Southern California Bight
2013 Regional Marine Monitoring Survey
(Bight'13)

Contaminant Impact Assessment Workplan

Prepared by:
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I. INTRODUCTION

The Southern California Bight (SCB; Figure I-1), an open embayment in the coast between Point Conception and Cape Colnett (south of Ensenada), Baja California, is an important and unique ecological resource. The SCB is a transitional area that is influenced by currents from cold, temperate ocean waters from the north and warm, tropical waters from the south. In addition, the SCB has a complex topography, with offshore islands, submarine canyons, ridges and basins, bays and estuaries, which provide a variety of habitats. The mixing of currents and the diverse habitats in the SCB allow for the coexistence of a broad spectrum of species, including more than 500 species of fish and several thousand species of invertebrates. The SCB is also a major migration route, with marine bird and mammal populations ranking among the most diverse in north temperate waters.

The coastal zone of the SCB is a substantial economic resource. Los Angeles/Long Beach Harbor is the largest commercial port in the United States, and San Diego Harbor is home to one of the largest US Naval facilities in the country. More than 100 million people visit southern California beaches and coastal areas annually, bringing an estimated $9B into the economy. Recreational activities include diving, swimming, surfing, and boating, with about 40,000 pleasure boats docked in 13 coastal marinas within the region (NRC 1990). Recreational fishing brings in more than $500M per year.

The SCB is one of the most densely populated coastal regions in the country, which creates stress upon its marine environment. Over 21 million people inhabit coastal Southern California (US Census Bureau 2010). Population growth generally results in conversion of open land into non-permeable surfaces. More than 75% of southern California's bays and estuaries have already been dredged and filled for conversion into harbors and marinas (Horn and Allen 1985). This "hardening of the coast" increases the rate of runoff and can impact water quality through addition of sediment, toxic chemicals, pathogens and nutrients to the ocean. Besides the impacts of land conversion, the SCB is already home to fifteen municipal wastewater treatment facilities, eight power generating stations, 10 industrial treatment facilities, and 18 oil platforms that discharge to the open coast.

Each year, local, state, and federal agencies spend in excess of $31M to monitor the environmental quality of natural resources in the SCB (Schiff et al 2001). At least 75% of this monitoring is associated with National Pollutant Discharge Elimination System (NPDES) permits and is intended to assess compliance of waste discharge with the state and federal regulations, which set water quality standards for effluent and receiving waters. Some of this information has played a significant role in management decisions in the SCB.

While these monitoring programs have provided important information, they were designed to evaluate impacts near individual discharges. Today, resource managers are being encouraged to develop management strategies for the entire SCB. To accomplish this task, managers need regionally-based information to assess the cumulative impacts of contaminant inputs and to evaluate relative risk among different types of stressors. It is difficult to use existing data to evaluate regional issues because the monitoring was designed to be site-specific and is limited to specific geographic areas. The monitoring provides substantial data for some

areas, but there is little or no data for the areas in between. Beyond the spatial limitations, data from these programs are not easily merged to examine relative risk. The parameters measured often differ among programs. Even when the same parameters are measured, the methodologies used to collect the data often differ and interlaboratory quality assurance (QA) exercises to assess data comparability are rare.

Previous Regional Monitoring Studies

There have been four previous regional monitoring efforts to begin addressing environmental concerns at larger spatial scales (Table 1). The first regional monitoring survey in 1994, called the Southern California Bight Pilot Project (SCBPP), was a compilation of 12 agencies that cooperatively sampled 261 sites along the continental shelf between Point Conception and the United States/Mexico border. The second regional monitoring survey, called the Southern California Bight 1998 Regional Monitoring Project (Bight'98), was comprised of 64 agencies that cooperatively sampled 416 sites between Point Conception and Punta Banda, Mexico and included new habitats such as ports, bays, and marinas. The third regional monitoring survey, called the Southern California Bight 2003 Regional Monitoring Project (Bight'03), was comprised of 65 agencies that cooperatively sampled 391 sites between Point Conception and the United States/Mexico border, and expanded the number of habitats from Bight'03 to include estuaries and deep ocean basins. The fourth regional monitoring survey, called the 2008 Southern California Bight Regional Marine Monitoring Program (Bight '08), was comprised of 61 organizations that sampled 383 sites between Point Conception and the United States/Mexico border, and included new contaminants of emerging concern.

Table I-1. Summary of previous Regional Survey Monitoring Programs.

Strata	1994 (Pilot Project)	1998 (Bight '98)	2003 (Bight'03)	2008 (Bight'08)
Inner Shelf	X	X	X	X
Middle Shelf	X	X	X	X
Outer Shelf	X	X	X	X
Upper Slope			X	X
Lower Slope and Basin			X	X
Channel Islands		X	X	X
River Mouths	X	X		
Mexico		X		
Estuaries			X	X
Marinas			X	X
Ports			X	X
Bays			X	X

Benefits derived from the previous surveys included the development of new useful technical tools that could only be developed with regional data sets and participation by multiple organizations. For example, the project produced iron-normalization curves for the SCB, allowing distinction between natural and anthropogenic contributions of metals in sediments

(Schiff and Weisberg 1998). A Benthic Response Index was developed that integrates complex benthic infaunal data into an easily interpreted form that describes the degree of perturbation at a site (Bergen *et al.* 1998). These types of tools have culminated in management tools such as the State of California's sediment quality objectives (Beegan and Bay 2012). The Bight Regional Surveys have also improved the comparability among the monitoring organizations in the SCB. The quality assurance and quality control (QA/QC) significantly improved following laboratory intercalibration exercises for chemistry, group training for field crews, and taxonomic resolution for biologists. The Regional Marine Monitoring Program has also produced a series of manuals containing standardized field, laboratory and data management activities that increased continuity of data and data reporting among participants, even after the regional monitoring surveys were completed. Many of these manuals are now mandated in NPDES monitoring and reporting programs regionwide.

2013 Survey

The proposed Southern California Bight 2013 Regional Marine Monitoring Project (Bight'13) is a continuation of the successful cooperative regional-scale monitoring begun in southern California. Bight'13 builds upon the previous successes and expands on the 2008 survey by including new participants, answering additional questions, and measuring more parameters. Thirty four organizations, including international and volunteer organizations, have agreed to participate (Table I-2). The inclusion of multiple participants, many of them new to regional monitoring, provides several benefits. Cooperative interactions among many organizations with different perspectives and interests, including a combination of regulators and dischargers, ensure that an appropriate set of regional-scale questions will be addressed by the study.

The Bight'13 Survey is organized into six technical components: 1) Contaminant Impact Assessment (formerly Coastal ecology); 2) Shoreline microbiology; 3) Water quality; 4) Marine Protected Areas, and; 5) Trash and debris. The CIA focuses on sediment contaminants and associated impacts on benthic infauna and demersal fish. This work plan provides a summary of the project design. The work plan is supported by five companion documents detailing Field Methods and Logistics, Benthic Laboratory Manual, Toxicology Laboratory Manual, Quality Assurance Plan (QAP), and Information Management Plan. Separate work plans are also available for the other elements of Bight'13.

FIGURE I-1. Map of the Southern California Bight.

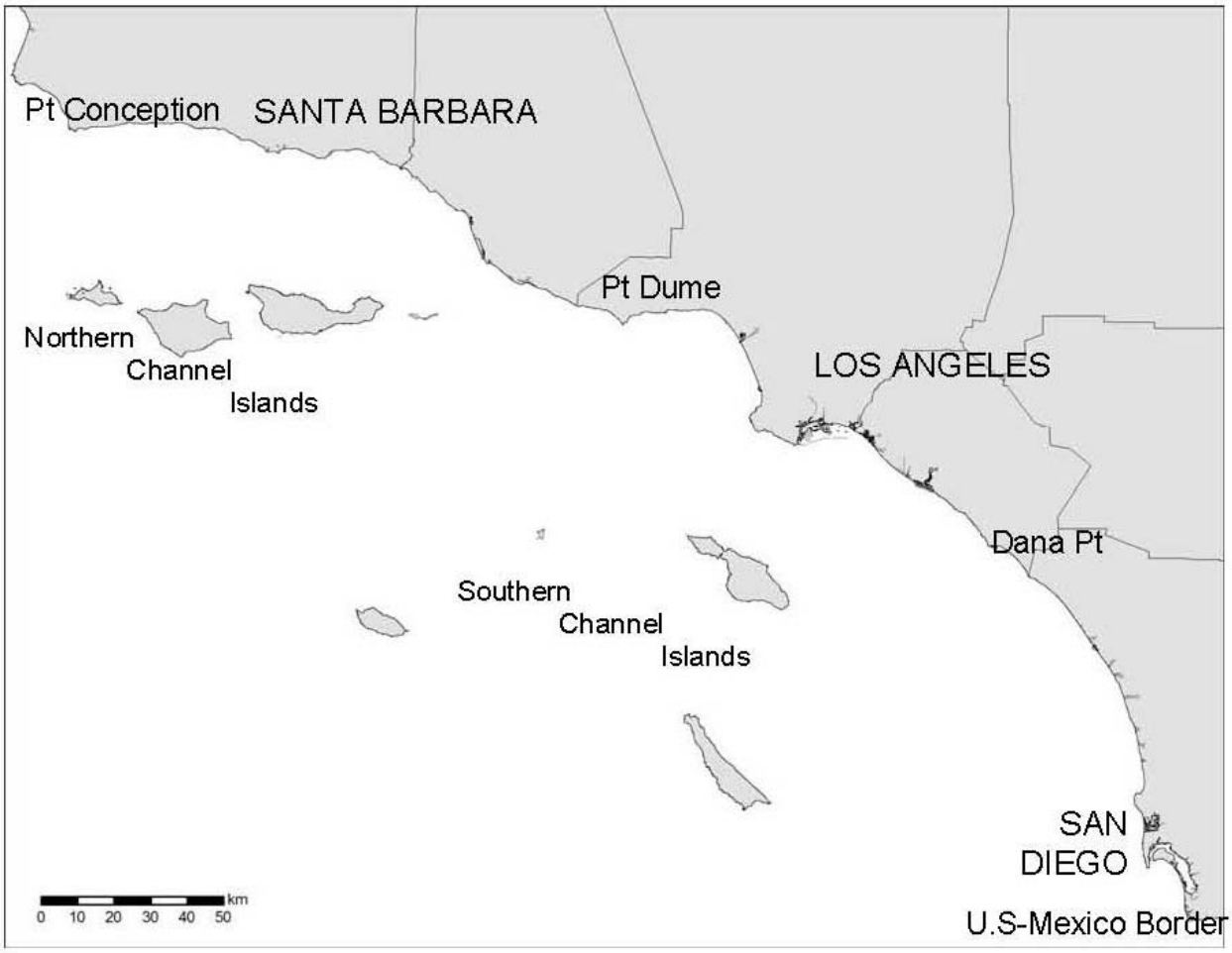

TABLE I-2. Participants in the Bight'13 Regional Marine Monitoring Program, Contaminant Impact Assessment component.

AES Corporation
Aquatic Bioassay and Consulting Laboratories (ABCL)
Calscience Environmental Laboratories, Inc.
Channel Islands National Marine Sanctuary (CINMS)
Chevron USA Products Company
City of Los Angeles Environmental Monitoring Division (CLAEMD)
City of Oceanside
City of Oxnard
City of San Diego
Encina Wastewater Authority
City of Los Angeles, Department of Water and Power (LADWP)
Los Angeles Regional Water Quality Control Board
Los Angeles County Sanitation Districts (LACSD)
MBC Applied Environmental Sciences
Minerals Management Service
National Oceanic and Atmospheric Administration (NOAA)
Nautilus Environmental, Inc.
NES Energy, Inc.
NRG Energy, Inc.
Orange County Sanitation District (OCSD)
Physis Environmental Laboratories, Inc.
Port of Long Beach
Port of Los Angeles
San Diego County Dept. of Environmental Health and Municipal Co-permittees
San Diego Regional Water Quality Control Board (SDRWQCB)
San Diego State University (SDSU)
San Diego Unified Port District
San Elijo Joint Powers Authority
Santa Ana Regional Water Quality Control Board
State Water Resources Control Board
Universidad Autónoma de Baja California (UACB)
U.S. Fish and Wildlife Service
Weck Laboratories, Inc.
Weston Solutions, Inc.

II. STUDY DESIGN

A. Study Objectives

The overall goal of the contaminant impact assessment component of Bight'13 is to assess the condition of the benthic environment and the health of the biological resources in the SCB. To accomplish this goal, Bight'13 will focus on three primary objectives:

1. What is the extent and magnitude of direct impact from sediment contaminants?
2. What is the trend in extent and magnitude of direct impacts from sediment contaminants?
3. What is the indirect risk of sediment contaminants to seabirds?

Direct impacts refer to ecological changes resulting from exposure to contaminated sediment. The first objective, estimating the area (i.e., number of acres) in which ecological conditions differ from reference conditions, is a departure from traditional approaches to environmental monitoring that generally focus on estimating average condition. Estimating the areal extent of ecological change offers several advantages. First, it provides a more direct assessment of status. For instance, identifying that the average Shannon-Weiner (H') benthic diversity in the SCB provides less useful information for environmental managers than does identifying what percentage of the area in the SCB has impaired biological communities. A corollary to this concept is the assessment of regional reference condition. Since most monitoring programs in the SCB are site specific, assessment of regional reference condition allows managers to compare individual sites to the breadth and depth of natural variation in the SCB.

There are two sub-objectives within the areal extent and magnitude objective. The first sub-objective is to determine if the areal extent and magnitude vary among geographic regions. If we answer this question, then managers can determine if specific areas are in worse condition than others, such as areas near anthropogenic inputs versus those areas distant from inputs. Therefore, Bight'13 will compare conditions among 11 geographic areas of interest (Table II-1). These subpopulations were selected to represent a range of natural and potentially affected habitats, and are inclusive of all the habitats sampled in Bight'08, with the except for the Channel Island stratum. However, Bight'13 has two new strata never focused on previously; submarine canyons and marine protected areas (MPA). Canyons bisect the continental shelf, much like a river canyon on land, which may serve as a conduit of pollutants from the nearshore to the lower slope and basin. MPAs are a new management area in the SCB. MPAs have restricted fishing and are intended to protect ecosystem integrity and provide protected stock for improving recreational and commercial fisheries. Comparison of the relative condition among strata provides information about the geographic distribution of impacts and may indicate the relative risk among a variety of pollutant discharges. Comparison of conditions may be conducted by comparing the extent of area exceeding a threshold of concern or by comparison of mean condition.

The second sub-objective within the areal extent and magnitude objective is to assess the relationship between biological responses and direct contaminant exposure. Such associations provide the information necessary for risk assessment, and for developing efficient regional strategies for protecting the environment by identifying the predominant types of stress in the SCB

ecosystem. Therefore, this sub-objective will be accomplished by simultaneously collecting numerous measures of biological response, contaminant exposure and habitat condition (Table II-2) to better identify when exposure has reached a level of concern. Measuring multiple indicators also permits us to identify the most likely type of exposure leading to biological response.

The second primary objective is to assess trends in estimates of areal extent and magnitude. If habitats of concern improve over time, then this demonstrates the effectiveness of cumulative management actions. If habitats of concern worsen, then this demonstrates the need for management actions to occur. However, if some habitats improve and others worsen, then the average condition might not change. By estimating the areal extent of alteration, we will be better able to describe these changes. We have designed the Bight'13 to build upon three previous surveys to assess trends in areal extent and magnitude. This will be accomplished by revisiting a subset of randomly sampled sites from 1998, 2003, and 2008.

The third objective is to assess bioaccumulation in higher order predators. Bioaccumulation in fish has been routinely measured in previous Bight surveys. In each survey, fish tissues were routinely contaminated with chlorinated hydrocarbons, and an attempt was made to estimate the risk of these contaminated fish to higher order predators such as birds. In Bight'13, bioaccumulation in birds will be measured directly by examining concentrations in eggs. This represents the first such study at a regional scale in the SCB.

B. Sampling Design

The CIA sampling design for Bight'13 will be divided into two components. These include: 1) areal extent, magnitude, and trends; and 2) bioaccumulation.

Areal Extent, Magnitude, and Trends

The areal extent, magnitude, and trends component of Bight'13 will involve sampling 397 sites for sediments in the SCB between July 1 and September 30, 2013. The summer period was chosen for the study because it represents a period of steady weather during which the indicators we measure are expected to remain stable.

Maps of the sampling sites are provided in Appendix A. Sites were selected using a stratified random approach, with the strata corresponding to the subpopulations of interest in Table II-1. Stratification ensures that an appropriate number of samples are allocated to characterize each population of interest with adequate precision. We aimed to allocate thirty sites to each strata because this yields a 90% confidence interval of about ± 10% around estimates of areal extent (assuming a binomial probability distribution and $p = 0.2$; Figure II-1). This level of desired precision was selected because differences in response of less than 10% among subpopulations are unlikely to yield different management decisions.

Sites were selected randomly within strata, rather than by investigator pre-selection, to ensure that they are representative and can be extrapolated to the response of the entire strata. Although sites were selected randomly, a systematic component was added to the selection process to minimize clustering of sample sites. The systematic element was accomplished by

using an extension of the sampling design used in the SCBPP and in EPA's Environmental Monitoring and Assessment Program (EMAP) (Stevens 1997). A hexagonal grid was randomly placed over a map of the sampling area, a subsample of hexagons chosen from this population, and one sample was obtained at a randomly selected site within each grid cell. The hexagonal grid structure ensures systematic separation of the sampling, while the random selection of sites within grid cells ensures an unbiased estimate of ecological condition. Further details about this site selection process are provided in Appendix B.

One of the design attributes of Bight'13 is to maximize the coincidence of indicators, allowing us to relate biological response to chemical exposure and physical habitat condition. The number of sites sampled for each indicator group within each strata is presented in Table II-3. To maximize overlap of indicators, sites that receive fewer indicator measurements were randomly chosen (with a systematic element) as a subset of the sites at which all indicators are measured.

Approximately half of the sites in each of seven strata are revisits of previously sampled sites in order to help assess trends. These strata include the 5-30m, 30-120m, and 120-200m depth zones on the coastal shelf as well as marinas, ports, bays and estuaries. One quarter of the sites will be from Bight'98, one quarter will be from Bight'03, and the remaining one half will be new sites for Bight'13. All of these sites will be randomly selected and spatially unbiased so estimates of spatial extent are still valid.

C. Indicators

Bight'13 will measure multiple indicators (Table II-2) at each site in order to relate contaminant exposure, biological response, and habitat condition. Collecting measures of contaminant exposure with measurements of biological response at common sites allows investigators to identify and statistically model associations between altered ecological conditions and particular environmental stresses. Habitat indicators help discriminate between changes caused by anthropogenic and natural factors.

One design principle of Bight'13 is that these indicators will be measured using uniform sampling methods throughout the Bight. The probability-based sampling design provides a framework for integrating data into a comprehensive regional assessment, but the validity of such an assessment depends on ensuring that all the data that contribute to it are comparable. Below, we present a short description of the methods used to measure the Bight'13 indicators; more detailed descriptions of the methods can be found in the accompanying Field Methods and Quality Assurance Manuals for the project.

Contaminant Exposure

1. Sediment Chemistry: Chemical analysis of sediment samples provides an assessment of contaminant exposure for bottom dwelling animals. Sediment samples will be collected from the top 2 cm (coastal sites) or top 5 cm (embayments) of a Van Veen grab sample. The chemical analyte list includes both inorganic and organics (Table II-4) and was developed to include comparisons to local programs and to national monitoring datasets such as NOAA's Status and

Trends program. The constituent list and associated reporting limits was specifically developed for comparison to sediment quality guidelines such as the State of California's Sediment Quality Objectives (SWRCB 2008). All chemistry measurements will follow performance-based quality assurance guidelines described in the Bight'13 Quality Assurance Plan.

Organics

Organic compounds in sediments will be extracted with solvents and cleaned to remove interfering substances. PAHs will be analyzed by GC/MS. Organochlorine pesticides and polychlorinated biphenyls will be analyzed by GC/ECD, GC/MS, or GC/MS/MS. The accuracy of PCB measurements will be enhanced by measuring 41 individual congeners in all samples with elevated concentrations. The PCB congener list was selected to include compounds that are abundant in the environment and compounds with a high potential for toxicity. New to the Bight'13 survey will be standard measurements of PolyBrominated DipPhenyl Ethers (PBDEs). Thirteen PBDE congeners will be analyzed by GC/ECD, GC/MS, or GC/MS/MS. The PBDE congener list was selected to include compounds that were present in the original technical mixtures, are abundant in the environment and compounds, and have a high potential for bioaccumulation.

Inorganics

Metals in sediments will be analyzed by ICP, ICPMS, or atomic absorption spectrophotometry after strong acid digestion. Mercury will be analyzed by cold vapor technique. In addition to trace metals, the reference elements iron and aluminum will also be measured in each sample. Normalization of the trace metal data to reference element concentrations will enable anthropogenic contamination to be distinguished from natural variations in background concentrations.

2. Marine Debris: The amount of plastic, metal and other anthropogenic debris on the ocean bottom is a measure of human influence. Debris captured in trawls will be classified by type (e.g., plant material, plastic, and cans) and scored according to relative abundance. In addition, microplastics will be quantified from sediment samples. These small plastic particles (> 10 µm) will be enumerated from sediment samples under the microscope.

Biological Response

While indicators of contaminant exposure provide an important measure of the influence of anthropogenic materials on the marine and estuarine environments, it is the effect of this exposure upon biological processes that determines the significance of the contaminants. The effect of contaminant exposure will be examined through a variety of indicators:

3. Benthic Infauna: Benthic infauna (animals that live in the sediment) are an important part of the ocean food web. Because infauna generally reside in one location for most of their lives and are chronically exposed to sediment contaminants, they are an excellent indicator of environmental quality. Samples for infaunal analysis will be taken with a 0.1 m^2 modified Van Veen grab. Samples will be washed through a 1.0 mm stainless steel screen and preserved for identification to the lowest practical taxonomic unit.

4. Demersal fish and megabenthic invertebrate assemblages: Demersal fish and megabenthic invertebrates are more mobile than the benthic infauna, but are still closely associated with the bottom and chronically exposed to sediment contaminants. Demersal fish and megabenthic invertebrates will be collected with a semiballoon otter trawl with 7.6-m headrope length and a 1.3 cm cod-end mesh. Trawls will be towed for 10 min at 0.8-1.0 m/s along depth isobaths (5 min in harbors). All fish and most invertebrates will be identified to species, counted, and weighed.

5. Gross fish pathology: The presence and extent of external diseases (e.g. fin rot and tumors) and anomalies (e.g. skeletal deformities or abnormal coloration) will be recorded from fish collected in the trawls for assemblage analysis. Specimens with unusual or unidentified conditions will be returned to the laboratory for detailed examination.

6. Sediment toxicity: Toxicity tests provide a direct measure of the effect of contamination on benthic organisms. These tests complement sediment chemistry measurements by providing a measure of the combined toxic effect of the complex mixture of contaminants present in surficial sediments or in the porewater between sediment grains (interstitial water). The toxicity of bulk sediments will be assessed by measuring survival of the amphipod, *Eohaustorius estuarius*, after exposure for 10 days. In addition, the normal development of the bivalve, *Mytilus galloprovincialis,* will be measured using the sediment:seawater interface test. Both tests support the application of California's SQOs.

Habitat Condition

The distribution of biota is also affected by natural habitat factors, such as grain size and the amount of organic matter present. Habitat indicators will be measured to help distinguish the relative effects of natural and anthropogenic factors on biotic distribution.

7. Sediment grain size: Grain size will be measured with a laser diffraction technique, a method that provides greater resolution between particle size classes with less variability than conventional pipette techniques. Two instruments will be used: 1) A Horiba LA920 which measures 89 size classes of particles between 0.05-2,000 μm and 2) a Coulter LS230 that measures 116 size classes between 0.04-2000 μm.

8. Sediment Total Organic Carbon (TOC), Total Nitrogen (TN: TOC and TN will be measured with an Elemental Analyzer.

Bioaccumulation

A targeted sampling design will be used to examine bioaccumulation in bird eggs. A census of bird nesting sites will be sampled to assess the frequency of occurrence and magnitude of concentrations in four different avian guilds. There are 12 major nesting areas in the SCB (Figure II-2). The four guilds include pelagic foragers (Caspian tern), benthic forager

(Cormorant), mixed forager (Western gull), and species of special concern (California Least tern). Not all species or guilds are expected at every nesting site.

A minimum of six eggs per species will be collected per nesting area for chemical analysis. In the case of California Least Terns, two eggs may need to be composited for sufficient tissue mass for chemical analysis. Bird eggs will be analyzed for DDTs, PCBs, PBDEs, and total mercury using analytical methods described for sediment. Lipid content will be measured using the gravimetric method. Egg processing will require physical measurements of eggs (length, weight, shell thickness) prior to analysis. Egg contents will be homogenized prior to extraction.

Special Studies

The Bight program represents an excellent opportunity to add on special studies and research not routinely conducted for ongoing monitoring programs. Researchers are always looking to test new technology, evaluate new indicators, apply new methods, or explore unanswered questions in new locations. The Bight program comprises an enormous platform of core measurements with indicators typically measured on a routine basis. The merging of the Bight program with researchers provides a positive interaction for both parties. Researchers view the Bight program as a cost efficient vehicle to move their research forward. Bight participants get the added value of their research for essentially no cost. Incorporating new measurements and methods into the Bight program benefits regulated participants in the Bight program because it is not part of a permit requirement and can help determine if a perceived issue is actually a widespread environmental problem. Incorporating their special studies into the Bight program benefits researchers because it allows their work direct access to the important environmental decision makers in the SCB.

There are eight special studies planned for Bight'13 (Table II-5, Appendix D). The studies range across all 10 indicators being measured in Bight'13 incorporating contaminant exposure, biological response, and habitat condition. Nearly all of the special studies supplement existing indicators already being measured as part of the Bight program. For example, the study of chemicals of emerging concern (CECs) supplements existing chemical measurements or the use of gene microarrays to identify specific toxicants supplements the standard toxicity assays being conducted with the same species. Several of the special studies also provide integration among one another. For example, the study on CECs in sediment provides insight into the same CECs in tissues. Another example would be the relationship between exposure from traditional chemicals or CECs, and the biological response comparisons between fish, invertebrates, and the new biological screening tools. Individually, these indicators all provide useful information, but collectively they provide invaluable insight.

FIGURE II-1. 90% Confidence Intervals about an estimate of percent of area changed as a function of sample size.

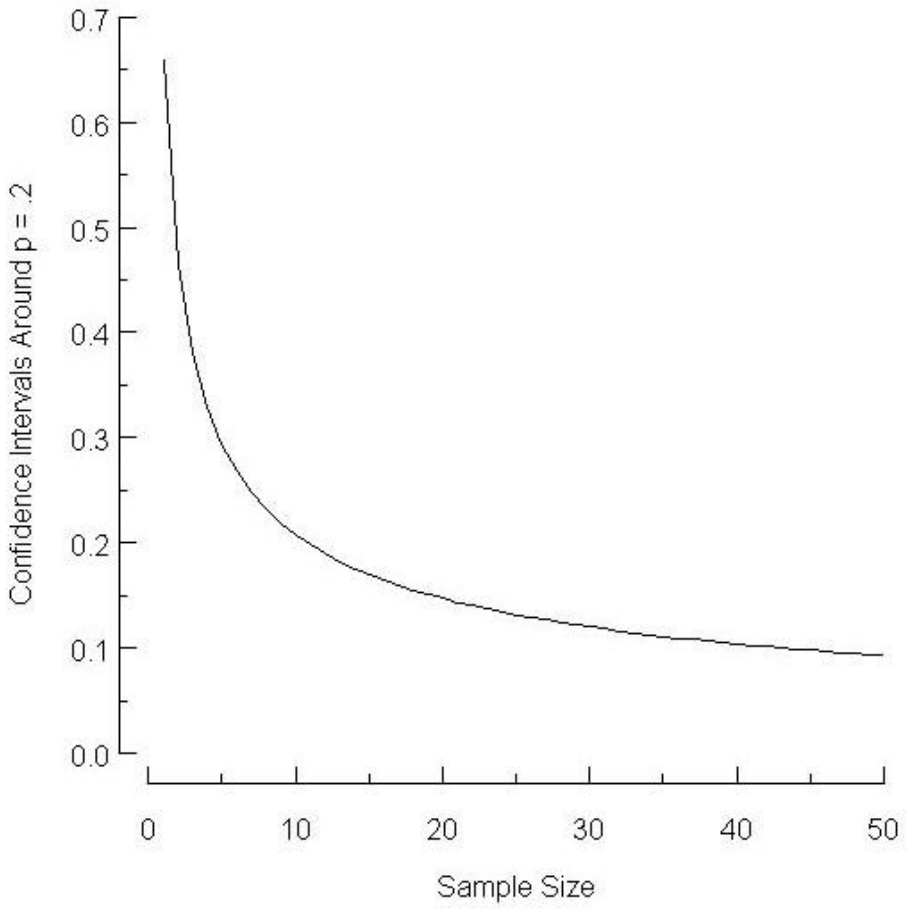

FIGURE II-2. Map of nesting areas in the SCB.

TABLE II-1. Subpopulations of interest in the areal extent, magnitude, and trends objectives of the Bight'13 Coastal Ecology study.

Offshore Areas

a. Inner shelf (5-30 m)
b. Mid-shelf (30-120 m)
c. Outer shelf (120-200 m)
d. Upper slope (200-500 m)
e. Lower slope and basin (500 – 1,000 m)
f. Submarine canyons (5 – 1,000 m)
g. Marine Protected Areas (5 – 500 m)

Embayment Areas

a. Estuaries
b. Ports
c. Bays
d. Marinas

TABLE II-2. Indicators to be measured in Bight'13.

Contaminant exposure
- Sediment chemistry
- Debris

Biological response
- Benthic infauna
- Fish assemblage
- Fish pathology
- Macroinvertebrate assemblage
- Sediment toxicity

Habitat
- Grain size
- Sediment organic carbon

TABLE II-3. Sample sizes in the subpopulations for Bight'13.

	Sediment Chemistry	Infauna	Trawl	Sed Tox
Offshore Strata				
5 to 30 m	30	30	30	10
30 to 120 m	31	31	30	10
120 to 200 m	30	30	30	10
200 to 500 m	40	40	31	
500 to 1000 m	21 [a]	21 [a]		
Submarine canyons	30	30		30
Marine Protected Areas	30	30	27	
Channel Islands		15 [a]		
Embayment Strata				
Marinas	43 [b]	43 [b]		43 [b]
Ports	45 [b]	45 [b]		45 [b]
Bays/Harbors	37 [b]	37 [b]	26	37 [b]
Estuaries/Lagoon	45 [b]	45 [b]		45 [b]
Target Sample Size	382	397	174	200

[a] revisit sites only
[b] local enhancements in the San Diego Region

TABLE II-4. Constituents that will be measured in sediment during Bight'13.

Trace Metals	PCB Congeners		Polycyclic Aromatic Hydrocarbons	PolyBrominated Diphenyl Ethers
Aluminum	PCB 18	PCB 157	Acenaphthene	BDE 17
Antimony	PCB 28	PCB 158	Acenaphthylene	BDE 28
Arsenic	PCB 37	PCB 167	Anthracene	BDE 47
Barium	PCB 44	PCB 168	Benz[a]anthracene	BDE 49
Beryllium	PCB 49	PCB 169	Benzo[a]pyrene	BDE 66
Cadmium	PCB 52	PCB 170	Benzo[b]fluoranthene	BDE 85
Chromium	PCB 66	PCB 177	Benzo[e]pyrene	BDE 99
Copper	PCB 70	PCB 180	Benzo[g,h,i]perylene	BDE 100
Iron	PCB 74	PCB 183	Benzo[k]fluoranthene	BDE 138
Lead	PCB 77	PCB 187	Biphenyl	BDE 153
Mercury	PCB 81	PCB 189	Chrysene	BDE 154
Nickel	PCB 87	PCB 194	Dibenz[a,h]anthracene	BDE 183
Selenium	PCB 99	PCB 201	Fluoranthene	BDE 209
Silver	PCB 101	PCB 206	Fluorene	
Zinc	PCB 105		Indeno(1,2,3-c,d)pyrene	
	PCB 110		Naphthalene	
	PCB 114		Perylene	
	PCB 118	**Chlorinated**	Phenanthrene	
	PCB 119	**Hydrocarbons**	Pyrene	
	PCB 123	cis-chlordane	2,6-Dimethylnaphthalene	
Other	PCB 126	trans-chlordane	1-Methylnapthalene	
Constituents	PCB 128	o.p'-DDT	2-Methylnapthalene	
Total Organic	PCB 138	p,p'-DDT	1-Methylphenanthrene	
Carbon	PCB 149	o.p'-DDD	1,6,7-Trimethylnaphthalene	
Total Nitrogen	PCB 151	p,p'-DDD		
Total Phosphorus	PCB 153	o.p'-DDE		
Grain Size	PCB 156	p.p'-DDE		
		p,p'-DDMU		
		cis-nonachlor		
		trans-nonachlor		
		oxychlordane		

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Table II-5. Integration of special studies with existing indicators. X=where there is overlap or correlation among measurements.

Special Study	Sediment Chemistry	Infauna	Demersal Fish	Sediment Toxicity	Sediment Grain Size	TOC
CECs	X	X	X	X		X
Bioanalytical screening tools	X	X	X	X		X
Sediment TIEs	X	X		X	X	X
Gene microarray	X	X		X	X	X
Multi-species toxicity testing	X	X		X	X	X
In-situ toxicity testing	X	X		X	X	X
DNA Barcoding	X	X				

APPENDIX A

Sample site maps

APPENDIX B

Sample Site Information

APPENDIX C

Sample Laboratory Assignments

APPENDIX D

Bight'13 Special Studies

Analysis of Contaminants of Emerging Concern in Sediment Samples

Collaborators: SCCWRP; Calscience Environmental Laboratories, Inc.; Physis Environmental Laboratories, Inc.; Weck Laboratories, Inc.

Background: Contaminants of emerging concern (CECs) are unregulated compounds that have been detected in the environment and may pose an ecological risk. They are not routinely monitored and typically little is known about their occurrence, fate, and risk. However, for certain CECs enough screening evidence has been collected to support an elevated prioritization for monitoring.

Statement of Problem: In 2012, the SWRCB convened an Expert Panel that used a risk-based screening framework identify a list of high-priority CECs for further investigation. These CEC were specifically recommended additional monitoring in various matrices. Among the analytes recommended for monitoring in marine sediment were: 1) perfluorooctane sulfonate (PFOS), a perfluorinated compound (PFC) used in stain repellants; 2) p-nonylphenol, an alkylphenol (AP) breakdown product of alkylphenol ethoxylate surfactants; 3) bifenthrin and permethrin, two pyrethroid pesticides; and 4) polybrominated diphenyl ethers (PBDEs), used as flame retardants. The Panel recommended these compounds be monitored in a regional program in order to provide the greatest information on extent of occurrence, and to use this information as additional input to the Panel's framework for CEC prioritization.

Objectives: The primary objective of this special study is to measure the recommended CECs at a subset of Bight '13 sediment stations. Specifically, we will quantify three classes PFCs, APs, and pyrethroids. PBDEs will be measured as a routine parameter by participating laboratories. This special study will answer the following questions.

1. What is the extent and magnitude of CEC concentrations?
2. How does the extent and magnitude of CEC concentrations vary by stratum?
3. How does the extent and magnitude of CEC concentrations correlate with legacy contaminant concentrations?
4. How does the extent and magnitude of CEC concentrations compare to potential sources of these constituents?

Task 1: Quality Assurance Preparation. Three laboratories (Calscience, Physis, Weck) have volunteered to measure the field samples for CECs. Sufficient QA must occur to be confident data from multiple laboratories can be combined. The QA will occur using three mechanisms: 1) an inter-calibration exercise prior to sample collection involving the three laboratories and SCCWRP; 2) ongoing QA during sample analysis; and 3) review of split sample analysis following laboratory data submittal.

Task 2: Sample Analysis. Up to 180 sediment samples will be analyzed for the three CEC classes.

Task 3: Integration with Bight '13 Chemical Analysis. CEC concentration data will be combined with the routine chemical concentration data for data analysis and reporting. CEC data analysis and conclusions will be reviewed by the Chemistry Technical Working Group and the CIA Planning Committee.

Products: There will be two products for this special study. The first will be a section in the Bight '13 Chemistry Report answering the four study objective questions. The data products will include maps of chemical concentrations (e.g., Figure 1), and tables or graphs of spatial extent and concentration by stratum (e.g., Table 1). These data will be summarized for the second product, a peer-reviewed journal publication on CECs in the southern California Bight. The manuscript will be an Appendix in the Bight '13 Chemistry Report.

Figure 1. Example spatial distribution map from Bight '08 showing 4 PBDE congeners representative of the 3 technical mixtures.

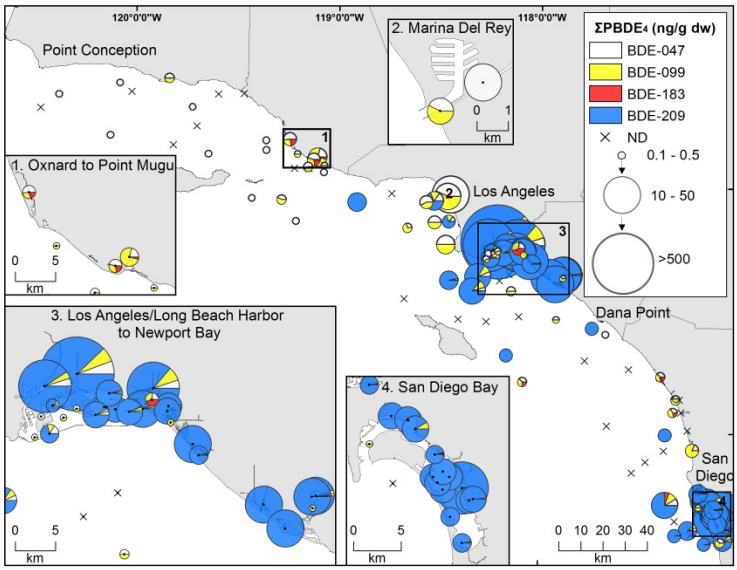


Table 1. Example table of PBDE area weighted geometric mean (AWGM) concentration by stratum.

Congener	Stratum	Number Detects	AWGM (ng/g dw)
BDE-47	Embayments	54	0.25 (0.20, 0.32)
	Offshore	32	0.12 (0.092, 0.15)
BDE-99	Embayments	50	0.24 (0.18, 0.33)
	Offshore	18	0.098 (0.074, 0.13)
BDE-183	Embayments	24	0.086 (0.071, 0.10)
	Offshore	3	NA
BDE-209	Embayments	36	7.9 (5.1, 12)
	Offshore	13	0.90 (0.69, 1.2)
$\Sigma PBDE_{13}$	Embayments	56	12 (8.0, 17)
	Offshore	36	2.0 (1.6, 2.5)

Bioanalytical Screening of Bight '13 Sediment Extracts

Collaborators: SCCWRP, UC Riverside, Univ. Florida, LACSD, Life Technologies, BDS

Background*:* High throughput in vitro toxicity bioassays developed by the EPA are being used to screen a large number of chemicals based on a mode of action paradigm. We are currently evaluating commercial versions of these assays for screening chemicals that initiate adverse outcome pathways in drinking and recycled water. Because these molecular initiation steps are thought to be conserved across both humans and species of wildlife, these bioassays hold promise as a more efficient screening tool for monitoring of thousands of chemicals that occur in receiving waters, compared to the traditional chemical-by-chemical approach.

Statement of Problem: In 2009, the SWRCB convened an Expert Panel to provide guidance for monitoring of contaminants of emerging concern (CECs). In addition to recommending chemical-specific (or "targeted") monitoring, the Panel recommended development of bioanalytical tools to screen for a broad suite of chemicals by mode of biological action. Although in vitro bioassays have been developed for high throughput screening of individual chemicals, they have not yet been evaluated and adapted for testing of complex mixtures or chemicals in matrices such as water or sediment. If successful, adaptation of a battery of cell-based bioassays that are linked to a diverse, relevant set of endpoints of concern for ecological receptors will greatly expand the scope and improve the efficiency of chemical monitoring in receiving waters.

Objectives: The primary objective of this special study is to measure the response of Bight sediment extracts using a battery of cell-based in vitro bioassays that integrate the response of chemicals based on a common mode of biological action. This special study will answer the following questions.

1. What is the response of a battery of cell-based in vitro bioassays to extracts of Bight sediment representing a range of chemical contamination?
2. How do bioassay responses correlate with the sediment concentrations of contaminants measured as part of the Bight '13 monitoring design?
3. How do bioassay responses correlate with legacy (i.e., routinely monitored) contaminant concentrations? How do they correlate with contaminants of emerging concern such as PBDEs and PFCs?

Task 1: Bioassay Optimization and QA. Candidate bioassays (Table 1) developed for water matrices will be tested for compatibility and response with organic extracts of marine sediment. Bioassay response will be compared for different sediment processing steps, including raw extracts and those treated to remove analytical interferences such as elemental sulfur. In addition, sediment extraction blanks will be analyzed to determine the background response, and minimize the interferences if necessary, by the bioassays. Once optimized, Special Study collaborators will analyze aliquots of split sample extracts to determine inter-laboratory precision and accuracy.

Task 2: Sample Extraction and Analysis. Up to 50 sediment samples will be tested with a battery of in vitro bioassays optimized from Task 1. The sediment samples will be obtained from archived material at participating laboratories following chemical analysis. The samples will be selected based on the range of chemical concentrations for a variety of constituents.

Task 3: Integration with Bight '13 Chemical Analysis. Bioassay results will be represented as bioassay equivalents (i.e., sample response is referenced to the response of a strong agonist pre-selected and standardized for each candidate endpoint). Sediment contaminant concentration data determined concurrently will be grouped by expected mode of action (e.g., as toxic equivalents or TEQs) and individually regressed against BEQs using a stepwise approach. Bioassay and sediment contaminant data analysis and conclusions will be reviewed first by the Special Study collaborators prior to review by the Chemistry Technical Working Group and the CIA Planning Committee.

Products: There will be two products for this special study. The first will be a section in the Bight '13 Chemistry Report answering the study objective questions. The data products will include a summary of bioassay response by Bight station and correlations of BEQs with chemical concentrations and toxic equivalents (TEQs). The results will also be written up into a peer-reviewed journal publication. The manuscript will be an Appendix in the Bight '13 Chemistry Report.

Table 1. Candidate cell-based bioassays to be optimized for testing of marine sediment extracts.

ENDPOINT	REFERENCE CHEMICAL	CANDIDATE BIOASSAY(S)
estrogenicity	17b-estradiol	estrogen receptor (ER+)
androgenicity	dihydrotestosterone	androgen receptor (AR)
progesterone activity	levonorgestrel	progesterone receptor (PR)
glucocorticoid activity	triclosan	glucocorticoid receptor (GR)
genotoxicity	mitomycin	p53 reporter gene
aryl hydrocarbon reactivity	PCB 126	aryl hydrocarbon receptor (AhR)
CEC-specific response	gemfibrozil	PPAR alpha receptor
cytotoxicity		Presto Blue

Sediment Toxicity Identification Evaluation in Embayments

Collaborators: SCCWRP, ABC Labs, LACSD, Nautilus Environmental

Background: Sediment quality surveys have historically shown that embayments have the highest contaminant concentrations and greatest incidence of sediment toxicity. Sediment toxicity was present throughout 50% of the embayment area sampled in the 2008 Bight regional survey. However, it is difficult to use these results to guide management actions to improve sediment quality because the cause of the toxicity cannot be determined from the test results. However, procedures for toxicity identification evaluation (TIE) are available to help determine the cause of toxicity in sediment. These procedures consist of applying various treatments to the sediment sample to reduce the effects of specific contaminants, followed by toxicity testing.

Statement of Problem: Sediment TIEs have been included in a limited basis in previous Bight surveys, but existing sediment TIE information is not sufficient to determine the primary causes of sediment toxicity in many southern California embayments. Sediment toxicity in many locations has not been evaluated using TIEs, or the TIEs were conducted long ago and may not accurately reflect current conditions.

Objectives: The goal of this special study is to characterize the likely toxicants in sediments from multiple southern California embayments using TIE methods. Three questions will be addressed:

1. What contaminant groups are likely responsible for toxicity in embayments?
2. Do the toxicity characteristics of whole sediment and pore water differ?
3. Are pyrethroid pesticides a contributor to sediment toxicity?

Task 1: Develop Standardized TIE Approach. Several laboratories will conduct the TIEs, each at different locations within the Bight. A subcommittee of the Toxicology Working Group, comprised of the test laboratories conducting the TIEs, will develop a standardized method for selecting sites and conducting the TIEs.

Task 2: Sample Analysis. We will apply TIE methods to both whole sediment and pore water for up to 10 sites. All tests will be conducted using the amphipod *Eohaustorius estuarius*. The whole sediment TIE methods will likely include: addition of carbon, addition of cation exchange resin, addition of piperonyl butoxide, amongst other treatments. Pore water will be extracted using centrifugation and likely treated with the following TIE methods: EDTA addition, C-18 solid phase extraction, addition of piperonyl butoxide, amongst other treatments. Changes in the toxicity of the sample following each type of treatment will indicate whether the toxicity has characteristics of a nonpolar organic, metal, or pyrethroid pesticide toxicant(s).

Task 3: Data Analysis. The relative change in toxicity due to each type of TIE treatment will be compared against treatment blanks to determine whether the treatments were effective in altering the toxicity of the sample. Patterns of response will be summarized graphically and compared among stations. The whole sediment and pore water response patterns will also be compared in order to determine if different types of toxicants are present in each matrix. The TIE results will also be compared to the available sediment chemistry data to assist in data interpretation.

Products: Three products are expected from the study:

1. A description of a standardized approach for sediment TIE studies. This document will be a resource for other agencies and programs wishing to conduct sediment TIEs in the future. Use of a standardized approach in this and other studies will result in greater comparability of the results.
2. A summary of the results will be included as an appendix to the Toxicology Committee Technical Report. The report will provide updated or new toxicant characterization information for several water bodies. The report will describe spatial or regional patterns in sediment toxicity characteristics that are important for planning management activities. These new data should complement current TMDL activities related to sediment quality.
3. Publication of the results in a peer-reviewed journal. This study will represent the most spatially extensive evaluation of the causes of sediment TIE survey in southern California and is likely to yield several findings of interest to the scientific community.

Gene Microarray Analysis of Sediment Toxicity Samples

Collaborators: SCCWRP and Bight '13 toxicity testing laboratories

Background: Previous Bight surveys have shown that sediment toxicity is present along the coast of Southern California. However, identifying the contaminants present in the sediments has remained challenging. Existing Toxicity Identification Evaluation (TIE) methods have had limited success partly because sediments contain complex mixtures of toxic chemicals and the magnitude of toxicity is often too low for successful TIE analysis. Recently, research has shown that molecular techniques like gene expression profiling (microarrays) can provide valuable information about sediment quality. Microarrays can help to isolate potential classes of contaminants present in the sediments based on changes in gene expression from organisms exposed to contaminated sediments.

The estuarine amphipod *Eohaustorius estuarius* is widely used for sediment toxicity testing. Scientists from SCCWRP and UC Berkeley have developed a cDNA microarray for this organism. Preliminary studies have shown that this microarray can be successfully used to discriminate between organisms exposed to various contaminants, resulting in potential new molecular-based method for TIE (molecular TIE).

Statement of Problem: The gene microarray approach has not been applied to a variety of field samples having real-world chemical and toxicity characteristics. Comparing the gene expression results to traditional toxicity and TIE results is a critical next step in developing and evaluating a molecular TIE method.

Objectives: The goal of this project is to apply molecular TIE methods to assess the toxicity of sediment samples collected during Bight'13 survey. The study has three objectives:

1. Characterize gene expression profiles in amphipods exposed to sediments with different levels of chemicals and chemical mixtures.
2. Investigate the relationship between gene expression changes and specific classes of contaminants.
3. Examine the correlation between transcriptomic data and results of TIE testing.

Task 1: Obtain Samples for Analysis. This study will use samples generated from the amphipod survival tests and TIE testing conducted during the Bight '13 survey. We will review toxicity station locations in the embayments and select several candidate sites for study. Criteria for site selection include: sites that vary in potential contaminant source inputs; sites exhibiting a gradient of response in base toxicity testing, and; plan for TIE testing. Collaborating laboratories will preserve surviving amphipods from their base sediment toxicity tests at the selected sites. Amphipods from multiple types of samples will be preserved: non-toxic stations, toxic stations, baseline TIE samples, TIE treatments, TIE blanks. SCCWRP will provide training in sample preservation for the laboratories.

Task 2: Sample Analysis. Replicate samples will be used for RNA extraction, labeling, and hybridization using the custom designed amphipod arrays. Sample processing and data analysis will be conducted at SCCWRP.

Task 3: Data Analysis. The data will be analyzed using several approaches. The first type of data analysis will be a One way ANOVA testing for differential gene expression of amphipods exposed to test sediments against controls. This will be used to identify the genes induced and suppressed, suggesting the classes of contaminants present in the sediment samples. The second type of data analysis will be cluster analysis to determine if the site replicates form distinct clusters. This analysis will be used to investigate whether the distance between clusters correlate with the level of toxicity measured by the TIE and chemistry methods. The third type of data analysis will be correlation analysis between transcriptomic data and other endpoints including TIE and chemistry data. This analysis will be used to establish the relationship between gene expression patterns and the presence of specific chemicals. Also, the correlation between transcriptomic data and TIE results will be evaluated for discrimination and sensitivity to different toxicants, especially those at marginally toxic concentrations.

Products: The products from this study are expected to include:

1. Figures and tables comparing gene expression data among stations and comparing the results to magnitude of toxicity, location, and TIE results. These will be presented to the Toxicology Technical Committee and to the CIA Planning Committee.
2. A summary of the results will be included in the Toxicology Technical Report.
3. Publication of the results in a peer-reviewed journal. This study will likely represent the first application of gene expression analysis to sediment toxicity monitoring.

Alternative Toxicity Test Species Comparison

Collaborators: LACSD and Bight '13 toxicity testing laboratories

Background: The methods and interpretation approach described in California's Sediment Quality Objectives (SQO) policy provide the framework for sediment quality assessment in the Bight '13 survey. Multiple types of sediment toxicity tests are specified for use in the SQO framework, but most testing has used two methods: a 10-day amphipod survival test using the estuarine amphipod *Eohaustorius estuarius* and a 2-day embryo development test using the mussel *Mytilus galloprovincialis*. One of the recommendations resulting from the Bight '08 regional survey was to include other SQO toxicity test methods in future surveys. The integration of data from additional toxicity test methods makes for a more complete and robust assessment. An additional sublethal toxicity test recommended for use in the SQO program is a 28-day growth test using the polychaete worm *Neanthes arenaceodentata*. The *Neanthes* growth test has not been used to measure toxicity in southern California in previous regional surveys, thus the performance of this test relative to other SQO toxicity methods is not well documented.

Statement of Problem: Most recent sediment quality assessments have used only two of the recommended SQO test species: the *E. estuarius* amphipod survival and *M. galloprovincialis* embryo development tests. Information on the response of other SQO toxicity test methods to Bight embayment sediments is needed in order to provide a more robust assessment of sediment quality and better understand the relative responsiveness of the various test methods. Such information will assist environmental managers in planning and interpreting sediment quality assessment studies.

Objectives: The objective of this study is to compare the data between the acute *Eohaustorius estuarius* and the sublethal *Mytilus galloprovincialis* sediment toxicity tests that will be routinely conducted during the Bight '13 survey with the alternative *Neanthes* sublethal test. This special study will answer the following questions:

1. To what extent do the three methods agree with respect to toxicity category?
2. What is the relative sensitivity of each toxicity test method?
3. How does the precision of the test compare among methods?
4. Is the integrated sediment toxicity category influenced by the use of data from three (as compared to two) test methods?

Task 1: Select Stations. A subset of up to 20 embayment stations will be tested using the *Neanthes* method. Stations will be selected in coordination with the Toxicology Committee in order to provide an optimal design with respect to test timing, and expected variation in sediment toxicity and other sediment characteristics.

Task 2: Sample Analysis. *Neanthes* sediment toxicity tests will be conducted by LACSD in accordance with the methods specified for the SQO program. Concurrent sediment toxicity tests using the *Eohaustorius* and *Mytilus* tests will be conducted by other laboratories as part of the overall Bight '13 survey.

Task 3: Data Analysis. The SQO toxicity category, percentage response, coefficient of variation among replicates, and percent minimum significant difference (pMSD) will be calculated and compared for each test method.

Products: There will be two products for this special study. The first product will be a section in the Bight '13 Sediment Toxicity Report that summarizes the results and answers the four study objective questions. The results will also be described in detail in a peer-reviewed journal publication on comparative sediment toxicity response in the southern California Bight. The manuscript will be an Appendix in the Bight '13 Sediment Toxicity Report.

In Situ Toxicity Testing using the SEA Ring

Collaborators: AMEC, SSC Pacific (SPAWAR), Nautilus, Anchor QEA, Port of Long Beach, Port of Los Angeles

Background: The Sediment Ecotoxicity Assessment Ring (SEA Ring) technology provides a robust standardized method to conduct a variety of biological effects tests and bioaccumulation exposures *in situ* (Figures 1 and 2). The technology can be used to evaluate a wide range of exposure pathways including direct contact with surficial sediments, the sediment-water interface, and overlying waters. The SEA Ring also has the ability to integrate these biological effects measures with real time water quality data sondes, passive sampling devices, and other physicochemical tools to better assess the relationships observed between biological responses, physical water quality parameters, and contaminant dynamics. The SEA Ring provides a potential improvement over traditional laboratory-based biological effects tests by preserving sample integrity and capturing natural dynamics that are difficult or impossible to mimic in a laboratory setting.

Statement of Problem: Existing tools for characterizing environmental effects often rely on unrealistic and disjointed independent lines of evidence for exposure, uptake, and response potentially resulting in inaccurate sediment or water quality management decisions. This problem is particularly acute for applications where the exposure is sensitive to disturbance or cannot be easily recreated in the laboratory. This is because typical sediment collection methods disturb vertical stratification which can impact alter the bioavailability of certain compounds (i.e., trace metals) that are highly sensitive to redox conditions.

In situ assessment technologies provide an alternative to laboratory testing that can overcome issues related to sample disturbance. However, implementation and acceptance of *in situ* assessment technologies have been limited to a range of research and applied studies. Application in regional studies or other regulatory programs has been limited by their perceived lack of experimental control and the complexity of their application relative to laboratory methods. For these *in situ* exposure methods to gain acceptance there is a need to improve and standardize methods, and to simplify field application to provide robust, repeatable measures that result in acceptable quality control.

Objectives: The primary objective of this special study is to measure *in situ* toxicity at a subset of Bight '13 sediment stations. Results will be compared to laboratory-based responses in concurrent side-by-side exposures. Specifically, we will quantify: 1) survival of amphipods (*Eohaustorius estuarius*) following a 10-day exposure to whole sediments, and; 2) normal/abnormal development of mussel embryos over a 48-hour sediment-water interface exposure. This special study will answer the following four questions:

1. Does the SEA-Ring provide a reliable methodology to assess *in situ* toxicity with species required for California's Phase I Sediment Quality Objective (SQO) framework?

2. Are laboratory-based amphipod and bivalve toxicity tests predictive of effects observed during field exposures to undisturbed sediment under natural conditions at selected locations in the southern California Bight?
3. What is the extent of small scale spatial variability for effects-based measures at select locations, and how might this affect interpretation of laboratory-based tests that rely on a single composite sample from each station.

Task 1: Site Selection. Specific sites will be selected by participating stakeholders based on the final Bight '13 sample draw. Sites will be selected based on expected toxicity from previous studies. Multiple locations at each site will be tested as replicates to assess small scale spatial variability.

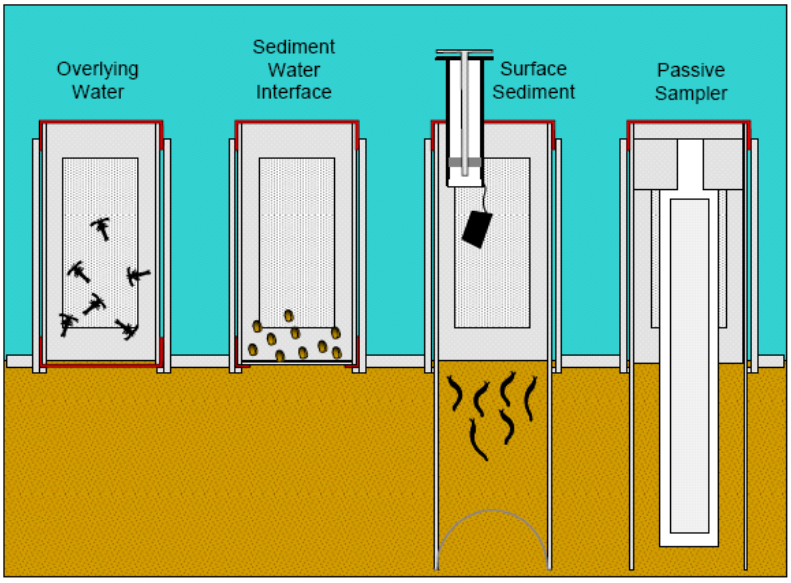
Task 2: Field and Laboratory Testing. A minimum of three to four Bight'13 locations will be assessed using the SEA-Rings. The field efforts will require approval and coordination with a number of agencies (i.e. Ports, Cities, Harbor Police, Coast Guard, and possibly Fish and Wildlife). Surface sediment samples near each SEA Ring location will be collected adjacent to each field replicate location for concurrent side-by-side tests in the laboratory using the same test species. Surface sediments will be collected, processed, and tested using the final Bight '13-approved methodologies and QA/QC.

Task 4: Integration with Bight '13 Toxicity, Chemical, and Benthic Community Analysis. *In situ* and concurrent laboratory-based toxicity data and associated real-time water quality measurements will be combined with the site-associated chemical concentration and benthic community data for data analysis and reporting. SQO scores will be calculated for field-based and laboratory-based measures for comparison. The data analysis and conclusions will be reviewed by the Toxicity Technical Working Group and the CIA Planning Committee.

Products: There will be two products for this special study. The first will be a section in the Bight '13 Toxicity Report answering the primary study objective questions. The data products will include a detailed write-up of methods, results, and conclusions; as well as graphical and tabular summaries of the data. These data will be summarized for the second product, a peer-reviewed journal publication on *in situ* toxicity in the southern California Bight. The manuscript will be an Appendix in the Bight '13 Toxicity Report.

Figure 1. SEA Ring showing test chamber configuration for whole sediments and deployment/ retrieval technique for shallow waters using an attached pole.

Figure 2. Diagram showing exposure pathways that can be assessed at the sediment surface using the SEA Ring.



Effects of Macrobenthic Preservation Techniques on Efficacy of Molecular and Morphological Taxonomy

Collaborators: SCCWRP, US EPA National Exposure Research Laboratory, field sampling crews, and appropriate expert taxonomists

Background: Traditionally, macrobenthic-based assessments of habitat quality in the Southern California Bight and other regions around the World are based upon species identification and community structure, which has been derived via morphological-based taxonomy. The reliable and consistent identification of some taxa can be problematic, however, due to their rarity of collection, phenotypic plasticity, or the obscure nature of their distinguishing characters. Molecular techniques that use genetic composition for identification are not affected by these problems of rarity or morphology. As such, using molecular taxonomic approaches in concert with traditional approaches may provide a solution to understanding the true identity of cryptic taxa. By clarifying species identities and providing greater resolution in community structure, improved habitat assessment tools might be developed that can provide greater discrimination and accuracy of habitat quality for ecosystem managers.

Statement of Problem: As a science, biological-based assessment of habitat quality is in the beginning of a transitional phase changing from a complete reliance on morphological taxonomy towards the addition of molecular-based taxonomic data. Beyond differences in the way the two approaches identify a given specimen, there are different, potentially incompatible, ways in which the fauna are preserved after collection from the field and before they are identified.

Traditionally, specimens destined for morphological-based taxonomic identification are first fixed with formalin, which preserves the tissue, and then transferred to ethanol, which dehydrates the tissue and acts as an anti-microbial agent. Samples preserved in this fashion are rigid, yet flexible, and are thereby suitable for the staining and manipulation with forceps required to typically identify an individual. Conversely, as part of the tissue fixation process, formalin fragments the genetic material, greatly reducing the ability to identify the individual with molecular methods (e.g., PCR). Specimens destined for molecular-based taxonomy are preserved in 95% ethanol, which dehydrates the sample, acts as an antimicrobial agent, and most importantly keeps the genetic material intact. However, the dehydrating properties of the ethanol can distort the tissue and can make many different taxa (e.g., arthropods) brittle and difficult to manipulate for morphological identification.

If molecular-based taxonomy is going to be used in future bioassessments, it will need to be calibrated and rectified with the existing assessment tools that use only morphology-based taxonomy. This rectification process will involve the assessment of a sample using both molecular and morphological techniques; ideally on the same exact sample and component fauna. Because of the apparent incompatibilities of the two different preservation techniques detailed above, this may require doubling the sampling effort to evaluate the molecular and morphological approaches side-by-side. However, there may be a middle ground of limited exposure to formalin combined with advanced genetic sequencing techniques that may facilitate the identification of a specimen by both morphological and molecular techniques.

Objectives: The primary objective of this study is to test the efficacy of taxonomic identification of different marine macrobenthic taxa by both morphological and molecular methods on specimens exposed to formalin for differing durations of time. Specimens from five different families of macrobenthos will be exposed to differing levels of formalin from 0 hours (direct placement in ethanol) to 48+ hours (standard exposure time). Individuals of each taxon will be identified morphologically and the quality/ease of identification will be evaluated. These same individuals will then be identified using barcoding of mitochondrial CO1 DNA and will be evaluated by the success rate of genetic identifications.

Task 1: Establishing target taxa. Five taxa frequently found in previous Bight surveys and which cover a range of body types (e.g., shelled, exoskeleton, soft tissue) will be selected. The ability to accurately predict the presence of any given taxon in a benthic sample is limited, so the exact taxa used in the experiment may vary from the initial target taxa.

Task 2: Collection of specimens. Replicate samples with the highest probability of containing the target taxa will be collected and preserved in the different formalin exposure treatments.

Task 3: Traditional sample processing. Samples will be sorted and identified to family level.

Task 4: Molecular sample processing. Tissue will be removed from fifteen individuals from each taxon and each treatment. DNA will be extracted from each individual and amplified via PCR at SCCWRP. If pieces of DNA large enough for identification can be amplified, then this material will be sent to US EPA NERL in Cincinnati, OH, for genetic barcoding.

Task 5: Evaluation of success. Success of morphological taxonomy will be evaluated using a categorical grading of specimen quality (e.g., fragility, distortion) and ease of identification by morphologically-based taxonomists. Success of molecular taxonomy will be evaluated as % of specimens that could be identified.

Product: The final product of this study will be a peer reviewed journal publication detailing the results of the experiments and providing guidance on, and general feasibility of, integration of molecular and morphologically derived macrobenthic taxonomy for use in biological assessments. This manuscript will also appear as an appendix to the Bight '13 Benthic Report. If the process is deemed successful, a new series of field and lab protocols for sampling and identifying marine macrobenthic fauna will also be created for use in future monitoring/sampling programs using both molecular and morphological taxonomic approaches.

Adaptation to Hypoxic, high CO_2 Environments – Phenotypic Plasticity in Echinoderms Across the So. California Continental Margin

Collaborators: SCCWRP, Bight '13 Trawl Team, and Levin Lab, Scripps Institution of Oceanography

Background: Near-bottom water hypoxia has shoaled throughout the Southern California Bight (SCB) over recent decades concurrent with worldwide expansion of Oxygen Minimum Zones. This may have profound effects on demersal fish and invertebrate species or communities whose depth distributions are limited by availability of dissolved oxygen (DO). Bight'03 and Bight'08 conducted trawling at Continental Slope depths (200-500m) for the first time. The invertebrate species presence/absence and peak density data collected by the Bight'13 Program may coincide with shoaling hypoxic boundary levels and other physiological or chemically relevant parameters such as pH, CO_2, temperature, and food availability.

Statement of Problem: Changes in megafaunal invertebrate community structure across oxygen gradients have not been analyzed despite trends in shoaling hypoxia throughout the SCB. This understanding will inform future testable hypotheses about differentiating anthropogenic impacts from naturally variable changes in community structure. Furthermore, knowledge of deep-sea invertebrate phenotypic plasticity in naturally hypoxic, high CO_2 environments will provide critical understanding of the environmental and physiological constraints on zonation in the contexts of evolution and adaptation.

Objectives: This special study aims to describe the historical trends in megafaunal depth distributions in response to environmental changes. In addition, megafauna samples will be collected to describe inter- and intraspecific variation in phenotypes (e.g. size, biomineral composition, stable isotopic ratios) of invertebrates throughout their observed depth distributions. Finally, energy budget models will be made for certain echinoderm species in order to model their present and future depth distributions. This special study will address the following questions:

1. Have depth distributions of slope and shelf megafauna shoaled concurrently with shoaling hypoxia and hypercapnia (high CO_2)?
2. Have depth ranges of selected upper slope and shelf megafauna contracted over the past 20 years?
3. How do phenotypes vary across depths and various chemical gradients with respect to DO, pH, pCO_2, temperature, and Total organic matter?

Task 1: Select Stations. A subset of Bight '13 trawl stations will be selected from the following depth strata: Upper Slope (200-500m), Outer Shelf (120-200m), and Middle Shelf (30-120m).

Task 2: Obtain Samples for Analysis. Echinoderms (*Lytechinus pictus*, *Strongylocentrotus fragilis*, *Astropectin virilli*, *Brissopsis pacifica*, and *Brisaster latifrons*) will be randomly sampled from each selected trawl, frozen on the ships, and returned to the Levin lab at Scripps Institution of Oceanography (SIO) for further analyses. Selected trawls will be equipped with a Dissolved Oxygen sensor to accompany the temperature and pressure sensors. When logistically possible, live organisms will be kept in seawater aquaria for seawater chemistry manipulation

experiments to be carried out at SIO. These will examine the effect of hypoxia, hypercapnia, and acidification on growth, survival, and physiology of adult and larval echinoderms.

Task 3: Sample Analyses. Samples will be analyzed via physiological assays for anaerobic metabolic endmembers (e.g. octopine, succinate), morphology, and biomineral composition. Frozen tissue samples will be analyzed for stable isotopes of carbon, nitrogen, and sulfur. Biomineral composition of calcified hardparts will be analyzed using X-Ray Diffraction methodology. All measurements will be compared across depth strata and concomitant concentrations of dissolved oxygen, temperature, and carbonate chemistry. Ancillary Bight '13 data required to inform the individual-based models include total organic matter and grain size. These measurements will be used to populate an individual-based model that describes the present-day depth distribution of each species based on key environmental variables. This information will lead to testable hypotheses about species range shifts in the context of future ocean acidification and ocean deoxygenation.

Task 4: Data Analysis and Proof of Concept. Species peak densities will be correlated with historical DO concentrations and other chemical measurements extrapolated from quarterly cruises conducted by the California Cooperative Oceanic Fisheries Investigations (CALCOFI). DO measurements from trawls will be compared to CALCOFI measurements via correlation analysis to test the hypothesis that near-bottom DO can be extrapolated from historical CALCOFI measurements without significant error. Correlation analyses of species density will be carried out with various interannual climatic indices such as the Multivariate ENSO Index and the North Pacific Gyre Oscillation.

Products: The final product of this study will be a PhD dissertation and peer reviewed journal publication detailing the species distributions relative to hypoxia and hypacnia, and the results of the confirmatory sample analysis. This manuscript will also appear as an appendix to the Bight '13 Benthic Report. This study should produce several findings of interest to the scientific community, and may raise additional questions for regulated and regulatory agencies for future activities.

Trophic Transfer of Bioaccumulative Compounds

Collaborators: SCCWRP, SDSU, USFW, CIAgent, RHMP, SDRWQCB

Background: Bioaccumulative compounds such as DDTs, PCBs, PBDEs, and Hg are known to magnify through the food chain. Previous Bight surveys have quantified levels of these compounds in fish. Concentrations in fish tissue were high enough to exceed wildlife risk thresholds. In order to forego assumptions that infer risk to wildlife, Bight'13 is measuring bioaccumulative compounds such as DDTs, PCBs, PBDEs, and Hg in birds (specifically bird eggs). This will be the first ever bight-wide survey of bioaccumulative compounds in bird eggs. Several species are being sampled that represent various feeding strategies including surface and diving species.

Statement of Problem: Bight'13 will be sampling bioaccumulative compounds in sediment and birds, representing opposite ends of the food chain. Intermediate trophic levels will not be quantified. Thus, the transfer of bioaccumulative compounds from sediments to birds will be unknown, hindering our understanding of contaminant trophic transfer. This becomes especially problematic for future use of the data, such as assessing indirect effects of contaminants associated with the SWRCB's development of indirect impacts to wildlife.

Objectives: The objective of this special study is to answer the question: What is the transfer of bioaccumulative compounds through the food chain? Two food chain pathways will be quantified; benthic and water column. Five embayment will be sampled; San Diego Bay, Mission Bay, Oceanside, Dana Point, Newport Bay. The goal will be to generate data to:

- calculate empirical contaminant transfer ratios
- calibrate and validate bioaccumulation models for sediment quality objectives
- compare contaminant transfer and wildlife risk at different locations.

Task 1: Sampling for water column pathway.

Since contaminants are not stationary and wildlife do not feed in exactly the same location, we will use a "zone" approach for collecting samples (Table 1). Each zone will consist of between one and three replicate sites. Some sites may be sampled more than once. The water column pathway will consist of three trophic levels:

- Dissolved water column concentrations
- Plankton composites
- Planktivorous fish

Dissolved water column concentrations will be sampled using an active in situ sampler comprised of a battery-powered pump and SPE extraction disk. Plankton will be collected using a standard plankton net. Planktivorous fish will be collected using a common seine. The fish species targeted will include anchovy, sardine, and topsmelt. Secondary species will include shiner perch.

Task 2: Sampling for benthic pathway.

Since contaminants are not stationary and wildlife do not feed in exactly the same location, we will use a "zone" approach for collecting samples (Table 1). Each zone will consist of between one and three replicate sites. Some sites may be sampled more than once. The water column pathway will consist of three trophic levels:

- Sediment concentrations
- Infaunal composites
- Demersal fish

Bulk sediment concentrations will be sampled during the regular Bight monitoring. Infauna will be collected at the same time and locations as sediment samples. Soft-bodied organisms, such as polychaetes and mollusk parts (i.e., siphons), will be live-sorted and composited for laboratory analysis. Benthic fish will be collected using a common seine and trawling. The fish species targeted will include gobies, killifish, and one flatfish (either turbot of halibut). Secondary species include croakers, sand bass, queenfish, or mullet.

Table 1. Number of sampling zones and sites within zones (replicates) by accumulation pathway for bioaccumulation monitoring.

Embayment	Water Column Pathway		Benthic Pathway	
	# Zones	# Sites/Zone	# Zones	# Sites/Zone
San Diego Bay	3	3	3	3
Mission Bay	-	-	1	1
Oceanside	-	-	1	1
Dana Point	-	-	1	1
Newport Bay	2	3	2	3
TOTAL	5	15	8	18

Task 3: Laboratory Analyses.

All samples will be analyzed for DDTs, PCBs, PBDEs, and mercury. Since we are focused on wildlife consumption, fish will be homogenized whole and combined in composites of five fish. If sufficient resources exist, either additional species composites or individual fish of target species will be analyzed. Target analyte lists and data quality objectives will mimic the existing

Bight'13 QA Plan for sediment and bird egg tissues, so that the data across multiple trophic levels can be combined for data analysis.

Products: There will be two products for this special study. The first product will be a section in the Bight '13 Contaminant Impact Assessment Synthesis Report that summarizes the results and answers the study question. The results will also be described in detail in a peer-reviewed journal publication on wildlife risk in the southern California Bight. The manuscript will be reviewed and approved by the CIA Planning Committee.