Southern California Bight
2023 Regional Marine Monitoring Program
(Bight '23)

Shellfish Assessment Field and Laboratory Plan

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I. INTRODUCTION

Organisms that live at the land-sea interface account for more than 80 percent of marine aquaculture production in the United States (NOAA Fisheries, 2020). These economically important organisms, which include bivalves such as oysters and mussels, are also ecologically important as a food source in marine ecosystems and provide critical habitat and shoreline stabilization while also serving to improve water quality (Rullens et. al. 2019, Theuerkauf et al. 2021). Living at the land-sea interface, these organisms are exposed to pressures from the ocean, such as changing ocean climate and marine harmful algal bloom (HAB) toxins, as well as contaminants and toxins coming downstream from the land. However, the extent and magnitude of these varying factors, their relative risk, and what factors may allow managers to predict it, are poorly understood. There is a strong management need to develop monitoring programs assessing freshwater-marine interface for ecological or public health risks, which is an increasing priority in many parts of the United States.

The Southern California Bight (SCB) is an ideal place to begin to understand this risk (Figure I-1). The coast of California constitutes one of the longest and most economically valuable coastlines in the U.S. Along its ~5,500 km coastline are some of the country's biggest ports, most significant fisheries, densest urban areas, extensive recreation and tourism industries, and areas of cultural significance. The development of the SCB coast is subject to numerous human pressures which can impact water quality through addition of sediment, toxic chemicals, pathogens and nutrients to the ocean. Furthermore, the coast of California is also experiencing increasing frequency and severity of both marine and freshwater harmful algal blooms (HABs). However, the effect of these pressures on shellfish populations and whether they are safe for consumption is unknown.

The purpose of the Southern California Bight 2023 Regional Monitoring Program's (Bight '23) Shellfish Assessment is to characterize these relative risks on bivalves throughout the region. Bight '23 is a continuation of the successful cooperative regional-scale monitoring in Southern California, building upon the previous successes and expanding on the 2018 program by including new participants, answering additional questions, adding new elements, and measuring more parameters. Forty-eight organizations, including international and volunteer organizations, have agreed to participate (Table I-2). The inclusion of multiple participants, some 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 '23 Program is organized into seven technical components: 1) Sediment Quality (formerly Contaminant Impact Assessment/ Coastal Ecology); 2) Microbiology; 3) Water Quality (formerly Nutrients/Ocean Acidification); 4) Harmful Algal Blooms; and 5) Trash and Microplastics, 6) Estuaries, and 7) Submerged Aquatic Vegetation. The Shellfish Assessment is leveraged across several of these elements: Sediment Quality, Harmful Algal Blooms, Trash and Microplastics, Estuaries, and Microbiology. With each element providing effort to assess the various factors potentially impacting shellfish and human health and ecological risks of consuming local shellfish. This Workplan provides a summary of the

Shellfish Study project design, sampling and sample preparation protocols. Additional information on sample analysis is addressed in workplans and Quality Assurance Plans developed for the leveraged Elements and is summarized in brief in this document.

FIGURE I-1. Map of the Southern California Bight

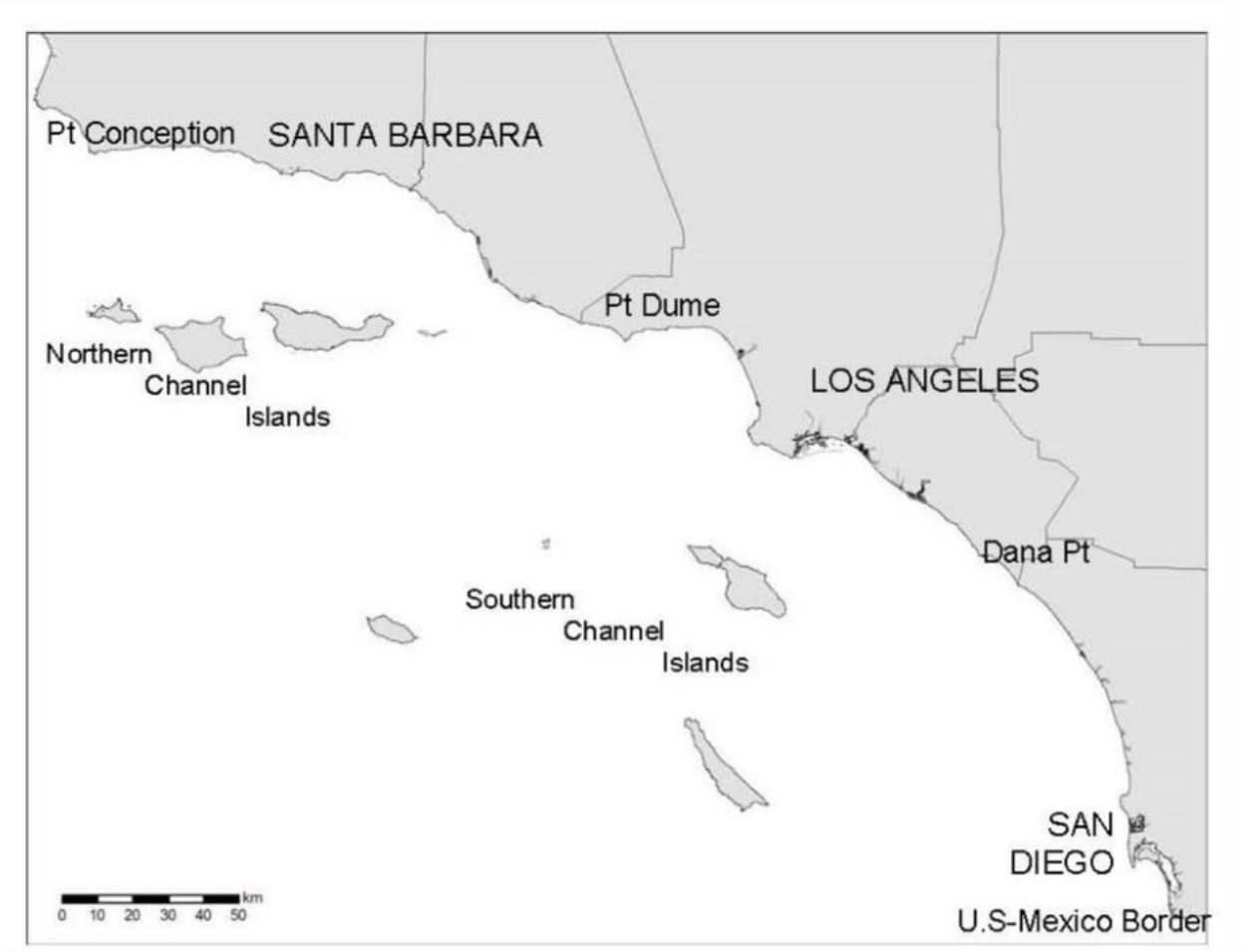

TABLE I-2. Participants in the Bight '23 Regional Monitoring Program, Shellfish Assessment.

AES Corporation
Anchor QEA
Aquatic Bioassay and Consulting Laboratories (ABC)
Bureau of Ocean Energy Management (BOEM)
Calscience Environmental Laboratories, Inc.
Channel Islands National Marine Sanctuary (CINMS)
Chevron USA Products Company
City of Los Angeles, Department of Water and Power (LADWP)
City of Los Angeles Watershed Protection District
City of Los Angeles Environmental Monitoring Division (CLA-EMD)
City of Oceanside
City of Oxnard
City of San Diego
Ballona Creek Watershed Management Group (City of Los Angeles, Los Angeles County Flood Control District, Los Angeles County, City of Beverley Hills, City of Culver City, City of Inglewood, City of Santa Monica, City of West Hollywood)
Eco-Analysts
Encina Wastewater Authority
Enthalpy
Greater Los Angeles and Long Beach Harbor Waters Regional Monitoring Coalition (RMC)
Los Angeles Regional Water Quality Control Board (LARWQCB)
Los Angeles County Public Works
Los Angeles County Sanitation Districts (LACSD)
MBC Aquatic Sciences (MBC)
National Oceanic and Atmospheric Administration (NOAA)
Naval Information Warfare Center Pacific
NES Energy, Inc.
NRG Energy, Inc.
Orange County Sanitation District (OC San)
Orange County Public Works
Oregon State University
Pacific EcoRisk
PHYSIS Environmental Laboratories, Inc.
Port of Long Beach
Port of Los Angeles
Port of San Diego
Riverside County Flood Control and Water Conservation District
San Diego County Dept. of Environmental Health and Municipal Co-permittees
San Diego Regional Harbor Monitoring Program (RHMP)
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
Southern California Coastal Water Research Project (SCCWRP)
State Water Resources Control Board
University of California, Santa Cruz
University of California, Riverside
U.S. Fish and Wildlife Service (USFWS)
U.S. Geological Survey (USGS)
Vantuna Research Group, Occidental College
Weck Laboratories, Inc.
Weston Solutions, Inc.
WSP

II. STUDY DESIGN

A. Study Objective

The Shellfish Bioaccumulation sub-element is leveraged across multiple Bight '23 Program Elements (Sediment Quality, Harmful Algal Blooms, Trash and Microplastics, Pathogens and Estuaries) to answer one question:

1. What is the extent and magnitude of impacts on Southern California Bight shellfish?

Impacts refer to human health or ecosystem health impacts resulting from consumption of shellfish tissues. Workplans for each of the leveraged Bight Program Elements detail the specific questions each element hopes to address in the shellfish assessment. In addition, a synthesis of all program findings will be published, providing a comprehensive assessment of the shellfish safety in the Southern California Bight.

B. Sampling Design

The purpose of this component is to assess regional shellfish for human and ecosystem health impacts throughout the SCB using multiple metrics. The Sediment Quality planning and technical committees will be responsible for the measurement of concentrations of legacy contaminants and emerging contaminants (PFAS), the Harmful Algal Blooms committee will assess HABs toxins (cyanotoxins [microcystins and anatoxin-a] and domoic acid), the Trash and Microplastics Committee will assess microplastic concentrations, and the Microbiology Committee will assess pathogens (Norovirus, Enterococci, and Vibrio) in collected shellfish throughout the Southern California Bight.

There will be a minimum of three sampling events that will occur at 20-30 stations across the SCB over a one-year period. Sampling events will occur during winter (wet season), spring (coastal upwelling), and the late summer/early fall (dry season). This design allows us to capture the "baseline" concentration of contaminants in the bivalves during the summer, as well as concentrations after upwelling events and flushing from the rainy season.

B1. Site Selection.

This component has both a spatial extent and magnitude component in its design. Site selection followed five basic guiding principles:

1. Include sites all along the Bight coastline
2. Target species that people eat
3. Sample locations where species are collected
4. Measure tissues that are consumed
5. Analyze constituents that represent potential risk to human consumers and ecological impacts

Sampling locations for this study will focus on known shellfish beds that are popular for subsistence and recreational harvesting, spaced along the SCB coastline, including sites sampled during the Mussel Watch Program and other shellfish contaminant surveys. Sites located near estuaries included in the Bight '23 Estuaries Assessment will be prioritized because those sites will have ancillary information about freshwater flow and chemistry that will be helpful in interpretation.

A targeted sampling design will be used to examine bioaccumulation in shellfish. Roughly 30 locations will be targeted from Point Conception to the US/Mexico International Border for this study and only sites with sufficient shellfish numbers will be sampled. Sampling locations are inclusive of 200m radius from the target location. This study aims to sample locations with a broad gradient of environmental stressors and responses, from minimally disturbed (reference sites) to very disturbed locations and will thus incorporate locations with different land use (agricultural, urban, and open space). Wherever possible, multiple taxa of bivalves (both oysters and mussels) will be collected from the same site or within the same estuarine/harbor complex. Ancillary field data such as temperature and salinity will be collected during sampling for shellfish, along with water samples for chlorophyll *a* concentration analysis.

Sampling will be conducted by the Bight '23 Shellfish Technical Committee. Sample collection will occur between Winter and Summer of 2024, targeting 3 sampling periods over a one-year period. Sampling will partially leverage the sampling effort of the Bight '23 Estuary Study group, which plans to sample in estuaries across the Southern California Bight in the fall of 2023, and potentially in the Spring of 2024.

B2. Species Selection

Selecting species to monitor is complicated due to the relatively high diversity of species, variation in habitat type and quality, variation in contamination, and the varying ecological attributes of potential indicator species. The following criteria were used to select target species:

1. Popular for consumption.
2. Widely distributed. Range of preferred species will extend the length of the SCB.
3. Representative of different depuration rates.
4. Continuity with existing monitoring efforts.

Two taxa have been selected for Bight '23: mussels and oysters. Wherever possible, oysters (*Crassostrea gigas*) and mussels (*Mytilus californianus* and/or *Mytilus galloprovincialis*; wild mussels are also sometimes hybridized) will be collected to allow for comparison of concentrations across bivalve taxa at or near the same confluence. Both bivalve species are among the most pervasive in coastal confluence zones in California.

A minimum of 10 individuals will be composited and splits of the composite will be used for analysis of each indicator listed below. Medium to large market sized organisms (≥ 3-4 inches) will be collected for composites. The same composite will be analyzed for tissue contaminants, HABs toxins, and pathogens, but not for microplastics, which will be measured from individuals collected at the same time and place. For each composite, roughly 70 grams of

tissue will be blended and then subsequently split for individual analyses of legacy contaminants, PFAS, HABs toxins, and pathogens. There will be a maximum of 200 composites total for this element of Bight '23 (30 sites x 2 species x 3 sampling period x 5% replication).

C. Indicators

Bight '23 will measure multiple indicators (Table II-1) to characterize both singular and relative risk of human and ecosystem health impacts. In order to integrate the data into a comprehensive regional assessment, quality assurance and quality control practices are built into the measurements to ensure that all data are comparable. Sample collection and analysis protocols are standardized to ensure comparability. Below, we present a description of the methods used to measure the Bight '23 shellfish indicators; more detailed descriptions of the methods can be found in the accompanying Workplans and Quality Assurance Manuals for each leveraged element.

C1. Tissue Contaminant Chemistry

The State of California OEHHA has provided guidelines for the evaluation of contaminant data (Table II-2). Each composite sample will be analyzed for polychlorinated biphenyls (PCBs) congeners, Dichlorodiphenyltrichloroethane isomers and metabolites (DDTs), Chlordanes, Per- and polyfluoroalkyl substances (PFAS), mercury, arsenic, and selenium (Table II-1). Total lipids will also be analyzed. These chemical analyses of shellfish tissue samples will provide an assessment of contaminant exposure. Tissue composites will be formed from 10-30 individuals based on size and wet tissue weight. The chemical analyte list includes both inorganics and organics and was developed to include comparisons to local programs and to state and national monitoring datasets such as California's SWAMP or NOAA's Status and Trends program. All chemistry measurements will follow performance-based quality assurance guidelines described in the Bight '23 Quality Assurance Plan.

1a. Organics

Organic compounds in tissues will be extracted with solvents and cleaned to remove interfering substances. Chlordanes, DDTs, and PCBs will be analyzed by GC/ECD, GC/MS, or GC/MS/MS. The PCB congener list was selected to include compounds that are abundant in the environment and compounds with a high potential for toxicity. Per- and polyfluoroalkyl substances (PFAS), a constituent of emerging concern, will be measured using LC/MS/MS.

1b. Inorganics

Metals i.e., mercury, arsenic, and selenium, will be analyzed by ICP, ICPMS, or atomic absorption spectrophotometry after strong acid digestion. Methyl mercury will be analyzed by cold vapor technique.

Reporting levels shall be consistent with OEHHA (Klasing and Brodberg, 2017) and SWAMP bioaccumulation monitoring (2021) thresholds for comparative purposes. Quality assurance activities shall focus on accuracy, precision, sensitivity, and comparability as described in the Bight '23 Sediment Quality Assurance Plan.

C2. Harmful Algal Bloom Toxins

The assessment of shellfish toxin levels will be made according to the State of California's OEHHA recommendations for cyanotoxins and the U.S. Food and Drug Administration (FDA) safe-to-eat level for DA (Table II-3). Reporting levels shall be consistent with OEHHA and FDA thresholds for comparative purposes. The samples will be analyzed for Microcystins (MCYs) (MCY congeners MC-LR, MC-RR, MC-YR, MC-LA, MC-LF, MC-WR, MC-LY, and dmLR), domoic acid (DA), and anatoxin-a (for late summer/early fall samples) using liquid chromatography/mass spectrometry (LC-MS). Chlorophyll *a* analysis will be conducted via the non-acidification method using fluorometric detection of the pigment, following the methods described in Seubert et al., (2013). All algal toxin samples will be analyzed at the University of California, Santa Cruz, and chlorophyll *a* samples will be analyzed at SCCWRP. Since there is only one participating laboratory performing the analysis, there is no need for an interlaboratory comparison. Quality assurance activities shall focus on accuracy, precision, sensitivity, and comparability. Quality control protocols including the use of laboratory replicates, blanks, and matrix spikes, will be followed where appropriate.

C3. Pathogens

The assessment of shellfish pathogens will be made according to U.S. Food and Drug Administration (FDA) thresholds for *vibrio parahaemolyticus* (Table II-4). No more than 12 hours after shellfish collection, 5g of homogenized shellfish tissue from the tissue composite will be immediately frozen at -80°C until further analysis. Thawed shellfish tissue will be extracted using the extraction kit chosen by the Technical Advisory Committee, with modifications from SCCWRP. DNA extracts will be immediately frozen at -80°C until further analysis; extracts can be frozen at -20°C for short-term (less than 2 months) but samples must stored at -80°C for long-term storage. Absolute gene copy number of pathogens (Table II-5) in shellfish tissue will be quantified using digital droplet PCR (ddPCR). Following the manufacturer's protocol for the QX200/QX600 (Bio-Rad, Hercules, CA), manual or automatic droplet generation will precede the following thermocycling conditions: hold at 95°C for 10 min, 40 cycles of 94°C for 30s, 60°C 1 min, and a final enzyme deactivation step at 98°C for 10 min, then droplet fluorescence will be read by QX200/QX600 Droplet Reader. Gene copies per gram shellfish tissue will be calculated in R (R Core Team, 2021; https://github.com/kylielanglois/SCCWRP/tree/main/ddPCR) by SCCWRP personnel.

A method blank will be extracted during every extraction event to ensure no contamination of reagents or personnel bias. Quality control measures suggested by Cao et al. (2015) and Steele et al. (2018) will be followed during ddPCR. If multiple labs participate in the extraction and analysis of pathogens, quality assurance between labs will be determined by an inter-lab calibration study; for more details see Bight '23 Microbiology Workplan.

C4. Microplastics

Microplastics, plastic particles <5mm in size, will be quantified and characterized in shellfish tissue. At least five shellfish of each species present will be collected at each site to create a composite sample. This composite is separate and distinct from the composite for other indicators because it must be handled in a clean lab to avoid contamination by air-borne plastic particles. At a pre-selected subset of sites, triplicate samples (i.e., three samples comprised of five pooled organisms each) will be collected to assess variation amongst individual samples. All suspected plastic particles ≥125 µm will be quantified and analyzed for size, morphology, and color according to Hampton et al. (2023). To confirm plastic particles and determine polymer type, up to 75 particles per sample will be randomly subsampled for spectroscopic analysis according to De Frond et al. (2023). Particle sizes 20-125 µm will be extracted and concentrated on filters for future potential analysis.

Quality control procedures include specialized background contamination mitigation procedures as well as the employment of field and laboratory blanks to track contamination rates during collection, cleaning, shucking, processing, and analysis. In addition, all laboratories processing and analyzing samples are required to participate in an interlaboratory comparability exercise prior to the study. During this exercise, laboratories must demonstrate acceptable levels of background contamination and particle recovery. For more details, see the Bight '23 Trash and Microplastics Workplan.

TABLE II-1. Constituents that will be measured in shellfish during Bight '23.

Analyte Type	Class	Analytes		Committed Effort
Tissue Chemistry	Trace Metals	Mercury Arsenic	Selenium	Yes
	PCB Congeners	PCB 8 PCB 18 PCB 28 PCB 37 PCB 44 PCB 49 PCB 52 PCB 66 PCB 70 PCB 74 PCB 77 PCB 81 PCB 87 PCB 99 PCB 101 PCB 105 PCB 110 PCB 114	PCB 128 PCB 138 PCB 149 PCB 151 PCB 153 PCB 156 PCB 157 PCB 158 PCB 167 PCB 168 PCB 169 PCB 170 PCB 177 PCB 180 PCB 183 PCB 187 PCB 189 PCB 194	Yes

		PCB 118 PCB 119 PCB 123 PCB 126	PCB 195 PCB 201 PCB 206	
	Chlorinated Hydrocarbons	4,4'-DDT 2,4'-DDT 4,4'-DDD 2,4'-DDD 4,4'-DDE 2,4'-DDE 4,4'-DDMU	alpha-Chlordane gamma-Chlordane *cis*-nonachlor *trans*-nonachlor oxychlordane	Yes
	Per- and Polyfluorinated Substances (PFAS)	PFOS	PFOA	Yes
HABs Toxins	Marine HABs	Domoic Acid		Yes
	Freshwater HABs	Microcystin		Yes
		Anatoxin-a		No
Microplastics	Microplastics	Microplastics		Yes*
Pathogens	Pathogens	Norovirus		Yes
		Enterococci		No
		Vibrio		Yes

* Effort committed for one sample event.

Table II-2. State of California Office of Environmental Health and Hazard Assessment Advisory Tissue Level (ATL).

Contaminant (ng/wet g)	**Number 8 oz Meals Per Week**		
	<Three	**<Two**	**<One**
DDTs*	520	1000	2100
methylMercury (women 18-45, child 1-17)	70	150	440
methylMercury (women >45, men)	220	440	1310
Selenium	2,500	4,900	15,000
PCBs*	21	42	120

*Congeners as listed in Table II-1

Table II-3. Algal toxin and cyanotoxin assessment levels from the State of California Office of Environmental Health and Hazard Assessment (OEHHA) for cyanotoxins and the U.S. Food and Drug Administration (FDA).

Toxin	Assessment Level (ng/g) ww	Issuing Agency
Domoic Acid	20,000	FDA
Microcystins	10	OEHHA
Anatoxin-a	5,000	OEHHA

Tabel II-4. U.S. Food and Drug Administration (FDA) shellfish pathogen assessment levels

Toxin	Assessment Level	Issuing Agency
V. parahaemolyticus	10,000 cells / g tissue	FDA/ISSC
V. vulnificus	NA	NA
Norovirus G1/G2	NA	NA

TABLE II-5. Primer and probe sets for ddPCR to measure pathogens in shellfish tissue during Bight '23.

Gene target	Target shorthand	Forward primer (3'-5')	Reverse Primer (5'-3')	Probe (5'-3')
Enterococcus 23S	ENT	GAGAAAT+TCCAA+ACGAACTTG	CAGTGCTCTACCTCCATCATT	[FAM]CGGAACCGA/ZEN/CTACTTTGGGTGTCCGT[3IABKFQ]
Norovirus G2	QNIFS	ATGTTCAGRTGGATGAGRTTCTCWGA	TCGACGCCATCTTCATTCACA	[FAM]AGCACGTGGGAGGGGATCG[TAMRA]
Norovirus G1	NV1LC	CGCTGGATGCGNTTCCAT	CCTTAGACGCCATCATCATTTAC	[FAM]TGGACAGGA/ZEN/GAYCGCRATCT[3IABkFQ]
V. vulnificus hemolysin	VVHA	TGTTTATGGTGAGAACGGTGACA	TTCTTTATCTAGGCCCCAAACTTG	[FAM]CCGTTAACCGAACCACCCGCAA[BHQ]
V. parahaemolyticus toxT	TOXR	GAACCAGAAGCGCCAGTAGT	AAACAAGCAGTACGCAAATCG	[FAM]TCACAGCAGAAGCCACAGGTGC[BHQ]

III. SAMPLE COLLECTION

Organisms must be collected during low tide and within 3 weeks of the target date. A minimum of 15 oysters and/or 40 mussels will be collected at each site (or for selected sites, both species). Shellfish individuals must be kept cold during the whole shipment process, from collection to delivery. Shellfish will be stored on ice and shucked within 48 hours of collection, homogenized, and split into four sample containers (contaminants, PFAS, HAB toxins, and pathogens). An additional 5 oysters and 10 mussels will be collected for microplastics and delivered to the microplastics analytical lab for processing during the summer sampling event, which should be added to the individual total for that period.

A. Health and Safety

- Storms producing high surf are prevalent during winter and spring collections. Extreme caution must be observed during sampling to prevent injury or loss of life from high wave conditions. Safety first. Abandon site for another day if surf conditions look dangerous.
- Teams MUST consist of at least two people. Teams are strongly encouraged to carry a throwable life-line in case one team member is swept off.
- Expect to get wet from wave splash and/or rainstorms. Air temperatures may be cold so wear thermal protection. Rocks may be slippery so wear appropriate foot gear. Use extreme caution while jumping, landing, or climbing on (in-between) rocks.
- Carry gear and collected mussels in a backpack to free your hands for balance and stability while traversing rocky habitats.
- Mussel and oyster beds may have sharp edges and objects. Wear gloves to protect your hands when necessary.

B. Cautions

- Equipment used to collect and store mussels should be clean to avoid external contamination.
- The animals (shells) should be thoroughly rinsed in water at the site to remove mud and debris which are sources of contamination to their tissue inside. In high energy areas, collect site water in a container then move to a safe location before cleaning organisms.
- Shellfish should be wrapped in foil and immediately placed in prelabled bags on ice or other cold media. Bivalves exposed to fresh water will open prematurely and degrade or contaminate their tissue. Keep bivalves cold and well drained and avoid contact with freshwater.
- Hold samples over weekend periods in cold location (not frozen) until individuals can be delivered and composited at SCCWRP.

C. Equipment and Supplies

- Backpack

- Ice chest
- Ice
- Plastic bags, zip lock style
- Buckets
- Gloves
- 500 mL amber plastic bottle for water sample collection
- Salinity sample container
- Thermometer
- Refractometer
- Multi-sensor meter (includes temperature and salinity, optional)
- Datasheet
- Pencil or pen with indelible ink
- Chain of Custody forms
- For oysters: chisel and mallet
- Collection permit
- Waders/rubber boots

D. Collection Procedure

- Select a day and time to arrive at a sampling location and provide proper collection permit notification. Coordinate sampling day in advance with SCCWRP to allow for prompt sample delivery and processing. Samples must be collected within the sampling window for each seasonal sampling event (Table III-1). Bivalves should be easy to access and could be considered as the height of earliest access. Water depth reference is Mean Low Low Water (MLLW). Actual tidal heights will vary according to weather condition and time. Use a tide calendar to plan sample collection. Recommended sampling times are during the minus tide time series.
- Clean and prepare sampling gear, back packs, ice chests, plastic bags, ice, temperature/salinity meters, backup thermometers, salinity sample containers, and datasheets. Calibrate any sensors according to manufacturer's recommendation. If needed, gather any access permission letters for the sampling site.
- Arrive at the specified sampling location within the site as described in Appendix B during low tide. Access problems may occur during storm and high surf conditions. Use best judgement and put safety first. Prepare to get wet. Use caution when traversing rocks and boulders because conditions may be wet and slippery. Remember that injuries can occur when humans fall on rocks or into the ocean. Boats can get damaged by swells pushing them onto rocks. Locate a bed of mussels or oysters and climb down to the location. This will have to be done at three locations within a site.
- Shellfish should not be collected from a single location; individuals should be gathered from an area within 200 m of the target coordinates. For example, wherever possible, establish 3 independent stations at each site, e.g., three stations within 100 m of shoreline near the site target lat/lon. Establish the center transect point as one location then radiate outward 20 – 40 m on either side to establish the two remaining locations. Collect similar numbers of individuals at each station for

the site composite. Sites designated as "field duplicates" will require twice the number of individuals.

- Mussel Collection: Mussels should be removed by hand (the byssal threads that the mussel uses for attachment to rocks are not that strong. Use gloves to protect your hands. If you encounter stubborn mussels, search for others easier to remove. Place animals in a bucket or similar container. If available, pass animal container to another team member for cleaning and storage.
    - At each site, the optimal mussel size is 5 – 8 cm (2 – 3 ¼ inches) which targets ~40 total mussels from a sampling site (~35 for composite (or until minimum required mass is obtained) and 5 for microplastics). Tissue chemistry analysis needs mass versus quantity. When animals are small, malnourished, or sick, their tissue mass is small compared to health animals. Some animals may die during transport so include extra mussels for microplastics and chemical analysis.
- Oyster Collection:
    - Oysters in mud can sometimes be seen, other times can only be found by dragging your hand or chisel through the mud. These can be picked up with your hand.
    - Oysters attached to rock or concrete: Rest the sharp end of the chisel in between the oyster and substrate at about a 45-degree angle. Hit the handle of the chisel with moderate force with rubber mallet to wedge it in between the oyster and substrate. If this does not work, hit it again with more force or try another angle. Note: Rocks can have irregular surfaces that make it hard to place the chisel between the rock and oyster so reposition frequently. Be wary- this does increase the chances of piercing the shell. Discard any organisms where shells are pierced.
    - At each site, the optimal oyster size is 7 – 18 cm (5 – 7 inches) which targets 15 total oysters from a sampling site (10 for composite and 5 for microplastics). Tissue chemistry analysis needs mass versus quantity. When animals are small, malnourished, or sick, their tissue mass is small compared to health animals. Some animals may die during transport so include extra mussels for microplastics and chemical analysis.
- At each station within a site collect roughly a third of individuals depending on size. Note GPS coordinates for each station. If unable to get enough animals from 3 stations, you may add additional stations.
- The specimens' shells should be thoroughly rinsed in water at the site to remove mud and debris which are sources of contamination of the tissues inside. In high energy areas, collect site water in a container then move to a safe location before cleaning mussels. Place animals in appropriately labeled bags on ice, whenever possible and store in an ice chest.
- Samples for microplastics and the composite (in different bags) can be placed side by side in an ice chest. Mixing between bags should be avoided. If field teams are sampling multiple sites within a day, sites cannot be co-mingled. Sites must be in separate containers (e.g., ice chest) or other barrier separated system. Oysters and Mussels should be kept in separate coolers.

- Measure surface water temperature, salinity, and take a discrete surface water sample for salinity (10 ml minimum). If a multi-sensor meter is unavailable, other acceptable measures are an outdoor thermometer placed in water or into a bucket of water for temperature and a refractometer for salinity. Salinity will also be measured by the analytical lab so it can be termed an optional field measure. A water sample for chlorophyll a must also be collected. Fill the provided amber plastic bottle with seawater and tape the lid securely. Place the bottle in the ice chest with the mussels. The water sample will be sent, with the mussels, to SCCWRP within 24 hours of collection. The datasheet has additional water column measures (e.g., dissolved oxygen) but those are optional.
- During and after bivalve collection, record information on waterproof site datasheet such as name, site code, date, time, water temperature, salinity, and check box that chlorophyll sample was taken. Estimate the "height of collection" as being the height above the water level at which mussels were collected (e.g., samples at water level are given a value of 0 ft). Estimate the "height of highest access" as being the height above the water level at which mussels are available for collection (e.g., mussels available at the high-water mark are given a value of 6 ft). Record the GPS coordinate (NAD 83 datum) at the midpoint of each station that has a bagged mussel sample. On the back of the sheet record relevant information that might influence contaminant levels, future collections or the health of the mussels. Typical observations might include notices of shellfish closures or prohibitions on fishing posted nearby, oil sheen on water, weathered oil on rocks, smell, known discharges into the area (nearby outfalls, recent oil spills, runoff from rainstorms, etc.), depauperate or declining populations, evidence of human harvesting, and/or limitations to accessibility. Use a pencil, preferable, or pen with indelible ink on datasheet. Place datasheet or card in one of the bags going back to the laboratory.
- Upon completing site requirements (mussel collection, measuring water temperature, salinity sample, and datasheet), gather equipment and travel back to your vehicle. Stowing gear in backpacks allows arm/hands to maintain body balance while traversing over rocks.
- At your vehicle, place samples in coolers and fill out chain of custody forms. Return samples to SCCWRP.

E. Sample handling, holding, packaging and delivery

- Two principles guide the transport process. First, keep the transit time (collection until delivery at SCCWRP) to a minimum. Samples should be delivered to SCCWRP within 24 hours of collection. Second, keep the samples cold on ice but not frozen, and well drained (no standing water, either fresh or seawater). Ice should be package in separate bags and is discussed below.
- The ideal scenario would be to collect the samples in the morning or early afternoon, pack them for shipping and deliver them (or have them picked up) to SCCWRP. However, this is not always possible for all tidal scenarios and sample locations, in that scenario, samples may be held cold overnight and delivered the next morning.

- Keep samples on ice until ready for delivery to SCCWRP. Samples must be delivered to SCCWRP within 24 hours of collection, ideally same day. Samples kept directly on ice (not in bag) is OK while collecting samples but melt water must be drained continually from the container. Note that contact with water will invariably cause the mussels to open and introduce possible contamination or depuration. If the water is fresh, it will kill the mussels or oysters, rendering them useless for the study. The preferred method is to place the mussels in a zip lock bag and place this on top or beneath another zip lock bag filled with ice. It is also OK to store the mussels in a refrigerator if an extended hold (overnight) is required. All sample bags should have the appropriate site and date marked on them with a waterproof pen. Don't put ice in the bags with the specimens. Don't allow them to freeze. Examine the bags of mussels to ensure that any entrained water has not leaked into the bivalves' container. If standing water is observed, drain it from the bivalves.

Table III-1. Sampling windows for each seasonal sampling event.

Season	Event	Sampling Window
Winter	Storm/runoff	January 1- February 28, 2024
Spring	Upwelling	March 15 - May 15, 2024
Summer	Warm/ocean stratification	August 1- September 30, 2024

Table III-2. Tissue weights for each sample type:

Sample Type	Tissue Wet Weight	Container	Storage
Legacy Contaminants	30 g	Glass Jar	-20 C
PFAS	10 g	HDPE jar	-20 C
Algal Toxins	20 g (2, 10 g aliquots)	2x 50mL Falcon Tube	-80 C
Pathogens	5 g	50 mL Falcon Tube	-80 C
Microplastics	5 individuals	Polypropylene Jar	-20 C

IV. SAMPLE PROCESSING

A. Shucking

- Individual oysters or mussels should be kept cold but dry during the shucking process.
- Each individual in the composite should be measured (longest length) and weighed. Length and weight of all individuals should be recorded on the lab datasheet.
- The total number of individuals will vary based on size. Wet weight will be needed for the composite.
- Prepare an area for oyster or mussel processing.
    - Wipe down lab bench and dissecting trays with EtOH.

  - Place lab pad over dissecting tray or surface.
  - Prep shucking knives (should be wrapped in foil and autoclaved between uses).
- Wash and sanitize oysters or mussels.
  - Wash each animal well (use a different brush per site). Be sure to remove any sediment/sand attached to the outside of the shellfish
  - Spray each animal with 70% EtOH and wipe down with paper towels.
- Place up to 5 oysters or 10 mussels on the dissecting tray at a time.
- Flame sterilize shucking knife and use to carefully shuck the shellfish.
- Cut/pull the oyster/mussel from the shell, being careful to retain as much liquid as possible.
- Carefully pour the contents of the oyster or mussel into a pre-labeled jar for an individual site and place on ice.
- When 10 oysters or 20 mussels have been processed from 1 site, place in the –80 deg C freezer if unable to homogenize immediately.

B. Homogenizing and Compositing:

- If homogenizing from a frozen sample, defrost sample in the dark before homogenizing.
- All samples will be homogenized with an Omni Tissue homogenizer with hard tissue plastic probe or stainless steel blender.
  - For Omni Tissue homogenizer: Use a single plasic probe will be used per composite, per site.
- Ensure the sample remains cool during homogenizing. Keep jar or tube on ice if needed during this step
- The homogenized tissue composite will then be aliquoted for subsequent analysis.
  - HAB toxins: Two aliquots, both 10 grams of tissue will be aliquoted into a 50 mL falcon tube
  - Legacy contaminants: one 30g aliquot into glass jar, make sure jar is no more than 2/3 full of tissue. Use multiple jars if necessary weight exceeds volume requirement.
  - PFAS: one 10g aliquot into HDPE jar, make sure jar is no more than 2/3 full of sediment. Use multiple jars if necessary weight exceeds volume requirement.
  - Pathogens: minimum give 1g aliquots into sterile 15ml Falcon tubes, immediately frozen at -80°C (-20°C acceptable for short-term storage)

C. Microplastics

- In general, the same shucking protocols are required for microplastics. All shellfish designated for microplastics analysis will be cleaned and shucked at SCCWRP in a clean environment to minimize possible background contamination.
- All laboratory personnel must wear cotton lab coats to prevent background contamination from clothing.
- After cleaning and immediately before shucking, each shellfish will be rinsed with microplastics-free water to remove any potential particles from the outside of the shell.
- Shellfish will be shucked under a fume hood or in a clean cabinet to mitigate background contamination from aerial deposition.

- During shucking, an air blank (i.e., open glass petri dish with filter paper) will be placed in the fume hood or clean cabinet to determine background contamination levels during shucking.
- The total length and mass of the viscera will be determined for each shellfish.
- Viscera from a total of five individuals will be pooled in polypropylene jars. Individuals will not be blended as for the composite to minimize contamination of the sample.
- Samples will be stored at -20°C prior to distribution to the assigned analytical laboratories.

V. Committed and Uncommitted Work Elements

This workplan describes the elements of the Bight '23 Shellfish Assessment that have been identified as priority needs. However, these priorities outstrip available resources. Therefore, the Bight Program commits to collecting data for some of these elements, identified as assigned analysis. In contrast, uncommitted elements may be implemented if additional resources become available, or non-Bight partners wish to contribute to the program. These elements include the "unassigned" analytes or sample locations.

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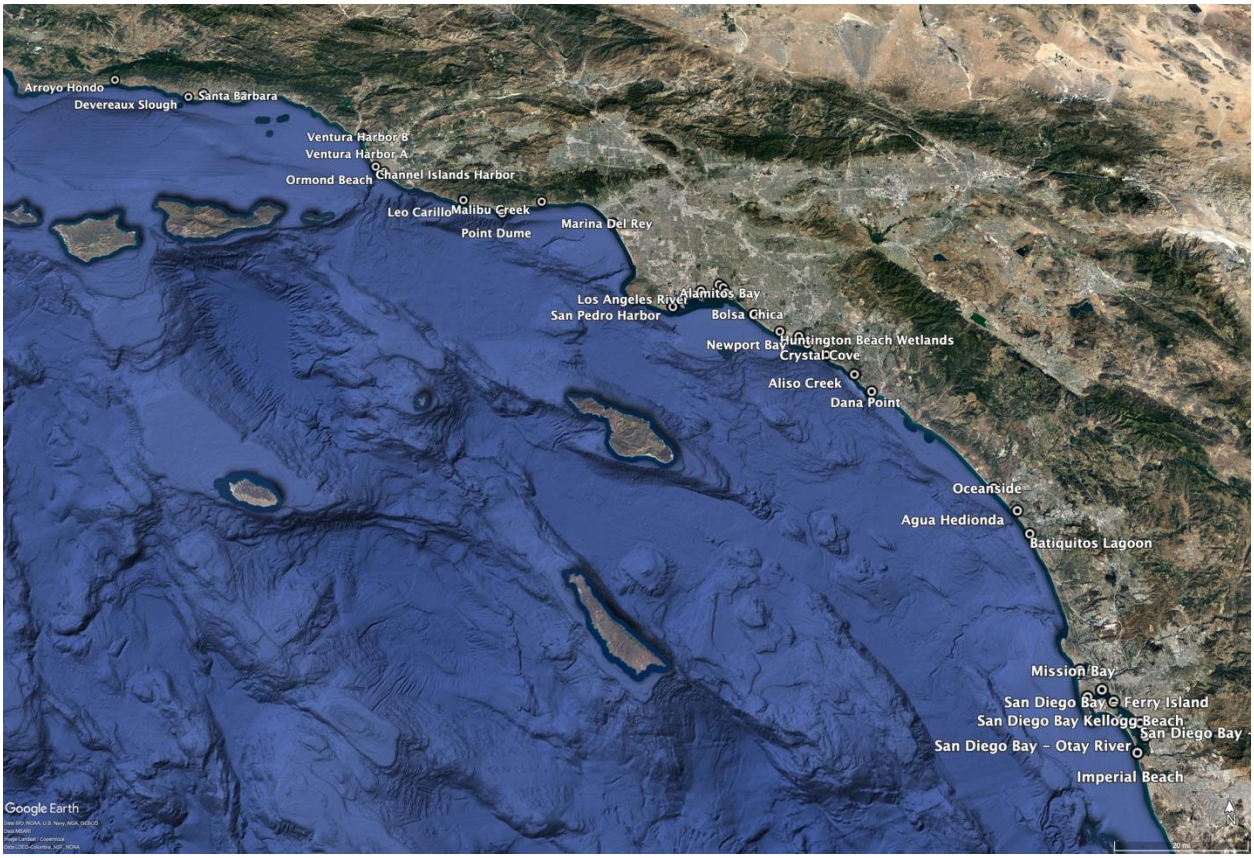
APPENDIX A

Sample Site Map

Proposed site locations for shellfish collection. Final site selection will be determined pending committed sampling effort for each site.



APPENDIX B

Sample Site Information

Proposed site locations for shellfish collection. Preliminary site reconnaissance was conducted for each site to determine if target shellfish taxa were present. Approximate latitude and longitude are indicated here but will be finalized by sampling partners.

General location	County	Latitude	Longitude	Shellfish Type	Assignment
Arroyo Hondo	Santa Barbara	34.473	-120.142	Mussels	MBC
Goleta Slough	Santa Barbara	34.417	-119.829	Mussels	MBC
Devereaux Slough*	Santa Barbara	34.407	-119.878	Mussels	MBC
Santa Barbara	Santa Barbara	34.407	-119.691	Mussels/Gigas	MBC
Ventura Harbor A	Ventura	34.264	-119.277	Mussels	LA Regional Board/SCCWRP
Ventura Harbor B	Ventura	34.258	-119.273	Mussels	LA Regional Board/SCCWRP
Ormond Beach	Ventura	34.141	-119.195	Mussels	MBC
Channel Islands Harbor	Ventura	34.158	-119.224	Mussels/Gigas	MBC
Point Dume	Los Angeles	34.001	-118.809	Mussels	MBC
Marina Del Rey	Los Angeles	33.962	-118.458	Mussels	LA Regional Board/SCCWRP
Los Angeles River	Los Angeles	33.753	-118.192	Mussels	LA Regional Board/SCCWRP
Alamitos Bay Inner	Los Angeles	33.762	-118.123	Gigas	LA Regional Board/SCCWRP
Alamitos Bay	Los Angeles	33.746	-118.116	Mussels	LA Regional Board/SCCWRP
San Pedro Harbor	Los Angeles	33.713	-118.283	Gigas	LA Regional Board/SCCWRP
San Pedro Harbor	Los Angeles	33.710	-118.281	Mussels	LA Regional Board/SCCWRP
Malibu Creek	Los Angeles	34.034	-118.683	Mussels	LA Regional Board/SCCWRP
Leo Carillo	Los Angeles	34.045	-118.934	Mussels	LA Regional Board/SCCWRP
Bolsa Chica	Orange	33.684	-118.036	Mussels	Chevron/Vantuna
Huntington Beach Wetlands	Orange	33.632	-117.961	Mussels	Chevron/Vantuna

Newport Bay	Orange	33.617	-117.905	Gigas	Chevron/Vantuna
Newport Beach	Orange	33.596	-117.882	Mussels	Chevron/Vantuna
Crystal Cove	Orange	33.564	-117.829	Mussels	OCPW
Aliso Creek	Orange	33.508	-117.751	Mussels	OCPW
Dana Point	Orange	33.461	-117.706	Mussels/Gigas	SD Regional Board
Oceanside	San Diego	33.202	-117.393	Mussels	SD Regional Board
Agua Hedionda	San Diego	33.144	-117.337	Mussels/Gigas	SD Regional Board
Batiquitos Lagoon	San Diego	33.087	-117.313	Mussels	SD Regional Board
Mission Bay	San Diego	32.769	-117.243	Mussels/Gigas	SD Regional Board
San Diego Bay	San Diego	32.725	-117.195	Gigas	SD Regional Board
Imperial Beach	San Diego	32.588	-117.134	Mussels	SD Regional Board
San Diego Bay - Pepper Park	San Diego	32.650	-117.111	Gigas	Port of SD
San Diego Bay - Otay River	San Diego	32.622	-117.104	Gigas	Port of SD
San Diego Bay Kellogg Beach	San Diego	32.710	-117.237	Mussels	Port of SD
San Diego Bay - Ferry Island	San Diego	32.698	-117.170	Mussels	Port of SD

*No sampling in Spring because of Snowy Plover.

APPENDIX C

Sample Laboratory Assignments

Table C1. Tissue Chemistry Laboratory Assignments

Analytes	Agency	# samples assigned
Legacy	CLA-EMD	70
Legacy	CSD	39
Legacy	LACSD	30
Legacy	OC SAN	60
CECs	PHYSIS	190

Table C2. Microplastics Laboratory Assignments

Analytes	Agency	# samples assigned
microplastics	SCCWRP	30
microplastics	UC Riverside	
microplastics	Cal State	
microplastics	OSU	

Table C3. Microplastics Laboratory Assignments

Analytes	Agency	# samples assigned
Domoic Acid	SCCWRP	130
Microcystin	UC Santa Cruz	130
Anatoxin a		unassigned

Table C4. Pathogens Laboratory Assignments

Analytes	Agency	# samples assigned
Norovirus/ Vibrio	OC San	30 (OC Sites)
Norovirus/ Vibrio	SCCWRP	110 (all other counties)

APPENDIX D

Bight '23 Uncommitted Work Elements

Uncommitted Field Effort:

1. Unassigned Shellfish Bioaccumulation Sample Collection- $1000 per site

Uncommitted Laboratory Effort:

1. Unassigned Shellfish Bioaccumulation Processing- $1000 per composite
2. Unassigned Shellfish Bioaccumulation contaminants- $250 per composite per analyte
3. Unassigned Shellfish Pathogens DDPCR analysis- $500 per sample per analyte
4. Unassigned Shellfish Toxin analysis- $500 per sample per analyte
5. Unassigned Shellfish Microplastics Analysis- $2000 per sample