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 (OMP). 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 2013b), Environmental Assessment Division Standard Operating Procedures (OCSD 2008), and the Environmental Sciences Laboratory Operating Procedures Manual (LOPM) (OCSD 2013a).

For 2012-13, the OMP was conducted under conditions stipulated in the District's 2012 NPDES discharge permit (Order No. R8-2012-0035, NPDES No. CA0110604).

COMPLIANCE DETERMINATIONS

Water quality compliance was assessed based on: (1) specific numeric criteria for dissolved oxygen (DO), pH, and three fecal indicator bacteria (FIB), namely total and fecal coliform and enterococci; and (2) narrative (non-numeric) criteria for transmissivity, floating particulates, oil and grease, water discoloration, beach grease, and excess nutrients. The sampling approach comprised a 2 × 2 km fixed-grid pattern with seven nearshore-offshore transects of four stations each (Table A-1). 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. Compliance evaluations for DO, pH, and transmissivity were 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 allowed comparisons of data from similar water depths. FIB compliance used corresponding COP bacterial standards at each Rec-1 station (Table A-1). The remaining compliance determinations for the remaining criteria were done based on presence/absence and level of potential effect at each station. Water quality sampling dates for 2012-13 are shown in Table A-2.

Dissolved Oxygen, pH, and Transmissivity

For each survey, the depth of the pycnocline layer was calculated 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 (transmissivity, dissolved oxygen, and pH) were then binned by water column stratum: above, within, or below the pycnocline; or top, middle, or bottom third when a pycnocline was absent. Mean values for each parameter were calculated by stratum and station. All binned observations (1 m intervals) within each stratum were used in the calculations. The number of observations usually differed from station to station and survey to survey due to different water and pycnocline depths.

Table A-1. OCSD ocean monitoring program station positions and nominal depths.

Orange County Sanitation District, California.

Station	Latitude	Longitude	Depth (m)
Offshore Water Quality			
1901 *	33° 33.682' N	117° 49.654' W	10
1902 *	33° 33.165' N	117° 49.944' W	60
1903 *	33° 32.762' N	117° 50.182' W	100
1904 *	33° 31.787' N	117° 50.734' W	405
1905 *	33° 30.810' N	117° 51.285' W	510
1906 *	33° 29.829' N	117° 51.842' W	550
2001 *	33° 35.335' N	117° 51.564' W	10
2002 *	33° 34.755' N	117° 51.844' W	60
2003 *	33° 34.565' N	117° 52.123' W	100
2004 *	33° 33.589' N	117° 52.657' W	345
2005 *	33° 32.613' N	117° 53.225' W	410
2006 *	33° 31.647' N	117° 53.793' W	470
2021 *	33° 35.771' N	117° 52.099' W	10
2022 *	33° 35.283' N	117° 52.379' W	53
2023 *	33° 34.796' N	117° 52.658' W	165
2024 *	33° 33.811' N	117° 53.179' W	300
2025 *	33° 32.851' N	117° 53.741' W	390
2026 *	33° 31.900' N	117° 54.301' W	432
2101 *	33° 36.183' N	117° 55.749' W	10
2102 *	33° 35.631' N	117° 56.206' W	26
2103 ****	33° 35.089' N	117° 56.678' W	110
2104 ****	33° 34.199' N	117° 57.414' W	143
2105 ***	33° 33.309' N	117° 58.150' W	280
2106 ***	33° 32.420' N	117° 58.885' W	309
2181 *	33° 36.877’ N	117° 56.752’ W	10
2182 *	33° 36.272’ N	117° 57.264’ W	15
2183 ****	33° 35.701’ N	117° 57.744’ W	36
2184 ***	33° 34.811’ N	117° 58.480’ W	51
2185 ***	33° 33.922’ N	117° 59.215’ W	114
2186 ***	33° 33.032’ N	117° 59.951’ W	247
2201 *	33° 37.493' N	117° 57.831' W	10
2202 *	33° 36.901' N	117° 58.314' W	16
2203 ****	33° 36.313' N	117° 58.810' W	25
2204 ***	33° 35.423' N	117° 59.546' W	39
2205 ***	33° 34.534' N	118° 00.282' W	57
2206 ***	33° 33.644' N	118° 01.018' W	185

Table A-1 Continues.

Table A-1 Continued.

Station	Latitude	Longitude	Depth (m)
Offshore Water Quality			
2221 *	33° 38.099' N	117° 58.908' W	10
2222 *	33° 37.522' N	117° 59.374' W	15
2223 ****	33° 36.924' N	117° 59.871' W	22
2224 ***	33° 36.035' N	118° 00.608' W	31
2225 ***	33° 35.146' N	118° 01.346' W	47
2226 ***	33° 34.257' N	118° 02.083' W	135
2301 *	33° 38.572' N	118° 00.064' W	10
2302 *	33° 38.053' N	118° 00.495' W	15
2303 ****	33° 37.537' N	118° 00.936' W	21
2304 ***	33° 36.649' N	118° 01.674' W	29
2305 ***	33° 35.760' N	118° 02.412' W	38
2306 ***	33° 34.871' N	118° 03.149' W	114
2349 *	33° 39.190' N	118° 01.135' W	10
2350 *	33° 38.667' N	118° 01.566' W	14
2351 ****	33° 38.151' N	118° 02.001' W	21
2352 **	33° 37.262' N	118° 02.739' W	29
2353 **	33° 36.373' N	118° 03.477' W	37
2354 **	33° 35.484' N	118° 04.214' W	123
2401 *	33° 39.920' N	118° 02.103' W	10
2402 *	33° 39.342' N	118° 02.593' W	16
2403 ****	33° 38.765' N	118° 03.072' W	21
2404 **	33° 37.875' N	118° 03.808' W	29
2405 **	33° 36.986' N	118° 04.544' W	37
2406 **	33° 36.096' N	118° 05.280' W	60
2451 *	33° 41.475' N	118° 03.944' W	10
2452 *	33° 40.739' N	118° 04.584' W	17
2453 *	33° 39.987' N	118° 05.204' W	22
2454 *	33° 39.098' N	118° 05.946' W	30
2455 *	33° 38.210' N	118° 06.675' W	36
2456 *	33° 37.318' N	118° 07.411' W	42

* = Central Bight WQ Regional Grid station only - No discrete samples.

** = Core Water Quality Station - No discrete samples.

*** = Core Water Quality Station - Ammonia samples.

**** = Core Water Quality Station - Ammonia and Bacteria (REC-1) samples.

Table A-1 Continues.

Table A-1 Continued.

Station	Latitude	Longitude	Depth (m)
Core Nearshore (Surfzone) Water Quality			
39N	33° 42.114' N	118° 03.321' W	Surf
33N	33° 41.281' N	118° 02.495' W	Surf
27N	33° 40.587' N	118° 01.712' W	Surf
21N	33° 39.843' N	118° 00.785' W	Surf
15N	33° 39.114' N	117° 59.846' W	Surf
12N	33° 38.854' N	117° 59.413' W	Surf
9N*	33° 38.565' N	117° 58.924' W	Surf
6N*	33° 38.331' N	117° 58.573' W	Surf
3N*	33° 38.018' N	117° 58.032' W	Surf
0*	33° 37.764' N	117° 57.598' W	Surf
3S	33° 37.619' N	117° 57.264' W	Surf
6S	33° 37.337' N	117° 56.704' W	Surf
9S	33° 37.033' N	117° 56.283' W	Surf
15S	33° 36.342' N	117° 55.459' W	Surf
21S	33° 36.059' N	117° 54.213' W	Surf
27S	33° 35.646' N	117° 52.910' W	Surf
29S	33° 35.559' N	117° 52.508' W	Surf
39S	33° 34.700' N	117° 51.946' W	Surf

All stations are monitored at least once per week.
* = Stations are monitored at least twice per week.

Table A-1 Continues.

Table A-1 Continued.

Station	Latitude	Longitude	Depth (m)
Regional Nearshore (Surfzone) Water Quality			
OSB02*	33° 44.420' N	118° 06.937' W	Surf
OSB03	33° 44.355' N	118° 06.449' W	Surf
OSB05	33° 44.296' N	118° 06.378' W	Surf
OSB04	33° 44.209' N	118° 06.121' W	Surf
OSB01	33° 43.603' N	118° 05.041' W	Surf
OSUB1	33° 42.986' N	118° 04.341' W	Surf
BCO-1	33° 40.994' N	118° 02.138' W	Surf
HB1**	33° 40.065' N	118° 01.937' W	Surf
HB2**	33° 40.022' N	118° 01.937' W	Surf
HB3**	33° 39.952' N	118° 00.933' W	Surf
HB4**	33° 39.680' N	118° 00.613' W	Surf
HB5**	33° 39.414' N	118° 00.310' W	Surf
TM	33° 37.994' N	117° 57.645' W	Surf
SAR-N	33° 37.870' N	117° 57.434' W	Surf
BGC**	33° 35.389' N	117° 52.121' W	Surf
PPC**	33° 34.933' N	117° 51.416' W	Surf
WFC**	33° 34.900' N	117° 51.334' W	Surf
ONB39**	33° 34.444' N	117° 50.410' W	Surf
MDC**	33° 33.838' N	117° 49.702' W	Surf
El MORO**	33° 33.593' N	117° 49.292' W	Surf

All stations are monitored at least once per week.
* = Stations are monitored at least twice per week.
** = When flowing, 2 additional samples are collected: 1) 25 yards upcoast and 2) 25 yards downcoast of freshwater-ocean interface. When flow is not observed at the interface, a single sample is collected 25 yards downcoast.

Table A-1 Continues.

Table A-1 Continued.

Station	Latitude	Longitude	Depth (m)
Benthic			
0 *	33° 34.573' N	118° 00.598' W	56
1 *	33° 34.657' N	118° 00.968' W	56
3 *	33° 34.434' N	118° 00.660' W	60
4 *	33° 34.498' N	117° 59.761' W	56
5 *	33° 34.749' N	118° 01.612' W	59
7	33° 35.325' N	118° 00.367' W	41
8	33° 35.164' N	117° 59.555' W	44
9 *	33° 34.363' N	117° 59.510' W	59
10	33° 34.902' N	118° 02.081' W	62
12 *	33° 34.385' N	117° 59.054' W	58
13	33° 35.307' N	118° 02.944' W	59
17	33° 33.961' N	118° 00.187' W	91
18	33° 34.064' N	118° 00.750' W	91
20	33° 34.599' N	118° 02.229' W	100
21	33° 35.313' N	118° 01.891' W	44
22	33° 35.204' N	117° 59.028' W	45
23	33° 33.968' N	117° 59.147' W	100
24	33° 33.563' N	118° 01.140' W	200
25	33° 33.924' N	118° 02.176' W	200
27	33° 33.326' N	117° 59.708' W	200
29	33° 35.033' N	118° 03.113' W	100
30	33° 35.493' N	118° 02.899' W	46
33	33° 34.349' N	117° 57.866' W	100
36	33° 35.308' N	117° 57.495' W	45
37	33° 34.832' N	117° 57.369' W	56
38	33° 34.634' N	117° 57.317' W	100
39	33° 33.283' N	117° 58.531' W	200
40	33° 32.496' N	117° 59.775' W	303
41	33° 32.690' N	118° 01.149' W	303
42	33° 33.098' N	118° 02.598' W	303
44	33° 34.586' N	118° 05.422' W	241
55	33° 36.739' N	118° 05.413' W	40
56	33° 35.665' N	118° 05.417' W	100
57	33° 34.970' N	118° 05.418' W	200
58	33° 33.365' N	118° 05.347' W	300
59	33° 36.070' N	118° 03.701' W	40
60	33° 35.532' N	118° 04.017' W	100

Table A-1 Continues.

Table A-1 Continued.

Station	Latitude	Longitude	Depth (m)
Benthic			
61	33° 35.011' N	118° 04.326' W	200
62	33° 34.069' N	118° 04.568' W	300
63	33° 34.173' N	118° 03.407' W	200
64	33° 33.484' N	118° 03.663' W	300
65	33° 33.859' N	117° 57.230' W	200
68 *	33° 34.848' N	118° 00.694' W	52
69 *	33° 34.794' N	118° 00.465' W	52
70 *	33° 34.736' N	118° 00.183' W	52
71 *	33° 34.687' N	117° 59.939' W	52
72 *	33° 34.674' N	118° 01.146' W	55
73 *	33° 34.596' N	118° 00.709' W	55
74 *	33° 34.616' N	118° 00.230' W	57
75 *	33° 34.559' N	117° 59.974' W	60
76 *	33° 34.459' N	118° 00.297' W	58
77 *	33° 34.373' N	117° 59.730' W	60
78 *	33° 34.329' N	118° 00.036' W	63
79 *	33° 34.383' N	118° 00.876' W	65
80 *	33° 34.324' N	118° 00.662' W	65
81 *	33° 34.263' N	118° 00.362' W	65
82 *	33° 34.207' N	118° 00.077' W	65
83	33° 34.239' N	118° 01.414' W	100
84 *	33° 34.648' N	118° 00.543' W	54
85 *	33° 34.532' N	118° 00.679' W	57
86 *	33° 34.560' N	118° 00.802' W	57
87 *	33° 34.401' N	118° 00.380' W	60
C *	33° 35.799' N	118° 03.855' W	56
C2	33° 36.125' N	117° 56.014' W	56
C4	33° 35.056' N	117° 55.833' W	187
C5	33° 33.920' N	117° 55.620' W	296
Control 1 *	33° 36.037' N	118° 05.387' W	59
ZB *	33° 34.545' N	118° 00.274' W	56

* = Semi-annual stations.

Table A-1 Continues.

Table A-1 Continued.

Station	Latitude	Longitude	Depth (m)
Trawl			
T0 *	33° 34.641' N	118° 00.567' W	55
T1 **	33° 35.688' N	117° 59.561' W	35
T2	33° 35.946' N	118° 02.785' W	36
T6	33° 33.771' N	118° 00.250' W	137
T10	33° 36.055' N	118° 05.199' W	60
T11 **	33° 34.868' N	118° 01.670' W	57
T12 **	33° 34.672' N	118° 03.200' W	137
T14	33° 35.160' N	118° 02.658' W	60
T17 **	33° 36.960' N	118° 05.273' W	36
T18	33° 35.394' N	118° 05.424' W	137
T19	33° 34.326' N	117° 59.856' W	60
T22 **	33° 34.336' N	117° 59.051' W	58
T23 **	33° 35.648' N	118° 01.274' W	36
T24	33° 34.245' N	118° 01.967' W	137
T25	33° 34.641' N	118° 00.567' W	55

***** = T0 sampled for historical purposes.

****** = Semi-annual trawl stations.

Table A-1 Continues.

Table A-1 Continued.

Rig-fishing	
RF1	**Zone 1 (outfall):** Inshore of the 60 m depth contour bounded by the coordinates 33° 36.272’ N / 117° 57.264’ W and 33° 37.522’ N / 117° 59.374’ W along the 15 m contour and 33° 34.698' N / 118° 01.713' W along the 80 m contour, and 33° 33.475' N / 117° 59.583' W along the 180 m contour.
RF2	**Zone 2 (farfield reference):** Inshore of the 60 m depth contour bounded by the coordinates 33° 34.535’ N / 117° 52.078’ W and 33° 32.381’ N / 117° 49.218’ W along the 15 m contour inshore and coordinates 33° 31.860’ N / 117° 49.894’ W and 33° 33.983’ N / 117° 52.699’ W along the 60 m contour off shore, and bordering the Laguna Beach State Marine Reserve.

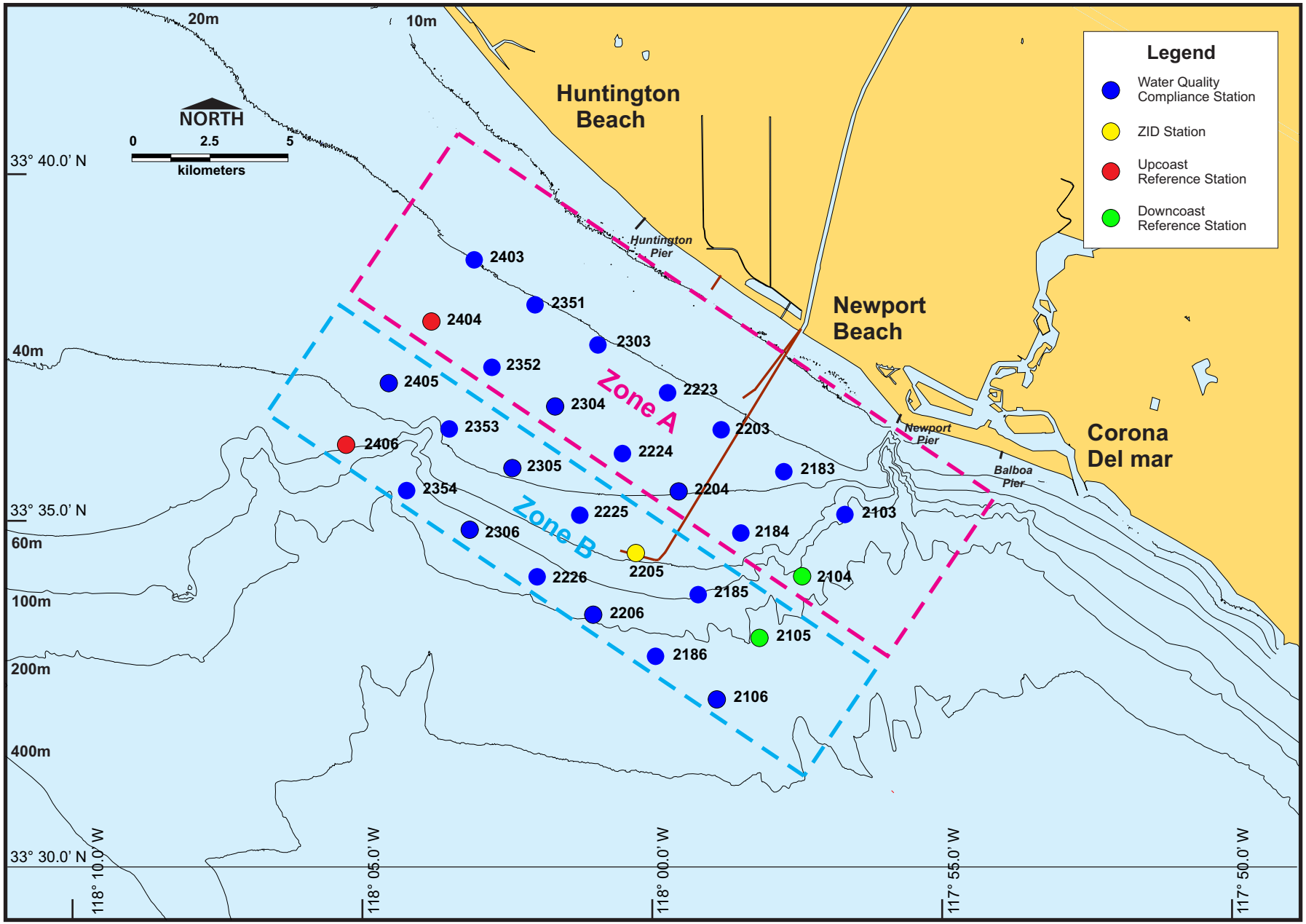

Figure A-1. **Water quality monitoring stations and zones used for compliance determinations.**

Orange County Sanitation District, California.

Table A-2. Sampling, equipment deployment, and training dates during 2012-13.

Orange County Sanitation District, California.

Quarter	Date	Cruise #	# of days	Purpose
Water Quality				
Summer	07/19/2012	OC-2012-032	1	Water Quality Compliance
	08/02/2012	OC-2012-031	1	REC-1 Water Quality Compliance
	08/06/2012	OC-2012-035	1	REC-1 Water Quality Compliance
	08/07/2012	OC-2012-036	1	Water Quality Compliance
	08/08/2012	OC-2012-037	1	Central Bight Water Quality
	08/09/2012	OC-2012-038	1	REC-1 WQ Cruise (# 3)/J-112 Pre-survey
	08/13/2012	OC-2012-039	1	REC-1 Water Quality Compliance/J-112 WQ Project
	09/10/2012	OC-2012-044	1	J-112 Water Quality Project
	09/11/2012	OC-2012-049	1	J-112 Water Quality Project
	09/12/2012	OC-2012-045	1	J-112 Water Quality Project
	09/13/2012	OC-2012-047	1	J-112 Water Quality Project
	09/17/2012	OC-2012-048	1	J-112 Water Quality Project
	09/18/2012	OC-2012-046	1	J-112 Water Quality Project
	09/19/2012	OC-2012-050	1	Water Quality Compliance
	09/24/2012	OC-2012-051	1	J-112 Water Quality Project
	09/25/2012	OC-2012-053	1	J-112 Water Quality Project
	09/27/2012	OC-2012-054	1	ECOHAB Project
Fall	10/01/2012	OC-2012-055	1	J-112 Water Quality Project
	10/02/2012	OC-2012-056	1	J-112 Water Quality Project
	10/03/2012	OC-2012-057	1	J-112 Water Quality Project
	10/09/2012	OC-2012-060	1	J-112 Water Quality Project
	10/10/2012	OC-2012-061	1	ECOHAB Project
	10/17/2012	OC-2012-069	1	ECOHAB Project
	10/30/2012	OC-2012-070	1	Water Quality Compliance
	11/06/2012	OC-2012-074	1	Water Quality Compliance
	11/07/2012	OC-2012-075	1	Central Bight Water Quality Cruise
	11/08/2012	OC-2012-073	1	REC-1 Water Quality Compliance
	11/13/2012	OC-2012-076	1	REC-1 Water Quality Compliance
	11/14/2012	OC-2012-077	1	REC-1 Water Quality Compliance
	12/04/2012	OC-2012-079	1	Water Quality Compliance
Winter	01/29/2013	OC-2013-001	1	Water Quality Compliance
	02/14/2013	OC-2013-004	1	REC-1 Water Quality Compliance
	02/25/2013	OC-2013-002	1	Central Bight Water Quality Cruise
	02/26/2013	OC-2013-003	1	Water Quality Compliance
	02/27/2013	OC-2013-005	1	REC-1 Water Quality Compliance
	02/28/2013	OC-2013-007	1	REC-1 Water Quality Compliance
	03/11/2013	OC-2013-006	1	Water Quality Compliance

Table A-2 Continues.

Table A-2 Continued.

Quarter	Date	Cruise #	# of days	Purpose
Water Quality				
Spring	04/24/2013	OC-2013-012	1	REC-1 Water Quality Compliance
	04/29/2013	OC-2013-014	1	Water Quality Compliance
	05/07/2013	OC-2013-015	1	REC-1 Water Quality Compliance
	05/09/2013	OC-2013-016	1	REC-1 Water Quality Compliance
	05/20/2013	OC-2013-018	1	Water Quality Compliance
	05/21/2013	OC-2013-017	1	Central Bight Water Quality Cruise
	06/13/2013	OC-2013-020	1	Water Quality Compliance
Sediment and Infauna				
Summer	07/10-17/2012	OC-2012-029	5	Annual and Semi-annual Benthic Infauna and Sediment Geochemistry
Summer	07/17/2012	OC-2012-030	1	J-112 Project Benthic Infauna and Sediment Geochem
Fall	11/19/2012	OC-2012-078	1	J-112 Project Benthic Infauna and Sediment Geochem
Winter	03/05-06/2013	OC-2013-008	2	Semi-annual Benthic Infauna, Sediment Geochemistry, and Sediment Toxicity
Trawls & Rig Fishing				
Summer	07/25-26/2012	OC-2012-034	2	Rig Fishing
Summer	07/30-08/01/2012	OC-2012-033	3	Annual Trawls
Winter	03/13-04/10/2013	OC-2013-009	3	Semi-Annual Trawls
Spring	04/10/2013	OC-2013-010	1	Research Trawls
Spring	05/23/2013	OC-2013-019	1	Research Trawls
Current Meters and Moorings				
Summer	08/14/2012	OC-2012-040	1	TRBM/ADCP
	08/16/2012	OC-2012-041	1	TRBM/ADCP
	08/30/2012	OC-2012-042	1	J-112 Project - MBARI Mooring
	09/05/2012	OC-2012-064	1	J-112 Project - AXYS Mooring
	09/06/2012	OC-2012-043	1	J-112 Project - AXYS Mooring
	09/07/2012	OC-2012-065	1	TRBM/ADCP
	09/09/2012	OC-2012-066	1	J-112 Project - Wave Rider/Wire Walker
	09/20/2012	OC-2012-052	1	J-112 Project - WQM Mooring
	09/26/2012	OC-2012-058	1	J-112 Project - Wave Rider
Fall	10/04/2012	OC-2012-059	1	J-112 Project - Wave Rider
	10/11/2012	OC-2012-067	1	J-112 Project - Wave Rider
	10/16/2012	OC-2012-068	1	J-112 Project - MBARI Mooring
	10/31/2012	OC-2012-071	1	TRBM/ADCP
	11/01/2012	OC-2012-072	1	J-112 Project - WQM Mooring
Spring	04/11/2013	OC-2013-011	1	J-112 Project - MBARI Mooring
	05/01/2013	OC-2013-013	1	Nerissa scientific winch/cable maintenance
Training				
Summer	08/21/2012	OC-2012-062	1	Training
	08/29/2012	OC-2012-063	1	Training

The selection of appropriate reference stations (i.e., upcoast or downcoast) for each survey day were determined based on current measurements (if available) and/or the presence or absence of ammonia at stations upcoast or downcoast from the outfall diffuser. Once reference stations were determined, the data were analyzed using in-house MATLAB (2007) routines to calculate out-of-range occurrences (OROs) for each sampling date and parameter. These OROs were based on comparing the mean data by stratum and test station with the corresponding reference station data to determine whether the following COP criteria were exceeded:

- Dissolved oxygen: cannot be depressed >10% below the mean;
- pH: cannot be greater than ± 0.2 pH units different than the mean; and
- Natural light (defined as transmissivity): shall not be significantly reduced, where statistically different from the mean is defined as the lower 95% confidence limit.

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

To determine whether an ORO was out-of-compliance (OOC), distributional maps were created that identified the reference stations for each sampling date and location of each ORO, including which stratum was out of range. Each ORO was then evaluated to determine if it represented a logical OOC event. These evaluations were based on: (A) evaluation of the wastewater plume location relative to depth using a combination of temperature, density, salinity, CDOM (colored dissolved organic matter), and when available, FIB and ammonia (NH3-N); (B) evaluation of features in the water column relative to naturally occurring events (i.e., high chlorophyll-*a* due to phytoplankton); and (C) unique characteristics of some stations that may not be comparable with permit-specified reference stations (2104/2105 or 2404/2406) 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 under certain conditions (e.g., winter storm surges). This can result in naturally decreased water clarity (transmissivity) that is unrelated to the wastewater discharge. An ORO can be in-compliance if, for example, a downcurrent station is different from the reference, but no intermediate (e.g., nearfield) stations exhibited OROs.

Once the total number of OOC events was summed by parameter, the percentage of OROs and OOCs were 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 over 12 survey dates per year are used. For Zone B, 432 comparisons are possible from 12 stations (not including the reference station), three strata, and 12 sampling dates. The total combined number of ORO and OOC events was 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).

Fecal Indicator Bacteria (FIB)

Three FIB—total coliform, fecal coliform (using *Escherichia coli*), and enterococci—were sampled at a subset of eight Zone A stations (Table A-1) located within waters designated by the RWQCB for water contact sports (i.e., waters designated as REC-1). These stations were sampled five times per quarter within a 30-day period with samples collected every 10 m, from the surface to 60 m or 2 m above the bottom (Table A-3). FIB counts at individual stations were averaged per survey and compliance for each FIB was determined using the following COP criteria (SWRCB 2010):

30-day Geometric Mean

- Total coliform density shall not exceed 1,000 per 100 ml.
- Fecal coliform density shall not exceed 200 per 100 ml.
- Enterococci density shall not exceed 35 per 100ml.

Single Sample Maximum

- Total coliform density shall not exceed 10,000 per 100 ml.
- Fecal coliform density shall not exceed 400 per 100ml.
- Enterococci density shall not exceed 104 per 100 ml.
- Total coliform density shall not exceed 1,000 per 100 ml when the fecal coliform/total coliform ratio exceeds 0.1.

Additionally, the District's permit includes the following USEPA Primary Recreation Criteria for *Enterococcus* (EPA 1994b).

- 30-day geometric mean: Density less than 35 per 100 mL
- Singles sample: Density less than 104 per 100 mL for designated bathing beaches
- Singles sample: Density less than 158 per 100 mL for moderate use
- Singles sample: Density less than 276 per 100 mL for light use
- Singles sample: Density less than 501 per 100 mL for infrequent use

For purposes of this report, compliance with the EPA criteria was based on infrequent use.

Determinations of fecal coliform compliance were accomplished by multiplying *E. coli* data by 1.1 to obtain a calculated fecal coliform value.

Nutrients and Aesthetics

Compliance for the floating particulates, oil and grease, and water discoloration, were determined based on presence/absence at the ocean surface for each station. Compliance with the excess nutrient criteria was based on evaluation of ammonia (NH3-N) compared to COP objectives for chronic (4 mg/L) and acute (6 mg/L) toxicity to marine organisms. Compliance was also evaluated by looking at potential spatial relationships between NH3-N distribution and phytoplankton (using chlorophyll-*a* fluorescence).

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
Nearshore (Surfzone)							
Shoreline Total Coliforms, Fecal Coliforms, and Enterococci	grab	Standard Methods 9222 B. Standard Methods 9222 D. EPA Method 1600.	Ice (<6°C)	125 mL HDPE (Sterile container)	8 hrs. (field + lab)	Ankle deep water	at least 10% of samples
Offshore							
Temperature	*in-situ* probe	(1)	none	none	not applicable	every 1 m *	at least 10% of stations
Salinity (conductivity)	*in-situ* probe	(2)	none	none	not applicable	every 1 m *	at least 10% of stations
pH	*in-situ* probe	(3)	none	none	not applicable	every 1 m *	at least 10% of stations
Dissolved Oxygen	*in-situ* probe	(4)	none	none	not applicable	every 1 m *	at least 10% of stations
Transmissivity	*in-situ* probe	(5)	none	none	not applicable	every 1 m *	at least 10% of stations
Photosynthetically Active Radiation (PAR)	*in-situ* probe	(6)	none	none	not applicable	every 1 m *	at least 10% of stations
Chlorophyll-*a* fluorescence	*in-situ* probe	(7)	none	none	not applicable	every 1 m *	at least 10% of stations
Color Dissolved Organic Matter (CDOM)	*in-situ* probe	(8)	none	none	not applicable	every 1 m *	at least 10% of stations
Ammonia (NH3-N)	Niskin	LOPM 350.1B Rev. B (EPA Method 350.1B Rev. A).	Ice (<6°C)	125 mL HDPE	28 days	Surface, 10m, 20m, 30m, 40m, 50m, 60m, Bottom	at least 10% of samples
Offshore Total Coliforms, Fecal Coliforms, *Escherichia coli*, and Enterococci	Niskin	Standard Methods 9223 B.	Ice (<6°C)	125 mL HDPE (Sterile container)	8 hrs. (field + lab)	Surface, 10m, 20m, 30m, 40m, 50m, 60m, Bottom	at least 10% of samples
Surface Observations	visual observations	permit specs.	none	none	not applicable	surface	none

(1) Calibrated to reference cells (0.0005°C accuracy) every year.
(2) Calibrated to IAPSO Standard and Guildline 8400B Autosal every year.
(3) Referenced and calibrated to NIST buffers of pH 7, 8, and 9 every survey.
(4) Referenced and calibrated each survey by comparison with the lab DO probe which is calibrated daily.
(5) Referenced and calibrated to known transmittance in air.
(6) Factory calibrated (Biospherical Instruments) once per year.
(7) Factory calibrated (Wetlabs) once per year.
(8) Factory calibrated (WetLabs) once per year.
* Sampled continuously but data processed to 1 m intervals.

WATER QUALITY MONITORING

Field Methods

Offshore Water Quality Monitoring

Permit-specified water quality studies were conducted monthly at 28 stations comprising a fixed-grid pattern (Tables A-1 and A-2, Figures A-1 and 3-1). Each survey included measurements of pressure (from which depth is calculated), water temperature, conductivity (from which salinity is calculated), dissolved oxygen (DO), pH, water clarity (light transmissivity, beam attenuation coefficient [beam-c], and photosynthetically active radiation [PAR]), chlorophyll-*a* fluorescence, and colored dissolved organic matter (CDOM). Profiling was conducted from 1 m below the surface to 2 m above the bottom or to a maximum depth of 75 m, when station water depths exceeded 75 m. Measurements were conducted using a Sea-Bird Electronics
SBE9/SBE 11 Deck Unit (SBE9/11) CTD (conductivity-temperature-depth) profiling system. SEASOFT (2012a) software was used for data acquisition, data display, and sensor calibration. PAR is measured in conjunction with chlorophyll-*a* because of the positive linkage between light intensity and photosynthesis per unit chlorophyll (Hardy 1993). A summary of the sampling methods are presented in Table A-3.

Visual observations of floatable materials or grease that might be of sewage origin were also conducted. Daily rainfall, sea state, and wind condition data were summarized from Newport Beach Fire and Marine Department and the District's Treatment Plant No. 2 records.

Discrete sampling for ammonia and FIB was conducted using a Sea-Bird Electronics Carousel Water Sampler (SBE32/SBE33) equipped with Niskin bottles. Sample depths are provided in Table A-3. Bacteriology samples were kept on wet ice in coolers and transported to the District's laboratory within 6-hours of collection for analysis.

Central Bight Regional Water Quality

An expanded grid of water quality stations was sampled quarterly as part of the District's Central Bight Regional Water Quality monitoring. 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 total sampling area extends from the Ventura River in the north to Crystal Cove State Beach in the south. Samples were collected using CTDs during a targeted 3- or 4-day period, over which sampling occurred at 216 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 depth to 2 m from the bottom or to a maximum depth of 75–100 m. Sampling and analytical methods were the same as those presented in Table A-3.

Data Analyses

Raw CTD data were processed using both SEASOFT (2012b) and third party (IGODS 2012) software. The steps include retaining downcast data and identifying outliers by flagging the data if it exceeded specific criteria limits. Flagged data were removed if it was considered to be due to instrument failures, electrical noise (e.g., large data spikes), or

physical interruptions of sensors (e.g., by bubbles) rather than by actual oceanographic events. After outlier removal, averaged 1 m depth values were prepared from the downcast scan data; if there were any missing 1 m depths, then the upcast data was used as a replacement. CTD and discrete data were then combined to create a single data file that contained all sampled stations for each survey day.

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 was derived from the normalized PAR data (Hardy 1993). Two August 2012 surveys (August 6 and 7) had pH data that showed consistent survey offsets compared to the other four August cruises. To correct for instrument bias, linear pH offset values were determined by using the depth averaged values for the four unaffected August surveys. The offset used for both August 6 and 7 was +0.35 pH units. Phytoplankton blooms were defined following Seubert *et al*. (2013). Major blooms were defined as values exceeding 2 standard deviations above the average for all stations and depths for the program year. These values were then excluded from the data set and a new average and standard deviation calculated. Minor blooms were then defined as those values exceeding 2 standard deviations above the new average.

Spatial and seasonal patterns in water quality data were summarized as 2- and 3-dimensional color plots of temperature, salinity, DO, pH, transmissivity, and two-dimensional displays of PAR and chlorophyll-*a*. The 3-dimensional plots were produced using IGODS (2012) software. Data were grouped into 15-m depth bins and standard descriptive statistics (e.g., minimums, means, and maximums) were computed both seasonally and for the year. Seasonal and annual scatter and box plots were also utilized.

BACTERIOLOGY/NUTRIENTS

Field Methods

Offshore Monitoring

Quarterly surveys were conducted in August and November 2012, and February and May 2013 (Table A-2). Primary water quality sampling occurred monthly at 28 sites that form a grid around the OCSD diffuser (see Figure 3-1). Samples were collected for ammonia at a subset of 22 stations and for bacteriology at eight REC-1 stations (Tables A-1) located within state waters (within 3 miles of the shoreline) for the purposes of determining compliance with Receiving Water Limitation V.A.1.a. Discrete samples for ammonia and bacteria (total coliform, *E. coli*, and enterococci) were collected at 10 m depth intervals from the surface (1 m below) to 2 m above the ocean floor or a maximum depth of 60 m (Table A-3). Three additional surveys were conducted to collect bacteriology and ammonia samples at the eight REC-1 water quality stations within 30 days of two of the three monthly water quality sampling days in order to calculate a 30-day geometric mean. During most surveys, additional bacteriological samples were collected at the outfall (Station 2205) and two nearfield stations (1 and 9).

Core Nearshore Monitoring

Nearshore (surfzone) samples for analysis of total and fecal coliform and enterococci bacteria were collected 1-2 days per week at 18 required stations (Table A-1; Figure 3-1). Two other stations located a few yards upstream of the mouths of Talbert Marsh (TM) and

Santa Ana River (SAR) were also sampled during the year. These two stations were selected as a measure to identify runoff influence impacting nearby stations. These two stations were collected as part of the regional program; however, data for these stations is presented with the core station results.

Samples were collected in ankle-deep waters, on an incoming wave, with the sampler downstream and away from the bottle, and the mouth of the bottle facing into the current. Sterile sample bottles were used and the sampler used aseptic techniques, making certain that the bottle did not touch the ocean bottom. After the sample was taken, the bottle was tightly capped and promptly stored on ice in the dark. Laboratory analysis began within 6 hours of sample collection. The occurrence and size of any grease particles at the high tide line was recorded 2 days per week at the same sampling locations.

Regional Nearshore Monitoring

In 2012, four Orange County, CA public agencies collaborated to provide a more integrated and efficient microbial ocean water quality monitoring program. Under this program, OCSD extended its monitoring efforts to include northern Laguna Beach, Sunset Beach, Seal Beach, and Orange County watersheds discharging to receiving waters. Eighteen additional sampling stations were added to OCSD's monitoring program with sampling frequencies ranging from 1-2 days per week (Table A-1). When creek/storm drain stations (denoted by ** in Table A-1) flowed to the ocean, three bacteriological samples were collected: 1) at the source, 2) 25 yards downcoast, and 3) 25 yards upcoast. When flow was absent, a single sample was collected 25 yards downcoast.

Samples were collected in ankle-deep waters, on an incoming wave, with the sampler downstream and away from the bottle, and the mouth of the bottle facing into the current. Sterile sample bottles were used and the sampler used aseptic techniques, making certain that the bottle did not touch the ocean bottom. After the sample was taken, the bottle was tightly capped and promptly stored on ice in the dark. Laboratory analysis began within 6 hours of sample collection.

Laboratory Methods

Laboratory analyses of ammonia 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 were summarized graphically in 2- and 3-dimensional color figures. The 3-dimensional plots were produced using IGODS (2012) software. Data was grouped into 15-m depth bins and standard descriptive statistics (e.g., minimums, means, and maximums) were computed both seasonally and for the year. Seasonal and annual scatter and box plots were also utilized.

SEDIMENT GEOCHEMISTRY

Field Methods

Sediment samples were collected for the OMP during July 2012 and March 2013 at 29 semi-annual stations and 39 annual stations (summer only) that ranged in depth from 40 to 303 m located on the San Pedro Shelf (Tables A-1 and A-2; Figure 4-1). Single samples were collected at all stations in each of the two surveys (Table A-4). In addition, 3 L of sediment was collected from nine stations in March 2013 for sediment toxicity testing.

Bottom sediments were collected using paired 0.1 m^2 Van Veen grab samplers. 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) (1986) protocols. All sediment samples (metals, organics, total organic carbon (TOC), grain size, and dissolved sulfides) were transported to the laboratory 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 through Laboratory Information Management Systems (LIMS) software.

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 Green and Schnitker (1974) and Standard Methods 20th Edition (American Public Health Association, American Water Works Association, and Water Environment Federation 1998).

Sediment toxicity, following EPA-recommended methods (EPA 1994a), was conducted on whole sediments collected from nine stations in March 2013, using 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 Water Quality Control Plan for Enclosed Bays and Estuaries – Part 1 Sediment Quality (SWRCB 2009; Bay *et al*. 2009). Stations which were significantly different when compared to the control, determined by a two sample t-test, were categorized as non-toxic when survival was 90-100% of the control, lowly toxic when survival was 82-89% of the control, and moderately toxic when survival was 59-81% of the control. Stations which were not significantly different when compared to the control were categorized as non-toxic when survival was 82-100% of the control and lowly toxic when survival was 59-81% of the control. All stations exhibiting survival less than 59% of the control were categorized as highly toxic.

Table A-4. Sediment composition, chemistry sampling, and benthic infaunal sampling summary.

Orange County Sanitation District, California.

Stations	Sampling Frequency	Sampling Interval	Field Replicates	Parameters
0, 1, 3, 4, 5, 9 12, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, C, CON, ZB	Semi-annual (summer and winter)	0-2 cm; from undisturbed grab sample	1 replicate per station	Metals Total Organic Carbon Dissolved Sulfides Grain size Chlorinated Pesticides[1] Polychlorinated Biphenyls (PCBs)[1] Polycyclic Aromatic Hydrocarbons (PAHs) Linear Alkyl Benzenes (LABs)[2]
		≥4 cm		Infauna
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, 83, C2, C4, C5	Annual (summer only)	0-2 cm; from undisturbed grab sample	1 replicate per station	Metals Total Organic Carbon Dissolved Sulfides Grain size Polycyclic Aromatic Hydrocarbons (PAHs) Linear Alkyl Benzenes (LABs)
		≥4 cm		Infauna

[1] Winter only
[2] Summer only

Table A-5. Sediment handling and analysis summary.

Orange County Sanitation District, California.

Parameter	OCSD Method	Method Reference	Preservation	Container	Holding Time
Dissolved Sulfides	LOPM 4500-S G Rev. B	Green and Schnitker (1974); Standard Methods 20th Ed.	Freeze	Plastic container	6 months
Chlorinated Pesticides	Sediment Fish PCB/Pesticides by GCMS	NS&T (NOAA 1993); EPA 8270	Freeze	Amber glass jar	6 months (analyze within 40 days of extraction)
Grain Size	EMSL Analytical (Plumb 1981)	Plumb (1981); EPA 3-284 and 3550	4° C	Plastic bag	6 months
Linear Alkyl Benzenes	Ocean Sediment Extraction and GCMS Analysis FOR PAH_LAB	NS&T (NOAA 1993); Eganhouse *et al*. (1983)	Freeze	Glass jar	12 months
Metals	EPA 200.8	NS&T (NOAA 1993); EPA 200.8	Freeze	Amber glass jar	6 months
Polychlorinated Biphenyls	Sediment Fish PCB/Pesticides by GCMS	NS&T (NOAA 1993); EPA 8270	Freeze	Amber Glass jar	6 months (analyze within 40 days of extraction)
Polycyclic Aromatic Hydrocarbons	Ocean Sediment Extraction and GCMS Analysis FOR PAH_LAB	NS&T (NOAA 1993); EPA 8270	Freeze	Glass jar	6 months (analyze within 40 days of extraction)
Total Organic Carbon	ALS Environmental (EPA 9060)	NS&T (NOAA 1993); ASTM D4129-05	Freeze	Glass jar	6 months

Table A-6. Parameters measured in sediment samples.

Orange County Sanitation District, California.

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

Table A-6 Continues.

Table A-6 Continued.

PAH Alkylated Homologues		
C1-Chrysenes	C2-Fluorenes	C3-Naphthalenes
C1-Dibenzothiophenes	C3-Fluorenes	C4-Naphthalenes
C2-Dibenzothiophenes	C1-Fluoranthenes/Pyrenes	C1-Phenanthrenes/Anthracenes
C3-Dibenzothiophenes	C1-Naphthalenes	C2-Phenanthrenes/Anthracenes
C1-Fluorenes	C2-Naphthalenes	C3-Phenanthrenes/Anthracenes
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		

Data Analyses

Linear regression was used to determine depth related factors and Pearson Product Moment Correlation for relationships to sediment physical attributes and chemical concentrations (e.g., total linear alkylbenzene-tLAB). Regression and correlation analyses were conducted using MINITAB 15 (2007) statistical software. Principal components analysis (PCA) is an ordination technique used to map stations in multiple-dimensions based on the similarity of their samples (i.e., sediment chemical concentrations). Correlation-based PCA was performed using the PRIMER (2001) statistical software package on samples collected in July 2012. Test parameters incorporated the sediment physical and chemical measures determined to be the best effluent footprint indicators. These were determined statistically by Dr. Kerry Ritter, former Biostatistician at the Southern California Coastal Water Research Project (Costa Mesa, CA), using District historical marine monitoring data. The parameters used were total linear alkylbenzenes (tLAB), cadmium, and zinc. Temporal trends were assessed graphically (qualitatively).

Total dichlorodipheynltrichloroethane (tDDT) represents the summed concentrations of o,p'- and p,p'- [2,4- and 4,4'-] isomers of DDD, DDE, and DDT and p,p'-DDMU, total polychlorinated biphenyls (tPCB) represents the summed concentrations of 45 congeners, and total chlorinated pesticides (tPest) represents the sum of, aldrin, dieldrin, endrin, cis- and trans-chlordane, gamma-BHC, heptachlor, heptachlor epoxide, hexachlorobenzene, Mirex, 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. Non-detected single analytes (e.g., individual metals) were assigned a value of one-half the detection limit for statistical analysis.

BENTHIC INFAUNA

Field Methods

The infaunal community was monitored by collecting marine sediments concurrently with sediment geochemistry samples (Tables A-1 and A-2, Figure 5-1). Samples were collected on the San Pedro Shelf in July 2012 and March 2013 at 29 semi-annual stations and 39 annual stations (summer only) that ranged in depth from 40 to 303 m. Single samples were collected at all stations in each of the two surveys (Table A-4).

Bottom sediments were collected using paired 0.1 m^2 Van Veen grab samplers. 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 depth of 5 cm. If after three attempts with a depth of < 5 cm at a station, the depth threshold was lowered to ≤ 4 cm. Acceptable samples were gently washed with filtered seawater through a 1.0 mm sieve. Retained organisms were rinsed into one liter plastic containers and anesthetized with 7% magnesium sulfate for approximately 30 minutes. To preserve the animals, full strength buffered formaldehyde was then added to achieve a 10%, by volume, solution and returned to the laboratory.

Laboratory Methods

After 3–10 days in formalin, samples were rinsed with water and transferred to 70% ethanol for long-term preservation. Samples were sent to Weston Solutions, Inc. and Marine Taxonomic Services, Inc. (San Marcos, CA) to be sorted to major taxonomic groups (Polychaeta, Mollusca, Crustacea, Echinodermata, and miscellaneous phyla). Removal of the organisms from the sediments was monitored to ensure that at least 95% of all organisms were successfully separated from the sediment matrix. QA/QC found that over 99% of all organisms had been recovered. Upon completion of sample sorting, the major taxonomic groups were distributed for identification and enumeration according to the schedule in Table A-7. Taxonomic differences were resolved and the database was edited accordingly; all samples exceeded the 90% threshold (see Appendix C).

Table A-7. Benthic infauna sample identification schedule.

Orange County Sanitation District, California.

Date	Survey	Taxonomic Groups				
		Annelida	Arthropoda	Mollusca	Echinodermata	Misc. Phyla
Summer 2012	Annual	OCSD	Contractor	OCSD	OCSD	OCSD
	Semi-annual	OCSD	OCSD	OCSD	OCSD	OCSD
Winter 2013	Annual	Contractor	Contractor	Contractor	Contractor	Contractor
	Semi-annual	OCSD	OCSD	OCSD	OCSD	OCSD

OCSD refers to in-house taxonomists.
Contractor refers to Marine Taxonomic Services, Inc.

Data Analyses

Infaunal community data was analyzed to determine if populations outside the ZID were affected by the outfall discharge. Six measures were used to assess infaunal community health and function: (1) total number of species, (2) total abundance of individuals, (3) Shannon-Wiener Diversity (H'), (4) Swartz's 75% Dominance Index (SDI), (5) Infaunal Trophic Index (ITI; Word 1978, 1990), and (6) Benthic Response Index (BRI; Smith *et al*. 2001). H' was calculated using $\log_e$ (Zar 1999). SDI was calculated as the minimum number of species with combined abundance equal to 75% of the individuals in the sample (Swartz 1978). 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. H' (Shannon and Weaver 1962) and SDI (Swartz *et al*. 1986) are sensitive to the distribution of species within a sample (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. Two pollution tolerant species, *Capitella capitata* Complex (polychaete) and

Euphilomedes carcharodonta (ostracod), were examined because their presence may indicate stressed, polluted, or organically enriched environments.

Spatial pattern analysis was conducted using the PRIMER (2001) multivariate statistical software package. Multivariate cluster analysis was performed on the July 2012 species abundance data to define those stations (station clusters) having similar species/abundance relationships (assemblages). Cluster analysis was performed using a hierarchical agglomerative clustering method. Abundance data was 4^{th} root transformed, a Bray-Curtis similarity matrix constructed, and a dendrogram generated using a group average cluster mode. The data were 4^{th} root transformed in order to down-weight the highly abundant species and incorporate the less common species (Clarke and Warwick 2001). The SIMPER ("similarity percentages") routine was also used to determine inter- and intra-group species differences. As a confirmatory step, ordination clustering using non-metric multidimensional scaling (MDS) analysis was performed on the same data set. Abundance data from the 51 middle shelf stations were used in this analysis. The outer shelf and upper slope/canyon stations were excluded from this analysis because cluster analysis should not be used when there are known environmental gradients (Clarke and Warwick 2001). Previous analyses have consistently shown that the outer shelf and slope/submarine canyon stations cluster apart from the middle shelf stations (OCSD 2010).

Correlation and regression analyses were conducted using MINITAB (2007) statistical software to test for relationships among factors (i.e., community measures vs. sediment characteristics and occurrence of indicator species vs. depth) at the middle shelf Zone 1 and 2 stations in July 2012. Station C2 was omitted from the analyses, as this station typically differs from other 60-m, non-ZID stations in sediment characteristics and contaminant concentrations (OCSD 2013c). Prior to analysis, the data were transformed as necessary (e.g. square-root, $\log_{10}$, rank, or arcsine), and significance was set at α = 0.05. Temporal trends were assessed graphically (qualitatively).

TRAWL COMMUNITIES

Field Methods

Demersal fish and epibenthic macroinvertebrates (EMI) species were collected in the summer (July/August) of 2012 and the winter (March/April) of 2013. Sampling was conducted at 15 stations: shallow (18m) Station T0; inner shelf (36 m) Stations T2, T6, T18, and T24; middle shelf (60 m) Stations T1, T11, T12, T17, T22, and T23; and outer shelf (137 m) Stations T10, T14, T19, and T25 (Tables A-1 and A-2; Figure 6-1). One haul was conducted at each station. Only middle shelf stations were sampled in both summer and winter surveys.

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 m (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 (450 m). Trawl depths and time on the bottom were determined using an attached SBE 39 pressure sensor on the trawl boards.

Upon retrieval of the trawl net, the contents were emptied into a large flow-through water tank and all fish and macroinvertebrate specimens were sorted by species into separate containers. Fish bioaccumulation specimens were counted, recorded, and removed for processing; then the remaining fish specimens were measured to the nearest millimeter (standard length) and weighed to the nearest gram. A minimum of 15 (randomly selected) specimens of each species were weighed and measured individually. If a sample contained substantially more than 15 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, parasites, and skeletal deformities. Invertebrates were sorted to species, counted, and batch weighed. In the event of a large haul of a single species (> 100), 100 were counted and batch weighed, and the remaining animals were batch weighed and enumerated later by back calculating using the weight of the first 100. Specimens that required further examination for positive identification were retained for final taxonomic identification at a later date.

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 in the OCSD Taxonomy Lab for reference and verification.

Data Analyses

Fish and EMI populations were summarized by total abundance and species, percent abundance, frequency of occurrence (per haul), mean abundance per haul, and mean occurrence per haul. In addition, number of species, number of individuals, biomass, and diversity indices including Shannon-Wiener Diversity Index (H') and Swartz's 75% Dominance Index were calculated for both fish and EMI. In some analyses, stations were grouped into the following categories to assess spatial outfall-related patterns: "outfall" or "nearfield" (Stations T1 and T22); and "farfield" (Station T11). When examining historical trends, nearfield Station T22 was excluded from this category because it was a new station included in the 2012 NPDES permit.

PRIMER (2001) multivariate statistical software was used to examine the spatial patterns of the fish assemblages in the District's monitoring area (Clarke 1993; Warwick 1993). Analyses included hierarchical clustering with group-average linking based on Bray-Curtis similarity indices, and ordination clustering of the data using non-metric multidimensional scaling (MDS). Data were truncated to include only the middle shelf (60 m) stations since

depth is a strong environmental factor in delineating species clusters (OCSD 2004, 2010). Clarke and Warwick (2001) warn that clustering is less useful and may be misleading where there is a strong environmental forcing, such as depth. Prior to the calculation of the Bray-Curtis indices, the data were square-root transformed in order to down-weight the highly abundant species and incorporate the importance of the less common species (Clarke and Warwick 2001). The SIMPER ("similarity percentages") routine was also used to determine inter- and intra-group species differences.

Community measures from Stations T1 and T11 were evaluated for long-term temporal and spatial patterns, and compared with regional reference conditions, such as 1994 Southern California Bight Pilot Project (SCBPP), the Bight'98, and Bight'03 regional monitoring programs (Allen *et al*. 1998, 2002, 2007, respectively).

Fish biointegrity in the District's monitoring area was assessed using the fish response index (FRI). The FRI is a multivariate weighted-average index produced from an ordination analysis of calibrated species abundance data (see Allen *et al*. 2001, 2006). The FRI was calculated for middle-shelf stations in 2012-13. For a historical perspective, the annual mean FRI was calculated from 1985 to 2012 for outfall Station T1 and farfield Station T11.

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*). Bigmouth sole was not used in this reporting year due to low sample size. 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

Fishes were collected during trawl surveys using an otter trawl (as described earlier). The sampling objective was to collect 10 individuals of each of two target species for muscle and liver tissue analysis at both outfall (T1) and farfield (T11) stations. Individual fish were weighed and measured in the field; placed in clean, plastic, resealable bags; and stored on wet ice in insulated coolers. All samples were subsequently transported under chain-of-custody protocols to the laboratory.

Laboratory Methods

Individual fish were dissected in the laboratory under clean conditions. Muscle and liver tissues were analyzed for various parameters listed in 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) (Table A-9). 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

Table A-8. Parameters measured in fish tissue.

Orange County Sanitation District, California.

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

Table A-9. Fish tissue handling and analysis summary.

Orange County Sanitation District, California.

Parameter	OCSD Method	Method Reference	Preservation	Container	Holding Time
Arsenic, Mercury, and Selenium	200.8B*	NS&T (NOAA 1993); EPA 200.8	Freeze	Ziplock bag	6 months
Chlorinated Pesticides	**	NS&T (NOAA 1993); EPA 8270	Freeze	Ziplock bag	6 months (analyze within 40 days of extraction)
DDTs	**	NS&T (NOAA 1993); EPA 8270	Freeze	Ziplock bag	6 months (analyze within 40 days of extraction)
Polychlorinated Biphenyl Congeners (PCBs)	**	NS&T (NOAA 1993); EPA 8270	Freeze	Ziplock bag	6 months (analyze within 40 days of extraction)
Lipids	***	EPA 9071	Freeze	Ziplock bag	

* Determination of Trace Metals in Sediments and Solids Using ICPMS
** Instrument Determination of Organochlorine Pesticides and PCB Congeners in Fish Tissue and Ocean Sediment
*** Extraction of Organochlorine Pesticides and PCB Congeners in Fish Tissue

capture detector, and lipids were determined gravimetrically. All concentrations are reported on a wet weight basis.

Total dichlorodipheynltrichloroethane (tDDT) represents the summed concentrations of o,p'- and p,p'- [2,4- and 4,4'-] isomers of DDD, DDE, and DDT and p,p'-DDMU, total polychlorinated biphenyls (tPCB) represents the summed concentrations of 44 congeners, and other total chlorinated pesticides (tPest) represents the sum of 8 compounds (cis- and trans-chlordane, cis- and trans-nonachlor, dieldrin, heptachlor, heptachlor epoxide, and oxychlordane). 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. Non-detected single analytes (e.g., individual metals) were assigned a value of one-half the detection limit for statistical analysis.

Data analysis consisted of summary statistics and qualitative comparisons only.

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 analysis consisted of summary statistics and qualitative comparisons only.

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