Southern California Bight
2018 Regional Marine Monitoring Survey
(Bight '18)

Harmful Algal Blooms Workplan

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I. INTRODUCTION

A. Setting and Background

The Southern California Bight (SCB; Figure I-1) is an open embayment in the coast between Point Conception and Cape Colnett (south of Ensenada), Baja California. Complex bathymetry and currents have resulted in a diversity of habitats and marine organisms, including more than 500 species of fish and several thousand species of invertebrates. The SCB is a major migration route for marine bird and mammal populations and is ranked among the most diverse ecosystems in north temperate waters. In addition to its ecological value, the coastal zone of the SCB is a substantial economic resource. The Los Angeles/Long Beach Harbor complex is the largest commercial port in the United States, while San Diego Harbor is home to one of the largest US Naval facilities in the country. In addition to being the home to more than 20 million people, (NRC 1990), southern California receives over 100 million visitors to its beaches and coastal areas annually. The combination of resident and transient populations has resulted in a highly developed urban environment that has greatly altered the natural landscape. The conversion of open land into impervious surfaces has included dredging and filling over 75% of bays and estuaries (Horn and Allen 1985) and extensive alternations of coastal streams and rivers (Brownlie and Taylor 1981, NRC 1990). This "hardening of the coast" changes both the timing and rate of runoff releases to coastal waters and can affect water quality through addition of sediment, toxic chemicals, pathogens, and nutrients. Besides input of urban runoff via storm drains and channelized rivers and streams, numerous municipal wastewater treatment facilities, power-generating stations, industrial treatment facilities, and oil platforms discharge to the SCB. At the same time, the SCB is situated on the southern portion of the California Current System, one of the world's four large eastern boundary upwelling systems (Chavez and Messie, 2009). Seasonal upwelling represents a natural source of nutrient-rich water that fuels primary productivity in the region.

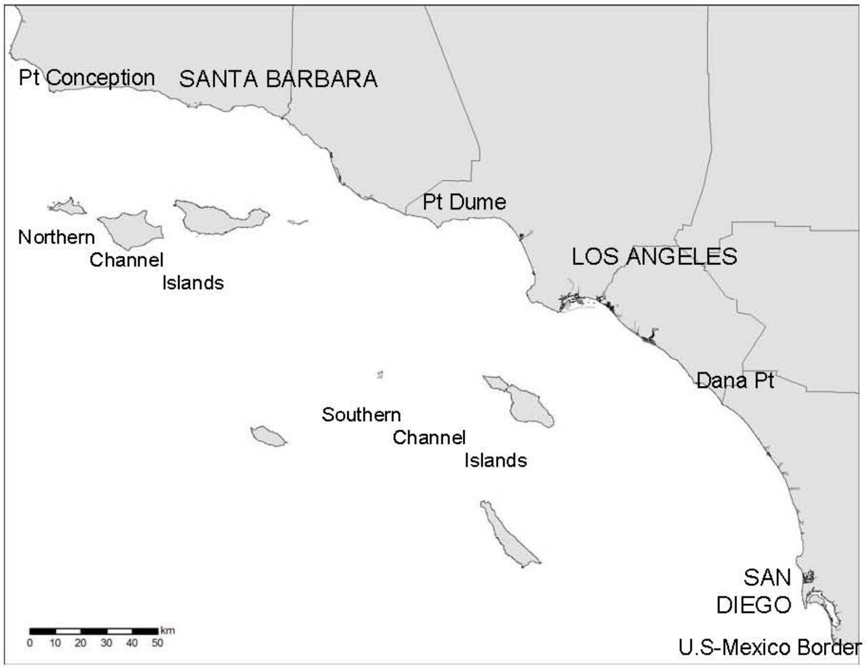
Harmful algal blooms (HABs) and associated algal toxins have been a persistent and escalating issue in California's coastal and inland waterbodies. Globally, HABs have increased in frequency, severity and spatial extent over the past decade, and anthropogenic nutrient inputs and warmer temperatures (i.e. climate change) are considered the most significant factors contributing to these increases (Smayda 1990, Hallegraeff 1993, 2004, Anderson et al. 2002, Glibert et al. 2005, Hudnell, 2008, Paerl et al, 2011, O'Neil et al., 2012). In marine and estuarine coastal waters, the diatom, *Pseudo-nitzschia* that produces the neurotoxin domoic acid (DA), is considered the leading HAB issue for much of the U.S. West Coast (Trainer et al., 2000, Kudela et al., 2008, Schnetzer et al., 2007, Lewitus et al., 2012, Smith et al., 2018). In response to recent DA events, the California Ocean Protection Council (OPC) and the Interagency Marine Harmful Algal Bloom Task Force (Task Force) have convened an OPC Science Advisory Team (SAT) working group that have provided scientific recommendations and guidance (http://www.oceansciencetrust.org/wp-content/uploads/2016/11/HABs-and-CA-Fisheries-Science-Guidance-10.25.16.pdf). The Bight '18 HABs program will address Recommendation 5: Improve understanding of how biotoxins move through food webs.

Domoic acid has caused major socioeconomic impacts, including prolonged closures of key fish, bivalve and crab fisheries, health advisories, and marine wildlife illness and mortalities (Lefebvre et al., 2002, Scholin et al., 2000, McCabe et al., 2016). Southern California has some of the highest concentrations of DA recorded in the literature (Smith et al., 2018). While the pelagic impacts of DA have been well studied, the fate and persistence of DA has been historically understudied, but the large socioeconomic impacts in recent years have created interest from the California management communities to understand the long-term impacts and fate of DA to benthic communities.

Other HAB species, such as cyanobacteria, produce toxins in the freshwater and estuarine environments, which can be transported downstream through hydrological interconnections and cause issues in estuarine and marine waters. These toxins, called cyanotoxins, have caused direct impacts in the marine environment such as the mortality of over 30 threatened marine California Sea Otters (*Enhydra lutris*) due to ingestion of contaminated shellfish (Miller et al., 2010). Watershed studies in Monterey Bay have shown that this downstream transport of microcystins is a persistent and prevalent issue throughout the watershed (Gibble and Kudela 2014), and that cyanotoxins are prevalent throughout freshwater and estuarine environments in the SCB (Fetscher et al., 2015, Howard et al., 2017, Tatters et al., 2017, Tatters and Howard, unpublished data). Cyanotoxins have been shown to bioaccumulate in marine shellfish in CA and WA (Miller et al, 2010, Kudela, 2011, Gibble and Kudela, 2014, Preece et al., 2015a, 2015b, Gibble et al., 2016, Peacock et al., 2018). Due to the recognition that both marine and freshwater toxins are present in marine waters, recent studies have detected multiple freshwater and marine toxins simultaneously in marine shellfish in central CA (Peacock et al., 2018). To date, marine shellfish have not been investigated for the presence of cyanotoxins in the SCB.

Due to the transport of freshwater toxins into estuarine and marine environments, there is a new recognition that management and mitigation of HABs needs to occur cohesively across the freshwater to marine continuum due to the hydrologic interconnections and toxin impacts downstream of the bloom event origin (Paerl et al., 2016, Paerl et al., 2017, Paerl et al., 2018).

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



B. History of Bight Regional Surveys

To understand the cumulative impacts of anthropogenic nutrient discharges on HABs in the SCB, a cooperative, multi-agency regional monitoring program has been established that looks at the health of the southern California bight ecosystem as a whole. Prior to the inception of the Bight Regional Monitoring Program, coastal monitoring was conducted primarily around individual discharges related to National Pollutant Discharge Elimination System (NPDES) permits and was intended to assess compliance of waste discharge with the state and federal regulations, which set water quality standards for effluent and receiving waters. While these monitoring programs provided important information to evaluate impacts near individual discharges, they did not provide the regionally-based information to assess the cumulative impacts of contaminant inputs and to evaluate relative risk among different types of stressors needed by managers. The Bight Program was designed to fill this need. Other benefits derived from the Bight surveys included the development of new technical tools and increased standardization and comparability in field and laboratory methods that could only be developed with regional data sets and participation by multiple organizations.

To date, there have been five previous regional monitoring efforts to begin addressing environmental concerns at larger spatial scales in the SCB. The Bight Regional Monitoring Program is organized into technical components, each focusing on research with clear management implications. All Bight surveys to date have contained a component related to offshore water quality. This component of the Bight Regional Monitoring Program focuses on assessing condition of the water column in the near coastal ocean, building on the existing collaborations between the large discharging agencies to bring additional partners and expand the variety of parameters measured and questions addressed.

The first Offshore Water Quality Assessment was associated with the 1994 Southern California Bight Pilot Project (SCBPP), which included 12 agencies that sampled over 260 sites along the continental shelf between Point Conception and the United States/Mexico border. Findings showed natural latitudinal differences (e.g., colder water in the Northern strata) and that over 99% of the coastal waters met California Ocean Plan objectives for dissolved oxygen and light transmittance.

In 1998, 64 agencies undertook the Southern California Bight 1998 Regional Monitoring Project (Bight'98) and sampled sites between Point Conception and Punta Banda, Mexico that included new habitats such as ports, bays, and marinas. The Bight'98 water quality surveys looked at both dry and wet weather water quality and the relative inputs of offshore ocean outfalls versus urban stormwater runoff at over 500 stations.

The Southern California Bight 2003 Regional Monitoring Project (Bight'03), was comprised of 65 agencies that sampled between Point Conception and the United States/Mexico border. To better characterize stormwater flows, the Bight'03 water quality survey sampled four major SCB river systems at nearly 200 stations. Sampling occurred over multiple days (3-5) after a rainfall event and collected discrete samples for bacteria, toxicity, chlorophyll and phytoplankton both at the source and within the stormwater plumes with the goal of correlating these measures with standard satellite imagery (e.g., ocean color). While the offshore turbidity plumes observed by satellites were found to be extensive in time and space there, the measured water quality impact (e.g., toxicity and indicator bacteria exceeding recreational standards) was typically <10% of this area, and declined rapidly within 1-3 days following the rainfall event.

The Southern California Bight 2008 Regional Monitoring Project (Bight '08) was comprised of 65 agencies sampling the same geographic area as in 2003. Bight '08 Offshore Water Quality Study provided evidence that on small scales relevant to the development of algal blooms, anthropogenic nitrogen loads were equivalent to upwelled nitrogen loads in the heavily urbanized regions of the SCB (Howard et al. 2012). The discharged effluent of Publicly Owned Treatment Works (POTWs) was the main anthropogenic constituent that comprised the anthropogenic nitrogen loads, whereas riverine runoff and atmospheric deposition were determined to be 1-3 orders of magnitude smaller (Howard et al. 2012). Additionally, the results indicated that the extent of surface algal blooms has increased over the last decade, with chronic blooms documented in areas of the SCB co-located with major inputs of anthropogenic nutrients as well as longer residence times of coastal waters. The Bight'08 study also provided new insights into algal bloom development in that upwelling was documented to transport a subsurface algal bloom closer to shore and into surface waters, resulting in bloom intensification.

The Southern California Bight 2013 Regional Monitoring Project (Bight '13) was comprised of 34 organizations, sampling the same regions as the previous two surveys, with the inclusion of some new habitats. The water quality component of this survey was further broken into three research areas: an assessment of acidified waters in the SCB, an assessment of spatial and temporal patterns in subsurface chlorophyll a, and direct measurements of key rates and processes related to nutrient and carbon cycling (process studies). This survey found that a substantial portion of southern California continental shelf waters exhibit water column aragonite saturation states (a key measure of acidified conditions) to fall within a range critical for biological organisms. The study also found that global forcings had a significant impact on chlorophyll a in the SCB as well as on the key rates of nutrient and carbon cycling, including primary production and respiration, but that local impacts may also play a role at smaller scales in the nearshore environments.

C. 2018 Survey

The proposed Southern California Bight 2018 Regional Marine Monitoring Project (Bight '18) is a continuation of the successful cooperative regional-scale monitoring begun in southern California. Bight '18 builds upon the previous successes and expands on the 2013 survey by including new participants and answering additional questions on biological impacts. Fifty organizations have agreed to participate (Table I-1). 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 '18 Survey is organized into six technical components: 1) Harmful Algal Blooms; 2) Sediment Quality (formerly Contaminant Impact Assessment/ Coastal ecology); 3) Microbiology; 4) Ocean Acidification; 5) Trash; and 6) Protected Areas. The Water Quality group chose to split into Ocean Acidification and Harmful Algal Blooms, because the study design and approaches were sufficiently different to warrant separation. The Harmful Algal Bloom component will focus on assessing the aerial extent and magnitude of both the marine HAB toxin, domoic acid, and the freshwater HAB toxin, microcystin. This work plan provides a summary of the project design for this component. Separate work plans are also available for the other elements of Bight '18.

TABLE I-1. Participants in the Bight '18 Regional Marine Monitoring Program, Harmful Algal Blooms component.

AES Corporation
Amec Foster Wheeler / Wood
Anchor QEA
Aquatic Bioassay and Consulting Laboratories (ABCL)
Bureau of Ocean Energy Management (BOEM)
Calscience Environmental Laboratories, Inc.
Catalina Sea Ranch
Channel Islands National Marine Sanctuary (CINMS)
Chevron USA Products Company

City of Los Angeles Environmental Monitoring Division (CLAEMD)
City of Los Angeles, Department of Water and Power (LADWP)
City of Oceanside
City of Oxnard
City of San Diego
Dominguez Channel Watershed Management Group (City of Los Angeles, Los Angeles County Flood Control District, Los Angeles County, City of Lomita, City of Carson, City of El Segundo, City of Hawthore, City of Inglewood, City of Lawndale)
EcoAnalysts
Encina Wastewater Authority
Greater Harbor Waters Regional Monitoring Coalition
Los Angeles Regional Water Quality Control Board
Los Angeles County Department of Public Works
Los Angeles County Sanitation Districts (LACSD)
Los Angeles Waterkeeper
MBC Applied Environmental Sciences
National Oceanic and Atmospheric Administration (NOAA)
Nautilus Environmental, Inc.
NES Energy, Inc.
NRG Energy, Inc.
Orange County Coastkeeper
Orange County Sanitation District (OCSD)
Orange County Public Works
Pacific EcoRisk
Physis Environmental Laboratories, Inc.
Port of Long Beach
Port of Los Angeles
Riverside County Flood Control and Water Conservation District
San Diego County Dept. of Environmental Health and Municipal Co-permittees
San Diego Regional Water Quality Control Board (SDRWQCB)
San Diego Unified Port District
San Elijo Joint Powers Authority
Santa Ana Regional Water Quality Control Board
Santa Barbara Channel Keeper
Santa Monica Bay Restoration Commission
Southern California Coastal Ocean Observing System (SCCOOS)
Southern California Coastal Water Research Project
State Water Resources Control Board
U.S. Fish and Wildlife Service
U.S. Geological Survey
University of Southern California (USC)
Vantuna Research Group, Occidental College
Weck Laboratories, Inc.
Weston Solutions, Inc.

II. Study Design

A. Study Objectives

The overall goal of the Bight '18 Harmful Algal Blooms Study is to determine the extent and magnitude of HAB toxins in the SCB. There are two principal questions for the HABs Component:

1. What is the extent and magnitude of domoic acid in sediments during the summer of 2018?

2. What is the aerial extent of microcystins in deployed mussels before and after storm events in 2018?

These two principal questions form the two components of the Bight '18 HABs monitoring component. The first question will be addressed by a probabilistic sampling of the SCB shelf sediments, and will also compare the interannual variability of DA concentrations by comparison with 2017 results and an additional field survey of a subset of sites in the summer of 2019. There are also two sub-objectives within this first question. The first sub-objective is to determine how the concentration of DA changes throughout the year, both within the peak DA event season (March, April and May) as well as in the offpeak season. To address this sub-objective, a targeted monthly sampling will be conducted at 3 sites in Orange County for 1 year. The second sub-objective is to determine if benthic infauna bioaccumulate DA. Benthic infauna samples will be collected monthly from 2 of the 3 sites in Orange County for 1 year.

The second question on microcystins in mussels will be addressed with targeted sampling of caged mussels deployed in the watershed terminus of southern California watersheds.

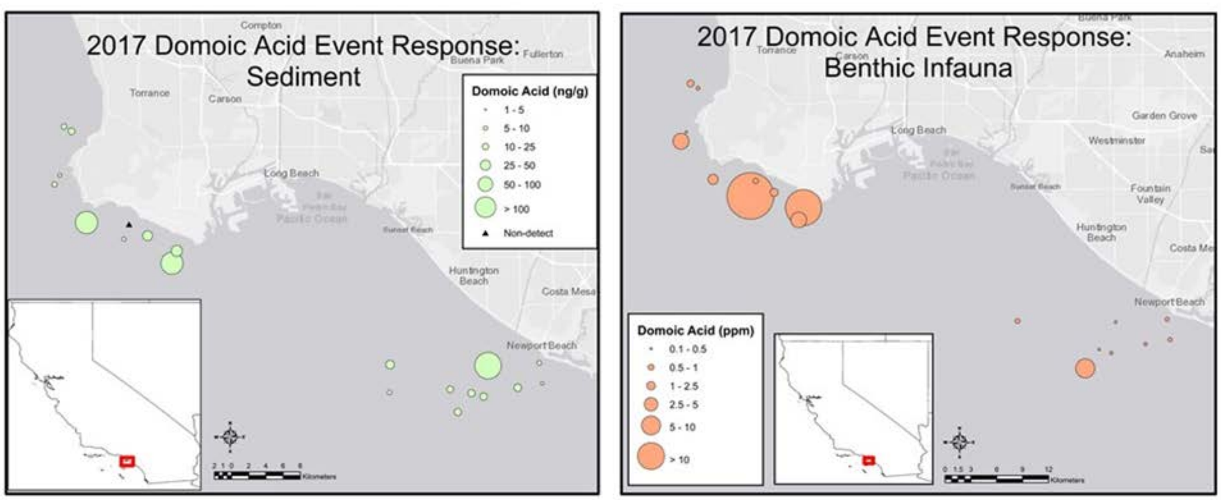
B. Sampling Design

The sampling design for Bight '18 HABs Program will be divided into two main components: 1) Assessment of DA in sediments, and 2) Assessment of microcystins in mussels.

B.1. Extent and Magnitude of Domoic Acid in Sediments

Annual blooms of the marine diatom genus *Pseudo-nitzschia* that produce DA domoic acid (DA) have been documented in southern California since 2003, with the occurrence of DA in shellfish tissue predating monitoring data in this region. The major socioeconomic impacts from DA events in recent years have been due to several species of benthic invertebrates, especially benthic shellfish (such as commercial crabs) to contain high concentrations of DA for up to a year after the bloom has senesced. In 2017, there was an unusual bird mortality event in Santa Barbara, Ventura and Los Angeles counties as well as an influx of sea lions to marine mammal rescue centers attributed to DA poisoning. Due to the interest in the fate of DA and impacts to the benthic communities and sediments, event response samples of sediment and benthic fauna were collected in 2017 from annual surveys by the Los Angeles County and Orange County Sanitation Districts. The sediment results detected DA in every sample except 1, with concentrations ranging from 3.1 to 168 ng/g sediment (Figure 2). These results are the highest DA concentrations from sediment recorded to date in California. Sediment samples collected from limited previous studies ranged from below detection to 85 ng/g sediment in Monterey Bay (Ziccarelli, 2014, Kudela, unpublished data), and studies conducted in the SCB detected concentrations ranging from no toxin detected to 8 ng/g (Santa Barbara Basin; Sekula-Wood et al., 2009), and 17 to 38 ng/g dry sediment weight (Santa Barbara Basin and San Pedro Shelf; Sekula-Wood et al., 2009, 2011). The benthic infauna samples were a composite of organisms collected from each location (such as crustaceans, polychaetes, echinoderms and mollusks). All the samples had measurable domoic acid that ranged from 0.1 ppm to 29 ppm (Figure 2). The Bight '18 program will provide a regional assessment of domoic acid in sediments, as well as a monthly time-series in Newport Beach, to determine how extensive DA contamination is in the SCB and how these concentrations change throughout the year.

Figure 2 Sediment and benthic infauna 2017 sample results showing domoic acid (ppb for sediment; left panel; ppm for benthic infauna samples, right panel).



Conceptual Design.

The magnitude and extent of domoic acid (DA) in SCB shelf sediments will involve sampling 90 sites for sediments in the SCB between July 1 and September 30, 2018. This period was chosen because sampling effort could be leveraged with the Bight '18 Sediment Quality element. Measurements during the Bight '18 will be placed into an interannual context by comparing regional results to shelf sediments collected in 2017 after a DA event (Figure 2) and additional collections of a subset of 20 sites in 2019.

The planning committee recognized there may be some degradation of sediment DA if the summer sampling is temporally distant from the occurrence of the DA bloom event. Historical data illustrates that the spring months of March, April and May have the highest frequency of DA events (Smith et al., 2018). To account for the possible degradation of DA in the sediments, a temporally intensive time-series of sediment samples will be collected monthly at 3 sites in Orange County for 1 year (March 2018 through February 2019). DA events have been documented annually in Orange County for the last decade, thereby making this area an ideal location to evaluate the possible degradation of DA (Smith et al., 2017, Smith et al., 2018, SCCOOS HAB Monitoring Program: **http://www.sccoos.org/data/habs/**).

Additionally, benthic infauna samples collected after the 2017 DA bloom were all positive for DA (Figure 2). Therefore, monthly benthic infauna samples will be collected simultaneously with the sediment samples at 2 of the 3 sites in Orange County for 1 year. These samples will be sorted and placed into 3 different categories that reflect feeding source: sediment feeders (e.g. worms), filter feeders (e.g. bivalves/shellfish) and sediment surface feeders (e.g. echinoderms, gastropods, amphipods). This will provide insight into the source of DA contamination, either water or sediment.

2018 Site Selection.

Maps of the sampling sites are provided in Appendix A (dark blue are the inner shelf sites, red are the mid-shelf sites and yellow are the outer shelf sites). Sites were selected using a stratified random approach, with the strata corresponding to the subpopulations of interest as described by the Bight '18 Sediment Quality Workplan. The Bight '18 Harmful Algal Blooms component planning committee elected to measure DA in three of the Bight '18 Sediment Quality strata: inner-shelf, mid-shelf, and outer-shelf. 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 stratum because this yields a 90% confidence interval of about ± 10% around estimates of areal extent (assuming a binomial probability distribution and p= 0.2). 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 entire strata. Although sites were selected randomly, a systematic component was added to the selection process to minimize clustering of sample sites. Further details about this site selection process are provided in the Bight '18 Sediment Quality Workplan.

Interannual Context Site Selection.

Due to the large degree of interannual variability in domoic acid concentrations (Smith et al., 2017, 2018), additional sites will be sampled in 2019, to place the 2017 and 2018 sample results into context. The 2017 sites were measured during regular sediment monitoring by OCSD and Los Angeles County Sanitation Districts (LACSD) during the summer of 2017, and are indicated on the map in Figure 2. Site selection for the 2019 sampling event will be determined following analysis of the 2018 sediment data, and will likely include a subset of sites measured in 2017 and sites measured in 2018 that fall within the regular monitoring grids of the POTWs.

Temporally Intensive Site Selection.

The three temporal-intensification sites are indicated on the sampling map in Appendix B and will be collected by the Orange County Sanitation District (OCSD). These sites were selected to represent an onshore-offshore gradient of DA concentrations in a region of the San Pedro Shelf where HAB events have been observed regularly for the last decade. These sites will be sampled monthly for a year from March 2018 through February 2019. Additionally, benthic infauna samples will be collected simultaneously at 2 of these sites (OCSD sites 24 and 28).

Sample Collection.

Sample collection procedures are documented in the Bight '18 Field Methods Manual. In summary, sediment samples will be collected from the top 2 cm of a Van Veen grab sample. Sediments will be subsampled from the grab sample, and ½ of a 250 mL amber glass jar will be filled with sample, and stored frozen until analysis. Benthic infauna samples will be sorted live in the field and categorized based on feeding source as described above.

Sample Analysis.

Sediment samples will be analyzed for DA using liquid chromatography/mass spectrometry (LC-MS) analysis of extracted sediment samples using the same methods as for the 2017 samples (Figure 2) and will be compatible with other regional efforts to measure DA in coastal sediments (such as Sekula-Wood et al., 2009, 2011, Ziccarelli, 2014, Kudela, unpublished data). Weck laboratories has agreed to measure DA in the 90 shelf sediments from the 2018 study.

The temporal intensification sites collected from March 2018 to February 2019 will also be sent to Weck Laboratories for analysis (resources provided by the Orange County Sanitation Department). The 2019 interannual context sites with either be analyzed at the Orange County Sanitation District (if domoic acid analysis is available in 2019) or to Weck Laboratories. Both laboratories will follow performance-based quality assurance guidelines described in the Bight '18 Quality Assurance Plan.

The benthic infauna samples will be analyzed using Mercury Science ELISA kits that were developed by the National Oceanographic and Atmospheric Association Centers for Coastal Ocean Science, National Ocean Service, the Northwest Fisheries Science Center with Mercury Science, Inc. Bight sample analysis will use the published methodology that validated Mercury Science ELISAs for the analysis of DA in shellfish tissues and in dissolved and particulate phytoplankton samples (Litaker et al., 2008).

Products.

There will be two data products for this study: (1) a cumulative distribution function graph of DA in SCB sediments by habitat type in 2018, and (2) a monthly time-series to determine temporal changes in DA concentrations in both sediment and benthic infauna samples collected from three OCSD sites. These data will be analyzed and written as a chapter in the

Bight '18 Harmful Algal Bloom Report, will be summarized for a section in the Bight '18 Chemistry Report and will be published in a peer-reviewed scientific journal.

B.2. Assessment of Microcystins in Mussels Before and After Storm Events

Cyanotoxins produced in fresh and estuarine waterbodies have been shown to be transported downstream of their origin, and to cause direct impacts in the marine environment, such as the mortality of threatened marine California Sea Otters. Recent studies have shown that cyanotoxins can bioaccumulate in marine shellfish, posing a human and wildlife health risk since cyanotoxins are not currently included in the California Marine Biotoxin Monitoring Program.

The Bight '18 HABs program will determine the concentration of microcystins in caged mussels at the end of the dry weather season and during the first major storm of the wet season, called "first flush".

Human health guidelines have been developed for microcystins in food (WHO, 2003, Ibelings and Chorus, 2007, Mulvenna et al., 2012, OEHHA, 2012). The World Health Organization (WHO) established a tolerable daily intake (TDI) of 0.04 µg/kg/day for microcystin-LR (WHO 2003). Based this TDI, a couple of different international groups have set guidance levels. Australian health guideline values were established for the No Observed Adverse Effect Level (NOAEL) of 40 µg/kg/day, and an acceptable daily limit of 51 µg/kg of mussel was set (Mulvenna et al., 2012). Ibeling and Chorus (2007) determined a seasonal daily exposure TDI for microcystins in seafood for adults (300 µg/kg/day) and children (40 µg/kg/day). In California, there is regulatory guidance for microcystins in fish tissue that has been established by the Office of Environmental Health and Hazard Assessment (OEHHA) of 10 ng microcystins per gram of fish (OEHHA, 2012) and other studies have used this guidance value to provide context for microcystin concentrations detected in mussels (Gibble et al., 2016, Peacock et al., 2018).

Conceptual Design.

The purpose of this component is to assess the accumulation of microcystins in deployed mussels at the terminus of the watershed for 30 drainage systems along the SCB coast. These sites will be measured every two weeks for four months from the end of the dry season (September-October 2018) through the start of the wet season (December 2018-January 2019). This design allows us to capture the "baseline," dry weather concentration of microcystins in mussels and the "first flush" delivered by the first storm event that "flush" the upper watersheds of toxins, delivering them to the marine environment. The two-week site revisit strategy is based on the fact that mussels have been shown to have minimal depuration of microcystins within two-weeks of exposure; however, after two-weeks depuration rates increase and exposure risk becomes more difficult to interpret (Gibble et al., 2016).

To address the potential depuration of microcystins in mussels over the course of the two weeks following exposure, a temporally intensive sampling will occur at four of the thirty sites. These sites will be visited weekly over the same four-month deployment time frame to evaluate the variability in depuration rates of microcystins from mussels.

Site Selection

The 30 watersheds with highest nutrient fluxes to the SCB, as determined during the Bight '08 Estuarine Eutrophication study (http://ftp.sccwrp.org/pub/download/DOCUMENTS/TechnicalReports/711_B08EE.pdf) were selected because watersheds with high nutrient fluxes are typically associated with more frequent cyanobacterial blooms. This sampling strategy is expected to provide a "worst case scenario" for cyanotoxin loading into the marine environment. The deployment location for each terminus was defined as the "base" of the watershed in water with an average salinity of at least 25 ppt. The four sites for the temporal intensification study were selected based on existing weekly sampling effort, which could be leveraged for this component. A map of the proposed sampling sites is provided in Appendix C and will be finalized after the site reconnaissance is complete.

Sample Deployment and Collection

Mussels will be provided by the Catalina Sea Ranch, an offshore aquaculture facility located 6 miles offshore of San Pedro. Initial sub-samples will be collected and analyzed to ensure the mussels do not have detectable microcystins prior to deployment. Mussels are to be adapted to lower salinity seawater in the laboratory at SCCWRP to ensure that the mussels are not unduly stressed when deployed *in situ* (for any sites that have lower than 33 ppt salinity). Mussels will be deployed in mesh bags and attached to a support structure for the duration of the deployments. Field teams will collect a mesh bag during each recovery period (every one or two weeks depending on the site). Mussels will be shucked, and the tissue will be collected, stored frozen, and delivered to Dr. Dave Caron's laboratory at the University of Southern California (USC) for analysis.

Sample Analysis.

Mussel tissue from each bag will be homogenized from each site for each time period and prepared for analysis using standard methods employed in other studies (Preece et al., 2015a, 2015b). The samples will be analyzed using the Abraxis direct monoclonal (DM) ELISA (model PN 522015) since it has been successfully used for mussel tissue in other relevant studies (Preece et al., 2015a, 2015b). All samples will be analyzed at University of Southern California (Dr. David Caron's Laboratory), and since there is only one participating laboratory performing the analysis, there is no need for an interlaboratory comparison.

Products.

The main data product for this study will be a cumulative distribution function graph of microcystins detected in mussel tissue for dry and wet weather. These data will be summarized for a chapter in the Bight '18 Harmful Algal Bloom Report and published in a peer-reviewed journal.

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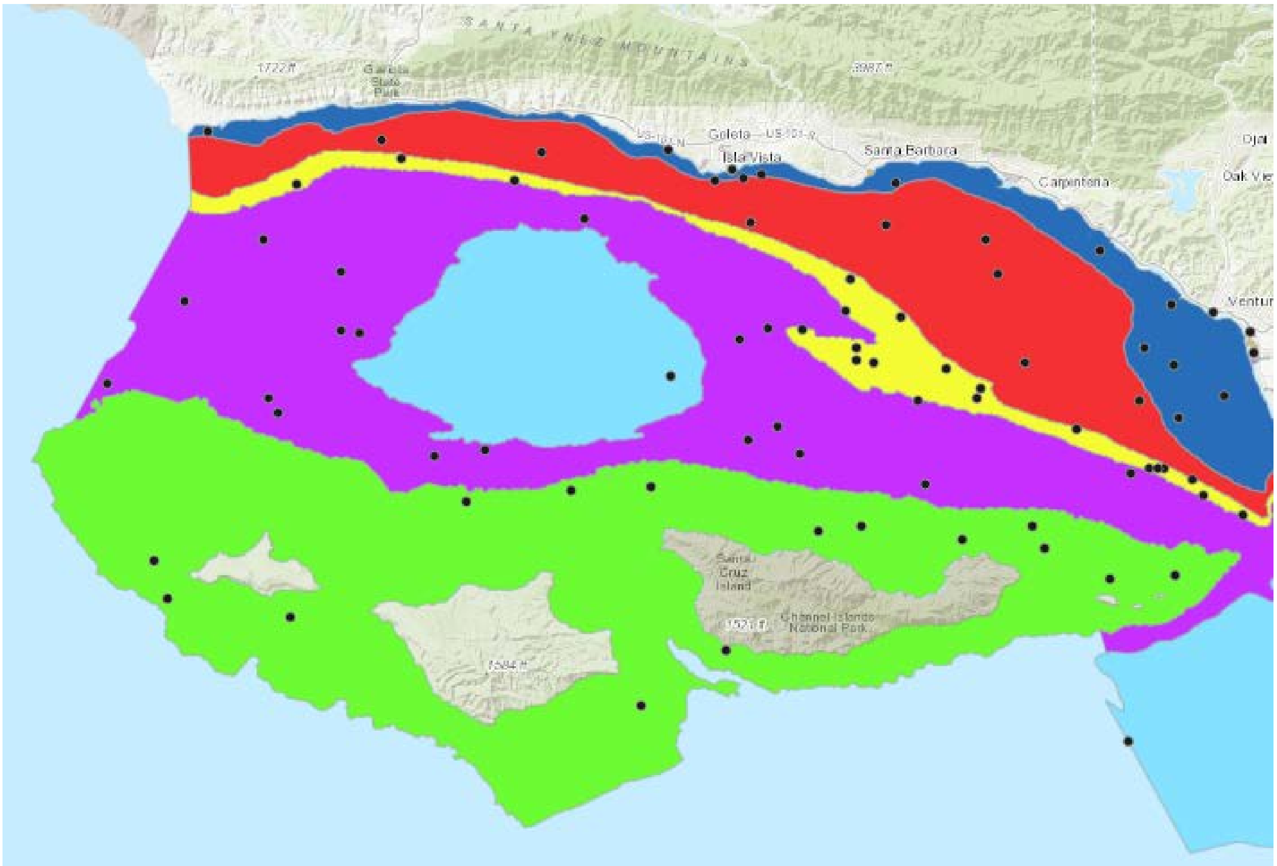
APPENDIX A

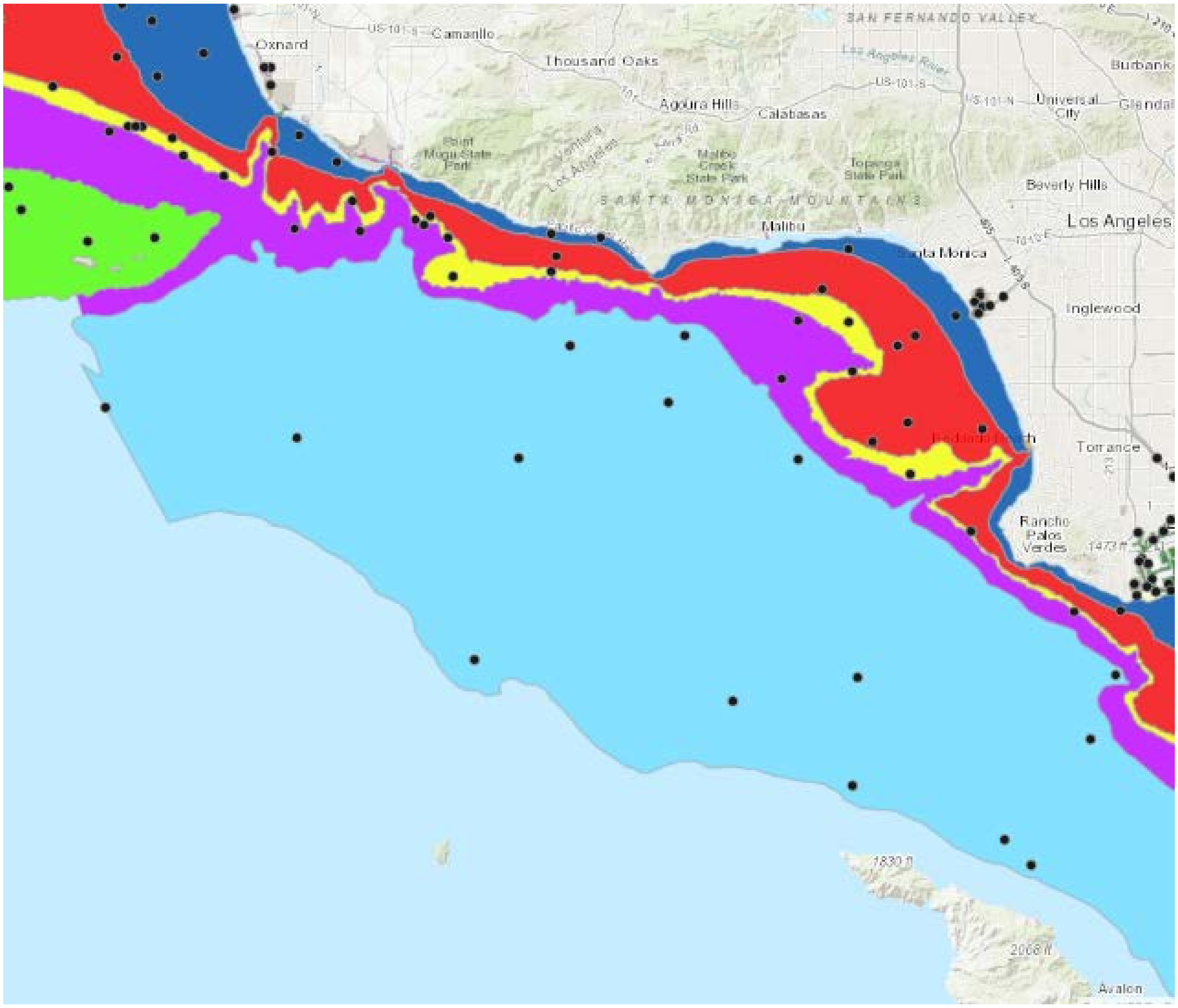
Sample Site Maps

There will be 3 strata sampled for domoic acid, the inner shelf strata (dark blue), the mid-shelf strata (red) and the outer shelf strata (yellow).

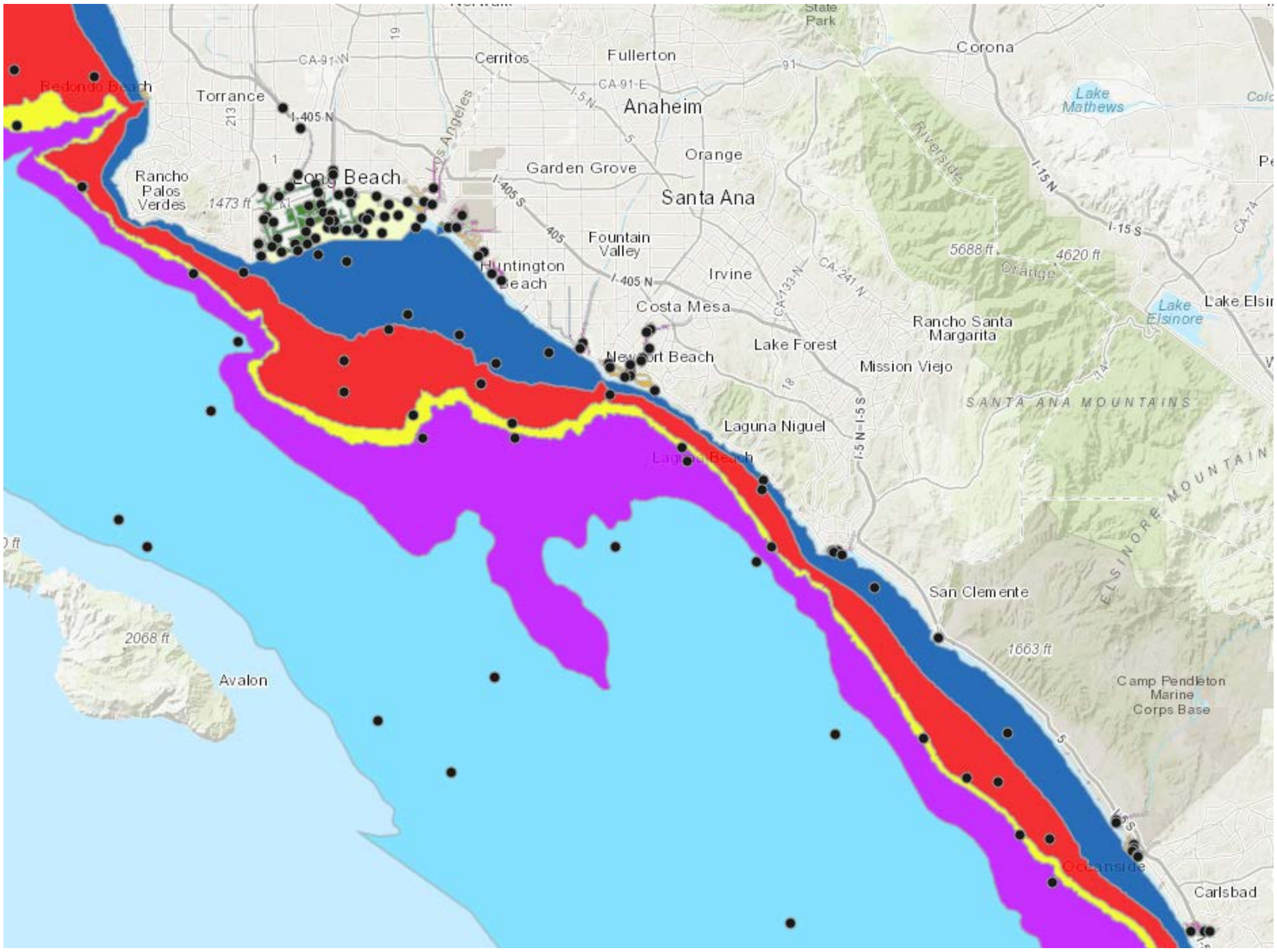
Santa Barbara Channel



Hueneme to Santa Monica Bay



San Pedro Shelf and Channel



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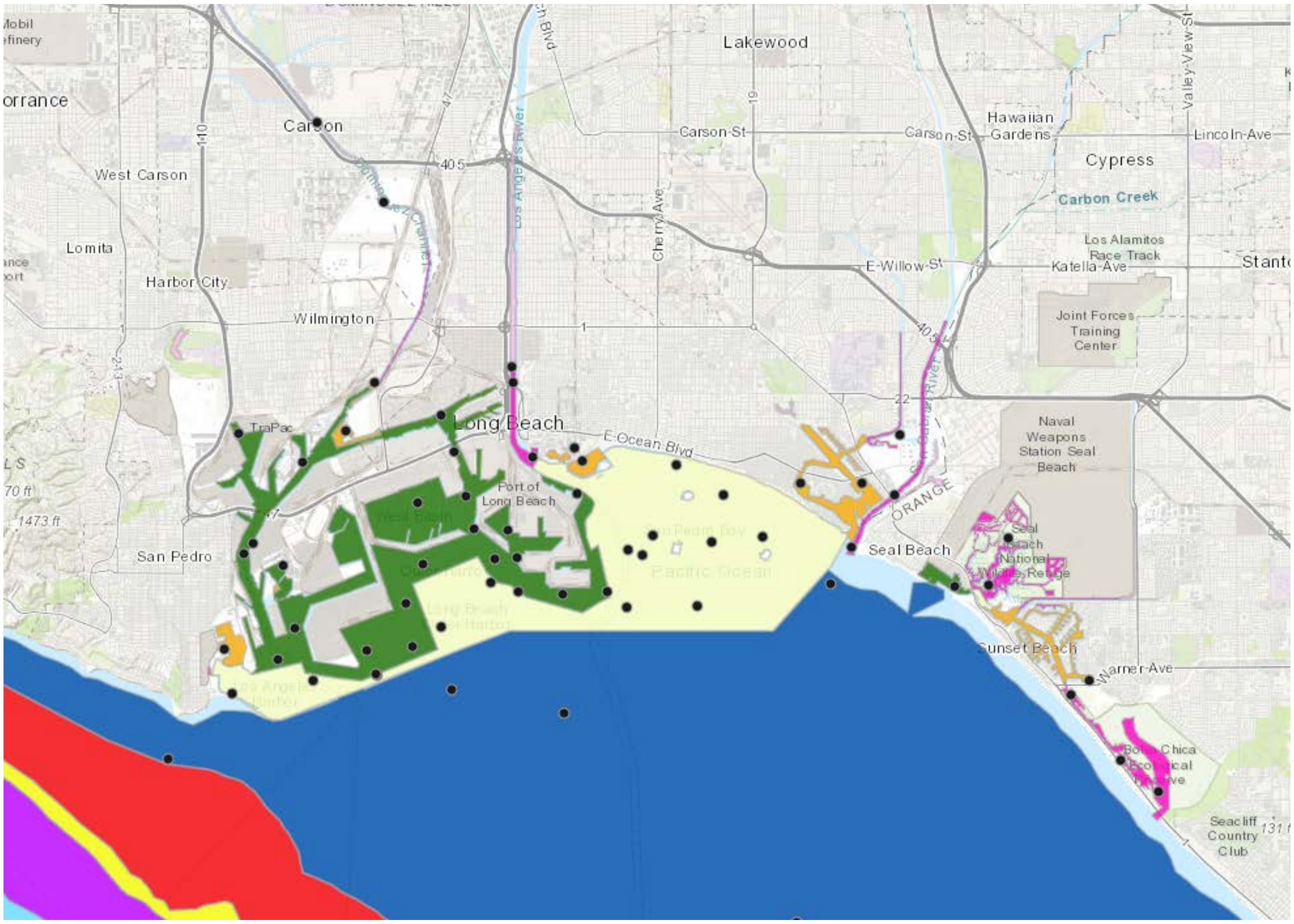
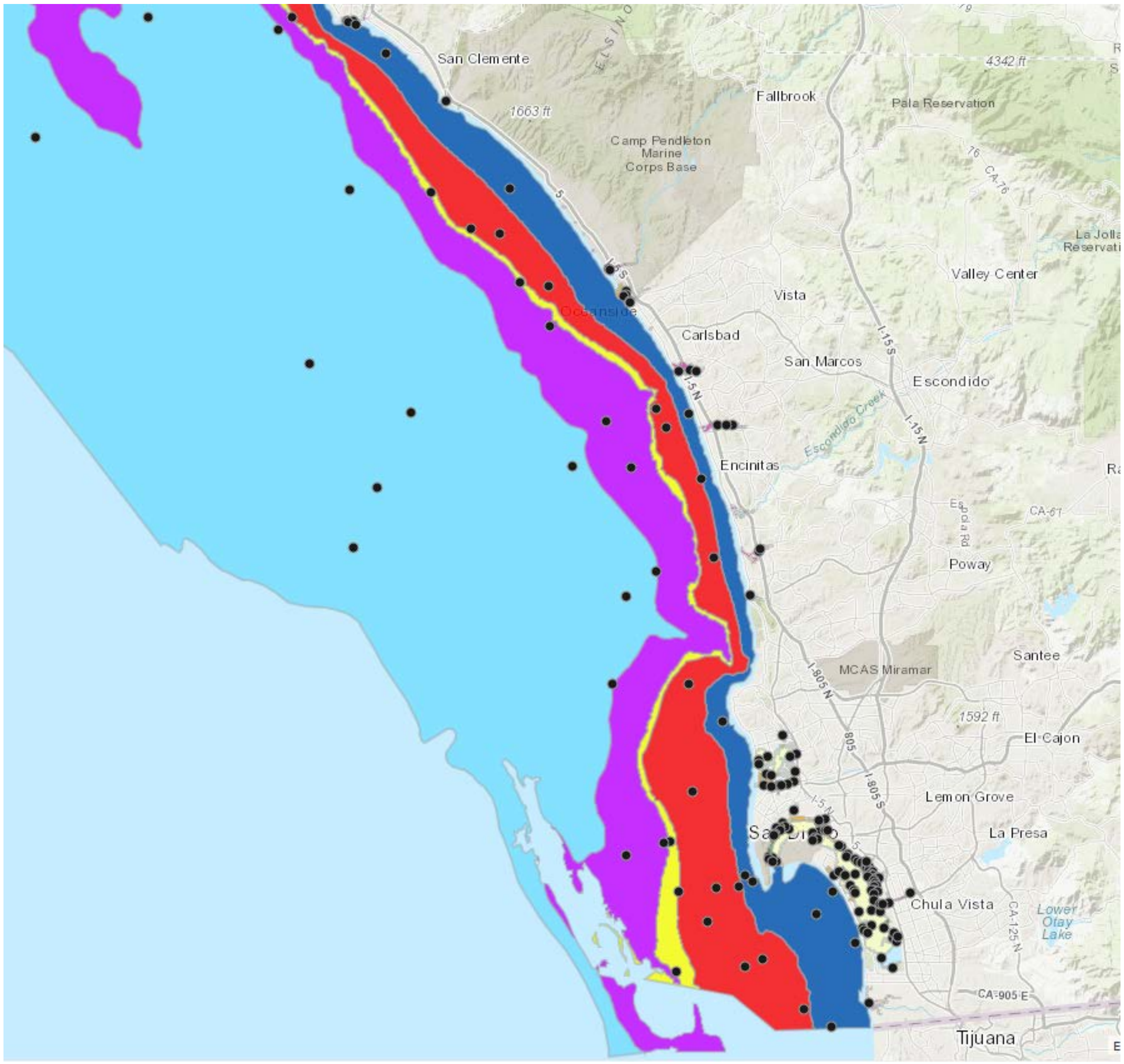


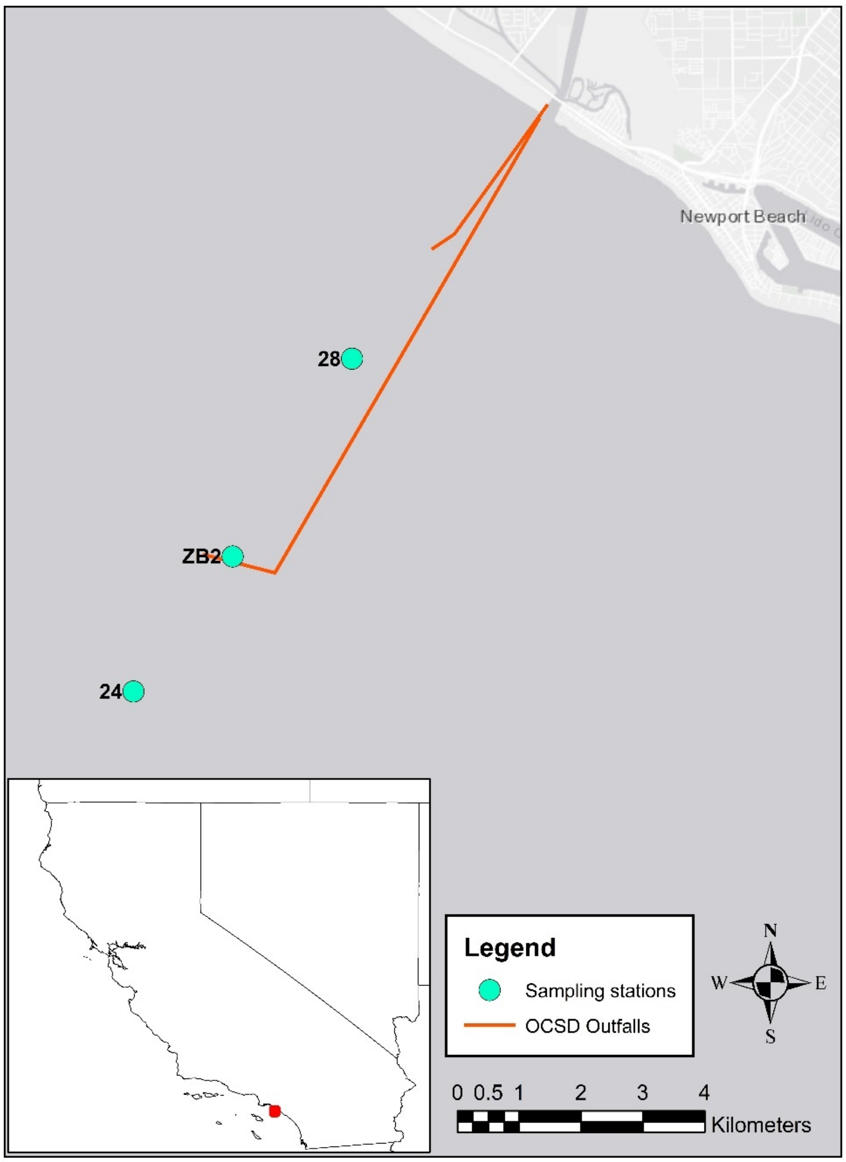
San Diego County



San Diego Bay

APPENDIX B

Site locations of the monthly sampling sites in Orange County for sediments (all) and benthic infauna (sites 28 and 24 only).



APPENDIX C

Proposed site locations for the microcystins in mussel component. Final site locations will be determined after site reconnaissance is completed.