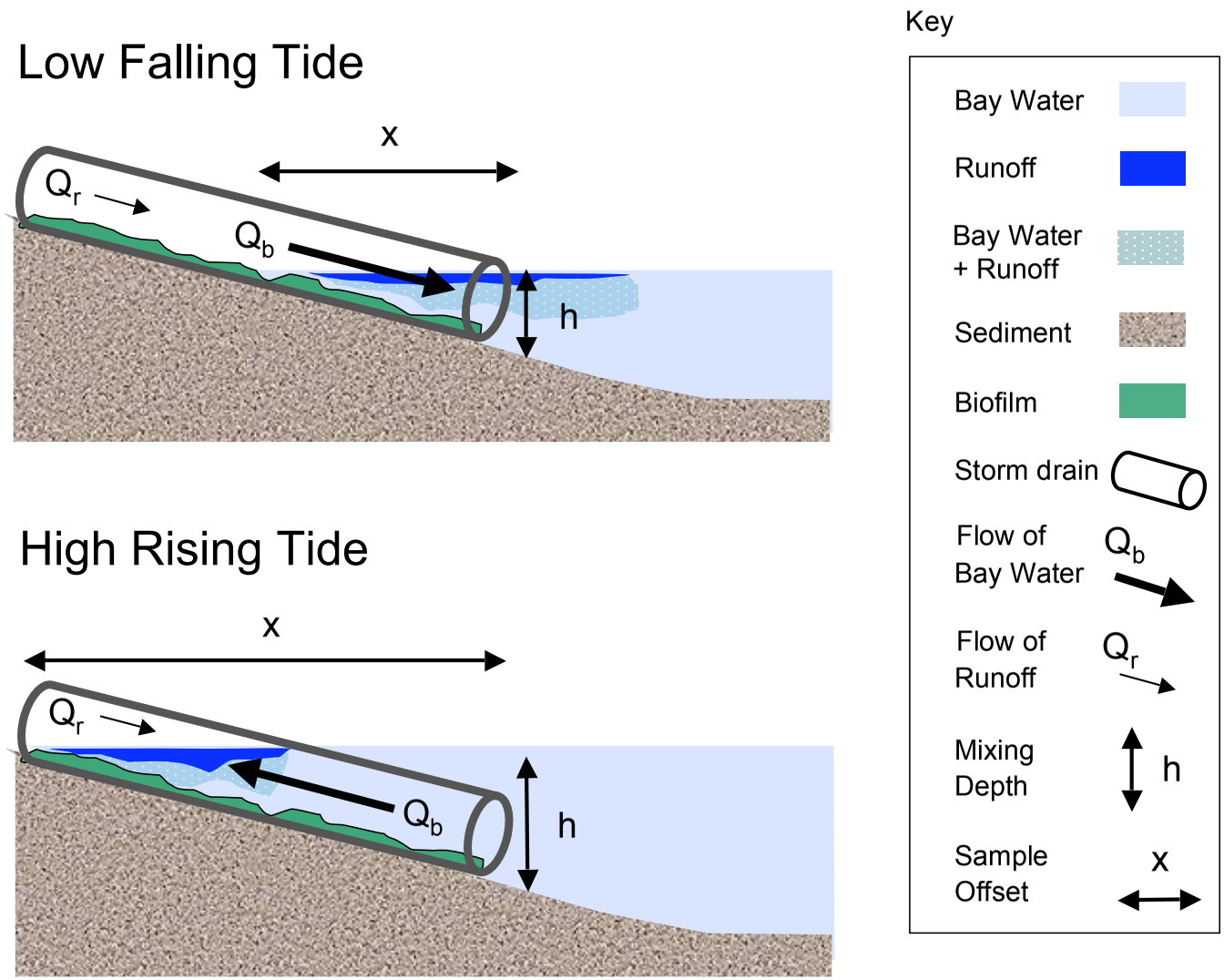

Figure 7-7. Conceptual model of how runoff in storm drains might impact water quality along the shoreline in Lower Bay. Because of density differences between the runoff and Bay water, runoff may accumulate at the free surface inside the drain during high tide (dark blue, bottom panel), and then be released to the Bay when the free surface drops below the upper lip of the outfall pipe during low tide (dark blue, top panel). Mixing of runoff and Bay water in the pipe will generate a brackish mixture (grey blue in top and bottom panels) that can also be released during ebb tides.

The cross-shore variability in salinity is small compared to the variability in salinity measured at different sampling sites in Lower Bay. Four regions, in particular, have relatively low salinity: (1) Turning Basin; (2) North Lido Isle; (3) at a few locations on North Balboa Peninsula; and (4) East and West Balboa Island (Figs. 7A-9 and 7A-10, Appendix 7A). With the exception of the West Balboa Island, these are the same regions where FIB concentrations frequently exceeded marine bathing water single-sample standards (Figs. 7-5 and 7-6). It is noteworthy that the salinity of samples collected in the Turning Basin, and at a few sites on the north shore of the Balboa Peninsula and on the Balboa Island, are low during both low and high tide, perhaps reflecting the impact of runoff on water quality in these regions (e.g., Arches drain at the Turning Basin).

7.4.3. Discussion of Synoptic Data

Most drains in Lower Bay are small (median 18 inches), drain relatively small areas (median 10 acres), and are variably submerged at different stages of the tides (see the distribution of storm drain characteristics described in Chapter 8). During dry weather this combination of features may lead to the scenario pictured in Fig. 7-7. In this conceptual model, the up-slope side of the drain receives relatively small and intermittent flows of nuisance runoff (e.g., from over irrigation, car washing, etc), while the down-slope side of the drain experiences relatively large periodic flows of Bay water into the storm-drain during flood tides, and out of the storm drain during ebb tides. In this scenario, water quality along the shoreline is worse during low tides, and better during flood tides, for the following reasons:

1) Because the flow of runoff is much smaller than the flow of ocean water, the tides can act as a tide gate, forcing runoff to accumulate in the storm-drain pipes during flood tides (i.e., Q_b and Q_r are oriented in opposition), and releasing runoff to near shore waters during ebb tides (i.e., Q_b and Q_r are oriented down slope).
2) During ebb tides, the depth of water at the storm drain outlet decreases with time, and thus contaminants flowing out of the storm drain mix into an increasingly shallower water column (i.e., h is decreasing). The impact of runoff on nearshore water quality is thus magnified because FIB and other contaminants in the storm drain undergo minimal dilution as they mix into near shore waters.
3) During ebb tides, the water's edge moves progressively closer to the storm drain outlet with time (i.e., the horizontal distance, or offset x, decreases). Thus, the pollutant source (the storm drain outlet) moves closer to potential receptors precisely when pollutants are flowing out of the storm drain, and dilution is minimal.

Two scenarios can be envisioned for how runoff might be released from storm drains during ebb tides (Fig. 7-7). In the first scenario, runoff and Bay water are thoroughly mixed in the drainpipe during rising tides, and a brackish mixture of runoff contaminated water flows into the Bay during ebb tides. In this first scenario, the Bay water-runoff mixture can exit the storm drainpipe during ebb tides, even if the tide level never falls

below the upper lip of the pipe (see light blue area labeled Baywater+Runoff in Fig. 7-7). In the second scenario, runoff accumulates at the free surface inside the pipe, and is released only when the tide level drops below the upper lip of the drainpipe (see dark blue area labeled Runoff in Fig. 7-7). In reality, a combination of these two scenarios probably occurs (i.e., inside the drain pipe there is both mixing of the runoff with Bay water, and runoff lensing at the free surface).

FIB may also grow inside storm drains, on detritus and leaf litter deposited there and/or in association with biofilms (as represented by the green layer at the bottom of the pipe in the conceptual model, Fig. 7-7). Initial studies funded by the State Water Resources Control Board and carried out at UCI found that slurries of biofilm and water collected from storm drains in Lower Bay did not have significantly higher FIB concentrations compared to the overlying water[6]. On the other hand, if FIB grow in storm drains, as has been suggested (D. Ferguson and D. Moore, personal communication), this might explain why, during the synoptic study, high FIB concentrations are sometimes associated with low salinity waters (implying the presence of runoff) while in other cases high FIB concentrations are associated with high salinity waters (implying no runoff).

The conceptual model pictured in Fig. 7-7 is consistent with the physical characteristics of many storm drains in Lower Bay, and would explain key results of the synoptic study, including: (1) FIB concentrations are higher, and water quality exceedences more frequent, during low tides; (2) the apparent relationship between low salinity (indicative of runoff) and high FIB concentrations.

7.5. OCHCA's Water Quality Monitoring of Newport Bay

The synoptic study described above provides a relatively fine scale description of water quality along the shoreline in Newport Bay. However, because the effort was limited to a total of four different sampling events, not much can be said about how water quality varies along the shoreline over time. To address the later issue, and to assess if historical monitoring of water quality in the Bay also follows a tidal pattern, in this section we present an analysis of the routine FIB monitoring data collected by the Orange County Health Care Agency (OCHCA).

7.5.1. Methods

OCHCA collects water samples from 35 sites in Newport Bay and analyzes the samples for TC, FC, and ENT concentrations on a once weekly basis (Fig. 7-8; see also site coordinates and names, Tables 7B-1, Appendix 7B). OCHCA's water quality monitoring data were obtained for the time period 3/1/01 through 2/22/05. OCHCA provided, for each of their 35 Newport Bay sampling sites, the date and time samples were collected, together with the concentrations of TC, FC, and ENT. Altogether, approximately 30,000 FIB measurements were incorporated into this study.

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[6] Pednekar, A., S.B. Grant, L.Candelaria (2007) "Assessing the seasonal impact of storm drains on water quality in Western Newport Bay, southern California" A report to the State Water Resources Control Board.

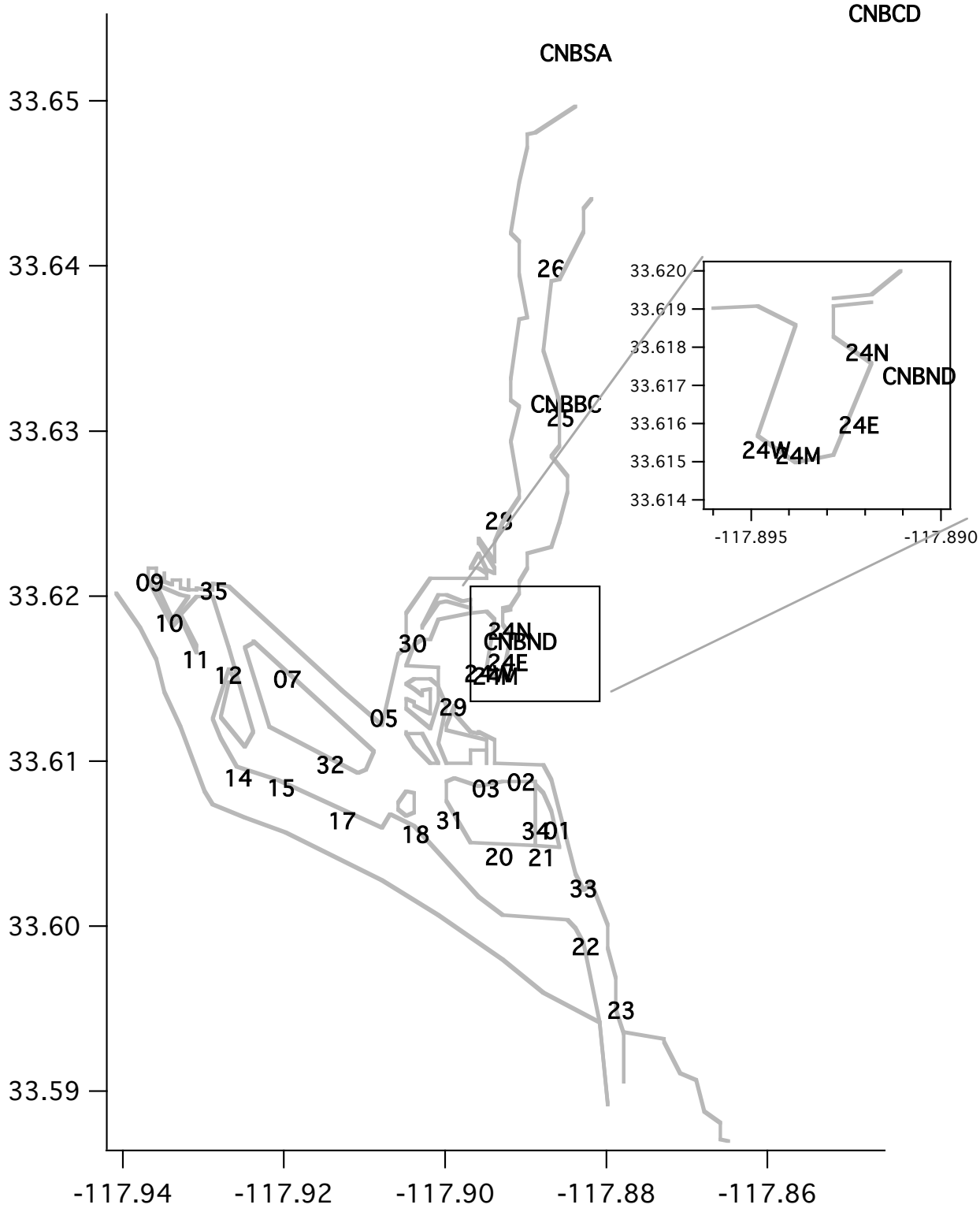

Figure 7-8. A map of the 35 water quality sampling sites in Newport Bay. OCHCA collects water samples from these sites once per week, and tests the water for TC, FC, and ENT concentrations. Shown are the OCHCA station I.D. numbers; note that the "BNB" prefix on these station I.D. numbers has been removed for clarity.

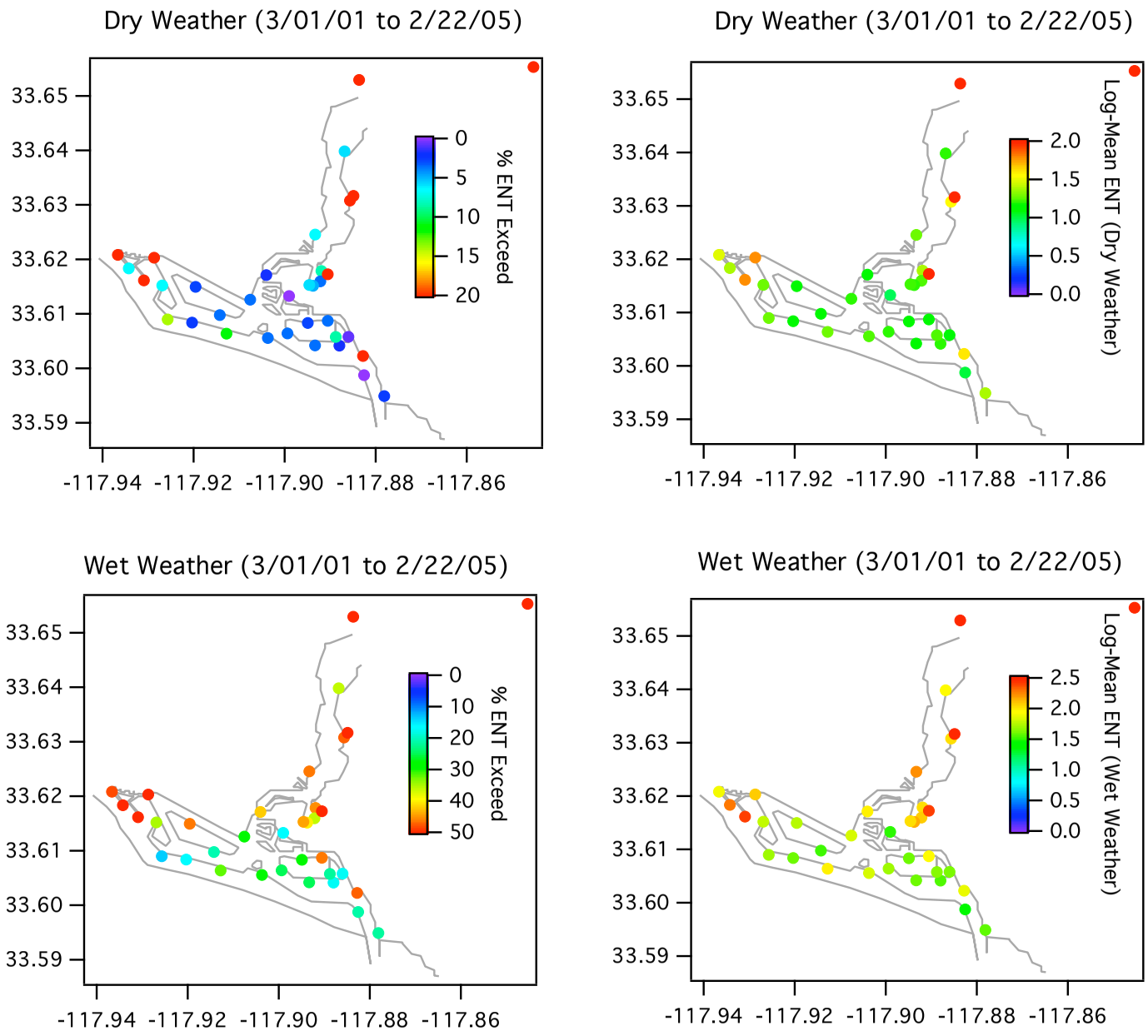

Figure 7-9. Map of OCHCA sampling sites in Newport Bay, where the color denotes the frequency with which water samples exceed the marine bathing water standard of 104 MPN/100 mL (left panels) or the log-mean concentration of ENT (right panels).

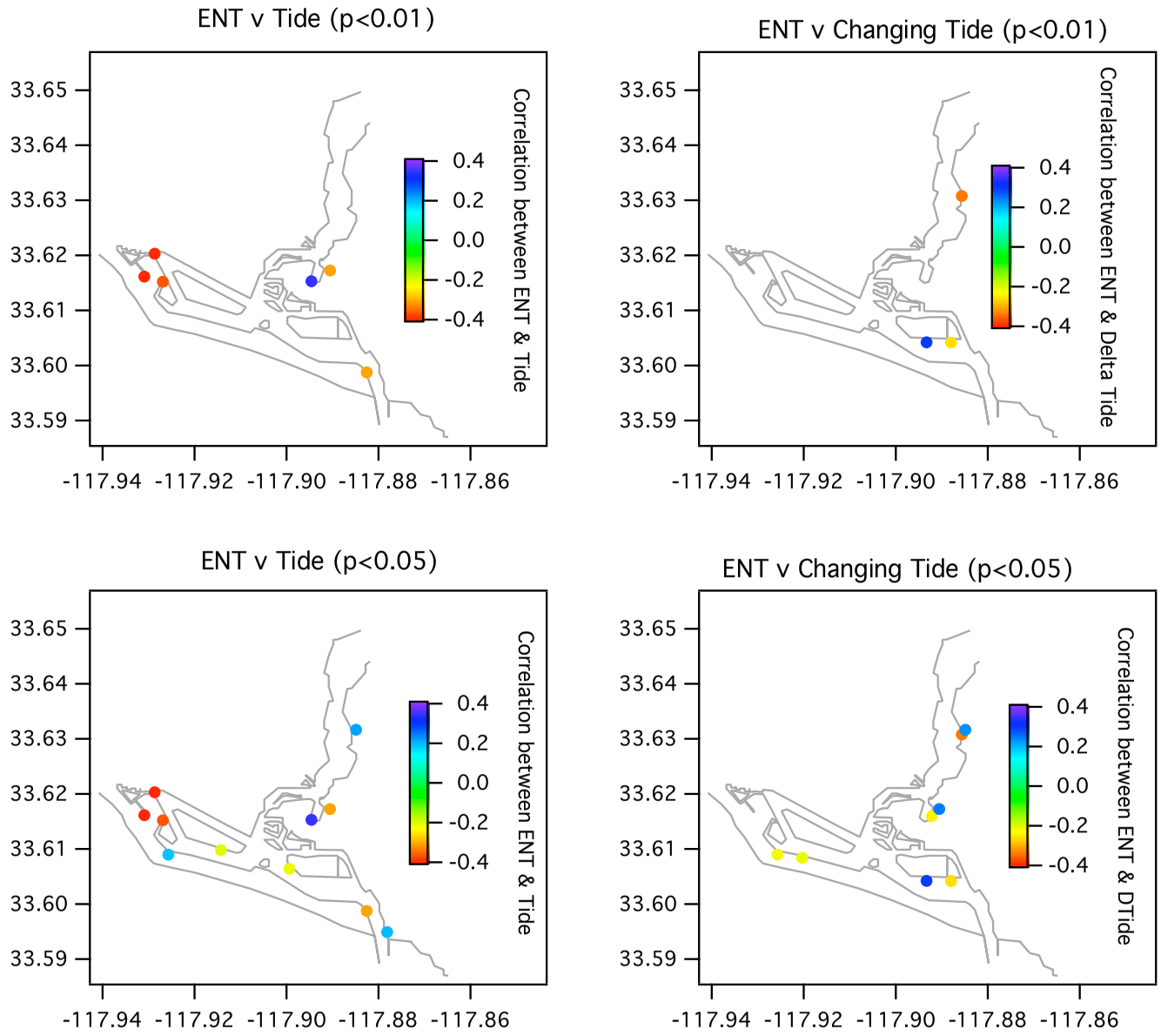

Figure 7-10. Map of OCHCA sampling sites in Newport Bay, where the color denotes the Spearman Rank correlation between ENT and tide (left panels), and ENT and changing tides (right panel). The upper and bottom panels denote correlations with significance cutoffs of p<0.01 (top panels) and p<0.05 (bottom panels).

For each sample collected, two parameters were determined: (1) the tide level (m, MSSL) at the time of sampling (referred to here as TIDE), and (2) the change in tide level (m/h) over the hour preceding the time of sampling (referred to here as DELTA TIDE). Statistical tests were then conducted to determine if the ENT concentration measured in the samples is significantly correlated with either TIDE or DELTA TIDE.

Calculations were carried out as follows.

1. All sampling times provided by OCHCA were corrected to local standard time by subtracting 1 hour from the recorded time when the sample date fell in one of the daylight savings time periods listed in Table 7B-2 (see Appendix 7B).
2. Tide levels predicted for the entrance to Newport Harbor (id # 9410580) were downloaded from the NOAA website (http://tidesandcurrents.noaa.gov) for the time period 3/1/01 through 2/22/05. The predictions had a sampling frequency of 1/6 min^{-1}, for a total of 380,145 predictions.
3. Using the sampling times provided by OCHCA, predicted tide levels from the NOAA website were interpolated to obtain TIDE values for every sample collected from the 35 Newport Bay sampling sites for the time period 3/1/01 through 2/22/05.
4. NOAA predicted tide levels were interpolated to obtain DELTA TIDE values for every sample collected from Newport Bay by OCHCA over the time frame 3/1/01 through 2/22/05. DELTA TIDE was obtained by subtracting the tide level predicted at the sampling time, from the tide level predicted one hour prior to the sampling time. The sign of DELTA TIDE indicates whether the tide is rising (DELTA TIDE > 0) or falling (DELTA TIDE < 0) at the time of sampling.
5. All samples collected by OCHCA from Newport Bay over the time frame 3/1/01 through 2/22/05 were separated into two groups: a) samples collected when rain advisories were in effect, and b) samples collected when no rain advisories were in effect. Rain advisories periods were obtained from OCHCA, and are listed in Table 7B-3 (Appendix 7B).
6. Spearman Rank Correlations (denoted "Sp"), a non-parametric statistic for the evaluation of correlation between two different variables, were calculated between the ENT concentration measured in a sample, and the sample's TIDE and DELTA TIDE values. These correlations were carried out separately for samples collected when rain advisories were in effect, and for samples collected when no rain advisories were in effect.

Steps 1) through 5) were implemented using the graphics and data analysis software Igor (Wavemetrics, Oswego, OR). Step 6) was implemented using Statistical Package for the Social Sciences (SPSS v. 13, SPSS Inc., Chicago, Ill).

7.5.1. Results

Sampling Times and Tidal Conditions. The 35 sampling sites in Newport Bay were sampled by OCHCA approximately 100 and 20 times during dry and wet weather,

respectively (see Fig. 7-8 for sampling locations and names of OCHCA codes for sampling sites). Over the nearly four year period encompassed by this study—from March 6, 2001 to February 22, 2005—virtually all samples were collected by OCHCA between 6:00AM and noon (Fig. 7B-1, Appendix 7B). Sampling times at any particular site varied relatively little from week-to-week; i.e., every sampling site in Newport Bay was sampled at nearly the same time of day every week.

While the sampling time at a particular site was relatively invariant, tide conditions at the time of sampling varied significantly for all sites, ranging from large positive (>2 m, MLLW) to negative (<0 m, MLLW) tides, and from rapidly rising (>0.4 m/h) to rapidly falling (<-0.4 m/h) tides (Fig. 7B-2, Appendix 7B). Hence, if water quality in Newport Bay is affected by either tide level (TIDE) or changing tide level (DELTA TIDE), such effects should be evident in the water quality data collected by OCHCA.

Dry Weather ENT Concentrations. During dry weather periods, ENT concentrations exhibit considerable site-to-site variability, as assessed by both the log-mean ENT concentration and the frequency that samples exceed the California single-sample marine bathing water standard for ENT (upper panels, Fig. 7-9). ENT concentrations and exceedence rates during dry weather are highest at the following sampling sites: Western Newport Bay (BNB09, BNB11, BNB35), Big Canyon Creek (CNBBC), Vaughn's Launch (BNB25), Santa Ana Delhi Channel (CNBSA), San Diego Creek (CNBCD), a drain in the Dunes Recreation area (CNBND), and Harbor Patrol Beach (BNB33).

Wet Weather ENT Concentrations During storm advisories, the log-mean ENT concentration shifts upward at most sites by about 0.5 log units, although some sites experience more of an increase than others (lower panels, Fig. 7-9). Wet weather log-mean ENT concentration and single-sample exceedence rates are highest at the following sites: Western Newport Bay, Turning Basin, and Rhine Channel (BNB09, BNB10, BNB11, BNB35), Big Canyon Creek (CNBBC), Vaugh's Launch (BNB25), Santa Ana Delhi Channel (CNBSA), San Diego Creek (CNBCD), the ski zone in Upper Bay (BNB26), the Dunes Recreation area (CNBND, BNB24N, BNB24E, BNB24M, BNB24W), Onyx Beach Avenue on the north shore of Balboa Island (BNB02), Harbor Patrol Beach (BNB33), Via Genoa Beach on Lido Isle (BNB07), and Rocky Point Beach (BNB23).

Dry Weather Correlations between ENT and TIDE. ENT concentrations are significantly ($p<0.01$ or $p<0.05$) and modestly ($Sp > 0.3$ or $Sp < -0.3$) correlated with TIDE values at 6 sites in Newport Bay, including three sites in Western Newport Bay (BNB35, BNB11, and BNB12), a beach site and drain in the Dunes Recreation area (BNB24W and CNBND), and a site near the Harbor Entrance (BNB 22) (top left panel, Fig. 7-9). The three sites in Western Newport Bay all exhibit modest negative correlations between ENT and TIDE (i.e., $Sp = -0.3$ to -0.5), suggesting that ENT concentrations are higher when tide levels are low. Negative correlations between tide level and ENT concentrations are also observed at BNB 22 and CNBND. The Dunes Recreation area (BNB24W), on the other hand, exhibits a positive correlation between ENT and tide level, implying that ENT concentrations there are higher when the tide level is high. At

the $p<0.05$ level of significance, ENT is significantly correlated with TIDE over more stations (bottom left panel, Fig. 7-9), although the correlations are relatively weak at most of these sites (generally $Sp<0.3$ and $Sp>-0.3$). The negative correlations between ENT and TIDE observed at most sites, particularly those in Western Newport Bay, are consistent with the storm drain model presented in the last model, which predicts that runoff mixed with Bay water flows out of the drains into the Bay at low tides. The positive correlation between ENT and TIDE observed at a few sites (e.g., BNB24W), may indicate that the source of FIB is located at higher elevations on the beach; e.g., wrack lines and bird droppings.

Wet Weather Correlations between ENT and TIDE During rain advisories, none of the sampling stations exhibit a significant ($p<0.01$) and moderate ($Sp>0.3$ or $Sp<-0.3$) correlation between ENT and TIDE (see Table 7B-4 in Appendix 7B). The lack of significant correlation could be due to the lower N values associated with rain advisories. Also, during rain advisories, the timing of the last rain event (relative to sampling) may have a greater impact on water quality at a site than does whether the tide is high or low at the time of sampling.

Dry Weather Correlations between ENT and DELTA TIDE. During dry weather, change in tide level (i.e., whether the tide is rising or falling at the time a sample is collected) is not a strong predictor of ENT measured in OCHCA water samples. Using a cut-off of $p<0.01$, only three sites exhibit a significant correlation between ENT and DELTA TIDE, including two sites on the southern shore of Balboa Island (BNB 20 and 21) and one site on the eastern shore in Upper Bay (BNB 25) (upper right panel, Fig. 7-9). The number of significant correlations increases if the cut-off is raised to $p<0.05$ (lower right panel, Fig. 7-9), but the correlations are weak in all cases. Further, the nature of the correlation between FIB and DELTA TIDE appears to be site specific, with some sites indicating higher ENT during rising tides (positive correlations), and other sites indicating higher ENT during falling tides (negative correlations).

Wet Weather Correlations between ENT and DELTA TIDE. During rain advisories, none of the sampling stations exhibit a significant ($p<0.01$) and moderate correlation between ENT and DELTA TIDE (see Table 7B-4 in Appendix 7B).

7.6. FIB in Runoff Collected from Storm Drains and Irrigation Water

From the results of the synoptic study and the historical analysis of OCHCA data described above, it appears that dry and wet weather runoff may be a significant source of FIB in the near shore waters of Lower Bay. To better quantify the magnitude of this FIB source, 21 storm drains in Lower Bay were singled out for detailed study. Two samples were collected at each storm drain site: (1) a mixture of runoff and Bay water collected from near, or at, the outlet of the drainpipe; and (2) irrigation runoff collected from the curb of the nearby street. The entire study was conducted twice, once during a dry weather period and once during a wet weather period.

The inclusion of irrigation runoff in the sampling design (item 2 above) was motivated by the realization that ecological transformations in the storm sewer system (e.g., selection of certain strains or species of FIB suited for survival and/or regrowth in the drain environment) could affect FIB speciation and concentration. Hence, the concentrations of FIB in irrigation water collected from the street level may differ from the concentrations of FIB that flow into the Bay from the drainpipe outlet.

7.6.1. Methods

Storm drain and irrigation runoff sampling sites were selected to collectively capture a variety of geographical areas and land-use types around Lower Bay, including the mainland side of Lower Bay, Balboa Island, Lido Isle, and the Balboa Peninsula (Fig. 7-11). The drains also had to be easily accessible by car, and the drainpipe outlet needed to be accessible by foot at low tide. Latitudes and longitudes for all sampling sites are included in a table (see Table 7C-1, Appendix 7C).

Sampling was conducted in the early morning hours to coincide with a peak in irrigation runoff from the surrounding landscape (personal observation, K. McLaughlin) and during low tide so that runoff would likely affect Bay water quality in the immediate vicinity of the drainpipe outlet (see Fig. 7-7 for a conceptual picture of how Bay water intrudes into storm drains in Lower Bay during rising tides). Storm drain runoff was collected by sampling the mixture of runoff and Bay water near the outlet of the drainpipe outlet; in a few cases, the end of the drainpipe was exposed at low tide, and in these cases runoff flowing out of the drainpipe was sampled directly. In the results section, we refer to these samples as "near-drain" samples. All near-drain samples were collected in 100 mL sterile bottles. Irrigation runoff was sampled from the curb at a nearby street (usually located immediately adjacent to the storm drain). Ponded or flowing runoff in the street curb was siphoned into a sterile 50 mL syringe, and the siphoned water was then slowly dispensed into a sterile 100 mL sample bottle. All water samples were analyzed for FIB (TC, EC, and ENT), salinity, and pH using procedures described in Chapter 2.

7.6.2. Results

Timing of Runoff Sampling. Near-drain and irrigation samples were collected at all 21 sites during two separate sampling excursions, once on November 16 (2006) and again on January 31 (2007). As indicated in the inset of Fig. 7-11, rain fell both the day before, and the day of, the January 31st sampling (0.04 and 0.01 inches of rain were recorded at Newport Beach on January 30 and 31, respectively). No rainfall was recorded on November 16, although a small amount of rainfall was recorded in the Newport Beach area several days earlier (0.08 and 0.01 inches of rain recorded by the Newport gauge on November 14 and 15th, respectively). In the analysis presented below, we refer to the November 16 and January 31 sampling events as the "dry weather" and "wet weather" sampling events, respectively. However, the rain history suggests that runoff samples collected on November 16th may have been impacted by the very small amount of rainfall that occurred several days earlier.

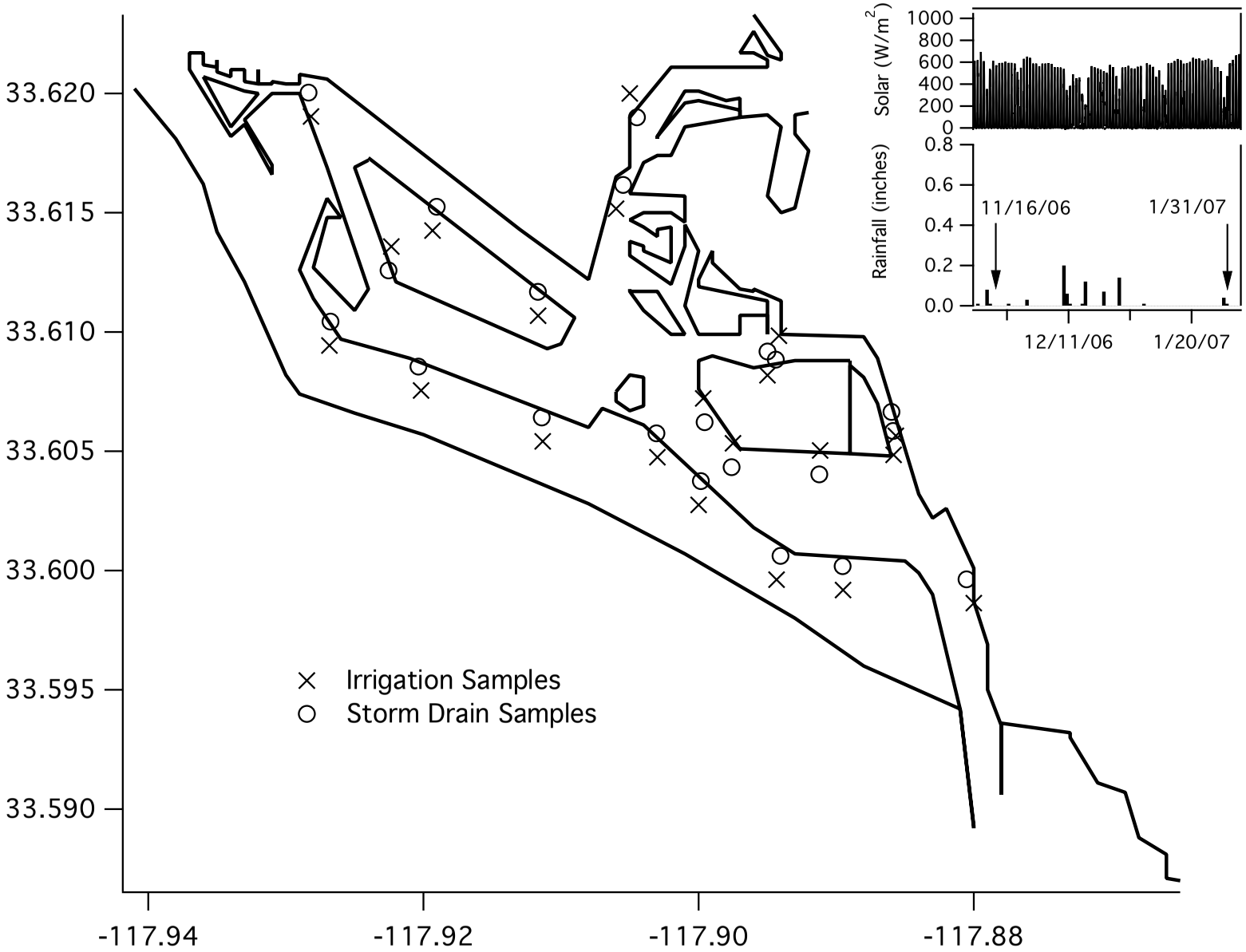

Figure 7-11. Map of near-drain and irrigation sampling sites in Newport Bay. Inset shows measured precipitation and solar irradiance during the two sampling events, on 11/16/06 and 1/31/07.

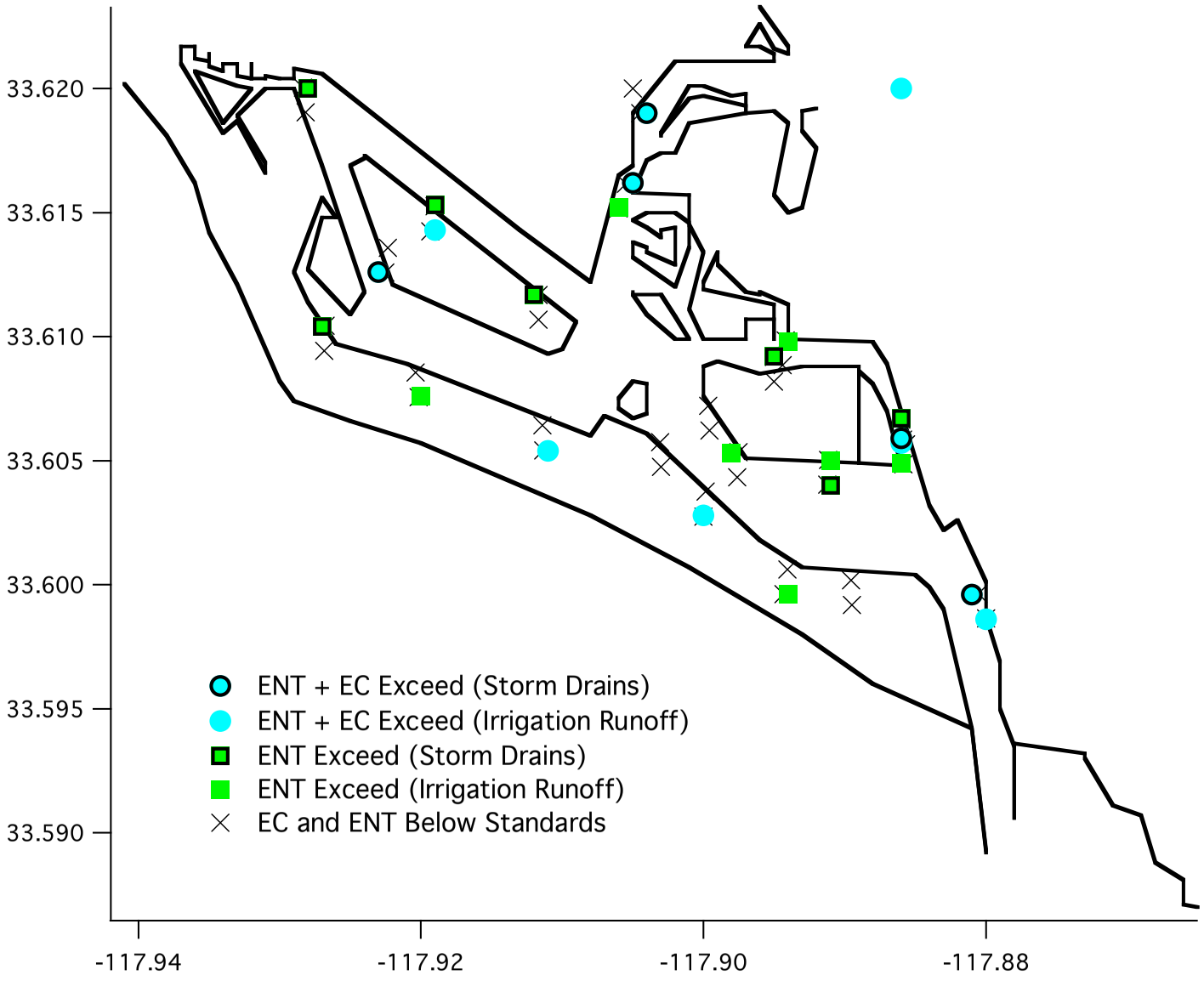

Figure 7-12. Map of Lower Bay showing where water samples exceeded single-sample standards for ENT (green squares), or both EC and ENT (light blue circles) during the dry weather sampling of near-drain and irrigation runoff on November 16 (2006). Crosses denote the location of samples that did not exceed either EC or ENT standards. No samples exceeded just the EC standards.

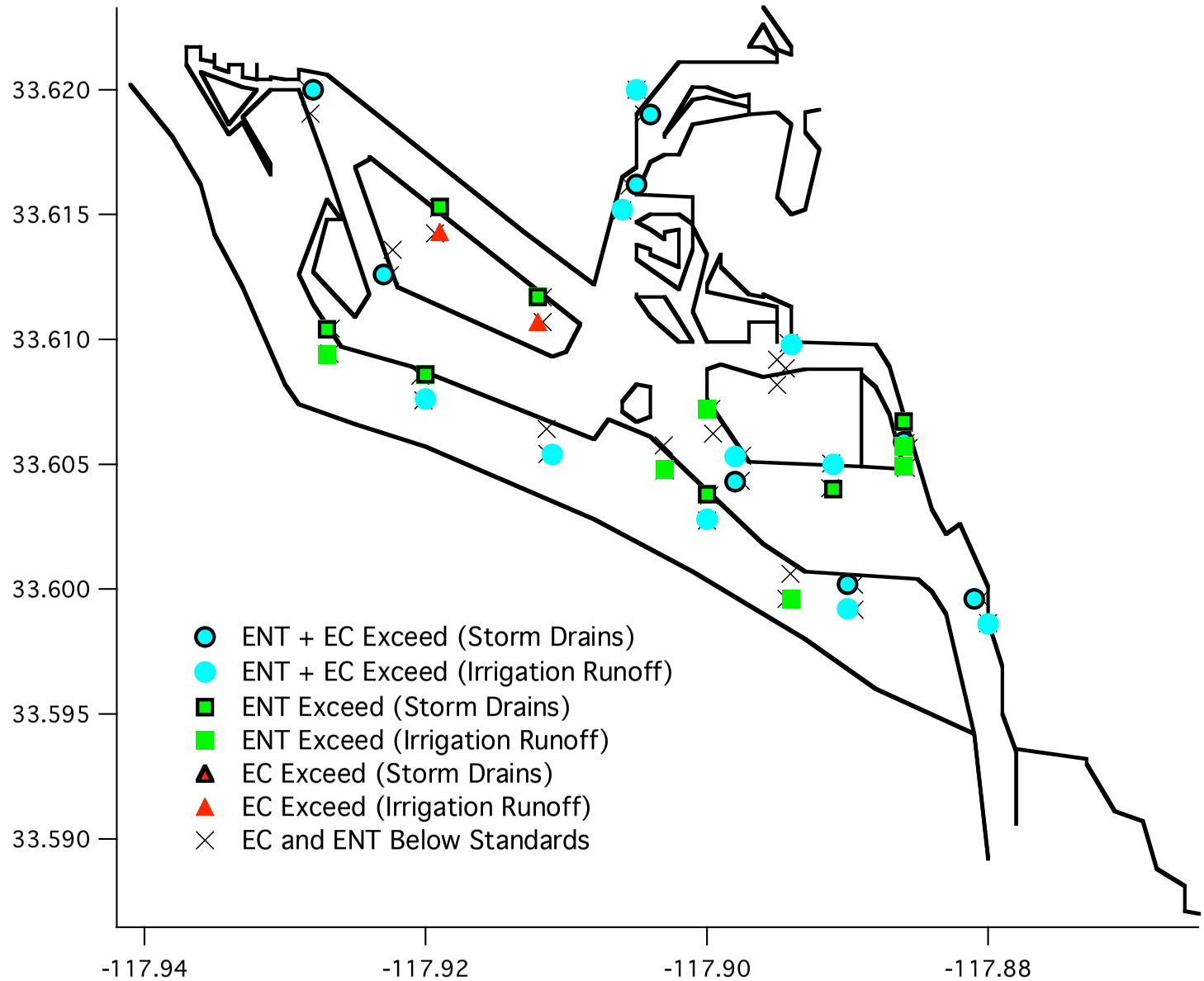

Figure 7-13. Map of Lower Bay showing where water samples exceeded single-sample standards for ENT (green squares), EC (red triangles), or both EC and ENT (light blue circles) during the wet weather sampling of near-drain and irrigation runoff on January 31 (2007). Crosses denote the location of samples that did not exceed either EC or ENT standards.

Table 7-3. FIB measurement statistics for the Lower Bay runoff study.

		Enterococcus spp. (MPN/100 mL)			***E. coli (MPN/100 mL)***		
Sample Location	Weather	N	Log-mean[a]	% > s.s.s.[b]	N	Log-mean[a]	% > s.s.s.[b]
Near-Drain	Dry	21	2.2 +/- 1.4	57	21	2.1 +/- 0.9	24
Near-Drain	Wet	21	2.7 +/- 1.0	71	21	2.3 +/- 1.1	38
Irrigation	Dry	21	3.1 +/- 1.4	67	21	1.8 +/- 1.7	29
Irrigation	Wet	21	2.8 +/- 1.1	76	21	2.5 +/- 1.6	62

[a]Average and standard deviation of log-transformed FIB concentrations
[b]Percent of samples with FIB concentrations over California marine bathing single-sample standards (s.s.s.) and 2005 USEPA criteria for marine recreational waters.

Measurements on Near-Drain and Irrigation Water. Samples collected from near storm drain outlets have a broad range of salinities (from <2 to 32) and relatively uniform pH (from 7.7 to 8.3) (see Tables 7C-2 and 7C-3 and Figs. 7C-1, 7C-2, and 7C-3, Appendix 7C). Samples of irrigation runoff have lower salinities (< 2) and relatively broad pH range (from <7 to >8.5). Median salinity increases in the order: wet weather irrigation runoff < dry weather irrigation runoff << wet weather near-drain runoff < dry weather near-drain runoff. Median pH increases in the order: wet weather irrigation runoff < dry weather irrigation runoff < dry weather near-drain runoff = wet weather near-drain runoff.

Table 7-3 presents statistics on the FIB data collected during the near-drain and irrigation sampling; the raw FIB data are presented in both tabular and graphical form in Appendix 7C (Tables 7C-2 and 7C-3; Figs. 7C-4 and 7C-5). A relatively large percentage of samples collected during this study had EC and/or ENT concentrations above the California single-sample marine bathing water standards. The percentage of samples with ENT concentrations above the standards decreased in the order: wet weather irrigation runoff (76%) > wet weather near-drain samples (71%) > dry weather irrigation runoff (67%) > dry weather near-drain samples (57%). The percentage of samples with EC concentrations above the standards decreased in precisely the same order: wet weather irrigation runoff (62%) > wet weather near-drain runoff (38%) > dry weather irrigation runoff (29%) > dry weather near-drain runoff (24%).

It should also be noted that probability plots of the raw FIB data reveal a very broad distribution of FIB measurements, ranging from the lower limit of detection of 10

MPN/100 mL to > 24,000 MPN/100 mL (Appendix 7C). Compared to the synoptic study presented earlier, FIB concentrations measured during the near-drain study are generally higher, and a larger percentage of samples exceed single-sample standards for EC and/or ENT.

The next two maps of Lower Bay show near-drain and irrigation sites where water samples exceeded one or both single-sample standards for EC and ENT (Figs. 7-12 and 7-13). More near-drain and irrigation samples exceeded single-sample standards during wet weather (Fig. 7-13) compared to dry weather (Fig. 7-12). However, water quality exceedences occurred uniformly across Lower Bay (i.e., there was no single " hot spot" in the Bay which accounted for the majority of water quality exceedences). This result suggests that, regardless of its source, irrigation runoff typically has high concentrations of FIB. Furthermore, water samples collected from the Bay near storm drain outlets tend to have elevated FIB concentrations, particularly in cases where the presence of runoff in the samples is suggested by depressed salinity (see cross-plots of EC and ENT color coded by salinity, Fig. 7C-6, Appendix 7C).

7.7. Summary and Discussion

Sewage Contamination of Shallow Groundwater. A series of exploratory investigations were carried out to identify regions of the Lower Bay where bathing water quality might be impacted by the efflux of sewage-contaminated shallow groundwater. The main findings are:

1. Sewer collections lines in Lower Bay vary in age and composition. Older sewer lines, some of which were laid in the early 1920s, are composed primarily of vitrified clay pipe (VCP) with cement joints. Newer sewer lines are composed primarily of polyvinyl chloride (PVC) with rubber-gasket wedge-lock joints. The City of Newport Beach is implementing programs to replace the older sewer lines as new development and/or significant remodeling occurs.
2. Five sites in Lower Bay were selected for shallow groundwater testing, based on their proximity to sewer lines of various age and material composition, proximity to recreational beaches, and accessibility. Samples of shallow groundwater from these sites had oceanic salinities.
3. EC concentrations in the shallow groundwater were below the California single-sample standard for marine bathing waters at all five shallow groundwater testing sites. ENT concentrations, on the other hand, were above the California single-sample standard for marine bathing waters at two of the shallow groundwater testing sites. One site (at 9th street on the Balboa Peninsula, ENT = 292 MPN/100 mL) is near a sewer main constructed of VCP and installed in 1931. The other site (at Alvarado Place on the Balboa Peninsula, ENT= 1118 MPN/100 mL) is located near a sewer main constructed of PVC and installed in 1985.

Given that ENT concentrations were somewhat elevated at one site (9th Street) and significantly elevated at another site (Alvarado Place), and given that 2 of 5 shallow

groundwater sites had elevated FIB concentrations, further testing of the shallow groundwater in Lower Bay may be warranted.

Synoptic Study of Lower Newport Bay. The goal of the synoptic survey was to measure water quality at 80 to 90 locations along the shoreline in Lower Bay during a dry weather period in the summer, and to determine how water quality there is modulated by: (1) tide conditions (high tide vs. low tide), and (2) cross-shore location (water's edge, 100 feet bay-ward). The main findings are:

1. For a fixed tide condition (i.e., low or high tide), water samples collected closer to shore have lower salinity, lower pH, and higher EC and ENT concentrations.
2. For a fixed cross-shore location (i.e., water's edge or 100 feet bay-ward), samples collected at low tide have lower salinity, lower pH, and higher EC and ENT concentrations.
3. During low tide, more samples exceed the single-sample marine bathing water standards for EC and ENT. Regions prone to water quality exceedences include: (1) Turning Basin in West Newport (near the Arches Drain); (2) north-east shore of Lido Isle; (3) north-east shore of Balboa Peninsula; (4) east shore of Balboa Island (near the El Paseo Drain); and (5) Rhine Channel.
4. During low tide, the same four regions of Lower Bay have relatively low salinity.
5. During high tide, few samples exceeded single-sample standards for EC and ENT.
6. FIB contamination along the shoreline in Lower Bay may be affected by the episodic discharge of runoff to the Bay by tidal flow in storm drains.

These results demonstrate that water quality along the shoreline in Lower Bay is strongly modulated by the tides—with higher FIB concentrations (and more frequent FIB standard exceedences) occurring during low tides, and lower FIB concentrations (and less frequent FIB standard exceedences) occurring during high tides. This tidal signature, together with the association between elevated FIB concentrations and depressed salinity, suggest that runoff flowing into storm drains in Lower Bay may adversely impact water quality along the shoreline in Newport Bay.

OCHCA's ENT Monitoring Data for Newport Bay. The Orange County Health Care Agency (OCHCA) collects water samples weekly from 35 sites in Newport Bay and analyzes the samples for TC, FC, and ENT concentrations. These data were analyzed with two goals in mind: (1) to identify regions of the Bay where water quality is consistently poor during dry and/or wet weather, and (2) to determine if water quality is modulated by the tides at storm drain impacted sites. The main findings are:

1. During dry weather, ENT concentrations are highest at the following sampling sites: Western Newport Bay (BNB09, BNB11, BNB35), Big Canyon Creek (CNBBC), Vaughn's Launch (BNB25), Santa Ana Delhi Channel (CNBSA), San Diego Creek (CNBCD), a drain in the Dunes Recreation area (CNBND), and Harbor Patrol Beach (BNB33).
2. During storm advisories, the log-mean ENT concentration shifts upward at most sites by approximately 0.5 log units, although some sites experience more of an

increase than others. Sites with the greatest storm-related increase in ENT concentration include: BNB23 (Rocky Point Beach), BNB10 (38th Street Beach), BNB30 (De Anza, near the PCH bridge), BNB24M (Middle Station at Newport Dunes), and the San Diego Creek (CNBCD).

3. During dry weather, ENT concentrations are significantly and modestly negatively correlated with tide levels at 6 sites in Newport Bay, including three sites in Western Newport Bay and Rhine Channel (BNB35, BNB11, and BNB12), a beach site and drain in the Dunes Recreation area (BNB24W and CNBND), and a site near the Harbor Entrance (BNB 22). Negative correlations between tide level and ENT concentrations are also observed at BNB 22 and CNBND. The Dunes Recreation area (BNB24W), on the other hand, exhibits a positive correlation between ENT and tide level.
4. During rain advisories, none of the sampling stations exhibit a significant correlation between ENT and either tide level or change in tide level. Thits lack of significant correlation may be due to the relatively small sample size for the FIB measurements.

ENT concentrations are higher during low tides at several OCHCA monitoring sites in Newport Bay. These sites tend to be located in "dead-end" regions of the Bay—such as Western Newport Bay, Rhine Channel, and the Dunes Recreation area—with limited tidal mixing, long water residence times, and significant sources of runoff. Change in tide level (i.e., whether the tide is rising or falling at the time a sample is collected), on the other hand, does not appear to be a predictor of water quality at most sites.

Irrigation Runoff and Storm Drains. From the results of the synoptic study and the historical analysis of OCHCA data described above, it appears that dry and wet weather runoff can be a significant source of FIB in the nearshore waters of Lower Bay. To better quantify the magnitude of this FIB source, 21 storm drains in Lower Bay were singled out for detailed study. Two samples were collected at each storm drain site: (1) a mixture of runoff and Bay water collected from near, or at, the outlet of the drainpipe (called near-drain sites); and (2) irrigation runoff collected form the curb of the nearby street. The entire study was conducted twice, once during a dry weather period and once during a wet weather period. The main findings are:

1. A relatively large percentage of samples of both irrigation water and near-drain water had EC and/or ENT concentrations above the California single-sample marine bathing water standards (and US EPA's marine bathing water criteria).
2. The percentage of samples with ENT concentrations above water quality standards decreased in the order: wet weather irrigation runoff (76%) > wet weather near-drain samples (71%) > dry weather irrigation runoff (67%) > dry weather near-drain samples (57%).
3. The percentage of samples with EC concentrations above water quality standards decreased in precisely the same order: wet weather irrigation runoff (62%) > wet weather near-drain runoff (38%) > dry weather irrigation runoff (29%) > dry weather near-drain runoff (24%).

4. Nearly all storm drain samples with relatively low salinity (<25) also exceeded both the EC and ENT standards, implying that low salinity is a good predictor of poor bathing water quality near storm drain outlets in Lower Bay.
5. The main result described here—that low salinity is a risk factor for poor water quality around the outlet of storm drains in Lower Bay--is consistent with the conceptual model presented earlier of how runoff from stormdrains impacts water quality in Lower Bay.
6. The water quality exceedences occurred across all of Lower Bay (i.e., there was no single " hot spot" in the Bay which accounted for the majority of water quality exceedences).

From these data, it appears that dry and wet weather runoff is a significant source of FIB in the nearshore waters of Lower Bay, as evidenced by (1) the high concentrations of FIB measured in samples of irrigation runoff; (2) the high concentrations of FIB measured in samples of Bay water collected from near the outlet of storm drain during both dry and wet weather; and (3) the fact that, in samples of Bay water, elevated FIB concentrations are frequently associated with low salinity. By way of comparison, the following runoff drains that outlet to Newport Bay have low-flow diversions in place[7]:

1. Back Bay Drive Diversion (operated by the City of Newport Beach).
2. Four small plug type diversions for the storm drains in the Newport Dunes parking lot (operated by the Newport Dunes maintenance staff).

In addition to implicating dry weather runoff from storm drains in Lower Bay as a source of FIB pollution in near shore waters, these results also suggest that salinity may be a good (i.e., reliable, fast, and cheap) proxy for FIB contamination.

[7] This information kindly supplied by John Kappeler, City of Newport Beach.

[1]Chapter 8. FIB Loading from Urban and Natural Sources and Impact on Newport Bay Water Quality

8.1. Introduction and Overview.

This chapter provides first-order estimates of the impact that urban and natural sources of FIB have on shoreline water quality in Newport Bay. These estimates are intended to guide the assignment of Waste Load Allocations (WLAs) as part of the fecal coliform TMDL management plan. One difficulty encountered when developing WLAs for FIB is the fact that water quality exceedences—the critical condition of interest—occur in the very shallow (*ca.*, ankle depth) waters along the shoreline. As described earlier in Chapter 1, water quality along the shoreline is affected by both Bay-wide events (e.g., the impact of storm flow from the San Diego Creek on water quality over the entire Bay, see Chapter 2), and highly localized and transient shore-based non-point sources of FIB from storm drains, bird droppings and environmental growth on wrack lines and in beach sediments (see microcosm results and DITS study, Chapters 5 and 6).

To address the complexity just noted, in this chapter we evaluate the water quality impact of FIB loads from urban and natural sources at three distinct scales:

1. Bay Scale: Bay-wide water quality impact of FIB loading from San Diego Creek and Santa Ana Delhi Channel.
2. Beach Scale: Local water quality impact (in ankle depth waters) of FIB loading from distributed non-point sources along the shore.
3. Drain Scale: Local water quality impact (in ankle depth waters) of FIB loading from individual storm drains.

For each of these three scales, water quality impacts are evaluated using a two-step process. First, FIB loading rates are estimated for the specific FIB source of interest. In some cases this is a straightforward calculation, involving the multiplication of measured concentrations by measured flow rates (e.g., FIB loading from San Diego Creek). In other cases, FIB loading rates are estimated based on a model of how FIB are mobilized into the water column from a particular source (e.g., FIB loading from resuspension of contaminated sediment along the shoreline). Second, once the FIB loading rate associated with a specific source has been estimated, simple mass balance models are used to predict the consequent water quality impacts at an appropriate spatial scale. While more sophisticated models could be employed to translate FIB loads into shoreline FIB concentrations, the approach adopted here (of focusing on three distinct scales separately, and employing simple analytical models to convert loading estimates to FIB concentrations) conforms to EPA's Principle of Parsimony; namely, that the optimal WLA model is the simplest approach that captures "all the important estuarine phenomena affecting water quality" [2].

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[1] Prepared by Dr. S. Grant

[2] "EPA Technical Guidance Manual for Performing Wasteload Allocations, Book III: Estuaries – Part1: Estuaries and Wasteload Allocation Models", EPA Document Number EPA 823/R-92-002 (1990)

This chapter satisfies the following sub-tasks of the Prop 13 project, by reporting on FIB loading rates for urban and natural sources in Newport Bay:

- Task 2.1.1.4. Loading rates between Upper and Lower Bay; loading rates from watershed outlets; loading rates from natural and within-Bay sources.
- Task 2.1.3.7. Loading rates from natural sources, including turbulent resuspension of FIB contaminated sediment, release from bird droppings, debris mats, and wrack lines.
- Task 2.1.2.4 and 2.1.2.5. Loading rates from 20 Lower Bay storm drains of varying size.

8.2. Bay Scale: Water Quality Impact of FIB loading from San Diego Creek and Santa Ana Delhi Channel

In this section we utilize daily measurements of flow and FIB concentration in San Diego Creek and Santa Ana Delhi Channel to estimate daily loading rates to the head of the estuary. These loading rate data are then used as input to a simple analytical model that predicts the Bay-wide water quality degradation attributable to loading from San Diego Creek and Santa Ana Delhi Channel.

8.2.1. FIB loading rates from San Diego Creek and Santa Ana Delhi Channel

8.2.1.1. Methods

Flow Measurements. Volumetric flow rates for San Diego Creek and Santa Ana Delhi channel were provided by Orange County Public Works (OCPW). Provisional flow measurements from OCPW had a sampling frequency of 1/30 min^{-1}. For the loading rate calculations described below, these high frequency data were averaged to obtain daily-average flow rates for both creeks. At both sites, stream discharge was computed from measured stage using a Windows Software Hydrologic software program (XStream Measures, Auberry, CA).

Sampling of Tributaries and Water Quality Measurements. Water samples were collected from the San Diego Creek and Santa Ana Delhi channel on a near-daily basis for over nine months, from March 7 through November 29, 2006. Prior to March 7 and after November 29, tributary sampling occurred approximately weekly, coincident with BTO transect studies in Newport Bay (see Chapter 2). Tributary sites were accessed by car and on foot. Temperature of the water surface was recorded with an infrared gun (Raynger-ST, Raytek, Santa Cruz, CA); note that water temperature measurements were conducted intermittently. Water samples were collected from the surface of the water column, capped, placed on ice, and transported back to the laboratory at UCI for analysis. Back at UCI, water samples were analyzed for a suite of water quality parameters, as described earlier in Chapter 2. In this chapter we focus on measurements of FIB (*E. coli* and *Enterococcus* species, denoted here as EC and ENT, respectively), temperature, and salinity.

FIB Loading Rates. FIB loading rates L_{FIB} (units, bacteria per time) for San Diego Creek and Santa Ana Delhi were calculated by multiplying daily measurements of FIB concentration, C_{FIB}, by measurements of daily-average flow, Q_{creek}:

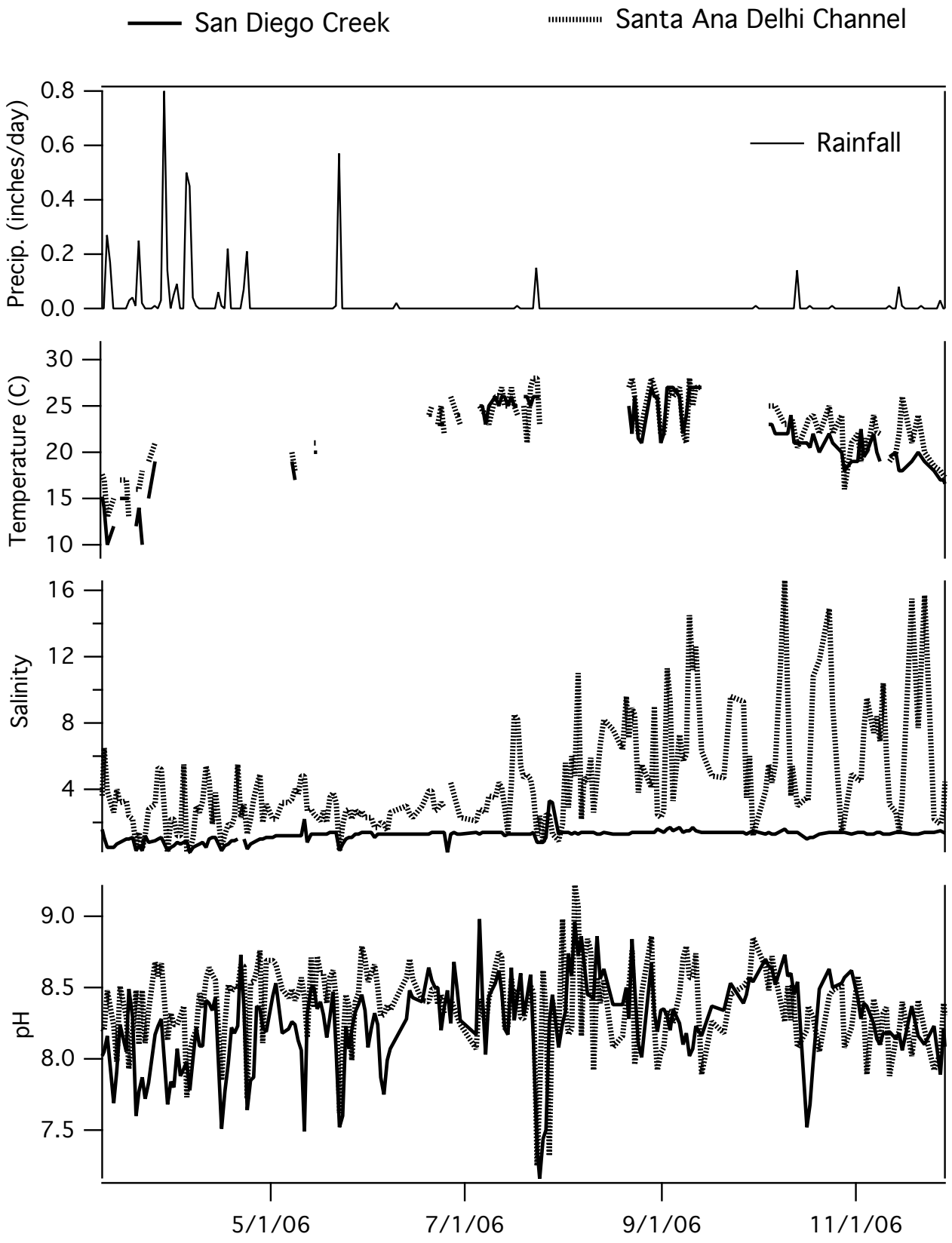

Figure 8-1. Time series measurements of temperature, salinity, and pH in San Diego Creek and Santa Ana Delhi Channel. Also shown is the rainfall history for Newport Beach.

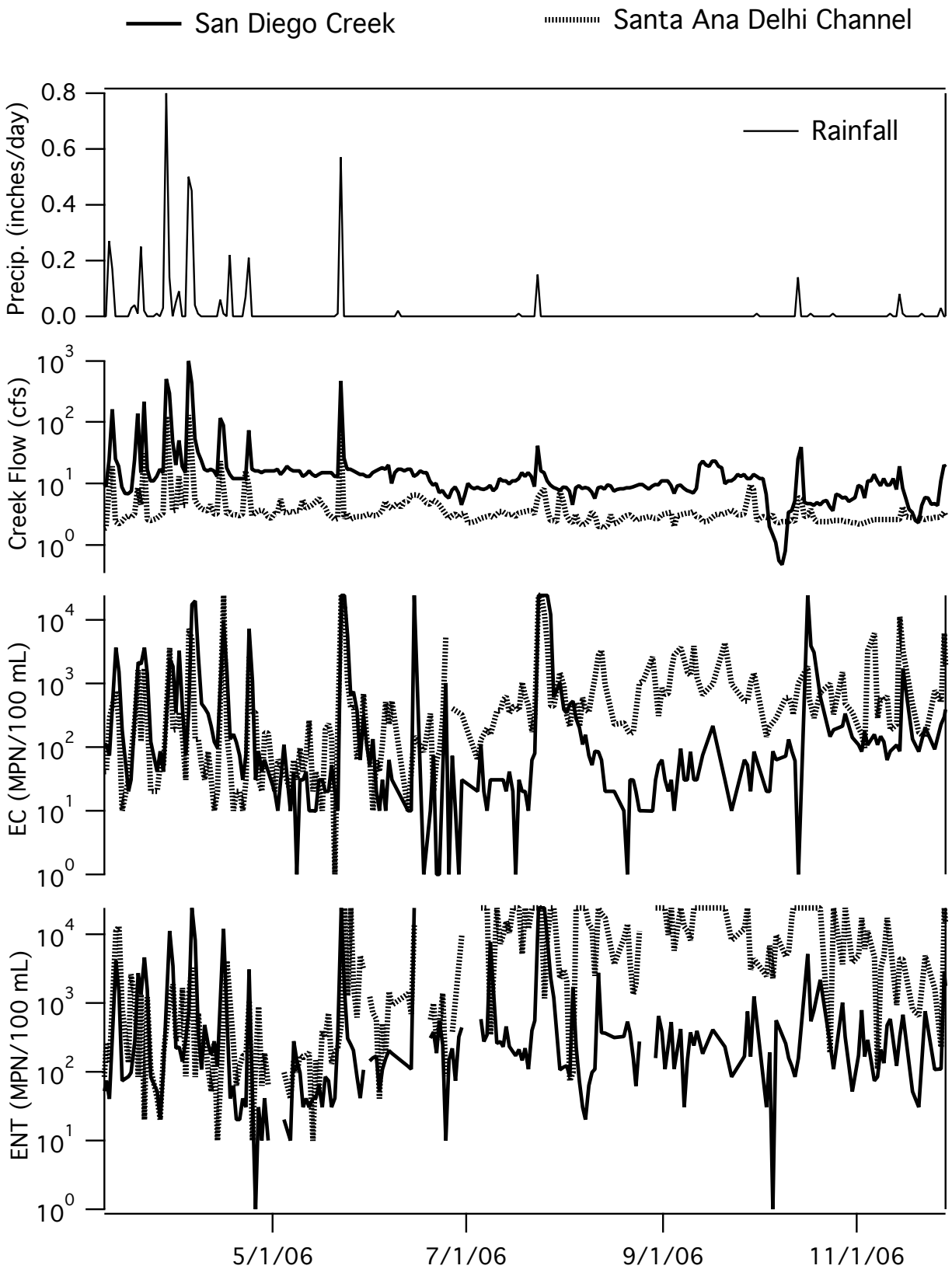

Figure 8-2. Time series measurements of *Escherichia coli* (EC) and enterococci bacteria (ENT) in San Diego Creek and Santa Ana Delhi channel. Also shown is the rainfall history for Newport Beach.

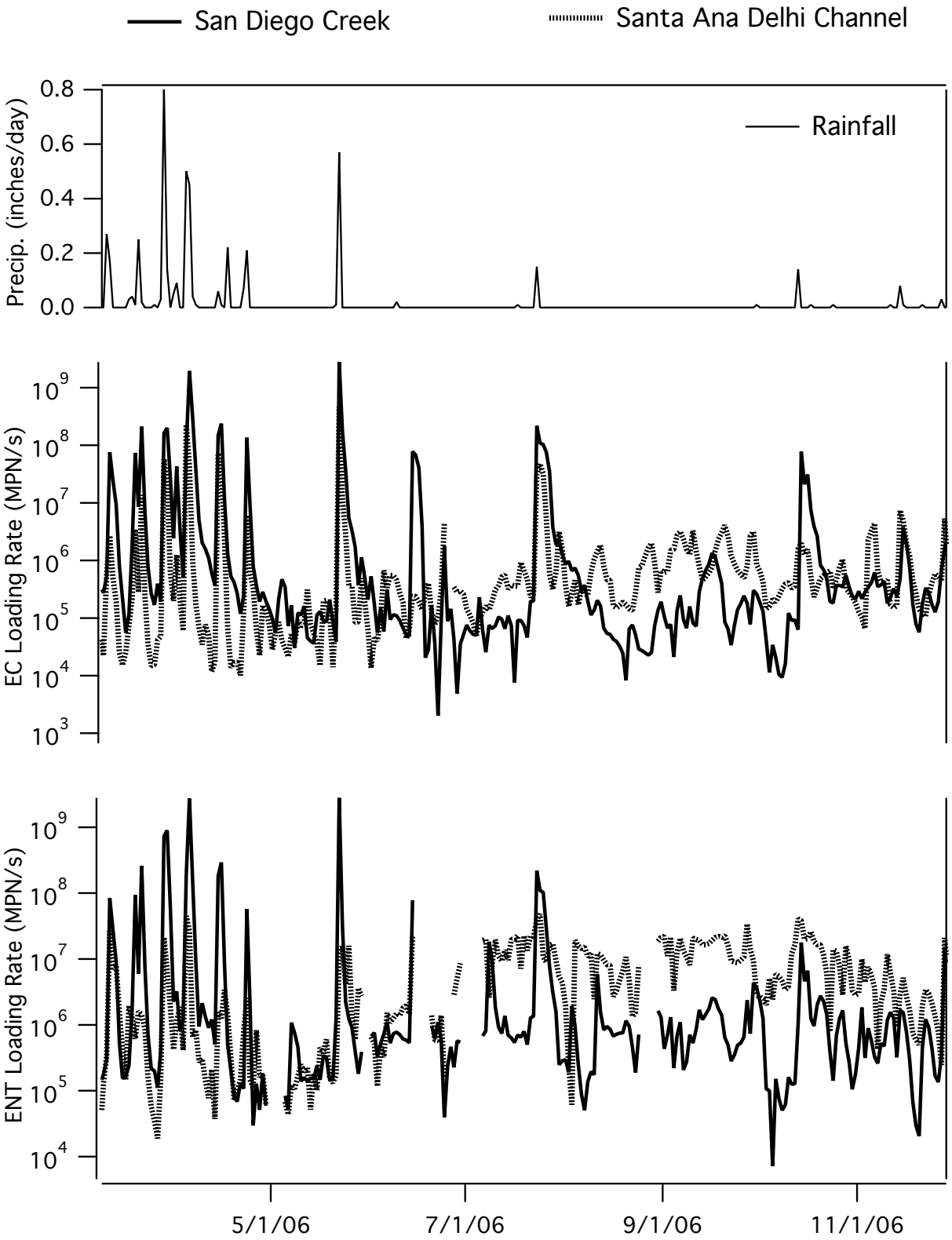

Figure 8-3. Time series estimates of *Escherichia coli* (EC) and enterococci bacteria (ENT) loading rates from San Diego Creek and Santa Ana Delhi channel. Also shown is the rainfall history for Newport Beach.

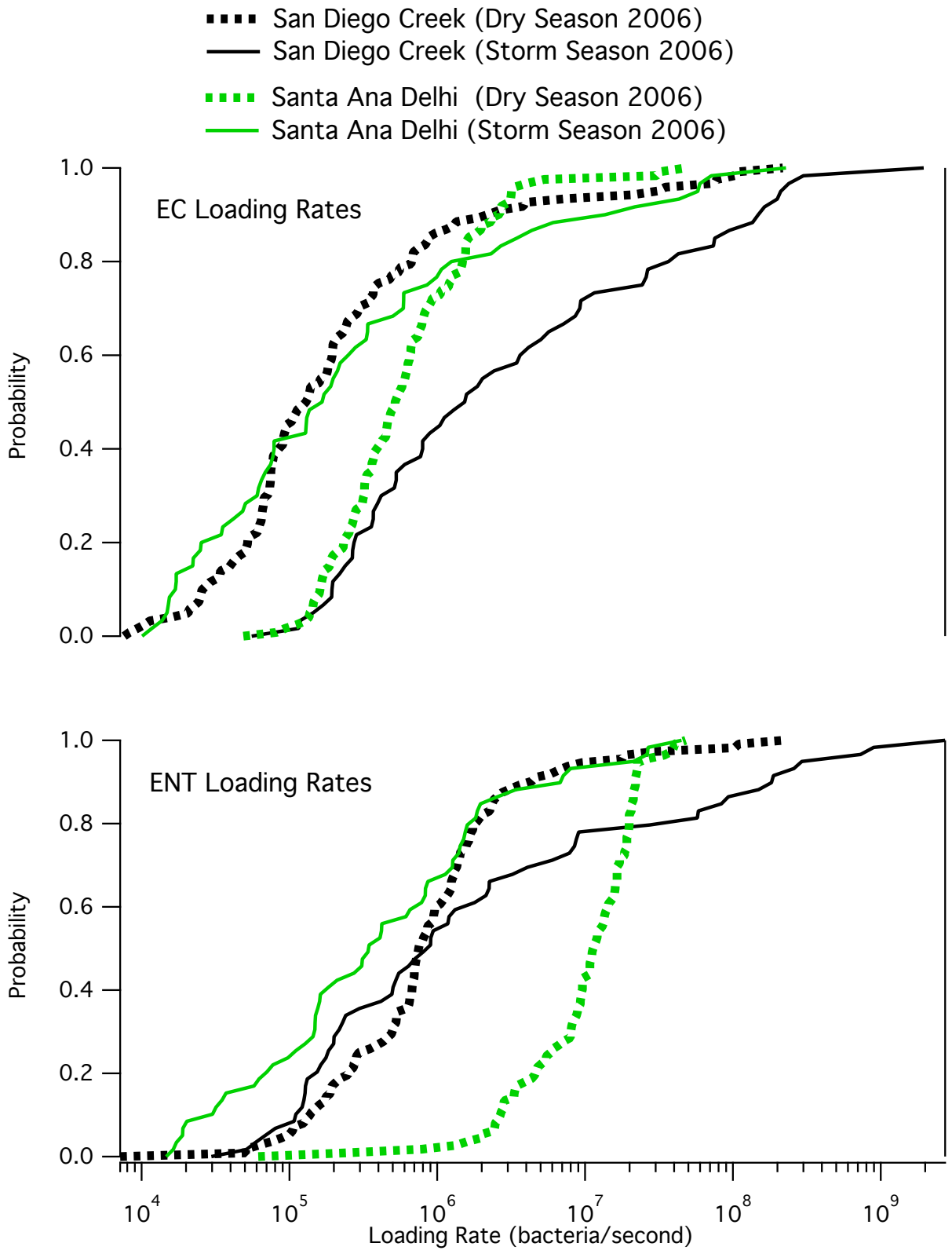

Figure 8-4. Probability plots of loading rates for *Escherichia coli* (EC) and enterococci bacteria (ENT), broken down by tributary site (San Diego Creek or Santa Ana Delhi Channel) and by season (Dry Season 2006 and Storm Season 2006).

$$L_{FIB} = C_{FIB} Q_{creek}$$

8.2.1.2. Results

<u>*Time series of salinity, temperature, and pH*</u>. Most samples (>90%) collected in San Diego Creek had very low salinities (< 3), consistent with our field observations that this sampling site does not experience tidal inundation (Fig. 8-1). Salinity measured near the outlet of the Santa Ana Delhi channel, on the other hand, fluctuated over a fairly narrow range (ca., 0 to 6) during and following the wet season (from March to August, 2006), and over a larger range (0 to 16) after August 2006 (Fig 8-1). Peak salinities observed after August appear to be caused by the intrusion of Bay water at the Santa Ana Delhi channel sampling site during spring tides, based on the fact that high salinity at this site is frequently correlated with high tides (see Appendix 8A).

Creek water temperature exhibits seasonal variability, with warmer temperatures (*ca*., 20 to 30°C) recorded in late summer to early fall, and cooler temperatures (*ca*., 10 to 20°C) recorded in late fall to late spring (Fig. 8-1). Temperature was consistently a few degrees higher at the Santa Ana Delhi channel site, and lower at San Diego Creek. As noted in Chapter 2, tributary sampling was carried out in the afternoon (between 14:00 and 16:00 PST) and in the order: San Diego Creek, Santa Ana Delhi Channel. Because there was a relatively short time delay between the sampling at the different creek sites (sampling at tributary sites was usually completed within one hour), creek-to-creek variations in temperature likely reflect differences in the heat budget at these locations, not a sampling artifact related to when during the day each creek was sampled.

Water samples collected from the two tributaries exhibit relatively similar pH, typically ranging between 7 and 9 (Fig. 8-1). The lowest tributary pH values were measured during storm events.

<u>*Time series of Volumetric Flow, EC, and ENT*</u>. Flow in San Diego Creek and Santa Ana Delhi channel is very flashy (Fig. 8-2). During dry weather there is a near constant flow of 10 and 3 cfs, respectively, in these two tributaries. This dry weather base flow apparently consists of return flow from irrigation and agricultural practices in the watershed, and nuisance runoff from, for example, car washing; a portion of the base flow in San Diego Creek may originate as groundwater[3]. During storms, flow in San Diego Creek and Santa Ana Delhi channel can increase dramatically; in some cases the peak flow during storms is 100 times the base (dry weather) flow rate. The flashy nature of storm water runoff in these creeks is typical of urban watersheds in southern California where a significant percentage of the land area has been made impervious by development[4].

The concentration of EC and ENT measured in the tributaries varies over multiple orders of magnitude, from <10 MPN/100 mL to >10,000 MPN/100 mL (Fig. 8-2). FIB concentration spikes generally coincide with rainfall events, suggesting that stormwater runoff mobilizes

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[3] French, C., L.Wu, T. Meixner, D. Haver, J. Kabashima, W.A. Jury (2006) "Modeling nitrogen transport in the Newport Bay/San Diego Creek watershed of southern California" *Agricultural Water Management* **81**: 199-215.

[4] Warrick, J.A., Rubin, D.M. (2007) "Suspended-sediment rating curve response to urbanization and wildfire, Santa Ana River, California" *J. Geophysical Research-Earth Surface* **112** (F2): Art. No. F02018.

Table 8-1. Loading rate percentiles by tributary source (San Diego Creek or Santa Ana Delhi channel), season (Dry Season 2006 or Wet Season 2006), and FIB group (EC or ENT). The season designations are the same as those used in Chapter 2.

	EC Loading Rate (MPN/s)			***ENT Loading Rate (MPN/s)***		
	10th	***50th***	***90th***	***10th***	***50th***	***90th***
San Diego Creek (Dry Season 2006)	2.6×10^4	1.3×10^5	2.3×10^6	1.4×10^5	7.5×10^5	4.4×10^6
San Diego Creek (Wet Season 2006)	1.9×10^5	1.5×10^6	1.5×10^8	1.1×10^5	9.0×10^5	1.8×10^8
Santa Ana Delhi (Dry Season 2006)	1.6×10^5	5.2×10^5	2.7×10^6	2.7×10^6	1.1×10^7	2.2×10^7
Santa Ana Delhi (Wet Season 2006)	1.7×10^4	1.7×10^5	1.4×10^7	3.0×10^4	3.5×10^5	6.8×10^6

significant numbers of these organisms into the surface water[5]. Further, spikes in EC and ENT do not appear to get lower with subsequent storms; i.e., there is not an obvious "seasonal first-flush" for FIB. Indeed, some of the highest peak concentrations of FIB occurred during storms in the middle of the March to May storm sequence (e.g., see EC peak during the storm on April 6, 2006). The concentration of FIB also exhibits a seasonal pattern. In the Santa Ana Delhi channel, EC and ENT concentrations increase steadily after July 1, frequently exceeding 1000 MPN/100 mL for EC and our upper-limit of detection of 24,000 MPN/100 mL for ENT.

<u>*FIB Loading from San Diego Creek and Santa Ana Delhi*</u>. During the storm season, the loading of EC and ENT from San Diego Creek is consistently greater than the loading of FIB from Santa Ana Delhi channel (Fig. 8-3). This trend reverses during the dry season, when FIB loading from Santa Ana Delhi channel is consistently larger than FIB loading from San Diego Creek (Fig. 8-3). For most of the 9-month study (80% of *E. coli* loading measurements, and nearly 100% of ENT loading measurements), loading of FIB from Santa Ana Delhi is significantly larger during the summer, and smaller during the winter.

The FIB loading rate data presented in Fig. 8-3 was used to generate probability plots (Fig. 8-4), after sub-dividing the data by tributary site (San Diego Creek or Santa Ana Delhi channel), season (Dry Season 2006 or Wet Season 2006), and FIB group (EC or ENT). The definition of the seasons conforms to the definition used in Chapter 2; namely, "Dry Season (2006)" denotes the period of time from July 1 to November 1, and "Storm Season (2006)" denotes the period of time March 1 and May 1. For each of the three sub-divisions, 10th, 50th (median), and 90th percentile loading rates were extracted from the probability curves in Fig. 8-4, and summarized in Table 8-1. The FIB loading rates listed in Table 8-1 were used as input to a simple pollutant mass transport model for Newport Bay, described next.

[5] Surbeck, C.Q., Jiang, S.C., Ahn, J.H., Grant, S.B. (2006) "Flow fingerprinting fecal pollution and suspended solids in stormwater runoff from an urban watershed" *Environmental Science and Technology*, **40**:4435-4441.-

8.2.2. Bay-wide water quality impact of FIB loading from San Diego Creek and Santa Ana Delhi Channel

8.2.2.1. Methods

1D Tidal Mixing Model for Newport Bay. A simple mass balance model is used to estimate the Bay-wide impacts of FIB loading from San Diego Creek and Santa Ana Delhi channel. The model assumes that FIB concentration in the Bay is determined by a steady-state balance between:

1. FIB loading at the head of the estuary (from San Diego Creek and/or Santa Ana Delhi channel).
2. Transport of FIB down-Bay by tidal dispersion, which is parameterized by a tidal dispersion coefficient that increases exponentially with distance down-bay.
3. Dilution of FIB by tidal dispersion and turbulence-driven vertical mixing over the cross-sectional area. The cross-sectional area is also assumed to increase exponentially with distance down-Bay.

Because FIB from the tributaries survive for long periods of time when mixed with Bay water (see microcosm results in Chapter 5), for this analysis we adopted the conservative assumption that FIB do not die-off in the Bay.

While published "box models" could be used to predict FIB concentrations in the Bay from creek loading rates, at the outset we decided that such models would be a poor description of water quality in Newport Bay because of the very strong longitudinal (down-bay) gradients documented for salinity, EC and ENT in Chapter 2 (box models assume that the Bay is well-mixed in all spatial dimensions). Instead, we derived a one-dimensional model for pollutant transport in Newport Bay, subject to the assumptions described above, that allows for pollutant concentrations to vary with longitudinal distance down-bay. Assuming that a tracer (e.g., salinity, FIB, etc) originates exclusively from a single creek, the predicted tracer concentration in the Bay is as follows:

$$\hat{C} = Pe\left(\exp\left(-x/\ell\right)\right)$$

$$\hat{C} \equiv \frac{C}{C_f} = \frac{\text{concentration of tracer in the estuary}}{\text{concentration of tracer in the creek}}$$

$$Pe = \text{Peclet Number} = \frac{\text{Advective transport}}{\text{Dispersive transport}} \equiv \frac{\left(Q_f / A_0\right)\ell}{K_0}$$

$$\ell = \text{decay length scale} \equiv \left(\ell_A^{-1} + \ell_K^{-1}\right)^{-1}$$

$$A_0 = \text{cross-sectional area at head of estuary}$$

ℓ_A = distance downbay over which the cross - sectional area increases

Q_f = volumetric flow rate of tributary

K_0 = tidal dispersion coefficient at head of estuary

ℓ_K = distance downbay over which the tidal dispersion coefficient increases

x = longitudinal distance down - Bay (the head of the estuary corresponds to $x = 0$)

As will be described in a journal manuscript, the above model correctly captures the decay in freshwater fraction with distance down-bay, based on salinity data collected during the February 2007 BTO studies (Chapter 2). Further, parameters obtained by fitting the model to the freshwater fraction data are physically reasonable and internally consistent. The model, as presented above, neglects residual circulation associated with addition of freshwater to the estuary, and is therefore restricted to cases where Peclet number is less than unity, $Pe << 1$. This assumption should not introduce significant error for all but the largest storms, during which our formulation breaks down anyway, because baroclinic circulation cannot be neglected (see Chapter 1).

To predict FIB concentrations in Newport Bay, the above equation was rearranged:

$$C = \frac{(L_{FIB}/A_0)\ell}{K_0}\exp(-x/\ell)$$

L_{FIB}= loading rate of FIB from San Diego Creek and/or Santa Ana Delhi Channel

This equation has three fitting parameters—the cross-sectional area and tidal dispersion coefficient at the head of the estuary (A_0 and K_0) and the length scale over which pollutants from the creeks are diluted with distance down-Bay (ℓ). These three parameters were estimated from physical measurements on Newport Bay (A_0) and fitting of the model to freshwater fraction data collected during the February Storm (2007) transect studies (ℓ and K_0):

$A_o = 45\text{m}^2$
$\ell = 2.3$ km
$K_0 = 45\ \text{m}^2/\text{s}$

Substituting these values into the model we obtain the following prediction for the concentration of FIB in Newport Bay:

$$C = \left(1.1\times10^{-4}\right)\left(L_{FIB}\right)\left(\frac{\text{s}}{100\text{mL}}\right)\exp(-x/2300\text{m})$$

where the units of L_{FIB} are bacteria/s or MPN/s and x is distance down-Bay from the head of the estuary (in meters).

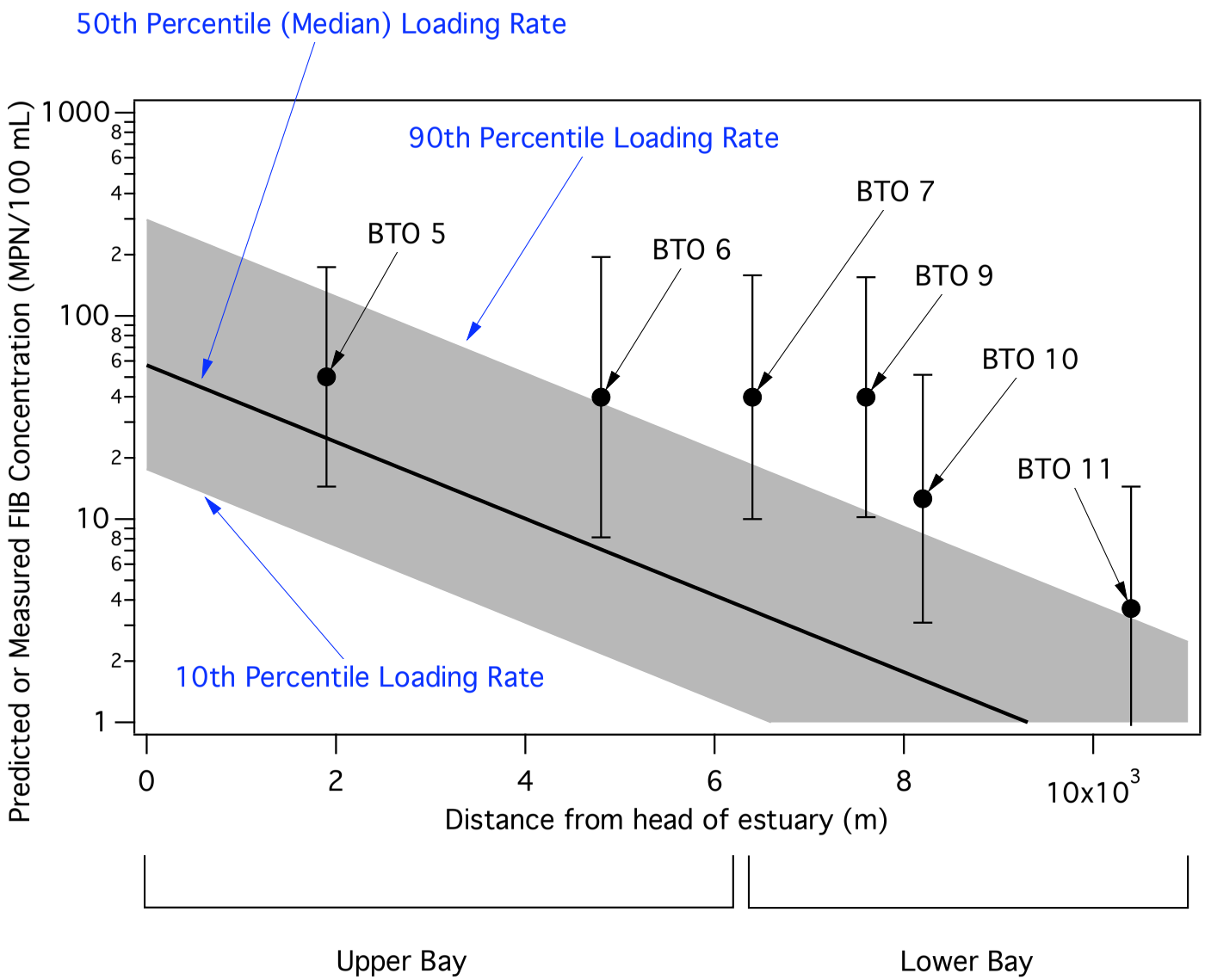

Figure 8-5. Example of predicted and measured FIB concentrations in Newport Bay. Black line denotes model prediction assuming 50th percentile FIB loading rate from San Diego Creek and/or Santa Ana Delhi Channel. The upper and lower edge of grey region represents predicted FIB concentrations assuming 10th and 90th percentile FIB loading rates. Points represent mean and standard deviation of measured FIB concentrations at BTO transect sites 5, 6, 7, 9, 10, and 11. BTO 4 was excluded because the water column is often stratified there. BTO 8 was excluded because it is located off the main tidal channel in West Newport Bay.

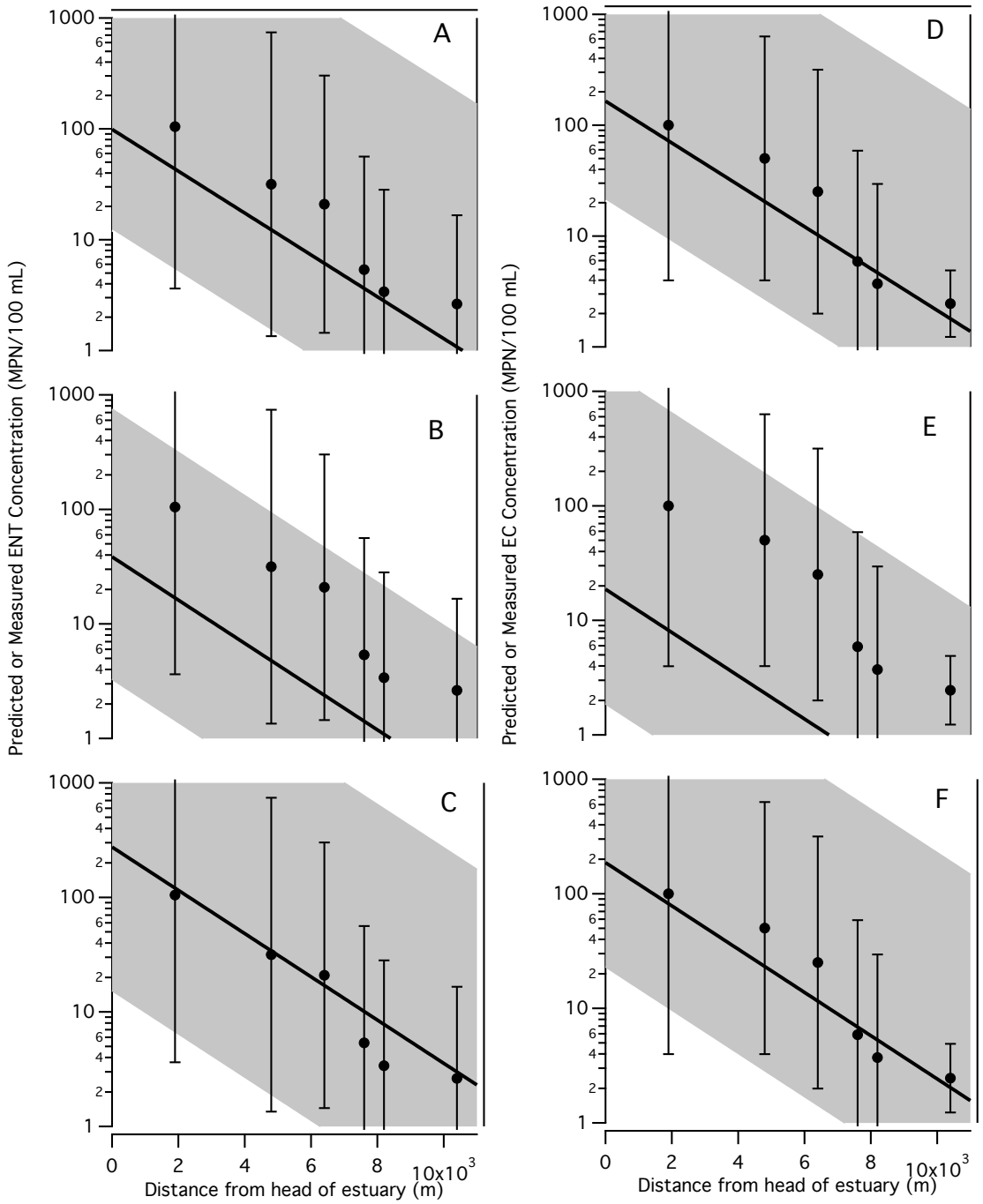

Figure 8-6. Comparison of predicted and measured FIB concentrations in Newport Bay during Storm Season (2006): **A**. ENT loading from San Diego Creek (SDC), **B**. ENT loading from Santa Ana Delhi (SAD), **C**. ENT loading from both SDC and SAD, **D**. EC loading from SDC, **E**. EC loading from SAD, **F**. EC loading from both SDC and SAD. Elements of graphs are annotated in Fig. 8-5.

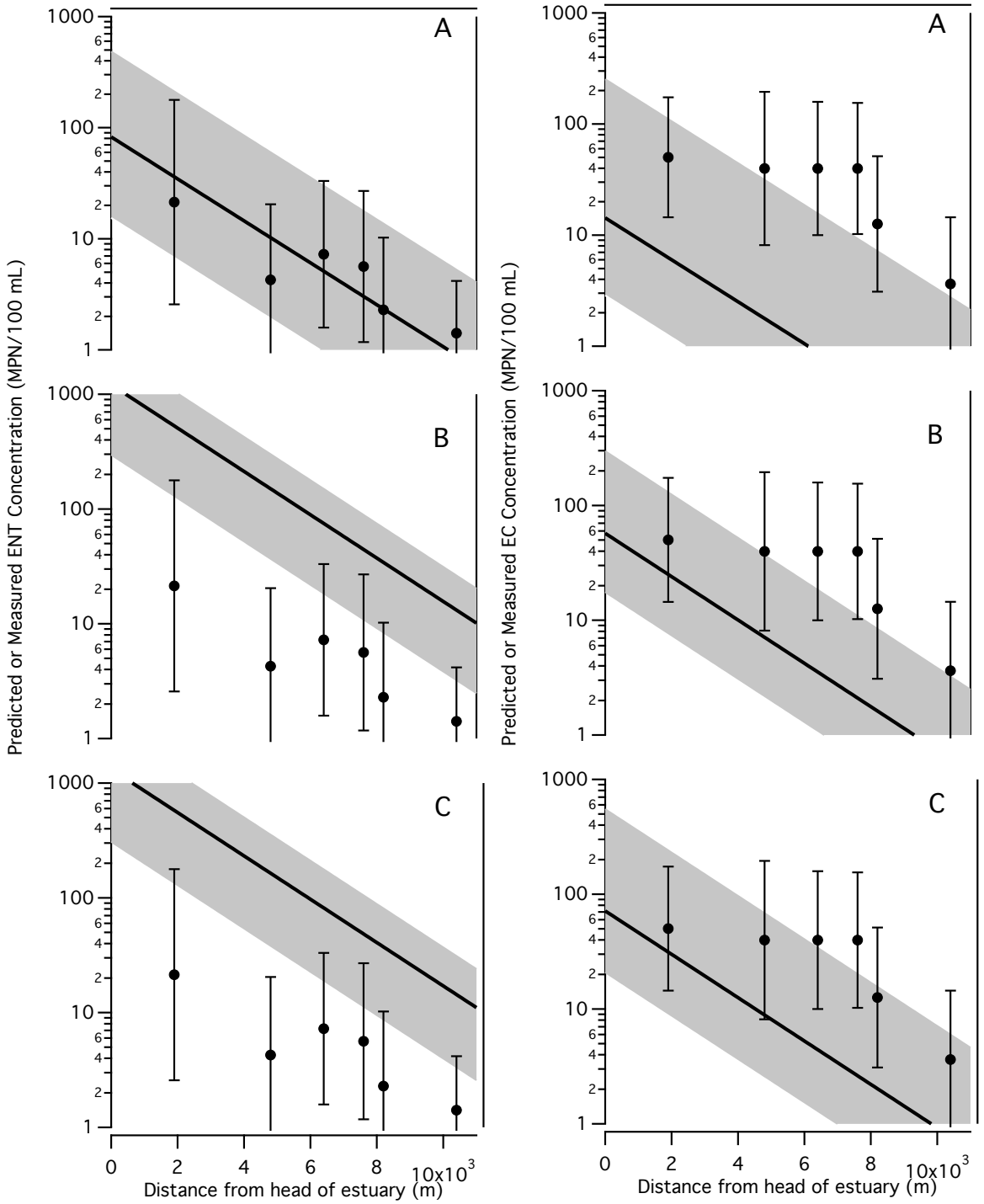

Figure 8-7. Comparison of predicted and measured FIB concentrations in Newport Bay during Dry Season (2006): **A**. ENT loading from San Diego Creek (SDC), **B**. ENT loading from Santa Ana Delhi (SAD), **C**. ENT loading from both SDC and SAD, **D**. EC loading from SDC, **E**. EC loading from SAD, **F**. EC loading from both SDC and SAD. Elements of graphs are annotated in Fig. 8-5.

In the next section we use this formula to predict the concentration of FIB throughout Newport Bay, given different scenarios for the loading of FIB from San Diego Creek and/or the Santa Ana Delhi channel. Because all fitting parameters for this model have been determined based on freshwater fraction data collected during the February 2007 sampling effort in Newport Bay (see discussion above), FIB concentrations at any distance (x) downstream from the head of the Bay can be predicted solely from measurements of FIB loading (L_{FIB}) from the one of the two tributaries (Table 8-1). In the following section, model-predictions are compared to FIB concentrations measured during the BTO transects described in Chapter 2 of this report. An annotated example of the graphs used to compare measured and model-predicted FIB concentrations is provided in Fig. 8-5.

8.2.2.2. Results

Measured and Model-Predicted FIB Concentrations in Newport Bay. During the Storm Season (2006), our mass balance model accurately predicts measured EC and ENT concentrations in the Bay when the model is run using, as input, the measured EC and ENT loading rate from San Diego Creek (Fig. 8-6A) or the combined EC and ENT loading rate from San Diego Creek and Santa Ana Delhi channel (Fig. 8-6C). When the model is run using only the loading rate from Santa Ana Delhi channel, the model significantly under-predicts measured EC and ENT concentrations in the Bay (Fig. 8-6B). These results are consistent with the idea that EC and ENT measured in Newport Bay's main tidal channel during the storm season originate primarily from San Diego Creek and, to a lesser extent, Santa Ana Delhi channel.

During the Dry Season (2006) period, different patterns emerge for EC and ENT (Fig. 8-7). Measured and model-predicted ENT concentrations closely agree, provided that we use as input to the model measured ENT loading rate from San Diego Creek (Fig. 8-7A). When the ENT loading rate from the Santa Ana Delhi channel (or the combined ENT loading rate from Santa Ana Delhi channel and San Diego Creek) are used, model-predicted ENT concentrations are significantly (i.e., 10 to 100 times) greater than measured ENT concentrations in the Bay (Fig. 8-7B). In addition, measured EC concentrations are typically larger than model-predicted EC concentrations, for all combinations of creek loading tested (Fig. 8-7D,E, and F). This last result is consistent with the idea that, during dry weather, another source (besides San Diego Creek and Santa Ana Delhi channel) contributes to EC contamination of Newport Bay.

Taking these model results at face value, they suggest that dry weather concentrations of ENT in the main tidal channel of Newport Bay are lower than would be expected, given the very high loading of ENT measured at our sampling site on the Santa Ana Delhi channel (see Table 8-1). This observation could have several possible explanations. First, one or more assumptions in the simple 1D model used here may render the model predictions inaccurate during dry weather. Second, the load of ENT from the Santa Ana Delhi channel may be attenuated between where the loading rate was measured (i.e., BTO 2, upper white arrow, Fig. 8-8) and the main tidal channel of Newport Bay (lower white arrow, Fig. 8-8). The former possibility will be evaluated as the model is vetted through peer review, and possibly followed up with more sophisticated (multi-dimensional) modeling efforts. To evaluate the latter possibility, and obtain more information about how bacteria from the Santa Ana Delhi channel make their way into Newport

Bay, several TAC members, led initially by Dr. Jack Skinner, conducted a field reconnaissance of the site. From this field reconnaissance, the following can be stated:

(1) Before runoff from the Santa Ana Delhi channel flows into Newport Bay's main tidal channel, it must first travel along a 1 km incised tidal mudflat channel referred to here as the "Santa Ana Delhi Tidal Channel". The Santa Ana Delhi Tidal Channel is located between the two white arrows in Fig. 8-8.
(2) Tidewaters intrude a significant distance up the Santa Ana Delhi Tidal Channel, including upstream of our Santa Ana Delhi sampling site (BTO 2). This explains why brackish salinities were measured in water samples collected from BTO 2 during the summer of 2006 (see Section 8.2.1.2, and the correlation between spring tides and high salinity at the Santa Ana Delhi sampling site, Appendix 8A).
(3) Water in the Santa Ana Delhi Tidal Channel does not appear to overflow its banks and spill into the surrounding mudflat. During a high tide on 5/29/09, for example, water in the Santa Ana Delhi Tidal Channel was well below the height of the channel levee (personal observation, Ray Hiemstra). A photograph of the lower reaches of the Santa Ana Delhi Channel during a high tide indicates that water in the channel is confined between the banks (Fig. 8-9).
(4) In the upper reaches of the Santa Ana Delhi channel, just downstream of our Santa Ana Delhi channel sampling site (BTO 2), the channel bottom forms a set of basins separated by shallow sills. One of these basin/sills in the upper reach of the Santa Ana Delhi Tidal Channel is shown in the photograph taken during a low tide on 4/30/09 (Fig. 8-11). While the depth of the basins and sills varies throughout the tidal cycle, when observed during a low tide, the basin illustrated in Fig. 8-11 was >2 m deep.

Taken together, these field observations suggest that the dry weather residence time of water in the tidally influenced portion of the Santa Ana Delhi channel is likely to be long (i.e., multiple tidal cycles) because: (1) the basins and sills will act to retard drainage of the channel during ebb tides, and (2) flow in the channel will probably be dominated by tidal action during dry weather, when the volume of freshwater from the Santa Ana Delhi channel is low. The long residence time of water in the Santa Ana Delhi Tidal Channel may act to attenuate the summertime loading of ENT from BTO 2, as our modeling suggests, by increasing the dilution of runoff with (relatively clean) Bay water, and increasing the time over which bacteria in the runoff die-off in the brackish waters of the tidal channel.

It is important to note that, even if the above scenario is correct, changes in the topography of the tidal channel (e.g., elimination of the basin and sills by scouring of the channel bottom during storms), could alter the residence time of water in the channel, and thus alter the loading of FIB to the main tidal channel of Newport Bay. Indeed, it appears that dry weather water quality in Newport Bay has improved over the last several years[6], which might be attributable to changing bottom topography in the Santa Ana Delhi channel and possibly the recent construction of detention basins in the lower reaches of the San Diego Creek (efforts are ongoing to determine the timing of alterations to the lower reach of San Diego Creek).

[6] "Report: Water quality is excellent" Daily Pilot Newspaper, May 21 (2009) edition.

Figure 8-8. Aerial photograph of Upper Newport Bay which illustrates that runoff from the Santa Ana Delhi channel must flow through a relatively extensive intertidal mudflat before discharging to the main tidal channel in Upper Newport Bay.

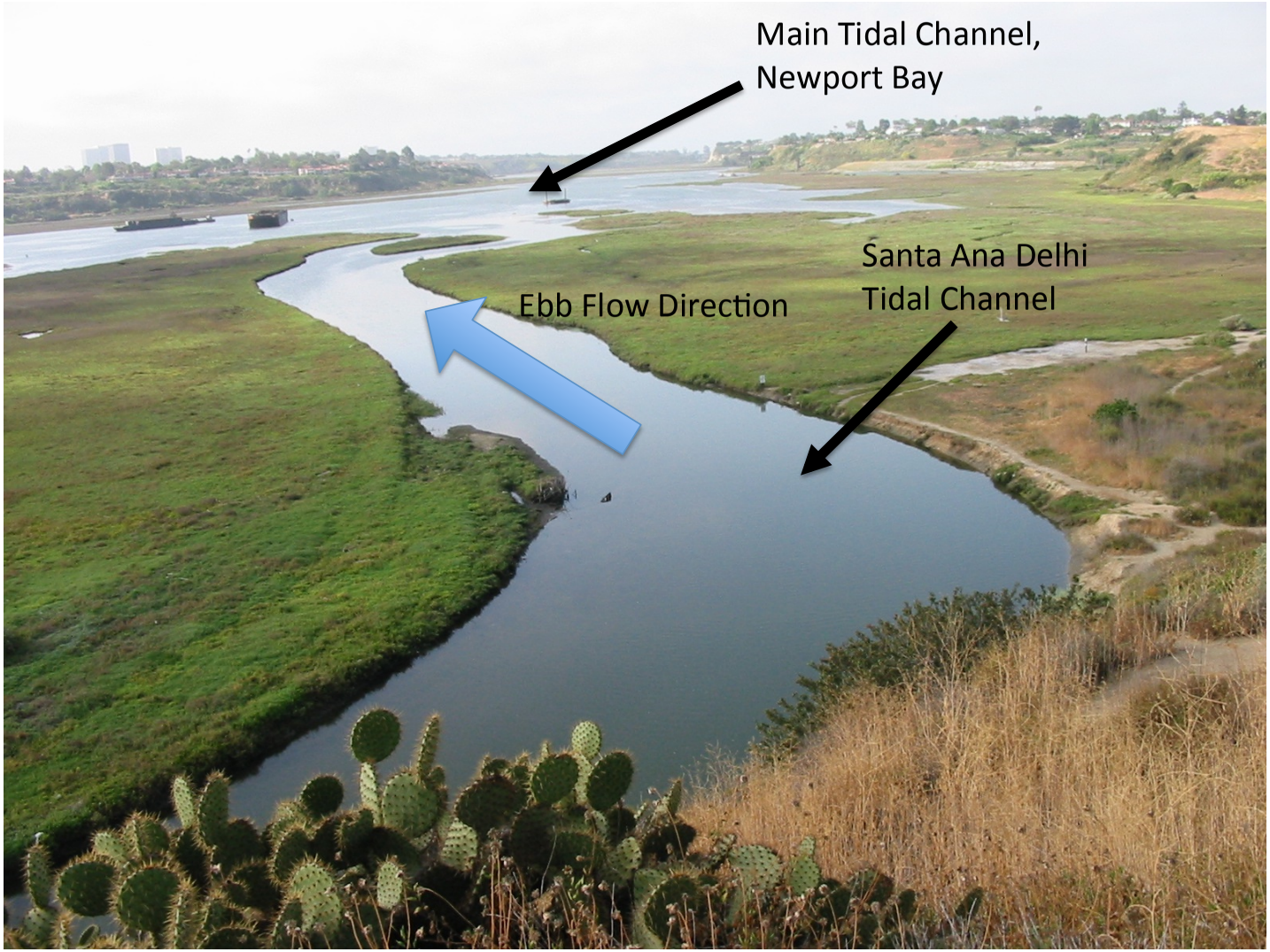

Figure 8-9. Photograph of the lower reach of the Santa Ana Delhi Tidal Channel, looking south from a trail south of the foot bridge. Photograph taken by Dr. Jack Skinner during a high tide (ca., +4.2' MSL) on May 5, 2009.

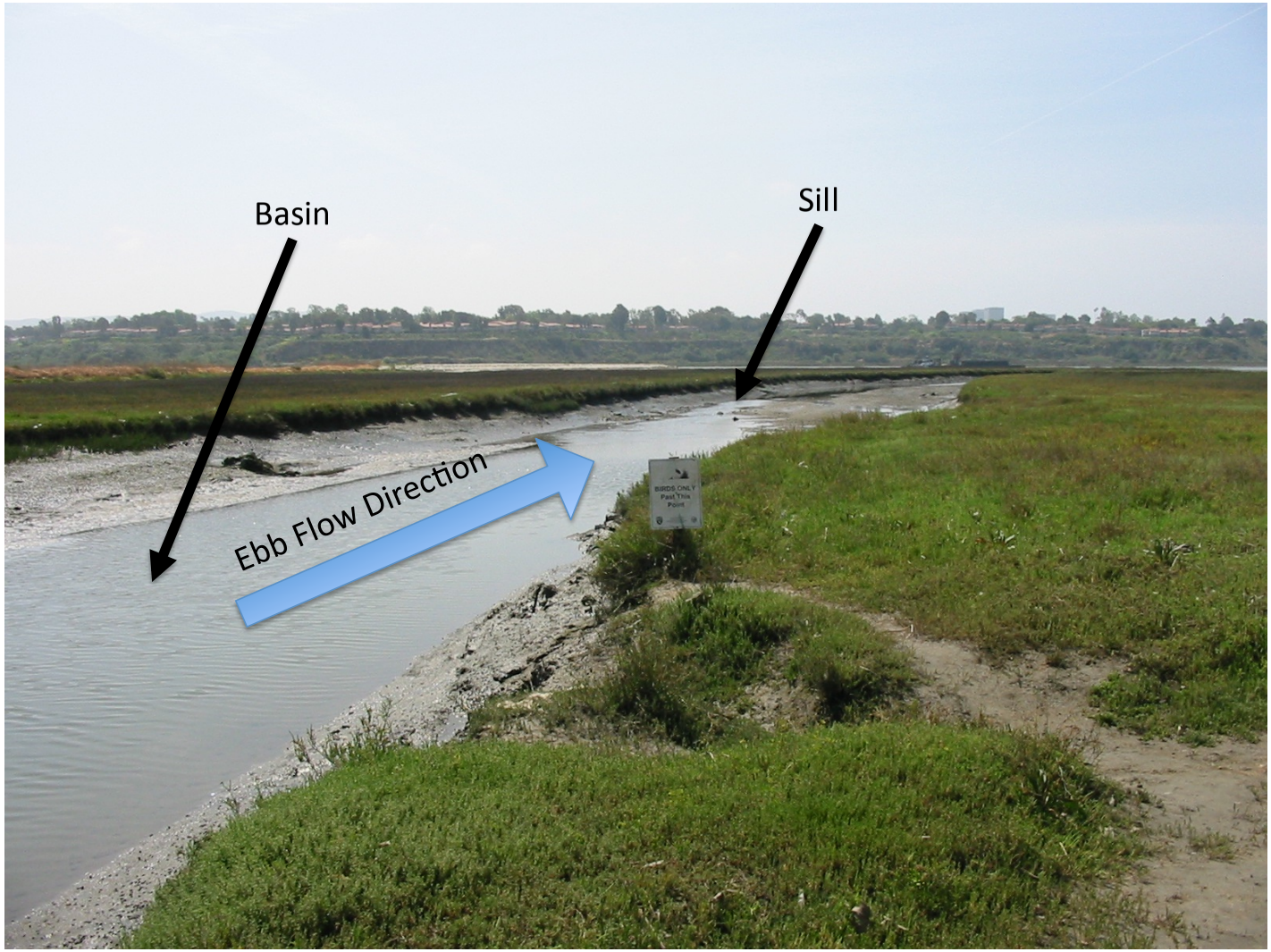

Figure 8-10. Photograph of the upper reach of the Santa Ana Delhi Tidal Channel, looking south from a trail just south of the foot bridge. taken Photograph take by Dr. Jack Skinner during a low tide (ca., -0.5' MSL) on April 30, 2009.

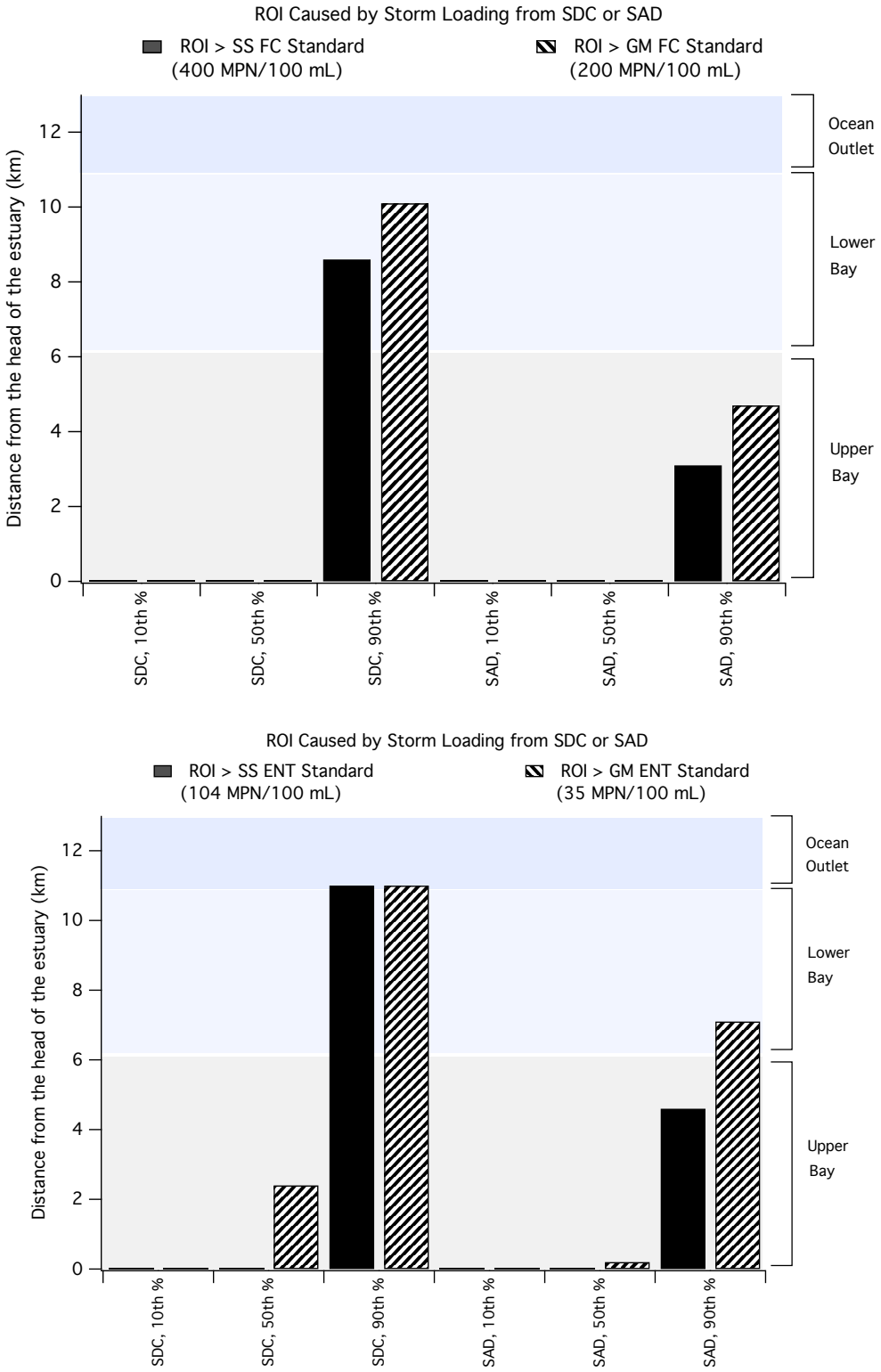

Figure 8-11. Model predicted regions of impact (ROI) for storm loading of Fecal coliform (FC, top panel) or ENT (bottom panel) from San Diego Creek (SDC) or Santa Ana Delhi (SAD). Black and hatched bars denote FIB concentrations above single-sample and geometric mean standards. Three different loading rates are shown (10th, 50th, and 90th percentiles). For this calculation, a 1:1 correspondence is assumed between FC and EC.

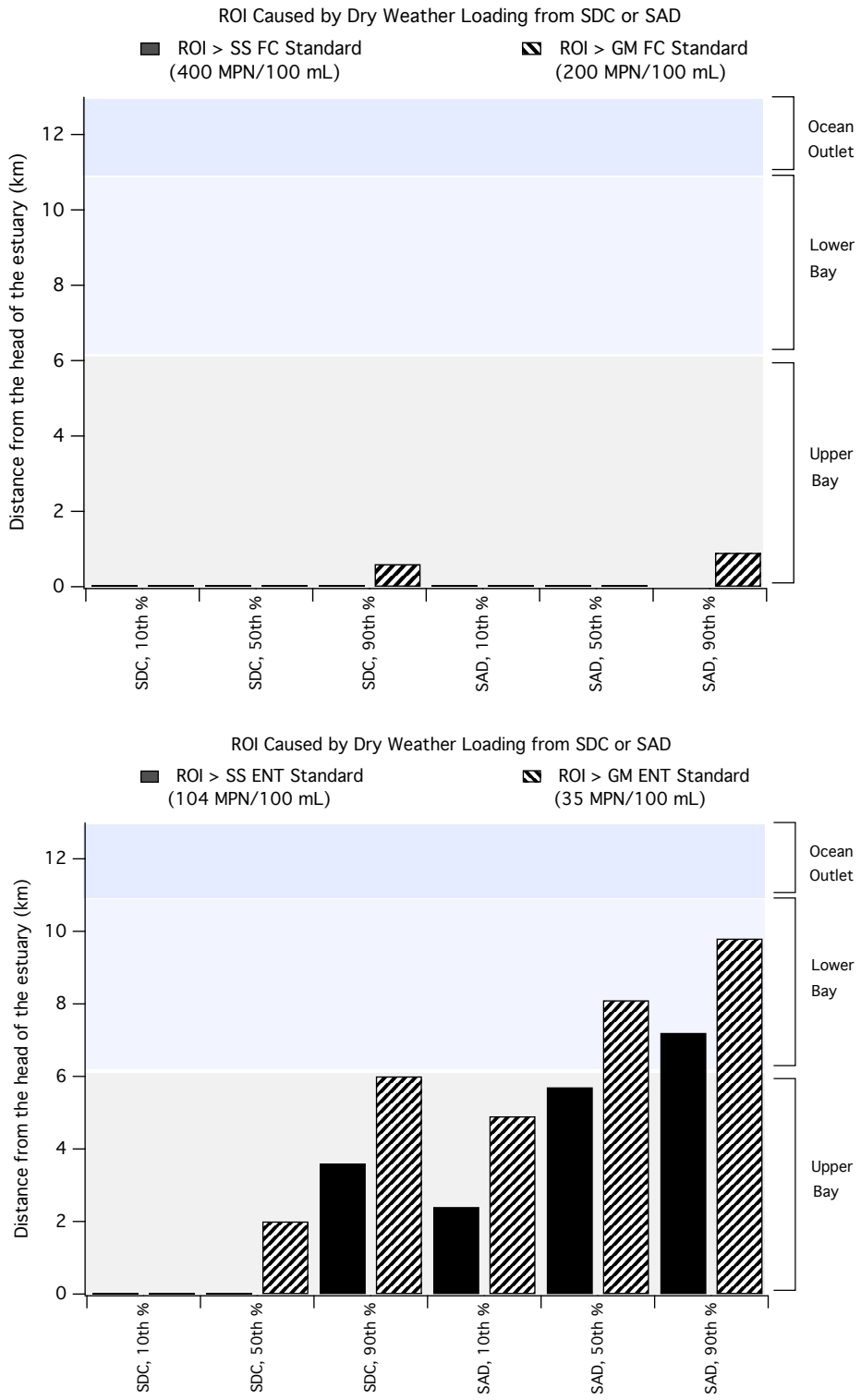

Figure 8-12. Model predicted regions of impact (ROI) for dry weather loading of Fecal Coliform (FC, top panel) or ENT (bottom panel) from San Diego Creek (SDC) or Santa Ana Delhi (SAD). Black and hatched bars denote FIB concentrations above single-sample and geometric mean standards. Three different loading rates are shown (10th, 50th, and 90th percentiles). For this calculation, a 1:1 correspondence is assumed between FC and EC.

Regions of Impact. The model results presented above were used to estimate regions of impact (ROI) in Newport Bay over which FIB concentrations are predicted to exceed California marine bathing water standards. These ROI results are presented for the Storm Season (2006) (Fig. 8-11) and Dry Season (2006) (Fig. 8-12), and for three different loading percentiles (10th, 50th, and 90th) measured at the two tributary sites, San Diego Creek and Santa Ana Delhi Channel.

During the storm season, the model predicts that FIB loading from San Diego Creek can affect water quality over a relatively large region of Newport Bay (Fig. 8-11). When 90th percentile FIB loadings from San Diego Creek are used as input to the model, the ROI extends from the head of the estuary far into Lower Bay; approximately 10 km for both Fecal Coliform (FC, assumed equivalent to EC) and ENT. For 50th percentile loading from San Diego Creek, on the other hand, the model predicts that no portion of the Bay exceeds standards for FC, and only the upper reaches of Upper Bay exceeds the geometric mean standard for ENT. For 10th percentile loading from San Diego Creek, FC and ENT concentrations are below single-sample and geometric mean standards everywhere in the Bay. A similar pattern is evident for wet weather FIB loading from the Santa Ana Delhi channel (Fig. 8-11). When the 90th percentile FIB loading from Santa Ana Delhi is used as input to the model, marine bathing water exceedences are limited to Upper Bay. When 10th and 50th percentile loading from Santa Ana Delhi are used as input to the model, no marine bathing water exceedences are predicted for the Bay.

Dry weather loading of ENT from San Diego Creek cause water quality exceedences in Upper Bay, but only when the 50th or 90th percentile loading rates are used as the model input (bottom panel, Fig. 8-12). The model predicts that dry weather loading of ENT from the Santa Ana Delhi channel causes water quality exceedences throughout the Bay (upper panel, Fig. 8-12). However, as noted above, comparison of measured and model predicted ENT concentrations in the main tidal channel of Newport Bay suggest that the very high dry weather loading of ENT measured in the Santa Ana Delhi channel does not make its way to Newport Bay's main tidal channel, possibly due to natural treatment that occurs as the dry weather runoff from this tributary flows through a mudflat channel (see discussion above).

Finally, it should be noted that, depending on location and season, 30 to 50% of the enterococci bacteria isolates from Newport Bay and its tributaries are species typically associated with fecal pollution (*E. faecalis*, *E. faecium*, *E. hirae*, *E. durans*, and *E. gallinarum)* while the rest are typically not associated with fecal pollution (*E. casseliflavus* or *E. mundtii*) (see Chapter 3). *E. faecalis* and *E. faecium* are the dominant enterococcal species in the gastrointestinal track of humans, and most enterococcal infections are caused by either *E. faecalis* (74-90%) or *E. faecium* (5-16%)[7]. The enterococci species composition in environmental waters can, at least in some cases, reflect the sources and magnitude of fecal pollution[8]. Based on the speciation results for Newport Bay, it could be argued that, for the ROI calculations above, the ENT loading rates from San Diego Creek and Santa Ana Delhi channel should be reduced in proportion to the fraction of enterococci species classified as "non-fecal", as suggested by one of the TAC members (Dr. Jack Skinner, personnel communication).

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[7] Varman, M., A.Chatterjee, W. Abuhammour (2008) "Enterococcal Infection" Emedicine: http://emedicine.medscape.com/article/971259-overview

[8] Petersen, A., A. Dalsgaard (2003) "Species composition and antimicrobial resistance genes of *Enterococcus* spp., isolated from integrated and traditional fish farms in Thailand" *Environmental Microbiology* **5**:395-402.

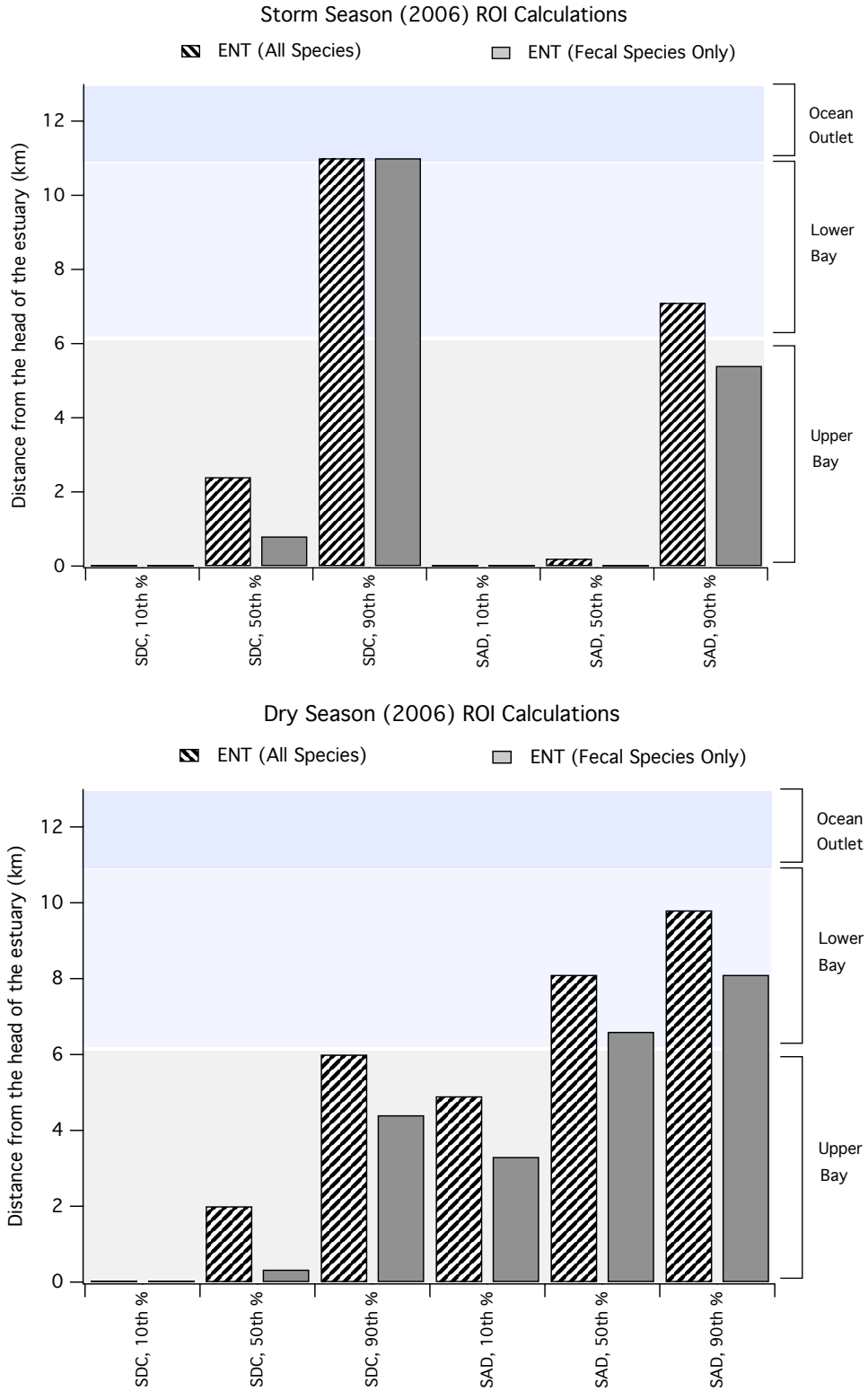

Figure 8-13. Model predicted regions of impact (ROI) for wet (top panel) and dry (bottom panel) weather loading of ENT from San Diego Creek (SDC) and Santa Ana Delhi (SAD) channel, assuming either all *Enterococcus* species (hatched bars), or only fecal species (solid bars) contribute to water quality impairment. For these calculations, the geometric mean standard was used as the trigger for water quality exceedences.

To investigate the effect of excluding non-fecal enterococci bacteria from the ROI calculations, in Fig. 8-13 we compare the ROI results before and after removing the non-fecal portion of the ENT loading from either San Diego Creek or Santa Ana Delhi channel. For the purposes of these calculations, we adopted the conservative assumption that 50% of the enterococci bacteria measured in these two tributaries were of non-fecal origin (implying that 50% are of fecal origin). Because of the exponential nature of our model, a 50% reduction in the loading rate from the tributaries results in less than a 50% reduction in the ROI (Fig. 8-13). In most cases, the ROI for fecal ENT is about 20 to 30% less than the ROI for all ENT. It should be noted that enterococci species we have categorized as "non-fecal", such as *E. casseliflavus*, are occasionally implicated as a cause of infection in humans[9]. Given the relatively minor reduction in ROI affected by reducing the ENT load from Newport Bay tributaries to only "fecal" species, together with the uncertain health effects associated with enterococci species that we have defined here as "non-fecal", the original ROI calculations (i.e., those displayed in Figs. 8-11 and 8-12) should probably be used for assigning waste load allocations for San Diego Creek and Santa Ana Delhi channel.

8.3. Beach Scale: Impacts of FIB loading from Shore-Based Non-Point Sources

In this section we focus on the non-point source contribution to FIB impairment in ankle depth waters along the shoreline in Newport Beach. Nonpoint sources considered here include bather shedding, bird droppings, and contaminated sediments. To model their impact on ankle depth water quality, we mathematically conceptualize these non-point sources as "line sources" oriented parallel to the shore and located where the water's edge meets the shore. The strength of the line sources is parameterized by a loading rate of FIB per unit length of beach, m', with units (bacteria/second)/(length of beach). The total loading rate of FIB to ankle depth waters from all three non-point sources can be written:

$$m'_{NPS} = m'_{bathers} + m'_{birds} + m'_{seds}$$

We begin by presenting first-order estimates of the loading rate terms on the right hand side of this last equation (Section 8.3.1.). A mathematical framework is then presented for translating the loading rates into estimates of FIB concentrations in ankle depth waters (Section 8.3.2). Our primary goal is to rank the relative contribution of non-point sources to fecal contamination of shoreline waters. For the purposes of this analysis we focus on ENT for the following reasons: (1) at several OCHCA sampling sites in Newport Bay, routine monitoring data frequently exceed marine bathing water standards for ENT (Chapter 7), and (2) ENT are recommended by the US EPA for assessment of human health risk at marine bathing beaches[10].

8.3.1. First-Order Estimates of Non-Point Source ENT Loading Rates

The individual loading rates on the right hand side of the last equation can be estimated as follows:

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[9] Iaria, C., G. Stassi, G.B. Costa, R.D. Leo, A. Toscano, A. Cascio (2005) "Enterococcal meningitis caused by *Enterococcus casseliflavus*. First case report" *BMC Infectious Diseases* v.5:1-3

[10] Arnone, R.D., J.P. Walling (2007) "Waterborne pathogens in urban watersheds" *J. of Water and Health* v**5**:149-162.

$m'_{bather} = S\dot{N}'$

$m'_{birds} = W'_{feces} C_{feces} / T_F$

$m'_{seds} = W'_{seds} C_{seds} / T_F$

where

S = FIB shed per bather

$\dot{N}'$ = Number of bathers wading into the water every hour per meter of beach (people/hour/m)

W'_{feces} = weight of bird feces deposited on intertidal sediments during low tide per meter of beach (g/m)

C_{feces} = Concentration of FIB in bird feces (FIB/g)

T_F = Time over which a single flood tide occurs (ca., 6 hours)

$W'_{seds} = V'_{seds}(1-\phi)\rho_{sand}$ = Weight of sand washed by the rising tide per meter of beach (g/m)

$V'_{seds} = d_w \eta_r / \sin(\tan^{-1} s)$ = Volume of sand washed by the rising tide per meter of beach (m^3/m)

d_w = Depth of sand washed by the rising tide (m)

η_r = Tide range (m)

s = Beach slope (unitless)

C_{seds} = Concentration of FIB in sediments (FIB/g)

<u>*Bather Shedding*</u>. To estimate loading from bather shedding we multiply the FIB shedding rate per person (S, FIB/person) into the number of bathers that wade into the water per meter of beach per hour ($\dot{N}'$). For bather shedding, we adopt the rate reported by Elmir et al[11] of 300,000 ENT bacteria per person:

S = 300,000 ENT/bather

[11] Elmir, S.M., M.E. Wright, A. Abdelzaher, H.M. Solo-Gabriele, L.E. Fleming, G.Miller, M. Rybolowik, M.P. Shih, S.P. Pillai, J.A. Cooper, E.A. Quaye (2007) "Quantitative evaluation of bacteria released by bathers in a marine water" *Water Res*. **41**:3-10.

Bird Droppings. To estimate FIB loading from bird droppings, we assume that FIB in bird droppings deposited on intertidal sediments at low tide ($W'_{feces}C_{feces}$) are washed into ankle depth waters during a single flood tide (T_F). For the concentration of FIB in bird feces we use the range of ENT measured in bird feces as part of microcosm studies described in Chapter 5 of this report.

$$T_F = 6 \text{ hours}, \; C_{feces} = \begin{cases} 4 \times 10^8 \text{ MPN/g (low range)} \\ 10^{10} \text{ MPN/g (high range)} \end{cases}$$

Tidal Washing of Contaminated Sediment. Intertidal sediments have been identified as a likely source of ENT in shoreline waters in Upper Bay (see Chapter 6), and the tidally influenced region of the nearby Santa Ana River[12]. To estimate a loading rate for ENT that might result from the tidal washing of intertidal sediments, we performed the following back-of-the-envelope calculation. For a tide range of η_r = 1 m, and a beach slope of s =0.18, from trigonometry the intertidal zone has a cross-shore dimension of $\eta_r / \sin\left[\tan^{-1} s\right]$=5.6 m. If we assume that the top d_w =1 cm of intertidal sediment is washed by the rising tide[13], each flood tide would wash a sediment volume of $V'_{sed} = d_w \eta_r / \sin\left[\tan^{-1} s\right] = 6 \times 10^4$ cm^3 per meter of beach. This corresponds to a sediment weight of $\rho_{sand} V'_{sed}(1-\phi) = 5 \times 10^4$ g per meter of beach, assuming a sediment porosity of ϕ =0.5 and sand density of approximately ρ_{sand} =1.8 g/cm^3. Thus, based on these calculations, the parameters for tidal washing of contaminated sediments become:

$$T_F = 6 \text{ hours}, \; W'_{seds} = 5 \times 10^4 \text{ g per meter of beach}$$

8.3.2. Contribution of Non-Point Sources to ENT Concentration in Ankle Depth Water

As will be described in a journal manuscript to be submitted later, the loading rates for shore-based non-point source pollution can be translated into an estimate of FIB concentration in ankle depth water by integrating Fick's Law of Diffusion across a prism of water along the shoreline:

$$C_{ankle} = \frac{3m'_{NPS}}{s\varepsilon} + C_{tidal\ channel}$$

$C_{tidal\ channel}$= the concentration of FIB in the main tidal channel

ε = turbulent diffusion coefficient (m^2/s)

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[12]Ferguson, D.M., D.F. Moore, M.A. Getrich, M. Zhowandai (2005) "Enumeration and speciation of enterococci found in marine and intertidal sediments and coastal water in southern California" *J. Appl. Microbiol.* **99**:598-608

[13] The term "washed" in this context means that all ENT associated with the sediment (including that in the pore fluids and attached to the sand) is liberated into the overlying water column. This washing process could occur by the turbulent resuspension of the sediment, or some other process (e.g., buoyant convection of ENT-laden pore fluids out of the sediment as relatively colder water rises over warm sand).

s = beach slope (dimensionless)

The concentration of FIB in the main tidal channel $C_{tidal\ channel}$ reflects the contribution of Bay-wide processes, such as FIB loading from the San Diego Creek, on water quality in the main tidal channel just offshore of the beach. For the purposes of evaluating shore-based non-point sources, in the analysis presented here we assume that all of the FIB originate from the shoreline, in which case $C_{tidal\ channel} = 0$. The concentration of FIB in ankle depth waters can then be estimated as follows:

$$C_{ankle} = \frac{3}{s\varepsilon}\left(m'_{bathers} + m'_{birds} + m'_{seds}\right)$$

Substituting the loading rate expressions into this last equation, we arrive at the following relationship between the concentration of FIB in ankle depth waters and the rate at which new bathers wade into the water per meter of beach per hour ($\dot{N}'$), the weight of bird droppings deposited at low tide on intertidal sediments per meter of beach (W'_{feces}), and the concentration of ENT in intertidal sediments (C_{seds}, in units of ENT per dry weight of sediment):

$$C_{ankle} = \frac{3}{s\varepsilon} \times$$
$$\left\{\left[300{,}000\ \text{ENT/bather}\right]\dot{N}' + \begin{Bmatrix} 7\times10^{8}\ \text{ENT/g/hour (low range)} \\ 2\times10^{9}\ \text{ENT/g/hour (high range)} \end{Bmatrix} W'_{feces} + \left[8\times10^{3}\,\text{g/m/hour}\right]C_{seds}\right\}$$

Based on this last expression, it appears that water quality in ankle depth waters is affected by physical properties of the beach site that determine the rate at which FIB in ankle depth waters diffuse cross-shore into deeper waters (beach slope s and turbulent diffusion coefficient ε), and three parameters for the loading rate from bathers ($\dot{N}'$), bird droppings (W'_{feces}), and contaminated sediments (C_{seds}). The relative significance of these three non-point sources to shoreline pollution at Newport Beach can be evaluated by defining a critical loading rate m'_c above which water quality in the shoreline exceeds the single-sample standard for ENT (104 MPN/100 mL):

$$C_{SSS} = \frac{3m'_c}{s\varepsilon} = 104\ \text{MPN/100 mL}$$

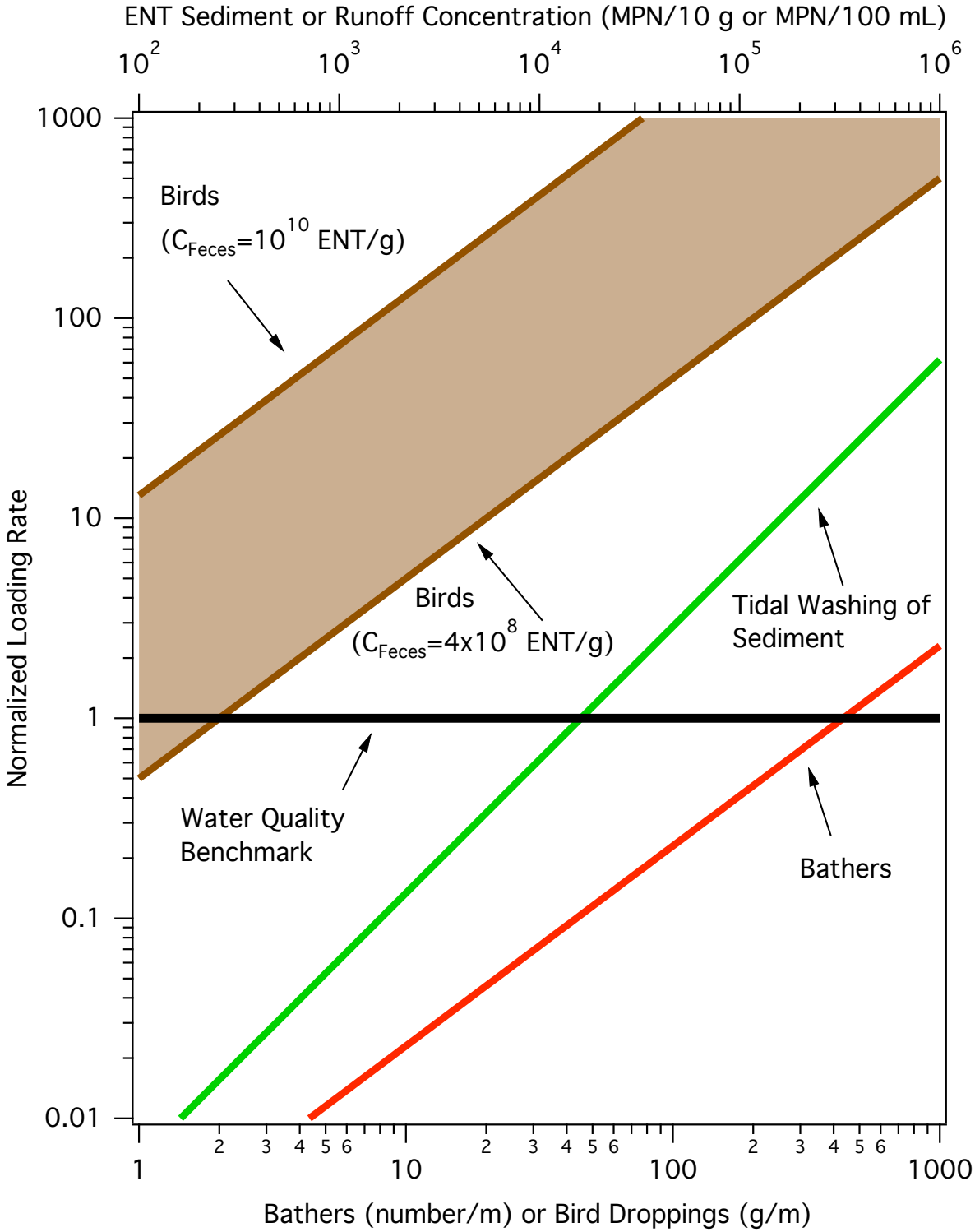

Figure 8-14. Model predictions of the contribution of shore-based non-point source pollution to ENT impairment of ankle depth waters in Newport Bay. Exceedences of the single-sample standard for ENT (104 MPN/100 mL) are indicated by values above the horizontal black line. Number of bathers and weight of bird droppings are shown on bottom horizontal axis. Concentration of ENT in sediments is shown on the top horizontal axis.

Taking the ratio of the last two expressions we obtain the following predictions for the concentration of ENT in ankle depth waters for the three shore-based non-point sources considered here:

$$\left[\frac{C_{ankle}}{C_{SSS}}\right]_{bathers} = \left[300{,}000 \text{ ENT/bather}\right]\frac{\dot{N}'}{m_c'}$$

$$\left[\frac{C_{ankle}}{C_{SSS}}\right]_{seds} = \left[8\times10^{3}\text{g/m/hour}\right]\frac{C_{seds}}{m_c'}$$

$$\left[\frac{C_{ankle}}{C_{SSS}}\right]_{birds} = \begin{Bmatrix} 7\times10^{8} \text{ ENT/g/hour (low range)} \\ 2\times10^{9} \text{ ENT/g/hour (high range)} \end{Bmatrix}\frac{W'_{feces}}{m_c'}$$

Using values for s and ε measured at one of the public beaches on the east side of Balboa Island ($s = 0.18$, ε = 0.5 m^2/s, and $s\varepsilon$ =0.1 m^2/s) we obtain the following estimate for the critical ENT loading rate above which a water quality violation occurs:

$m_c' = 1.3\times10^{8}$ ENT per hour per meter of beach

Predictions for the concentration of ENT in ankle depth waters, normalized relative to the single-sample standard of 104 MPN/100 mL, are plotted in Fig. 8-14. Each curve in the figure corresponds to a different shore-based non-point source, including bird droppings (two brown curves corresponding to the two different loading rates listed above), tidal washing of contaminated sediments (green curve), and bather shedding (red curve). The concentration of ENT in ankle depth water is predicted to exceed the single-sample standard at the point where a specific non-point source curve crosses the $C_{ankle}/C_{SSS} = 1$ line, indicated by the dark horizontal line in Fig. 8-14. From the location of the crossover points we conclude the following:

<u>*Bather shedding*</u>: An unrealistically large number of bathers (>400 per meter of beach) would need to wade into the water every hour to trigger a water quality violation in ankle depth water. While bather shedding may not cause a significant number of water quality violations, it could provide a pathway for the transmission of fecal-oral pathogens (see literature review, Chapter 1).

<u>*Bird droppings*</u>: Very few bird droppings (< 2 grams of feces per meter of beach) are needed to trigger water quality exceedences in ankle depth water. Given the very large loading rates predicted for this non-point source, bird droppings are likely to be the dominant cause of water quality violations at beach sites visited by even a modest number of birds (such as sites in Upper Newport Bay, see DITS study, Chapter 6).

<u>*Tidal Washing of Contaminated Sediment*</u>: Sediment concentrations of ENT >16,000 MPN/10g are needed to trigger a water quality violation in ankle depth water. This sediment concentration is well within the range (50th to 90th percentile) measured for sub-tidal and intertidal sediments in Newport Bay (Chapters 2 and 6). The threshold sediment concentration will be even lower at

beaches with relatively shallow bed slopes, because a larger volume of sediment is washed with every tide. For example, we measured a slope of $s = 0.05$ at one of the OCHCA monitoring sites in Upper Bay (BNB 26, data not shown). Going from a slope of $s = 0.18$ (used for the calculations illustrated in Fig. 8-14) to a slope of $s = 0.05$ (measured at BNB 26), lowers 4 fold the sediment concentration of ENT needed to trigger a water quality violation, to >4,000 MPN/10 g.

Based on the above results it appears that bird droppings and tidal washing of contaminated sediment are both likely causes of ENT marine bathing water standard exceedences at beach sites in Newport Bay. It is important to stress that the loading predictions presented in Fig. 8-14 are only as good as the analysis used to derive the loading equations, and the choice of parameter values used to plot the curves. Even if one assumes that the physics of mixing is well described by the 1D Fickian model employed here, many of the parameter values used to generate the curves in Fig. 8-14 (e.g., the weight of sand washed by the rising tides) are not well known at present. As such, these results should be regarded as more illustrative than conclusive. The mixing analysis that led to the calculations above will be submitted to an archival journal for peer review in the near future.

8.4. Drain Scale: Impacts of FIB loading from Small Drains

In this section we evaluate FIB loading from the many small drains distributed along the shoreline in Upper and Lower Bay. Based on field measurements presented in Chapter 7, it appears that these drains can impact ankle depth water quality. While the FIB loading rate from single drains is small in comparison to, for example, San Diego Creek, their large number and periodic spacing along the shore implies that these drains could, collectively, constitute a significant cause of water quality impairment in the ankle depth waters of Newport Bay. Below we present probability plots of the drainage areas, flow rates, and FIB loading rates from drains in Newport Bay.

8.4.1 Locations, Drainage Areas, and Pipe Diameters of Storm Drains in Newport Bay

As a first step toward characterizing the dry weather loading of FIB into Lower Bay from storm drains, an inventory of all drains discharging to Newport Bay was created. The inventory included the storm drain's location, drainage area, and pipe diameter (Table 8B-1, Appendix 8B). The 214 drains in the inventory are distributed throughout Newport Bay, although drain spacing is most dense in Lower Bay (Fig. 8-15).

Fig. 8-16 presents probability distributions of the drainage areas and drainpipe diameters for the 214 drains that discharge to Lower and Upper Newport Bay. Drainage areas vary over 3 log-cycles, from approximately 1000 to 3,000,000 m^2 (or 0.25 to 740 acres). The median drainage area for all storm drains discharging to Newport Bay is 40,500 m^2 (or 10 acres). The drainpipe diameters vary over approximately 1 log-cycle, from 0.1 to 3.7 m (or 4 to 146 inches). The median diameter for all storm drains discharging to Newport Bay is 0.46 m (or 18 inches). Although the diameter of storm drain outlets generally increases with increasing drainage area, there is not a one-to-one relationship between these two.

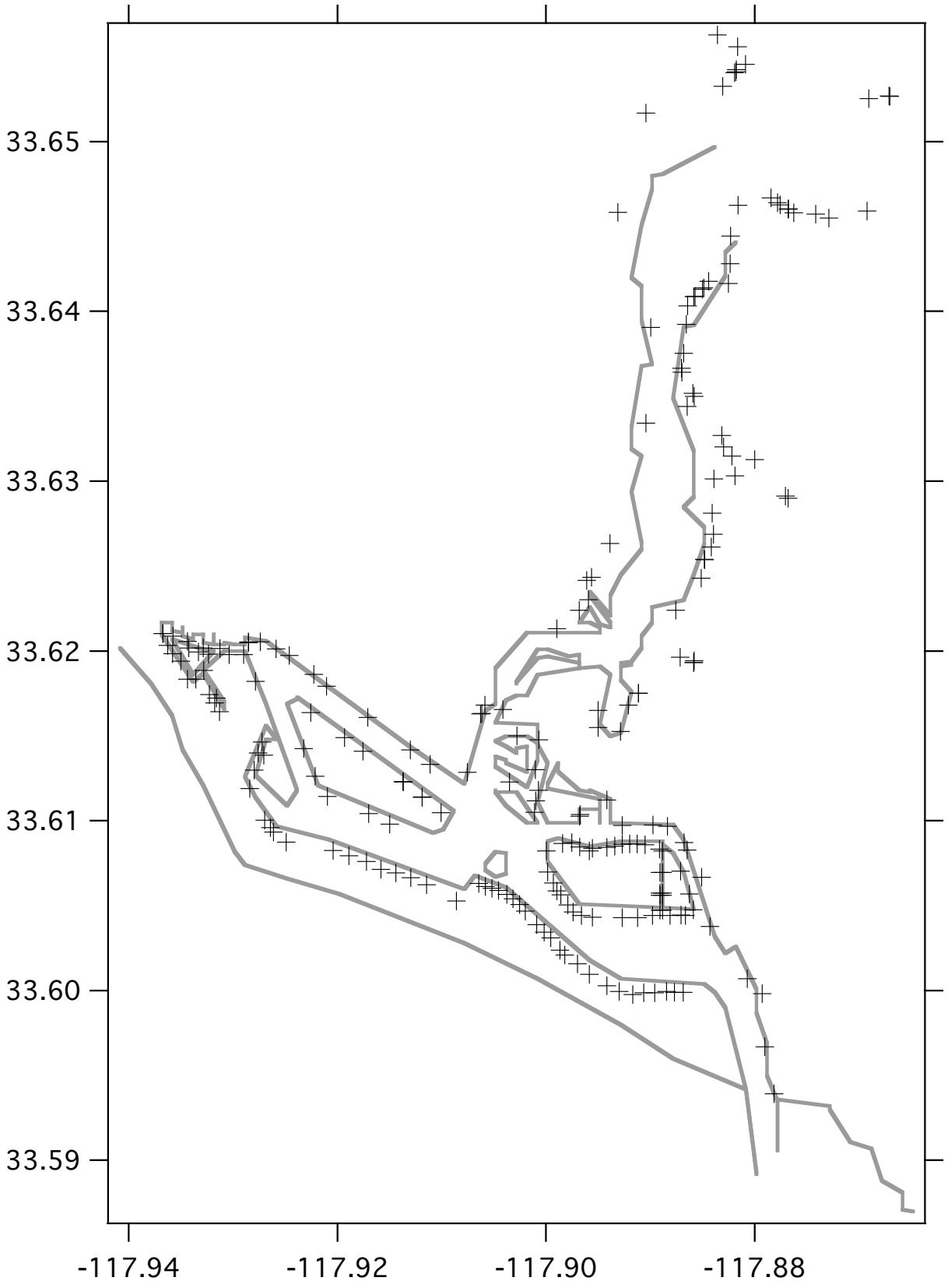

Figure 8-15. A map showing the location of small storm drains discharging to Newport Bay.

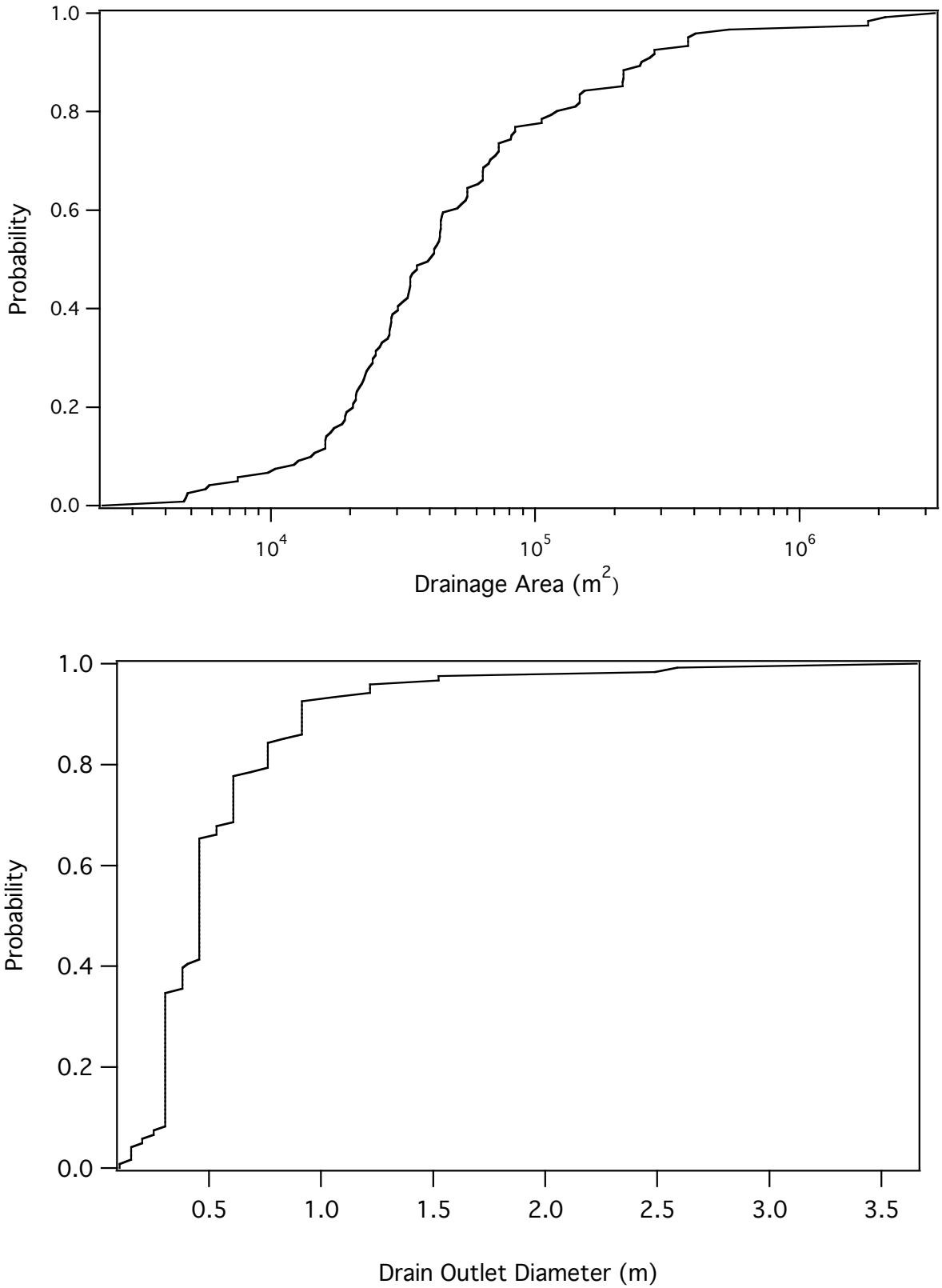

Figure 8-16. Probability distributions of the drainage area (top panel) and the diameter of the drain outlet (bottom panel) for the 215 drains that discharge to Newport Bay.

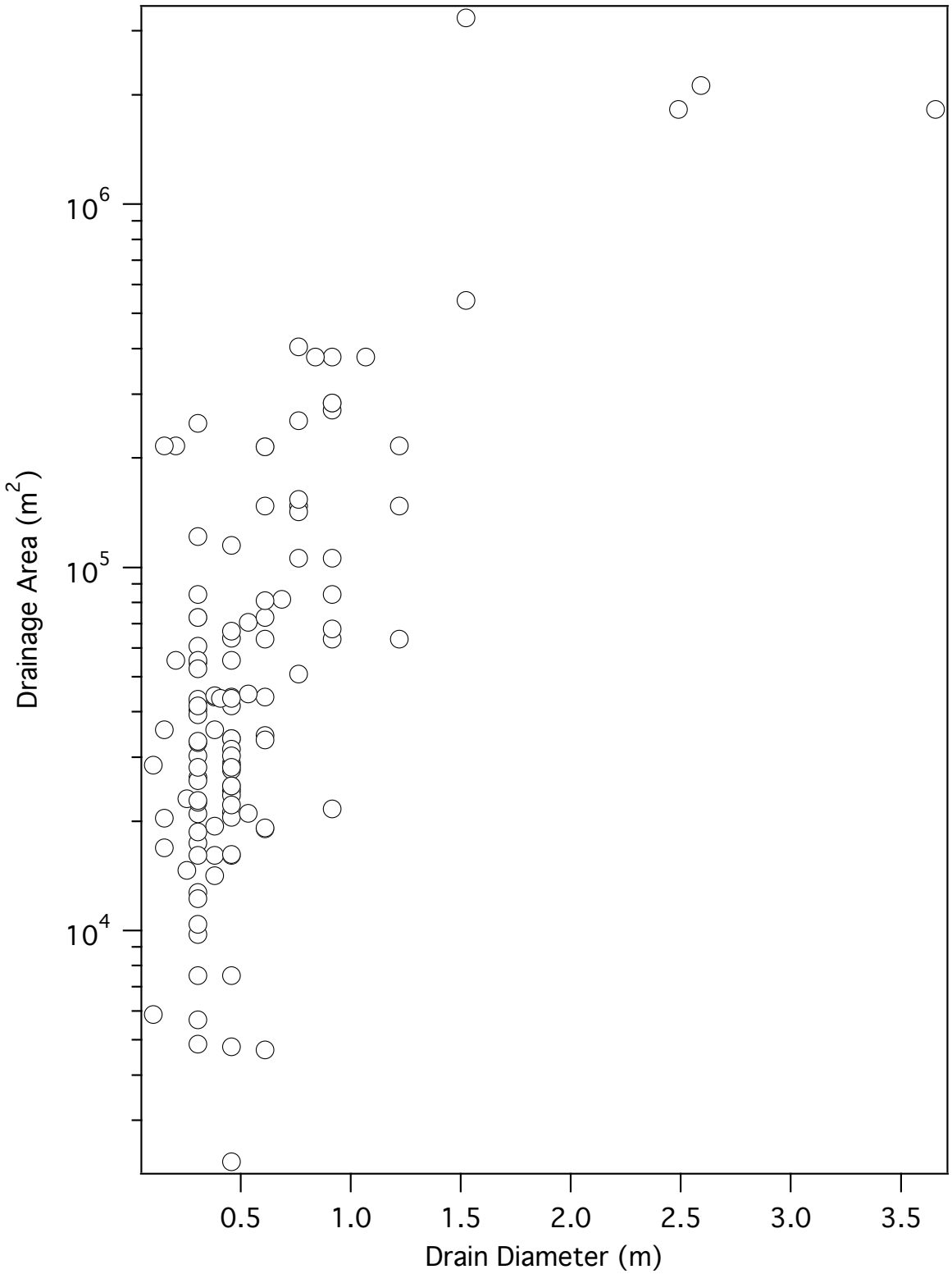

Figure 8-17. Relationship between drainage area and drain diameter for all storm drains discharging to Newport Bay.

parameters (Fig. 8-17). For example, storm drains with 0.3 m (12 inch) diameters service drainage areas ranging in size over two log-cycles, from 4800 to 250,000 m^2 (or 1.0 to 62 acres).

8.4.2 Measurement and Modeling of Dry Weather Flow Rates

Dry weather flow rates were estimated for all drains in Newport Bay using a two-step process. First, a mathematical relationship between dry weather flow and sub-drainage area was developed based on previously collected flow rate data. Second, this mathematical relationship was used to convert the sub-drainage areas reported for all 214 drains in Newport Bay into estimates of dry weather flow.

Measurement of Dry Weather Flow Rates. The relationship between dry weather flow rate and drainage area is shown graphically in Fig. 8-18. Note that the vertical axis in this graph spans nearly a million-fold change in dry weather flow rates, from 10 to 10 million gallons per day. At the highest end are dry weather flow rates measured in San Diego Creek and Santa Ana Delhi by Orange County personnel (squares in Fig. 8-18). Next highest are dry weather flow rates, measured by Everest Consultants, for a set of large storm drains that empty into Newport Bay[14] (circles in Fig. 8-18). The smallest dry weather flow rates, measured by the City of Newport Beach, for a set of small storm drains located on Balboa Island and the Newport Beach Peninsula in Lower Bay (triangles in Fig. 8-18) (see report prepared by the City of Newport Beach, Appendix 8C).

Modeling Dry Weather Flow Rates For the purposes of estimating flow rates for all 214 drains scattered around the perimeter of Newport Bay, it is necessary to develop a model that allows flow rates to be calculated from known drainage areas. As a starting point for developing such a model, we reasoned that dry weather flow should scale linearly with drainage area:

$$Q = FA$$

In this equation F represents the flux of dry weather runoff from the surrounding urban landscape. Relative to the log-log plot of flow rate against drainage area (Fig. 8-16), this last equation will plot as a line with slope unity and intercept $Q = F$ at $A = 1$. Based on the data plotted in Fig. 8-16, it appears that two separate flux values apply to these flow measurements—one for all drainages with areas greater than about 100 acres ($F = 97$ gallons/day/acre) and another for drainages with areas less than about 10 acres ($F = 11$ gallons/day/acre):

$$F = \begin{cases} 97 \text{ gallons/day/acre}, & A > 100 \text{ acres} \\ 11 \text{ gallons/day/acre}, & A < 10 \text{ acres} \end{cases}$$

For drainage areas intermediate between 10 and 100 acres, the flux value was obtained by linear interpolation.

[14] Everest International Consultants Inc., 2004. "City of Newport Beach storm drain diversion study-Main Report", prepared for the City of Newport beach, November, 2004

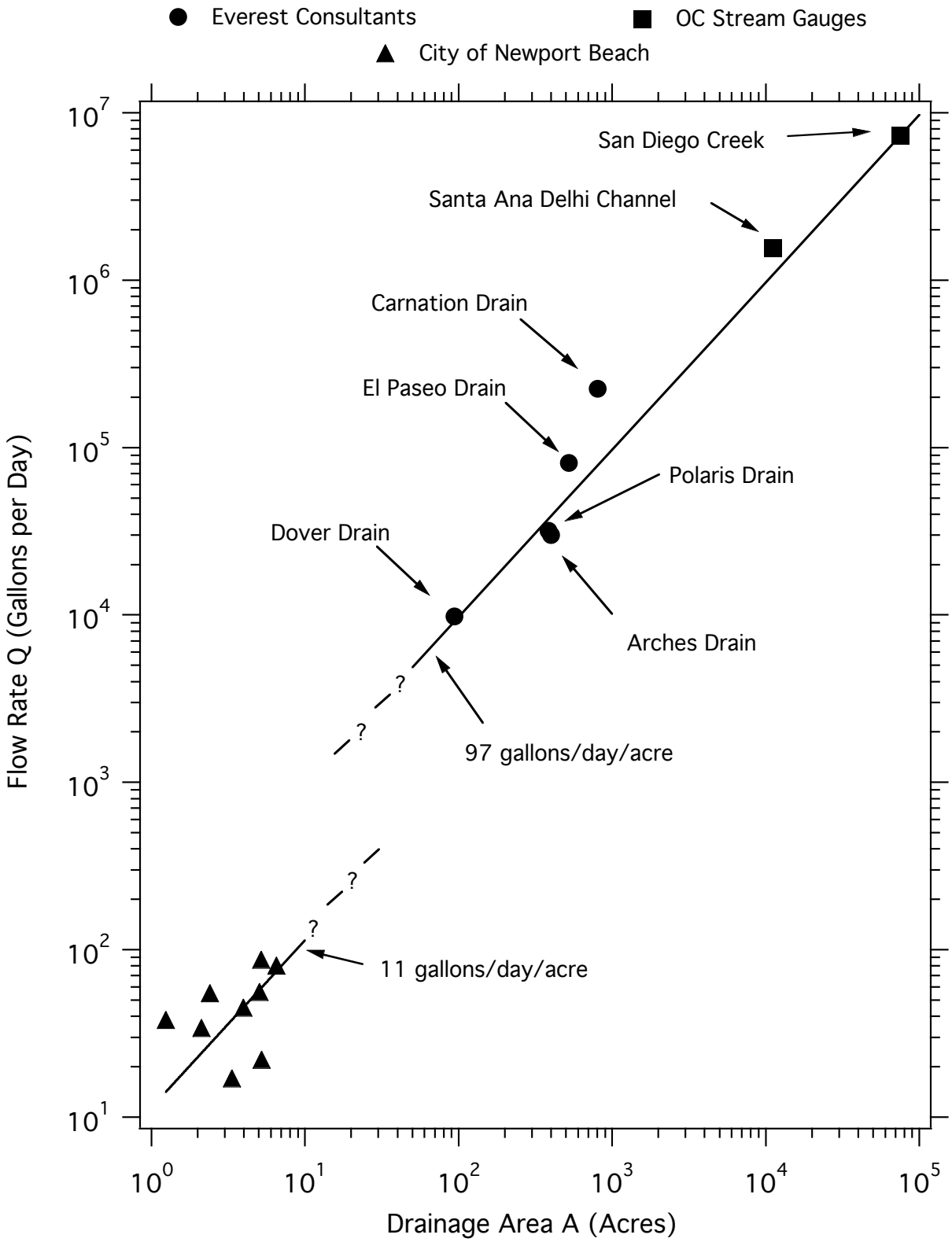

Figure 8-18. Relationship between average dry weather flow in storm drains and creeks discharging to Newport Bay, and the corresponding drainage areas.

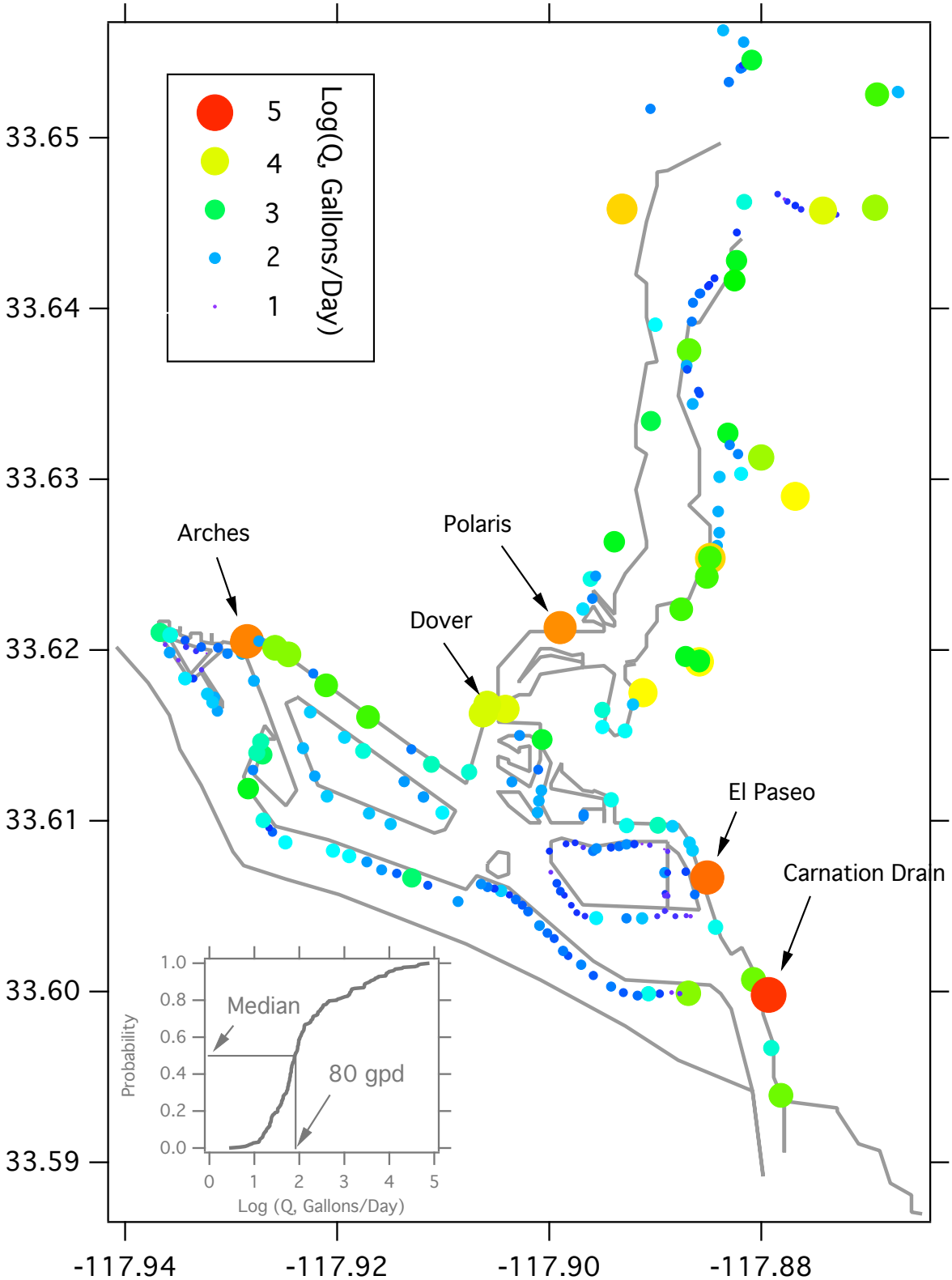

Figure 8-19. Map showing the estimated dry weather flow rates for all drains discharging to Upper and Lower Newport Bay. Inset is a probability graph of flow rates predicted for all drains. The median flow rate is 80 gallons per day.

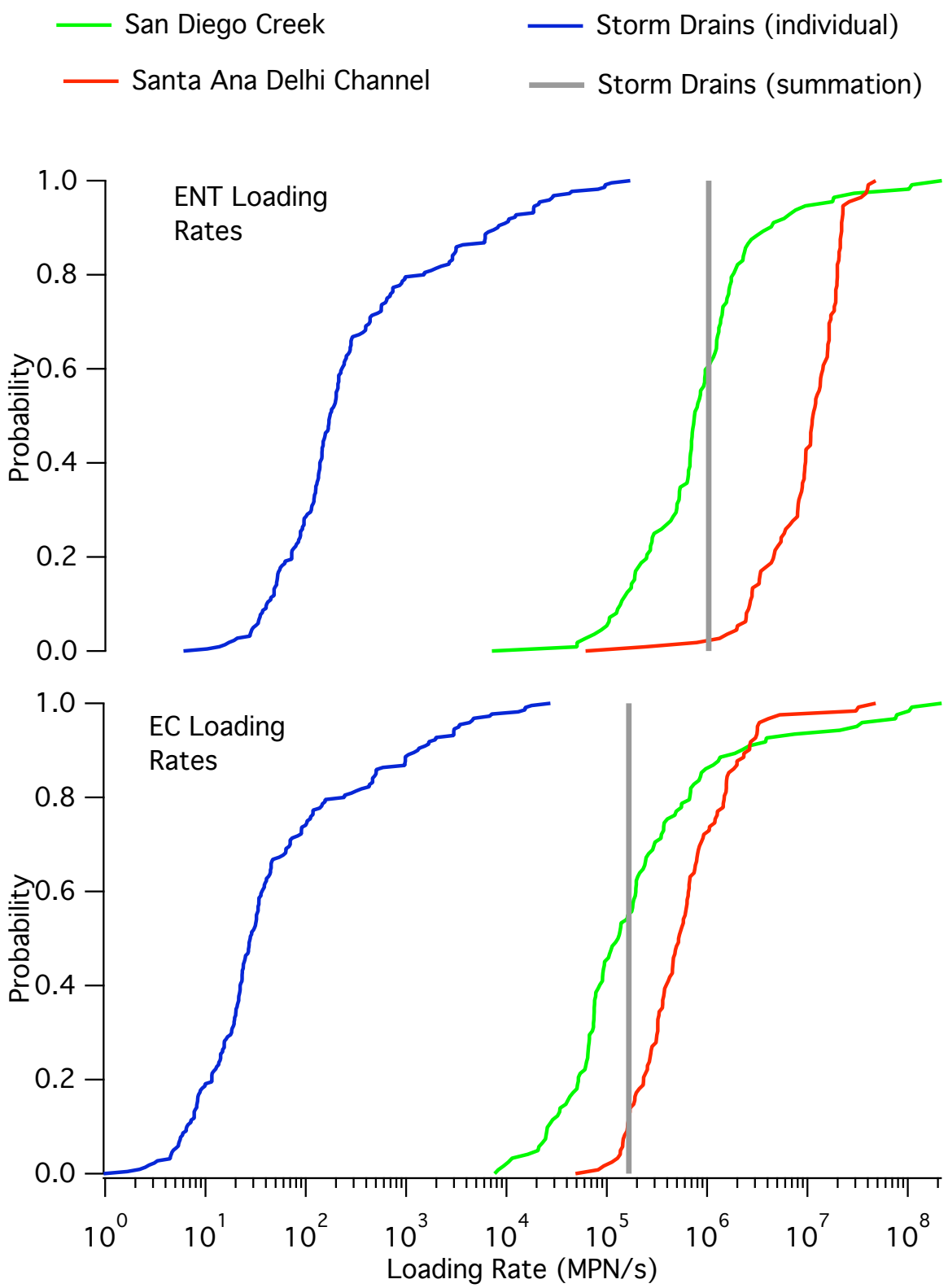

Figure 8-20. Probability distributions of dry weather FIB loading rates calculated for all storm drains (blue curves), and for San Diego Creek (green curves) and Santa Ana Delhi Channel (red curves). The total FIB loading rate from all storm drains discharging to Newport Bay is also shown (grey vertical lines).

8.4.3 Estimating Dry Weather Flow Rates for Storm Drains in Newport Bay

The above dry weather flow model was applied to the 214 drains that discharge to Newport Bay (Table 8B-1 and Fig. 8-19). Depending on the drain (and its associated drainage area), estimated dry weather runoff volume ranges over >4 log-cycles, from approximately 3 (=$10^{0.5}$) to 78,000 (=$10^{4.9}$) gallons per day; the median estimated dry weather runoff volume from all drains in Newport Bay is 80 (=$10^{1.9}$) gallons per day (inset, Fig. 8-19).

Drains estimated to discharge the largest volumes of dry weather runoff (>10,000 gallons per day) are located in regions of the Bay previously identified as having depressed salinity and high FIB concentrations (Chapter 7). For example, Arches drain in West Newport Bay is estimated to discharge approximately 40,000 gallons per day of runoff. Based on the synoptic study, water quality in this region of the Bay (Turning Basin) has lower salinity and higher FIB concentrations (compare Figs. 7-5 and 8-19). Likewise, the El Paseo drain discharges to the channel that separates the eastern shore of Balboa Island from the mainland. This particular drain has very high estimated dry weather flow rates (approximately 100,000 gallons per day) and, perhaps not surprisingly, the region of the Bay surrounding this storm drain has depressed salinity and high FIB concentrations (compare Figs. 7-5 and 8-19).

8.4.4 Estimating Dry Weather FIB Loading Rates from Storm Drains in Newport Bay

Based on the results presented in Chapter 7, it appears that storm drains in Lower Bay, particularly the bigger ones, can negatively impact local water quality in the Bay, as measured by both depressed salinity and elevated FIB concentrations. To better quantify, and perhaps prioritize, fecal pollution from these storm drains, dry weather FIB loading rates from storm drains were calculated and compared to dry weather FIB loading rates from San Diego Creek and the Santa Ana Delhi Channel reported earlier in this chapter. Dry weather loading rates were estimated for each storm drain by multiplying estimated dry weather flow rate into the median concentration of EC or ENT measured in all irrigation runoff samples collected during the dry weather storm drain survey described in Section 7.6. Median values of EC and ENT used for these calculations were 800 and 5000 MPN/100 mL.

FIB loading rates estimated for each of the 214 drains are presented as probability plots in Fig. 8-20. For comparison, the loading rates of San Diego Creek and Santa Ana Delhi Channel are also included in the figure. Based on the results presented in this plot, the dry weather loading of FIB from the largest drains (blue curves) is less than the smallest loading of FIB from the San Diego Creek and Santa Ana Delhi channel (green and red curves, respectively). However, when the FIB loading from all storm drains discharging to Newport Bay are summed (vertical grey lines in the figure), the resulting cumulative drain loading rates of FIB are comparable to the loading of FIB from the San Diego Creek and Santa Ana Delhi channel. It is important to stress that that cumulative loading of FIB from small drains is spread out over the length of the Newport Bay shoreline, whereas the loading from San Diego Creek and the Santa Ana Delhi channel is focused at the outlets of these two tributaries. Thus, while the loading of FIB from the tributaries can have a Bay-wide impact on water quality as indicated by the modeling exercise described in Section 8-2, this is unlikely to be the case for the cumulative loading from small storm drains. On the other hand, as noted in Chapter 7, the small storm drains can have localized impacts on

Bay water quality, particularly in the shallow (ankle depth) waters near where the drains discharge to the Bay, and routine water quality monitoring (and recreational contact) occur.

8.4.5 Estimating Wet Weather FIB Loading Rates from Storm Drains in Newport Bay

Wet weather loading rates from storm drains in Newport Bay will vary with the amount of precipitation that falls on a particular sub-drainage, and the period of time over which that rainfall occurs. Given a hyetograph for a particular storm, together with a suitable rainfall/runoff relationship (e.g., the Rational Formula), the FIB loading from a particular storm drain can be calculated from the data presented in this report—including the drainage areas associated with each storm drain (this chapter) and the concentration of FIB measured in wet weather runoff (Chapter 7). Indeed, wet weather flow from the drains in Newport Bay should exhibit a pattern similar to that Fig. 8-19, except the magnitude of the bubbles (i.e., the assigned flow rates to each bubble) will scale linearly with volume of rainfall that fell in a given subdrainage during a given storm. This observation follows from the fact that Fig. 8-19 as generated by assuming that the dry weather flow scales linearly with drainage area, and the same linear scaling of runoff and drainage area is assumed in the Rational Formula.

8.4.6 Water Quality Impact of FIB Loading Rates from Storm Drains in Newport Bay

The water quality impact of FIB loading from storm drains will no doubt be highly site specific, and depend on details such as: 1) the time frame over which FIB from the drains mix into near shore waters (c.f., the tidal trapping model, Chapter 7); 2) buoyancy associated with runoff as it mixes with brackish water from the Bay; 3) the number of dimensions over which pollutants in the runoff undergo mixing by turbulent diffusion (i.e., is the problem 1D, 2D, or 3D?); 4) the influence of tidal advective flow on the fate and transport of FIB in nearshore waters; and 5) non-conservative processes that can affect FIB concentrations, such as die-off, predation, and growth (see literature review in Chapter 1).

Relative to the question of dimensionality (item 3 above), it is interesting to note that in some regions of the Bay it may be possible to conceptualize the shoreline loading of FIB from runoff as a line source (e.g., see the continuous array of storm drains along the Newport Beach Peninsula, Fig. 8-19), in which case the simple Fickian Diffusion model presented in Section 8.3 of this chapter may suffice. In other cases (e.g., Arches Drain in West Newport Bay), it is likely that the mixing of FIB-laden runoff into this region of the Bay is at least a two-dimensional, and perhaps three-dimensional, problem, for which numerical solution methods are required.

8.5 Conclusions

The measurements and modeling studies documented in this chapter support the following conclusions:

A simple mathematical model of tidal transport and dilution in Newport Bay was developed and used to investigate the impact of San Diego Creek and Santa Ana Delhi channel on water quality in Upper and Lower Bay. During wet weather, the model accurately predicts FIB concentrations measured in the Bay during the BTO studies (Chapter 2), provided that we use as

model input the FIB loading from both San Diego Creek and Santa Ana Delhi channel. Thus, during wet weather, it appears that FIB concentrations in the main tidal channel of Newport Bay are controlled by the magnitude of FIB loading from these two tributaries.

During dry weather, the model accurately predicts measured ENT concentrations in the Bay, but only when we use as model input the FIB loading from San Diego Creek. When dry weather ENT loading from Santa Ana Delhi channel is used as input to the model, predicted ENT concentrations are significantly (10 to 100 times) greater than measured FIB concentrations in the Bay. Taking these model results at face value, they suggest that dry weather concentrations of ENT in the main tidal channel of Newport Bay are lower than would be expected, given the very high loading of ENT measured at our sampling site on the Santa Ana Delhi channel. This observation could have several possible explanations. First, one or more assumptions in the simple 1D model used here may render the model predictions inaccurate during dry weather. Second, the load of ENT from the Santa Ana Delhi channel may be attenuated between where the loading rate was measured on the Santa Ana Delhi channel (i.e., site BTO 2) and the main tidal channel of Newport Bay (where model predictions were compared to ENT measurements at BTO 4 through 11). The former possibility will be evaluated as the model is vetted through peer review, and possibly followed up with more sophisticated (multi-dimensional) modeling efforts. To evaluate the latter possibility, and obtain more information about how bacteria from the Santa Ana Delhi channel make their way into Newport Bay, several TAC members, led initially by Dr. Jack Skinner, conducted a field reconnaissance of the site. From this field reconnaissance, the following can be stated:

(1) Before runoff from the Santa Ana Delhi channel flows into Newport Bay's main tidal channel, it must first travel along a 1 km incised tidal mudflat channel referred to here as the "Santa Ana Delhi Tidal Channel".
(2) Tidewaters intrude a significant distance up the Santa Ana Delhi Tidal Channel, including upstream of our Santa Ana Delhi sampling site (BTO 2), which is consistent with the brackish salinities that were measured in water samples collected from BTO 2 during the summer of 2006.
(3) Water in the Santa Ana Delhi Tidal Channel does not appear to overflow its banks and spill into the surrounding mudflat. During a high tide on 5/29/09, for example, water in the Santa Ana Delhi Tidal Channel was well below the height of the channel levee (personal observation, Ray Hiemstra). A photograph of the lower reaches of the Santa Ana Delhi Channel during a high tide indicates that water in the channel is confined between the banks.
(4) In the upper reaches of the Santa Ana Delhi channel, just downstream of our Santa Ana Delhi channel sampling site (BTO 2), the channel bottom forms a set of basins separated by shallow sills. While the depth of the basins and sills varies throughout the tidal cycle, when observed during a low tide, one basin documented in the report was >2 m deep.

Taken together, these field observations suggest that the dry weather residence time of water in the tidally influenced portion of the Santa Ana Delhi channel is likely to be long (i.e., multiple tidal cycles) because: (1) the basins and sills will act to retard drainage of the channel during ebb tides, and (2) flow in the channel will probably be dominated by tidal action during dry weather, when the volume of freshwater from the Santa Ana Delhi channel is low. The long residence

time of water in the Santa Ana Delhi Tidal Channel may act to attenuate the summertime loading of ENT from BTO 2, as our modeling suggests, by increasing the dilution of runoff with (relatively clean) Bay water, and increasing the time over which bacteria in the runoff die-off in the brackish waters of the tidal channel.

It is important to note that, even if the above scenario is correct, changes in the topography of the tidal channel (e.g., elimination of the basin and sills by scouring of the channel bottom during storms), could alter the residence time of water in the channel, and thus alter the loading of FIB to the main tidal channel of Newport Bay. Indeed, it appears that dry weather water quality in Newport Bay has improved over the last several years, which might be attributable to changing bottom topography in the Santa Ana Delhi channel and possibly the recent construction of detention basins in the lower reaches of the San Diego Creek (efforts are ongoing to determine the timing of alterations to the lower reach of San Diego Creek). detention basins in the lower reaches of the San Diego Creek (efforts are ongoing to determine the timing of alterations to the lower reach of San Diego Creek).

Model predictions also indicate that dry weather loading of EC from San Diego Creek and Santa Ana Delhi channel is not sufficient to explain measured EC concentrations in the Bay, suggesting that another source—perhaps growth in a near-bed layer as suggested in previous chapters (see Chapter 2) —dominates during dry weather periods.

Model predictions were also carried out to define a region of impact (ROI)—defined as the region of the Bay where FIB concentrations are predicted to exceed marine bathing water standards—attributable to loading from San Diego Creek and Santa Ana Delhi channel. These ROI calculations indicate that, for all but the largest 90th percentile of storms, water quality violations attributable to FIB loading from the two tributaries are confined to Upper Bay.

Shore-based non-point sources of FIB, including bather shedding, bird droppings, and tidal washing of contaminated sediments, were also investigated using measured data and a simple (integrated) form of Fick's Law of Diffusion. The results indicate that bather shedding is unlikely to cause water quality exceedences along the shoreline in Newport Bay. Tidal washing of contaminated sediments and bird droppings, on the other hand, are very likely to cause exceedences of marine bathing water standards in ankle depth waters.

An inventory of storm drains in Newport Bay was conducted, and the following attributes were determined: drainage area, drain diameter, outfall location, and estimated dry weather flow rates. From these data, probability distributions were generated of FIB loadings from the many small and large drains in Newport Bay. The results suggest that the cumulative loading of FIB to the Newport Bay shoreline from all storm drains is comparable to the median (dry weather) loading rates measured in San Diego Creek and Santa Ana Delhi channel. However, this FIB loading is distributed around the perimeter of Newport Bay, and is thus a very diffuse source that will likely have only localized impact on shoreline water quality; i.e., with a few exceptions (such as Arches Drain), discharge of dry weather runoff from storm drains is unlikely to contaminate large regions of Newport Bay. Conversion of these loading rates into FIB concentrations along the shoreline is complicated by a number of issues, related to the timing of

the discharges, the geometry of the site, and the nature of mixing and transport that occurs after discharge.