appendix A

DETAILED METHODS

Appendix A
DETAILED METHODS

INTRODUCTION

This appendix contains a summary of the methods used for field sampling, laboratory testing, and data analysis used in the District's ocean monitoring program. The methods include those for calculations of water quality compliance with California Ocean Plan (COP) criteria, coastal oceanography, water quality monitoring, bacteriology/nutrients, sediment geochemistry, invertebrate and fish community analysis, fish health, and fish tissue contaminants. More detailed methods can be found in the program Quality Assurance Project Plan (QAPP) (OCSD 2007c), Environmental Assessment Division Standard Operating Procedures (OCSD 2007b), and the Environmental Sciences Laboratory Operating Procedures Manual (LOPM) (OCSD 2007a).

For 2007-08, the Ocean Monitoring Program (OMP) was conducted under conditions stipulated in the District's 2004 NPDES discharge permit (Order No. R8-2004-0062, NPDES No. CA CA0110604).

WATER QUALITY COMPLIANCE

Water quality compliance for the District's OMP is assessed based on (1) specific numeric criteria for dissolved oxygen and pH; and (2) narrative (non-numeric) criteria for transmissivity, floating particulates, oil and grease, water discoloration, beach grease, and excess nutrients. The sampling approach comprises a 2 km by 2 km fixed-grid pattern with seven nearshore-offshore transects of four stations each (Table A-1). Transect 7 also includes the historically relevant Newport Canyon Station C2. Station locations were defined as either Zone A (stations are along and inshore of the 3-mile limit) or Zone B (beyond the 3-mile limit) as shown in Figure A-1. Stations numbers ending in 03 and 04 comprise the Zone A stations; those ending in 05 and 06 are the Zone B stations. Compliance evaluations are based on statistical comparisons to the corresponding upcurrent Zone A or Zone B reference station (OCSD 1999). This matching of Zone A or Zone B stations allows comparisons of data from similar water depths.

Data Analyses

This section describes the procedures used since July 1998 to evaluate water quality compliance.

Field-collected water quality data are reviewed by the District for accuracy and completeness and then forwarded to a contractor for processing. Processing consists of first calculating the depth of the pycnocline layer for each station using temperature and salinity data. The pycnocline is defined as the depth layer where stability is greater than 0.05 kg/m^3 (Officer 1976). Data for each station and numeric compliance parameter

Table A-1. OCSD ocean monitoring program station locations.

Orange County Sanitation District, California.

Transect	Station	Latitude	Longitude	Depth (m)	Zone
Offshore Water Quality Monitoring Station Locations					
1	2403*	33° 38.765'	118° 03.072'	21	A
	2404	33° 37.875'	118° 03.808'	29	A
	2405	33° 36.986'	118° 04.544'	37	B
	2406	33° 36.096'	118° 05.280'	60	B
2	2351*	33° 38.151'	118° 02.001'	21	A
	2352	33° 37.262'	118° 02.739'	29	A
	2353	33° 36.373'	118° 03.477'	37	B
	2354	33° 35.484'	118° 04.214'	123	B
3	2303*	33° 37.537'	118° 00.936'	21	A
	2304	33° 36.649'	118° 01.674'	29	A
	2305	33° 35.760'	118° 02.412'	38	B
	2306	33° 34.871'	118° 03.149'	114	B
4	2223*	33° 36.924'	117° 59.871'	22	A
	2224	33° 36.035'	118° 00.608'	31	A
	2225	33° 35.146'	118° 01.346'	47	B
	2226	33° 34.257'	118° 02.083'	135	B
5	2203*	33° 36.313'	117° 58.810'	25	A
	2204	33° 35.423'	117° 59.546'	39	A
	2205	33° 34.534'	118° 00.282'	57	B
	2206	33° 33.644'	118° 01.018'	185	B
6	2183*	33° 35.701'	117° 57.744'	36	A
	2184	33° 34.811'	117° 58.480'	51	A
	2185	33° 33.922'	117° 59.215'	114	B
	2186	33° 33.032'	117° 59.951'	247	B
7	2103*	33° 35.089'	117° 56.678'	110	A
	2104*	33° 34.199'	117° 57.414'	143	A
	2105	33° 33.309'	117° 58.150'	280	B
	2106	33° 32.420'	117° 58.885'	309	B
	C2*	33° 36.125'	117° 56.014'	56	A

* = REC-1 sampling

Table A-1 Continues.

Table A-1 Continued.

Station	Latitude	Longitude	Depth (m)
Nearshore Water Quality Monitoring Station Locations			
39N	33° 42.114'	118° 03.321'	surf
33N	33° 41.281'	118° 02.495'	surf
27N	33° 40.587'	118° 01.712'	surf
21N	33° 39.843'	118° 00.785'	surf
15N	33° 39.114'	117° 59.846'	surf
9N	33° 38.565'	117° 58.924'	surf
6N	33° 38.331'	117° 58.573'	surf
3N	33° 38.018'	117° 58.032'	surf
0	33° 37.764'	117° 57.598'	surf
3S	33° 37.619'	117° 57.264'	surf
6S	33° 37.337'	117° 56.704'	surf
9S	33° 37.033'	117° 56.283'	surf
15S	33° 36.342'	117° 55.459'	surf
21S	33° 36.059'	117° 54.213'	surf
27S	33° 35.646'	117° 52.910'	surf
29S	33° 35.559'	117° 52.508'	surf
39S	33° 34.700'	117° 51.946'	surf
Quarterly Benthic Monitoring Station Locations			
0	33° 34.573'	118° 00.598'	56
1	33° 34.657'	118° 00.968'	56
4	33° 34.498'	117° 59.761'	56
5	33° 34.749'	118° 01.612'	59
9	33° 34.363'	117° 59.510'	59
12	33° 34.385'	117° 59.054'	58
C	33° 35.799'	118° 03.855'	56
Control 1	33° 36.037'	118° 05.387'	59
ZB	33° 34.545'	118° 00.274'	56
ZB2	33° 34.590'	118° 00.611'	56

Table A-1 Continues.

Table A-1 Continued.

Station	Latitude	Longitude	Depth (m)
Annual Benthic Monitoring Station Locations			
3	33° 34.434'	118° 00.660'	60
7	33° 35.325'	118° 00.367'	41
8	33° 35.164'	117° 59.555'	44
10	33° 34.902'	118° 02.081'	62
13	33° 35.307'	118° 02.944'	59
17	33° 33.961'	118° 00.187'	91
18	33° 34.064'	118° 00.750'	91
20	33° 34.599'	118° 02.229'	100
21	33° 35.313'	118° 01.891'	44
22	33° 35.204'	117° 59.028'	45
23	33° 33.968'	117° 59.147'	100
24	33° 33.563'	118° 01.140'	200
25	33° 33.924'	118° 02.176'	200
27	33° 33.326'	117° 59.708'	200
29	33° 35.033'	118° 03.113'	100
30	33° 35.493'	118° 02.899'	46
33	33° 34.349'	117° 57.866'	100
36	33° 35.308'	117° 57.495'	45
37	33° 34.832'	117° 57.369'	56
38	33° 34.634'	117° 57.317'	100
39	33° 33.283'	117° 58.531'	200
40	33° 32.496'	117° 59.775'	303
41	33° 32.690'	118° 01.149'	303
42	33° 33.098'	118° 02.598'	303
44	33° 34.586'	118° 05.422'	241
55	33° 36.739'	118° 05.413'	40
56	33° 35.665'	118° 05.417'	100
57	33° 34.970'	118° 05.418'	200
58	33° 33.365'	118° 05.347'	300
59	33° 36.070'	118° 03.701'	40
60	33° 35.532'	118° 04.017'	100
61	33° 35.011'	118° 04.326'	200
62	33° 34.069'	118° 04.568'	300
63	33° 34.173'	118° 03.407'	200
64	33° 33.484'	118° 03.663'	300
65	33° 33.859'	117° 57.230'	200
C2	33° 36.125'	117° 56.014'	56
C4	33° 35.056'	117° 55.833'	187
C5	33° 33.920'	117° 55.620'	296

Table A-1 Continues.

Table A-1 Continued.			
Station	**Latitude**	**Longitude**	**Depth (m)**
Trawl Station Locations			
T0*	33° 37.117'	117° 59.283'	18
T1	33° 34.641'	118° 00.567'	55
T2	33° 35.688'	117° 59.561'	35
T3	33° 34.856'	117° 57.345'	55
T6	33° 35.946'	118° 02.785'	36
T10	33° 33.771'	118° 00.250'	137
T11	33° 36.055'	118° 05.199'	60
T12	33° 34.868'	118° 01.670'	57
T13	33° 35.535'	118° 03.637'	60
T14	33° 34.672'	118° 03.200'	137
* = T0 sampled for historical purposes.			

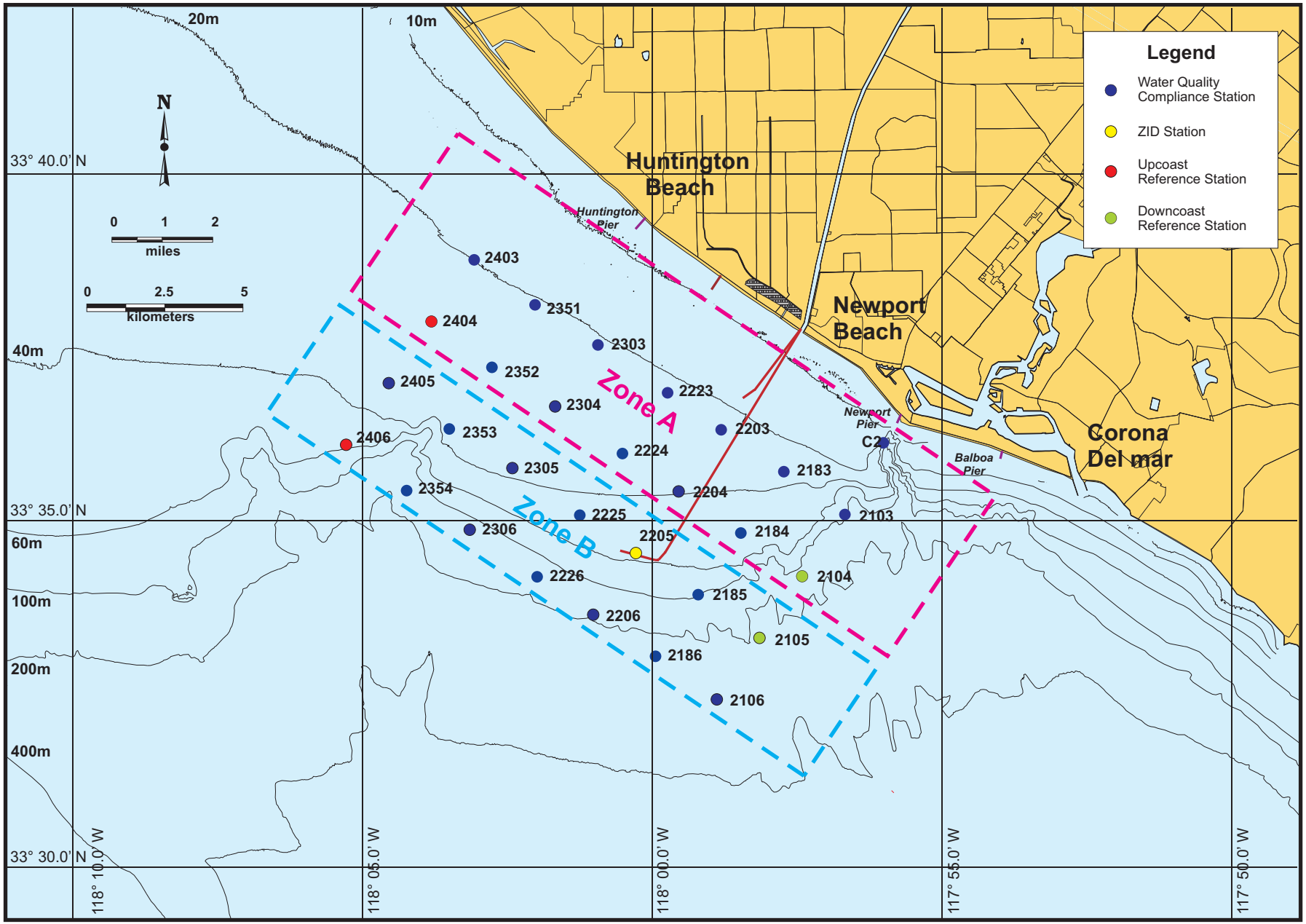

Figure A-1. **Water quality monitoring stations and zones used for compliance determinations.**

(transmissivity, dissolved oxygen, and pH) are then binned by water column stratum: above, within, or below the pycnocline; or top, middle, or bottom third when a pycnocline is absent. Mean values for each parameter are calculated by stratum and station. All binned observations (one-meter intervals) within each stratum are used in the calculations. The number of observations usually differs from station to station due to different water and pycnocline depths.

The selections of the appropriate reference stations (i.e., upcoast or downcoast) for each survey day are determined based on current measurements (if available) and/or on the presence or absence of ammonium at a station(s) upcoast or downcoast from the outfall diffuser. Once reference stations are determined, the data are analyzed using computer programs that calculate out-of-range occurrences (OROs) for each sampling date and parameter. These OROs are based on comparing the mean data by stratum by test station with the corresponding reference station data to determine whether the following COP criteria were exceeded:

- Dissolved oxygen: 10% or more below the mean;
- pH: greater than +/- 0.2 pH units different than the mean; and
- Natural light shall not be significantly reduced, defined as transmissivity: statistically different from the mean at a 95% confidence limit.

In accordance with permit specifications, the outfall station (2205) is not included in the comparisons because it is in the zone of initial dilution (ZID).

To determine whether an ORO is out-of-compliance (OOC), distributional maps are created that identify the reference stations for each sampling date and location of each ORO, including which stratum (above, within, or below the pycnocline for stratified conditions; surface, middle, and bottom for unstratified conditions) is out of range. Each ORO is then evaluated to determine if it represents a logical OOC event. These evaluations are based on (A) evaluation of the wastewater plume location relative to depth using a combination of temperature, salinity and CDOM, (B) evaluation of features in the water column relative to naturally occurring events (i.e., high chlorophyll-a due to red tide), and (C) unique characteristics of some stations that may not be comparable with permit-specified reference stations (2104/2105 or 2404/2406) during some surveys due to differences in water depth and/or variable oceanographic conditions. For example, Zone A stations (2103, 2203, 2303, and 2403) are located at shallower depths than reference station 2104. Waves and currents can cause greater mixing and resuspension of bottom sediments at shallower stations than deeper stations under certain conditions (e.g., winter storm surges). This can result in naturally decreased water clarity (transmissivity) that is unrelated to the wastewater discharge. Additionally, analysis of the water masses at Newport Canyon Station C2 shows that this area is very different from the rest of the survey area. Low transmissivity is often seen near the bottom. This low transmissivity water originates within the canyon and is advected offshore, often to Station 2103. Hence, OROs at this station are common but usually not linked to the effluent discharge. Furthermore, an ORO can be in-compliance if, for example, a downcurrent station is different from the reference, but no intermediate (e.g., nearfield) stations exhibit OROs.

Once the total number of OOC events is summed by parameter, the percentage of OROs and OOCs is calculated according to the total number of observations. In a typical year, Zone A, has a total of 504 possible comparisons if 14 stations (not including the reference station) and three strata on 12 survey dates per year are used. For Zone B, 432 comparisons are possible from 12 stations (not including the reference station), 3 strata, and 12 sampling dates. The total combined number of ORO and OOC events is then determined by summing the Zone A and Zone B results. If not all of the strata are present or additional surveys are conducted, the total number of comparisons in the analysis may be more or less than the target number of comparisons possible (936).

COASTAL OCEANOGRAPHY

Mooring locations and equipment used for measurement of ocean currents are summarized below.

Teledyne RD Instruments Workhorse Acoustic Doppler Current Profiler (ADCP).

Field Methods

Three Teledyne/RDI Workhorse Sentinel Acoustic Doppler Current Profilers (ADCP) were deployed across the shelf from the 78-inch outfall (~20 m) to the 120-inch outfall (~60 m) and a fourth ADCP was located downcoast of the 120-inch outfall near Newport Canyon (Table A-2, Figure 3-1). ADCPs were deployed in either a trawl resistant bottom mount or bottom tripod. Two of the ADCPs had a frequency of 300 kHz and two had a frequency of 600 kHz. All four ADCPs were equipped to measure water pressure and bottom temperature. Currents were sampled in one meter depth bins. Data were collected every six minutes using a burst sampling interval of one-minute duration. Compass calibrations and deployment parameters were done using WinSC version 1.29. A typical deployment lasted about three months. Raw data files were copied over from the instruments' compact flash memory cards.

Data Analyses

Current meter data were converted into scientific units using WinADCP 1.13. Pitch and roll values were checked to see if there was a serious tilt on the instrument during the deployment procedure. If the tilt exceeded 10°, current data was excluded from further analysis, and only temperature data was used. The time base for each instrument was checked for agreement with the projected number of records and for agreement with the logged deployment and recovery times for these sampling events. Pre- and post-deployment periods were truncated from the time series to remove invalid portions of the data when the ADCP was not positioned on the bottom (Figure A-2).

The remainder of the data processing was conducted in Matlab version 7.1. All non-usable data points and outliers were replaced with a "NaN" data flag. These data points were then replaced with linearly interpolated data before applying the low-pass filter. A Finite Impulse Response (FIR) filter was used to perform a zero-phase distortion, forward and reverse digital filter that minimizes start-up and ending transients by matching the initial conditions. A 40-hour low pass cutoff frequency was chosen to remove the tidal and higher frequency signals from the data.

Table A-2. Deployment summary for District's acoustic Doppler current profilers.

Orange County Sanitation District, California.

Quarter	Station	Deployment Date	Recovery Date	Latitude (N)	Longitude (W)	Depth (m)	Instrument Average Depth (m)	Sampling Interval
Summer	M18	07/01/2007	09/30/2007	33.5756	118.0232	60	62.85	6 min
	M19	Not deployed		n/a	n/a	n/a	n/a	n/a
	M20	07/01/2007	09/30/2007	33.6107	117.9789	20	21.92	6 min
	M21	07/01/2007	09/30/2007	33.5788	117.9571	60	67.86	6 min
Fall	M18	10/01/2007	12/31/2007	33.5756	118.0232	60	62.85	6 min
	M19	Not deployed		n/a	n/a	n/a	n/a	n/a
	M20	10/01/2007	12/31/2007	33.6107	117.9789	20	21.92	6 min
	M21	10/01/2007	12/31/2007	33.5788	117.9571	60	67.86	6 min
Winter	M18	01/01/2008	03/31/2008	33.5756	118.0229	60	64.57	6 min
	M19	01/01/2008	03/31/2008	33.5886	118.0073	40	41.93	6 min
	M20	01/01/2008	03/31/2008	33.6103	117.9789	20	21.28	6 min
	M21	01/01/2008	03/31/2008	33.5789	117.9579	60	62.73	6 min
Spring	M18	04/01/2008	06/30/2008	33.5756	118.0231	60	64.83	6 min
	M19	04/01/2008	06/30/2008	33.5890	118.0074	40	41.41	6 min
	M20	04/01/2008	06/30/2008	33.6107	117.9748	20	19.84	6 min
	M21	04/01/2008	06/30/2008	33.5786	117.9573	60	68.51	6 min

** Instruments have not been recovered

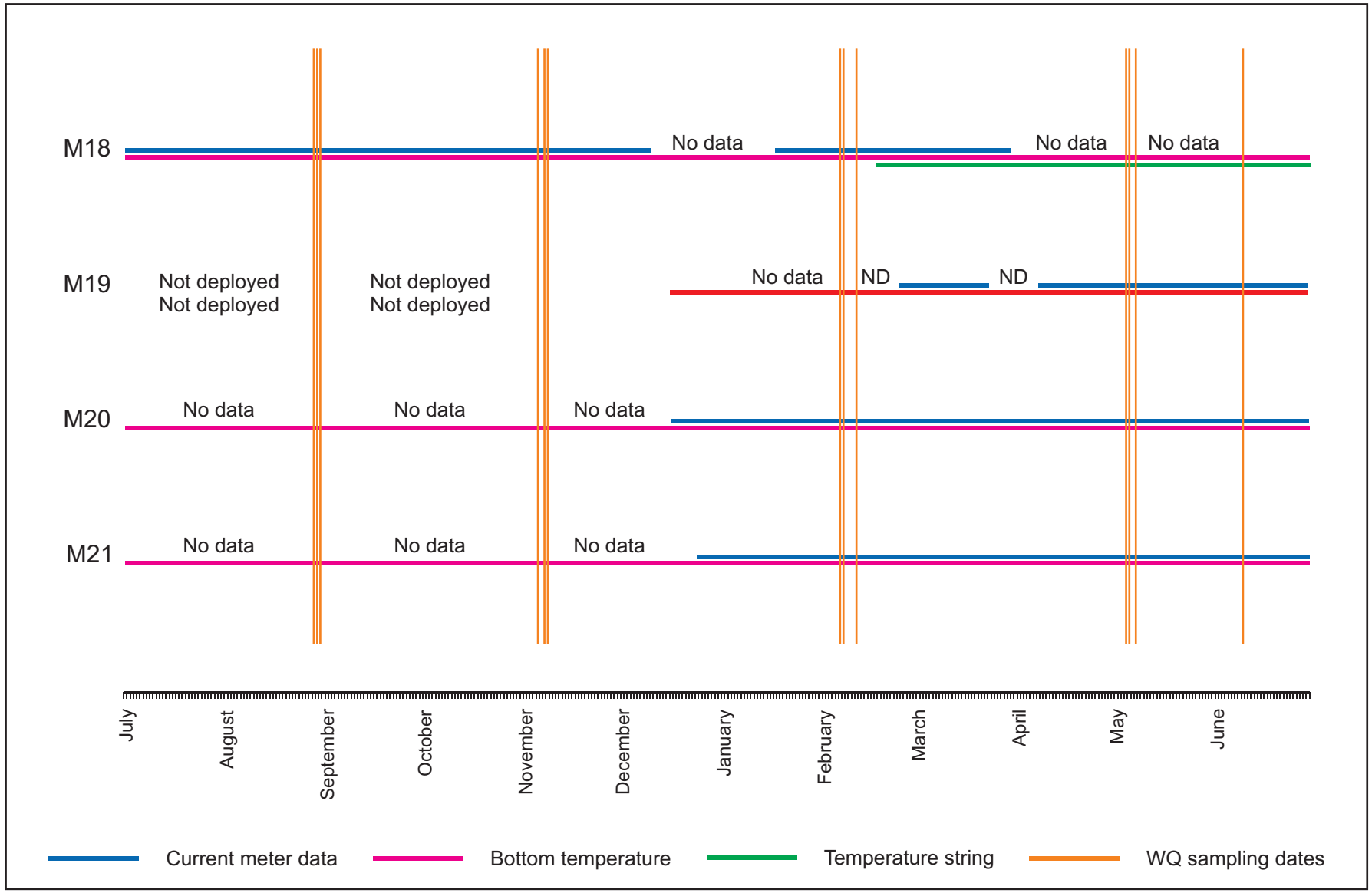

Figure A-2. **Timeline of deployment for the District's acoustic Doppler current profilers, bottom temperature sensors, and mid-water temperature sensors.**

Orange County Sanitation District, California.

Graphical data were shown as vector plots ("stick plots") with the lines pointing in the direction of the current and the length proportional to the current magnitude. Because current vectors generally tend to follow the local bathymetry, the frame-of-reference for the figures is rotated so that upward-directed "sticks" correspond to upcoast flows and sticks directed to the right correspond to onshore flows. For this presentation, the bathymetric orientation for the primary mooring line is 302°, and for Mooring M21 near Newport Canyon the orientation is 287°. These are consistent with bathymetry orientation values used for prior studies in the District's study region (OCSD 2004a).

WATER QUALITY MONITORING

Field Methods

Offshore Water Quality Monitoring

Permit-specified water quality studies were conducted three days per quarter at 29 stations comprising a fixed-grid pattern (Table A-1, Figure 3-1). Each survey included measurements of pressure (from which depth is calculated), water temperature, conductivity (from which salinity is calculated), dissolved oxygen, pH, water clarity, chlorophyll-*a*, colored dissolved organic matter (CDOM), and photosynthetically active radiation (PAR). Profiling was conducted from the surface (1 m) depth to 2 m from the bottom or to a maximum depth of 75 m. Measurements were conducted using a Sea-Bird Electronics SBE9-03/SBE 11 Deck Unit (SBE9/11) CTD (conductivity-temperature-depth) profiling system. SEASOFT software was used for data acquisition, data display, and sensor calibration. A summary of the sampling methods are presented in Table A-3. Light transmittance and PAR were measured for water clarity determinations. PAR is measured in conjunction with chlorophyll-*a* because increasing light intensity increases photosynthesis per unit chlorophyll (Hardy 1993).

Visual observations of water clarity (measured as Secchi-disc depth), water color, and floatable materials or grease that might be of sewage origin, were also conducted. Water color was determined using a Forel/Ule scale at one-half of the Secchi-disc depth. Daily rainfall, sea state, and wind condition data were summarized from Newport Beach Fire and Marine Department and District's Treatment Plant No. 2 records.

Discrete sampling for ammonium, total coliforms, fecal coliforms, *E. coli,* and *enterococci* was conducted using a Sea-Bird Electronics Carousel Water Sampler (SBE32/SBE33) equipped with Niskin bottles. Sample depths are provided in Table A-3. These samples were kept on wet ice in coolers, and transported to the District's Environmental Sciences Laboratory (ESL) for analysis. Bacteriology samples were transported to the ESL within six-hours of collection.

Central Bight Water Quality SPS

An expanded grid of water quality stations was sampled quarterly as part of the District's Central Bight Water Quality Strategic Process Studies (SPS). These additional stations were sampled concurrently with the permit specified stations, and in conjunction with the Los Angeles County Sanitation District (LACSD), the City of Los Angeles, and the City of Oxnard. The sampling area extends from Crystal Cove State Beach in the south to the Ventura River in the north. Samples were collected using CTDs during a targeted three- or

Table A-3. Water quality sample collection and analysis methods by parameter.

Orange County Sanitation District, California.

Parameter	Sampling Method	Method Reference	Preservation	Container	Holding Time	Sampling Interval	Field Replicates
Temperature	*in-situ* probe	(1)	none	none	not applicable	every 1 m[a]	at least 10% of stations
Salinity (conductivity)	*in-situ* probe	(2)	none	none	not applicable	every 1 m[a]	at least 10% of stations
pH	*in-situ* probe	(3)	none	none	not applicable	every 1 m[a]	at least 10% of stations
Dissolved Oxygen	*in-situ* probe	(4)	none	none	not applicable	every 1 m[a]	at least 10% of stations
Transmissivity	*in-situ* probe	(5)	none	none	not applicable	every 1 m[a]	at least 10% of stations
Photosynthetically Active Radiation (PAR)	*in-situ* probe	(6)	none	none	not applicable	every 1 m[a]	at least 10% of stations
Ammonium (NH_4^+)	Niskin	EPA Method 350.1B Rev. A	Ice	125 mL HDPE	28 days	Surface, 5m, 10m, 15m, 30m, 45m, 60m, Bottom	at least 10% of samples
Offshore Total Coliforms, Fecal Coliforms, *Escherichia coli*, and *Enterococci*	Niskin	EPA Method 9060 Rev. C	Ice	125 mL HDPE (Sterile container)	6 hrs	Surface, 5m, 10m, 15m, 30m, 45m, 60m, Bottom	at least 10% of samples
Shoreline Total Coliforms, Fecal Coliforms, and *Enterococci*	grab	EPA Method 9222 Rev. B EPA Method 9222 Rev. D EPA Method 1600	Ice	125 mL HDPE (Sterile container)	6 hrs	Ankle deep water	at least 10% of samples
Secchi Depth	visual observations	permit specs.	none	none	not applicable	surface	none
Forel/Ule Color Values	visual observations	permit specs.	none	none	not applicable	½ Secchi depth below surface	none
Surface Observations	visual observations	permit specs.	none	none	not applicable	surface	none

[a] Sampled continuously but data processed to 1 m intervals.

(1) Calibrated to reference cells (0.0005°C accuracy) every year.
(2) Calibrated to IAPSO Standard and Guildline 8400B Autosal every six months.
(3) Referenced and calibrated to NIST buffers of pH 7, 8, and 9 every survey.
(4) Referenced and calibrated to saturation table values.
(5) Referenced and calibrated to known transmittance in air.
(6) Factory calibrated (Biospherical Instruments) once per year.

four-day period, over which sampling occurred at 210 stations comprising a fixed-grid pattern (see Figure 3-2). Parameters measured included pressure, water temperature, conductivity, dissolved oxygen, pH, chlorophyll-*a*, CDOM, and water clarity. Profiling was conducted from the surface (1 m) depth to 2 m from the bottom or to a maximum depth of 100 m (75 m for OCSD). Sampling and analytical methods were the same as those presented in Table A-3.

Data Analyses

PAR data for each station were normalized to represent the percent of the respective surface PAR values at that station. The percent of light available to phytoplankton for photosynthesis is derived from the normalized PAR data (Hardy 1993). Spatial and seasonal patterns in water quality data are summarized in two- and three-dimensional color plots of temperature, salinity, dissolved oxygen, pH, transmissivity, and two-dimensional displays of PAR and chlorophyll-*a*. The three-dimensional plots were produced using IGODS computer software (OCSD 1996).

Analyses to determine compliance with COP criteria for dissolved oxygen, pH, and transmissivity were based on statistical significance testing of each permit-specified station. Designation of a reference station (Stations 2104 and 2404 for downcoast and upcoast Zone A locations and Stations 2105 and 2406 for downcoast and upcoast Zone B locations, respectively, Figure 3-1) for each survey was based on inspections of available current meter results and data plots to determine which direction (upcoast or downcoast) the wastewater plume was detected. These detections were based primarily on the occurrence of ammonium at depths below surface layers (e.g., 5–45 m). The reference was defined as the station direction (upcoast or downcoast) where no plume was evident at the time of sampling. Compliance analyses were conducted separately using both sets of reference stations when current direction and/or coliforms were not evident at either upcoast or downcoast directions from the outfall.

BACTERIOLOGY/NUTRIENTS

Field Methods

<u>Offshore Monitoring</u>

Quarterly surveys were conducted in August and November 2007, and February and May 2008. Primary water quality sampling occurred three times per quarter at 29 sites that form a grid around the OCSD diffuser (see Figure 3-1). Samples were collected for ammonium at a subset of 20 stations and for bacteriology at nine REC-1 stations (Table A-1, Figure 3-1) located within state waters (within three miles of the shoreline) for the purposes of determining compliance with Receiving Water Limitation C.2.a.1. Discrete samples for ammonium and bacteria (total coliform, *Escherichia coli*, and enterococci) were collected at 5 and 15 m depth intervals from the surface (1 m) to 2 m above the ocean floor or a maximum depth of 60 m (Table A-3). Two additional surveys are conducted to collect bacteriology and ammonium samples at the nine REC-1 water quality stations within 30 days of the three primary water quality sampling days in order to calculate a 30-day geometric mean. Fecal coliform densities are calculated from *E. coli* densities using a ratio of 1.1:1.0.

Nearshore Monitoring

Surfzone water samples for analysis of total and fecal coliform and enterococci bacteria were collected from approximately ankle deep water, five days per week, as specified in the District's NPDES permit, at 17 surfzone stations (Table A-1). The occurrence and size of any grease particles at the high tide line was recorded two days per week at the same sampling locations.

Laboratory Methods

Laboratory analyses of ammonium and bacteriology samples followed standard EPA guidelines, as listed in Table A-3. Quality assurance/quality control (QA/QC) procedures included analysis of laboratory blanks and duplicates. All data underwent at least three separate reviews prior to being included in the final database used for statistical analysis, comparison to standards, and data summaries.

Data Analyses

Offshore Bacteriology Monitoring

Spatial and seasonal patterns of several water quality parameters are summarized graphically in two- and three-dimensional color figures. The three-dimensional plots were produced using IGODS computer software (OCSD 1996). Prior to disinfection (see Chapter 1), these analysis included offshore bacteriology data (total coliform and *E. coli*); however, since the onset of disinfection in 2003, bacteriological values have become negligible and only the ammonium data are considered relative to other water quality parameters.

The permit identifies water contact standards for water within the nearshore zone, which correspond to the depth of the water column extending out three miles from shore (REC-1). Compliance evaluations of offshore bacteriological samples were conducted in accordance with the following Basin Plan (REC-1) criteria for recreational waters:

- Total coliform bacteria shall not exceed a geometric mean of 1000/100 mL MPN over a 30-day period provided that less than 20% of the samples exceed 1000 MPN/100 mL and no single sample can exceed 10,000/100 mL MPN when verified within 48 hours; and

- Fecal coliform bacteria shall not exceed a geometric mean of 200/100 mL MPN over a 30-day period, and not more than 10% of the samples in a 60-day period shall exceed 400/100 mL MPN.

Determinations of offshore bacteriology compliance with the fecal coliform water contact standard were accomplished by multiplying Colilert® *E. coli* data by 1.1 to obtain a calculated fecal coliform value.

SEDIMENT GEOCHEMISTRY

Field Methods

Sediment samples were collected for the ocean monitoring program during July and October 2007, and January and April 2008, at ten quarterly stations located along the 60-m bottom contour (Table A-1) (Figure 4-1). For the July survey, three replicate samples were collected from each station and analyzed separately while only one replicate sample was taken during the October, January, and April surveys, (Table A-4). Single samples were collected during July at 39 additional annual stations that ranged in depth from 40 to 302 m. In addition, 3 L of sediment were collected from each of the quarterly stations for sediment toxicity testing in January 2008.

Bottom sediments were collected using a paired 0.1 m^2 Van Veen grab sampler. The top 2 cm of the sample was collected for individual chemical and toxicity analyses using a stainless steel scoop. The sampler and scoop were rinsed thoroughly with filtered seawater prior to sample collection. Sample storage, preservation, and holding times followed specifications in the District's QAPP, as well as guidance based on EPA/301(h) protocols. All sediment chemistry samples (metals. organics, TOC, grain size, and dissolved sulfides) were transferred to the ESL for analysis. All sediment grain size samples were subsequently transferred to Weston Solutions, Inc. (Carlsbad, CA) for analysis. Sediment TOC samples were transferred to Columbia Analytical Services, Inc. (Kelso, WA) for analysis. All sample transfers were conducted and documented using required chain-of custody protocols.

Laboratory Methods

Sediment chemistry and grain size samples were processed and analyzed using performance-based and EPA-recommended methods (EPA 1986) that are listed in Table A-5. The measured sediment chemistry parameters are listed in Table A-6. Samples for dissolved sulfide were analyzed in accordance with procedures outlined in Schnitker and Green (1974) and Standard Methods 20th Edition (1998).

Sediment toxicity was tested in January 2008 using whole sediments for the 10-day *Eohaustorius estuarius* amphipod survival test. Amphipods were exposed to test and control sediments and the percent survival in each were determined. Toxicity threshold criteria were selected to be consistent with the Bight'98 Sediment Toxicity Program (Bay et al. 2000). A difference of 20–50% was considered a moderately toxic response, while differences greater than 50% were considered highly toxic.

Data Analyses

Differences in sediment grain size and chemistry data from the ten quarterly stations were analyzed statistically using analysis of variance (ANOVA). Station differences were identified using the Tukey Multiple Comparison Test. Prior to analysis, the data was tested for homogeneity of variance, and transformed (e.g. $\log_{10}$ or rank) when necessary. Regression analysis was used to determine depth related factors and relationships to invertebrate community measures. The purpose of these analyses was to evaluate whether sediment quality was uniform throughout the study area and to determine

Table A-4. Sediment sampling summary.

Orange County Sanitation District, California.

Stations	Sampling Frequency	Sampling Interval	Field Replicates	Parameters
0, 1, 4, 5, 9, 12, C, ZB, ZB2, CON	Quarterly (Summer)	0-2 cm; from undisturbed grab sample	3 replicates per station	Metals PAHs LABs (July only) Chlorinated Pesticides Polychlorinated Biphenyls Total Organic Carbon Dissolved Sulfides Grain size
0, 1, 4, 5, 9, 12, C, ZB, ZB2, CON	Quarterly (Fall, Winter, Spring)	0-2 cm; from undisturbed grab sample	1 replicate per station	Metals PAHs LABs (July only) Chlorinated Pesticides Polychlorinated Biphenyls Total Organic Carbon Dissolved Sulfides Grain size
3, 7, 8, 10, 13, 17, 18, 20, 21, 22, 23, 24, 25, 27, 29, 30, 33, 36, 37, 38, 39, 40, 41, 42, 44, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, C2, C4, C5	Annually (Summer)	0-2 cm; from undisturbed grab sample	1 replicate per station	Metals PAHs LABs Chlorinated Pesticides Polychlorinated Biphenyls Total Organic Carbon Dissolved Sulfides Grain size

Table A-5. Sediment handling and analysis summary.

Orange County Sanitation District, California.

Parameter	Method Reference	Preservation	Container	Holding Time
Dissolved Sulfides	Schnitker and Green (1974) Standard Methods 20th Ed.	Freeze	Glass jar	6 months
Chlorinated Pesticides	NS&T (NOAA 1993)	Freeze	Amber glass jar	6 months (analyze within 40 days of extraction)
Grain Size	EPA 3-284 and 3550	4° C	Plastic bag	6 months
Linear Alkyl Benzenes	Eganhouse et al. (1983)	Freeze	Glass jar	12 months
Metals	NS&T (NOAA 1993)	Freeze	Amber glass jar	6 months
Polychlorinated Biphenyls	NS&T (NOAA 1993)	Freeze	Glass jar	6 months (analyze within 40 days of extraction)
Polycyclic Aromatic Hydrocarbons	NS&T (NOAA 1993)	Freeze	Glass jar	6 months (analyze within 40 days of extraction)
Total Organic Carbon	EPA 415	Freeze	Glass jar	6 months

Table A-6. Parameters measured in sediments.

Orange County Sanitation District, California.

Metals		
Aluminum	Copper	Nickel
Arsenic	Iron	Selenium
Beryllium	Lead	Silver
Cadmium	Mercury	Zinc
Chromium		
Other Metrics		
Dissolved Sulfides	Total Organic Carbon	Grain Size
Chlorinated Pesticides		
Aldrin	Endosulfan sulfate	Mirex
alpha-BHC	Endrin	*trans*-Nonachlor
alpha-Chlordane	Endrin aldehyde	2,4'-DDD (o,p'-DDD)
beta-BHC	*gamma*-BHC	2,4'-DDE (o,p'-DDE)
cis-Nonachlor	*gamma*-Chlordane	2,4'-DDT (o,p'-DDT)
delta-BHC	Heptachlor	4,4'-DDD (p,p'-DDD)
Dieldrin	Heptachlor epoxide	4,4'-DDE (p,p'-DDE)
Endosulfan 1	Hexachlorobenzene	4,4'-DDT (p,p'-DDT)
Endosulfan 2	Methoxychlor	4,4'-DDMU
PCB Congeners		
PCB 8	PCB 110	PCB 167
PCB 18	PCB 114	PCB 168
PCB 28	PCB 118	PCB 169
PCB 37	PCB 119	PCB 170
PCB 44	PCB 123	PCB 177
PCB 49	PCB 126	PCB 180
PCB 52	PCB 128	PCB 183
PCB 66	PCB 138	PCB 187
PCB 70	PCB 149	PCB 189
PCB 74	PCB 151	PCB 194
PCB 77	PCB 153	PCB 195
PCB 81	PCB 153/168	PCB 200
PCB 87	PCB 156	PCB 201
PCB 99	PCB 157	PCB 206
PCB 101	PCB 158	PCB 209
PCB 105		
PAH Compounds		
1,6,7-Trimethylnaphthalene	Benzo[a]pyrene	Dibenzothiophene
1-Methylnaphthalene	Benzo[b]fluoranthene	Fluoranthene
1-Methylphenanthrene	Benzo[e]pyrene	Fluorene
2,6-Dimethylnaphthalene	Benzo[g,h,I]perylene	Indeno[1,2,3-c,d]pyrene
2-Methylnaphthalene	Benzo[k]fluoranthene	Naphthalene
Acenaphthene	Biphenyl	Perylene
Acenaphthylene	Chrysene	Phenanthrene
Anthracene	Dibenz[a,h]anthracene	Pyrene
Benz[a]anthracene		

Table A-6 Continues.

Table A-6 Continued.		
PAH Alkylated Homologues		
C1-Chrysenes	C1-Fluorenes	C3-Naphthalenes
C2-Chrysenes	C2-Fluorenes	C4-Naphthalenes
C3-Chrysenes	C3-Fluorenes	C1-Phenanthrenes/Anthracenes
C4-Chrysenes	C1-Fluoranthenes/Pyrenes	C2-Phenanthrenes/Anthracenes
C1-Dibenzothiophenes	C1-Naphthalenes	C3-Phenanthrenes/Anthracenes
C2-Dibenzothiophenes	C2-Naphthalenes	C4-Phenanthrenes/Anthracenes
C3-Dibenzothiophenes		
LAB Compounds		
2-Phenyldecane	2-Phenyltetradecane	4-Phenylundecane
3-Phenyldecane	3-Phenyltetradecane	5-Phenylundecane
4-Phenyldecane	4-Phenyltetradecane	6-Phenylundecane
5-Phenyldecane	5-Phenyltetradecane	2-Phenyldodecane
2-Phenyltridecane	6-Phenyltetradecane	3-Phenyldodecane
3-Phenyltridecane	7-Phenyltetradecane	4-Phenyldodecane
4-Phenyltridecane	2-Phenylundecane	5-Phenyldodecane
5-Phenyltridecane	3-Phenylundecane	6-Phenyldodecane
7+6-Phenyltridecane		

differences in concentration of contaminants from the outfall stations and the farfield station. ANOVA and regression analyses were conducted using MINITAB Release 14 statistical software.

Total DDT represents the summed concentrations of o,p'- and p,p'- [2,4- and 4,4'-] isomers of DDD, DDE, and DDT), total PCB represents the summed concentrations of 45 congeners, and total chlordane represents the sum of alpha- and cis-chlordane and cis- and trans-nonachlor. For summed concentrations, undetected components (i.e., concentrations below the analytical detection limits) were treated as zero. When all component concentrations were undetected, the corresponding total concentrations were assumed to be zero.

BENTHIC INFAUNA

Field Methods

The infaunal community was monitored by collecting marine sediments using a paired 0.1 m^2 modified Van Veen sediment grab sampler. Samples were collected concurrent with sediment geochemistry samples (Table A-1, Figure 5-1). Three replicate samples were collected at each of the ten quarterly stations, and a single replicate sample was collected at each of the 39 annual stations (Table A-7).

All infauna sediment samples were qualitatively and quantitatively assessed for acceptability prior to collection and processing. Samples were defined as acceptable if they had a volume of at least 4 L and a relatively undisturbed surface. These samples were gently washed with filtered seawater through a 1.0-mm sieve. Retained organisms were placed in glass jars and anesthetized with 7% magnesium sulfate for approximately 30 minutes. The samples were then fixed in a 10% buffered formalin solution and returned to the laboratory.

Laboratory Methods

After 3–10 days in formalin, samples were transferred to 70% ethanol for laboratory processing. Samples were sent to Weston Solutions, Inc. to be sorted to major taxonomic groups (polychaetes, molluscs, crustaceans, echinoderms, and minor phyla), using dissecting microscopes. Removal of the organisms from the sediments was monitored to insure that at least 95% of all organisms were successfully separated from the sediment matrix. QA/QC generally found that over 98% of all organisms had been recovered. Upon completion of sample sorting, the major taxonomic groups were distributed to qualified taxonomists for enumeration and speciation. Taxonomic QA/QC included 10% reanalysis of randomly selected samples by different taxonomists. Taxonomic differences were resolved and the database was edited accordingly. After completion of the counting and identifications, each taxonomic group was wet-weighed to the nearest 0.1 g.

Data Analyses

Infaunal community data was analyzed to determine whether differences in community measures exist between stations near the outfall discharge and farfield stations. The

Table A-7. Benthic infauna sampling summary.

Orange County Sanitation District, California.

Stations	Sampling Frequency	Sampling Volume	Field Replicates	Parameters
0, 1, 4, 5, 9, 12, C, ZB, ZB2, CON	Quarterly (Summer, Fall, Winter, Spring)	4 liters; from undisturbed grab sample	3 replicates per station	Infauna
3, 7, 8, 10, 13, 17, 18, 20, 21, 22, 23, 24, 25, 27, 29, 30, 33, 36, 37, 38, 39, 40, 41, 42, 44, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, C2, C4, C5	Annually (Summer)	4 liters; from undisturbed grab sample	1 replicate per station	Infauna

following variables and community parameters were analyzed: number of species/sample, number of individuals/sample (abundance), biomass/sample (major taxonomic group wet-weight), Infaunal Trophic Index (ITI) (Word 1978), Benthic Response Index (BRI) (Bergen et al. 1999), and diversity indices including Shannon-Wiener (H'), Margalef's Species Richness (SR), Pielou's Evenness (J'), and Schwartz' 75% Dominance Index (Dominance; number of species accounting for 75% of total abundance).

Diversity values are based upon the number of species and the equitability of their distribution, but these two attributes vary independently. Consequently, a large number of diversity indices have been developed. Shannon Wiener diversity (Shannon and Weaver, 1962) Pielou's evenness index (Pielou 1977) and Dominance are more sensitive to the distribution of species within a sample, while Margalef's Species Richness is more sensitive to the number of species (Tetra Tech 1985). The presence/absence of certain pollution sensitive and pollution tolerant indicator species were also determined to further assess sediment quality in the monitoring area. The pollution sensitive species included the red brittlestar (*Amphiodia urtica*) and amphipods in the genera *Ampelisca* and *Rhepoxynius*, which are commonly used in whole-sediment toxicity testing. Three pollution tolerant species, *Capitella* "*capitata*" complex (polychaete), *Euphilomedes carcharodonta* (ostracod), and *Parvilucina tenuisculpta* (bivalve mollusk) were examined because their presence may indicate stressed, polluted, or organically enriched environments.

Several analytical approaches were used: hypothesis testing using analysis of variance (ANOVA), pattern analysis via multivariate cluster and non-metric multidimensional scaling (MDS) analyses, and correlation and regression analyses to test for relationships among factors. Prior to analysis, the data was tested for homogeneity of variance and transformed (e.g. $\log_{10,}$ rank, or arcsine) when necessary. The ten quarterly stations were analyzed for differences between outfall and farfield upcoast stations in community measures using ANOVA at a $p \leq 0.05$ level of significance. Regression analysis was used to determine

depth-related factors. ANOVA, regression, and correlation analyses were conducted using MINITAB Release 14 statistical software. Cluster and MDS analyses were performed using PRIMER v6 statistical software.

Multivariate cluster analysis was used to analyze spatial patterns to define similar habitats (i.e., station clusters). This technique groups those stations (habitats) having the most similar species/abundance relationships (assemblages). Cluster analysis was performed on data from July 2007 using a hierarchical agglomerative clustering method. Abundance data from the first replicate from the quarterly stations and data from the 39 annual stations were used in this analysis. This provided the greatest spatial coverage and increased the number of stations used to 49. Abundance data were 4^{th} root transformed, a Bray-Curtis similarity matrix constructed, and a dendrogram generated using a group average cluster mode.

DEMERSAL FISH COMMUNITIES

Field Methods

Demersal fish and epibenthic macroinvertebrates (EMI) species were collected in the summer (July) of 2007 and the winter (January) of 2008. Sampling was conducted at nine permit stations: inner shelf (36 m) stations T2 and T6; middle shelf (55 m) stations T1, T3, T11, T12, and T13; and outer shelf (137 m) stations T10 and T14 (Table A-1, Figure 6-1). Two replicates were conducted at the inner and outer shelf stations and three replicates were conducted at the middle shelf stations. Additionally, two replicate samples were collected at T0 (18 m) in each survey to maintain a historical database, but the data are not presented in this report.

Trawling was conducted using a 7.6 meter (25 ft) wide, Marinovich, semi balloon otter trawl (2.54 cm mesh) with a 0.64 cm mesh cod-end liner, an 8.9-m chain-rigged foot rope, and 23 m long trawl bridles following regionally adopted methodology (Mearns and Allen 1978). The trawl wire scope varied from a ratio of approximately 5:1 at the shallowest stations to approximately 3:1 at the deepest station. To minimize catch variability due to weather and current conditions, which may affect the bottom-time duration of the trawl, trawls generally were taken along a constant depth at each station, and usually in the same direction.

Established survey methods for southern California require that a portion of the trawl track must pass within a 100-m circle that originates from the nominal sample station position and be within 10% of the station's depth. The speed of the trawl should range from 0.77 to 1.00 m/s or from 1.5 to 2.0 kts. Since 1985, the District has trawled a set distance of 450 meters (the distance that the net is actually on the bottom collecting fish and invertebrates). Station locations and trawling paths were determined using Global Positioning System (GPS) navigation. GPS was also used to control the speed of the trawl (2.0–2.5 knots over the bottom) and determine the distance sampled (0.45 km). Trawl depths and time on the bottom were determined using an attached pressure sensor.

Upon retrieval of the trawl net, the contents were emptied into a large flow-through water tank and all specimens were sorted by species into separate containers. After bioaccumulation specimens were counted, recorded, and removed for processing, the remaining specimens

were measured to the nearest millimeter (standard length) and weighed to the nearest gram. A minimum of 30 (randomly selected) specimens of each species were weighed and measured individually. If a sample contained substantially more than 30 individuals of a species, the excess specimens were enumerated in 1 cm size classes and a bulk weight was recorded. All specimens were examined for external tumors, other lesions, and parasites. Specimens retained for laboratory identification were weighed and measured in the laboratory. After identification, weighing, and measuring, all other specimens were returned to the sea.

Laboratory Methods

Specimens for the voucher collection and any animals that could not be identified in the field were preserved in 10% buffered formalin for subsequent laboratory analysis. A representative voucher collection of fish and macroinvertebrates is maintained for reference and verification.

Data Analyses

Data were summarized in terms of percent abundance (number of individuals/total of all individuals caught x 100), frequency of occurrence (number of occurrences/total number of trawls x 100) and mean abundance per occurrence (number of individuals/number of occurrences). In addition, the following community parameters were calculated for both fish and EMI: number of species/trawl, number of individuals/trawl, individuals/species, biomass (organism weight)/trawl, diversity indices including Shannon-Wiener (H'), Margalef's Species Richness (SR), Pielou's Evenness (J'), and 75% Dominance (Dominance), number of species accounting for 75% of total abundance). When depth or spatial differences were of concern, stations were grouped into the following categories: outfall stations included T1 and T12; shallow stations T2 and T6; deep stations T10 and T14; farfield downcoast station T3; and farfield upcoast stations T11 and T13.

Fish and EMI data was analyzed to determine whether differences exist between the outfall (Station T1) and farfield areas (Stations T11, upcoast; and T3, downcoast). The one-way analysis of variance (ANOVA) statistic was calculated to test the hypothesis that there are no significant ($p \leq 0.05$) differences between the outfall and farfield stations for each community measure (i.e., species richness, abundance, biomass, etc). ANOVA is a parametric test that assumes that the data analyzed: 1) are continuous, interval data comprising a whole population or sampled randomly from a population; 2) have a normal distribution; 3) are independent groups; and 4) have equal variances.

In order to meet the assumptions of the ANOVA test, data were tested for homogeneity of variance using the Bartlett's test for number of species, abundance, and biomass, and Levene's test for H', SR, DOM75, and J'. If the data were heterogeneous, they were transformed using a $\log_{10}$ calculation and retested for homogeneity of variance. If the data still failed the homogeneity test, they were rank transformed prior to testing with the ANOVA; however, the power of the ANOVA is substantially decreased with rank-transformed data. The Tukey multiple range test (Sokal and Rohlf 1981) was used to indicate station differences from results ANOVA testing of transformed data. All analyses were conducted using MINITAB Release 14 statistical software.

FISH TISSUE CONTAMINANTS

The District's permit lists three target fish species for analysis of muscle and liver tissue chemistry: English sole (*Parophrys vetulus*), hornyhead turbot (*Pleuronichthys verticalis*), and bigmouth sole (*Hippoglossina stomata*). Whole fish analysis was performed on one species, the Pacific sanddab (*Citharichthys sordidus*) to allow for comparison of the District's fish tissue data to data from other areas in the SCB. These species were selected because they are common demersal fish of the SCB, important to recreational fisheries, and used for other bioaccumulation studies. Muscle tissues were analyzed because they reflect the effects of chronic contaminant exposures and are typically consumed by humans. Liver tissues were analyzed because they typically have high lipid content and may accumulate relatively high concentrations of lipid-soluble contaminants that have been linked to pathological conditions, and therefore reflect fish health effects.

Field Methods

Fish were collected during trawl surveys using an otter trawl (described above). The sampling objective was to collect ten individuals of each of three target species for muscle and liver tissue analysis at both outfall (T1/T12) and farfield (T11/T13) sites. Individual fish were weighed and measured in the field, placed in clean, plastic, resealable bags, and stored on wet ice in insulated coolers. Pacific sanddabs were separated into three age classes (0, 1, and 2) based upon centimeter size classes 5–8, 9–13, and 14–16, respectively. Six individuals per size class per haul were collected for compositing in the laboratory. All samples were subsequently transferred under chain-of-custody protocols to the ESL.

Laboratory Methods

Individual fish were dissected and whole-fish tissues were homogenized using a blender in the laboratory under clean conditions. Muscle, liver, and whole fish tissues were analyzed for various parameters (Table A-8), including DDT and metabolites, chlorinated pesticides, PCBs (individual congeners), mercury, and lipids using methods consistent with NOAA National Status and Trends (NS&T) protocols (NOAA 1993). Method blanks, analytical quality control samples (duplicates, matrix spikes, and blank spikes), and standard reference materials were prepared and analyzed with each sample batch. Mercury was quantified by cold vapor atomic absorption spectrophotometry, organochlorines were measured using dual column gas chromatography with an electron capture detector, and lipids were determined gravimetrically. All concentrations are reported on a wet weight basis. For some liver samples, not all planned chemical analyses could be performed due to insufficient sample mass.

Total DDT represents the summed concentrations of o,p'- and p,p'- [2,4- and 4,4'-] isomers of DDD, DDE, and DDT), total PCB represents the summed concentrations of 45 congeners, and total chlordane represents the sum of alpha- and cis-chlordane and cis- and trans-nonachlor. For summed concentrations, undetected components (i.e., concentrations below the analytical detection limits) were treated as zero. When all component concentrations were undetected, the corresponding total concentrations were assumed to be zero.

Table A-8. Parameters measured in fish tissue.

Orange County Sanitation District, California.

Metals		
Mercury		
Chlorinated Pesticides		
Aldrin	Endosulfan sulfate	Mirex
alpha-BHC	Endrin	*trans*-Nonachlor
alpha-Chlordane	Endrin aldehyde	2,4'-DDD (o,p'-DDD)
beta-BHC	*gamma*-BHC	2,4'-DDE (o,p'-DDE)
cis-Nonachlor	*gamma*-Chlordane	2,4'-DDT (o,p'-DDT)
delta-BHC	Heptachlor	4,4'-DDD (p,p'-DDD)
Dieldrin	Heptachlor epoxide	4,4'-DDE (p,p'-DDE)
Endosulfan 1	Hexachlorobenzene	4,4'-DDT (p,p'-DDT)
Endosulfan 2	Methoxychlor	4,4'-DDMU
PCB Congeners		
PCB 8	PCB 110	PCB 167
PCB 18	PCB 114	PCB 168
PCB 28	PCB 118	PCB 169
PCB 37	PCB 119	PCB 170
PCB 44	PCB 123	PCB 177
PCB 49	PCB 126	PCB 180
PCB 52	PCB 128	PCB 183
PCB 66	PCB 138	PCB 187
PCB 70	PCB 149	PCB 189
PCB 74	PCB 151	PCB 194
PCB 77	PCB 153	PCB 195
PCB 81	PCB 153/168	PCB 200
PCB 87	PCB 156	PCB 201
PCB 99	PCB 157	PCB 206
PCB 101	PCB 158	PCB 209
PCB 105		
Other Parameters		
Lipids		

Data Analyses

Chemical contaminant data were analyzed to evaluate statistical differences between outfall and farfield stations in concentrations of mercury, pesticides, and PCBs as a function of fish size and tissue lipid content. Differences among sites were tested using two-sample comparisons: the null hypothesis (H_0) is - tissue contaminant concentrations in fish near the outfall are not significantly different from concentrations in fish from a farfield site relative to the District's outfall.

Prior to testing, all of the data (except mercury) were lipid-normalized. Differences among sites in the homogeneity of the variances in the data were evaluated using the Bartlett test. Differences between sites in the lipid-normalized concentrations and standard lengths of individual fish were also evaluated because contaminant concentrations may be related to tissue lipid content as well as the size/age distributions at each sampling location. Regression analysis was used to quantify statistical relationships between fish length, tissue lipid content, and contaminant concentrations. Station differences were determined using one-way ANOVA ($p \leq 0.05$).

FISH HEALTH

Field Methods

Assessment of the overall health of the fish population is also required by the NPDES permit. Consequently, all fish were visually inspected for large non-mobile external parasites, lesions, tumors, and other signs of disease (e.g., skeletal deformities).

Data Analyses

The low prevalence of parasites and other abnormalities in fish in the District's monitoring area precluded hypothesis testing; consequently, data analysis consisted of summary statistics and qualitative comparisons only.

FISH HISTOPATHOLOGY

Field Methods

Fish were collected during trawl surveys conducted in the summer (July) of 2005 using an otter trawl (described above). Up to 60 individuals of each target species were collected at each station. Sex, standard length (mm), weight (g), and any visible abnormalities of all individuals were recorded.

Laboratory Methods

Histopathological Analyses

Livers were dissected immediately from freshly collected live fish. Tissue sections approximately 2–3 mm thick were excised from the central portion of the liver. Occasional gross hepatic lesions, visible during dissections on other portions of the liver, were also

excised. Liver sections were placed in cassettes and immediately preserved in Dietrich's fixative for approximately 24 hours, followed by changes in 70% ethanol, as required. Samples were then dehydrated in an alcohol series, cleared in "Clear-Rite-3", and embedded in paraffin (Tissue Prep. 2). Sections were cut at 4 µm, placed on poly-1-lysine-coated slides, stained with Gill's and Weigert's hematoxylin, and counterstained with eosin Y. Two to three sections per liver were examined by E.M. Perkins. All lesion diagnoses were verified by Mark .S. Myers of the National Marine Fisheries Service, Northwest Fisheries Science Center. The presence and severity of lesion categories (Table A-7) were recorded, three of which; neoplasms (NEO), foci of cellular alteration (FCA), and hydropic vacuolation (HYDVAC), were designated as severe lesion categories. NEO included cholangioma, cholangiocellular carcinoma, and liver cell adenoma; FCA included basophilic, clear cell, and eosinophilic foci of cellular alteration; and HYDVAC was severe hydropic vacuolation of hepatocytes and biliary epithelial cells. Other lesion categories, such as non-neoplastic proliferative lesions (PROLIF), unique or specific degenerative/necrotic conditions (SDN), nonspecific necrotic lesions not associated with visible infectious agents (NECROSIS), intracytoplasmic storage disorders, and vascular lesions, were documented when observed. These lesion categories were consistent with those defined by the NBSP (Malins et al. 1987; Myers et al. 1994, 1999) and similar studies (Myers et al. 1987). Other minor lesion categories, such as intracytoplasmic storage disorders and vascular lesions, were documented when observed; however, these were rare. Representative slides of each of the lesion categories are archived in the National Registry of Tumors in Lower Animals, Department of Pathology, George Washington University Medical Center, Washington, D.C. (Harshbarger and Clark 1990).

Data Analyses

Due to the low prevalence of severe pathologies in fish in the District's monitoring area, hypothesis testing could not be conducted and data analysis consisted of summary statistics and qualitative comparisons only.

REFERENCES

Bay, S.M., D. Lapota, J. Anderson, J. Armstrong, T. Mikel, A.W. Jirik, and S. Asato. 2000. Southern California Bight 1998 Regional Monitoring Program: IV. *Sediment Toxicity*. Southern California Coastal Water Research Project, Westminster, CA. 49 pp.

Bergen, M., S.B. Weisberg, D. Cadien, A. Dalkey, D. Montagne, R.W. Smith, J.K. Stull, and R.G. Velarde. 1999. *Southern California Bight 1994 Pilot Project: IV. Benthos*. SCCWRP, Westminster, CA.

EPA (U.S. Environmental Protection Agency). 1986. *Quality Assurance and Quality Control (QA/QC) for 301(h) Monitoring Programs: (Guidance on field and laboratory methods.* Final Report TC-3953-04. EPA Office of Marine and Estuarine Protection, Washington, DC.

Green, E.J. and Schnitker, D. 1974. The Direct Titration of Water-soluble Sulfide in Estuarine Muds of Montsweag Bay, Maine. Marine Chemistry 2:111–124.

Hardy, J. 1993. Phytoplankton. Chapter 5, In: M.D. Dailey, D.J. Reish, and J.W. Anderson (eds.) Ecology of the Southern California Bight: A Synthesis and Interpretation. University of California Press, Berkeley, CA.

Mearns, A.J. and M.J. Allen. 1978. Use of small otter trawls in coastal biological surveys. U.S. Environ. Prot. Agcy., Environ. Res. Lab., Corvallis, OR. EPA-600/3-78-083. 33 pp.

MINITAB. 2000. MINITAB User's Guide 2: Data Analysis and Quality Tools. MINITAB, Inc.

NOAA (National Oceanic and Atmospheric Administration). 1993. *Sampling and analytical methods of the National Status and Trends Program National Benthic Surveillance and Mussel Watch Projects 1984-1992: Overview and summary of methods, Volume I.* NOAA Technical Memorandum NOS ORCA 71, Silver Spring, MD.

OCSD. 1996. *Science Report and Compliance Report, Ten Year Synthesis, 1985-1995. Marine Monitoring.* Fountain Valley, CA.

OCSD. 1999. *Annual Report, July 1997-June 1998. Marine Monitoring*. Fountain Valley, CA.

OCSD. 2007a. *Orange County Sanitation District – Environmental Sciences Laboratory. Laboratory Operating Procedures Manual.* Fountain Valley, CA.

OCSD. 2007b. *Orange County Sanitation District - Ocean Monitoring Program. Standard Operating Procedures.* Fountain Valley, CA.

OCSD. 2007c. *Orange County Sanitation District - Ocean Monitoring Program. Quality Assurance and Project Plan (QAPP), Phase II, Year 10 (2007-08).* Fountain Valley, CA.

Officer, C.B. (1976). *Physical Oceanography of Estuaries and Associated Coastal Waters*. New York: John Wiley.

Pielou, E.C. 1977. *Mathematical Ecology.* John Wiley and Sons, New York, NY, 384pp.

Shannon, C.H. and W. Weaver. 1962. *The Mathematical Theory of Communication.* Univ. Illinois, Urbana, IL. 177 pp.

Sokal, R.R. and F.J. Rohlf. 1981. *Biometry.* 2nd Ed. San Francisco, CA. W.H. Freeman and Company. 859 pp.

Standard Methods for the Examination of Water and Wastewater, 20th Edition, Method 4500-S^2 G (1998).

Tetra Tech, Inc. 1985. *Recommended Biological Indices for 301(h) Monitoring Programs. Final Report*. Prepared for Marine Operations Division. TC-3953-03. 17 pp.

Word, J. 1978. The infaunal trophic index. *Southern California Coastal Water Research Project Annual Report, 1979*. SCCWRP, Long Beach, CA.