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

Sediment Quality Assessment Field Operations Manual

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I. INTRODUCTION

Background

The Southern California Bight Pilot Project (SCBPP) was conducted in 1994 to begin addressing regional monitoring concerns. This project was the largest regional survey of environmental conditions on the mainland shelf in the Southern California Bight (SCB). It capitalized on the interest and cooperation existing in southern California and the resources available in current monitoring programs to develop an integrated and coordinated regional monitoring program that addressed the needs of the participating local, state, and federal agencies, and provided new management information. When completed, the SCBPP provided a first "snapshot" of the state of the SCB. Twelve participating agencies sampled 261 sites on the mainland shelf, which amassed a series of datasets that provided an unprecedented assessment of pollutant exposure, the status of biological resources, species diversity, and the presence of marine debris in the SCB.

Based on the initial success of the pilot program, another cooperative effort was scheduled to take place four years later. The Bight 1998 program continued the development of regional scale management information and followed the general plan of the SCBPP. Sixty-four organizations participated in the effort and the number of sites sampled grew to 416. New indicators were incorporated into the study, and the strata were expanded to include San Diego Bay, Catalina Island, the Northern Channel Islands, and historically sampled reference sites. Five years later, Bight 2003 continued to build on the cooperative interaction developed during the previous surveys. A total of 58 organizations were involved and a total of 388 sites were sampled. New strata were surveyed to include coastal estuaries, the upper continental slope (200-500 m), and the lower slope and inner basin (500-1000 m), using more parameters and new sampling methods. A fourth program, Bight 2008, took place five years later. Sixty-one organizations participated in the effort, sampling a variety of constituents at 383 sites located between Point Conception and the United States/Mexico border, including the newly added contaminants of emerging concern. The fifth survey, the 2013 Southern California Bight Regional Marine Monitoring Program (Bight'13), was comprised of 34 organizations that sampled 397 sites between Point Conception and the United States/Mexico border, and included submarine canyons and marine protected areas.

The Bight 2018 (Bight'18) survey will continue the cooperative trend developed during the prior surveys by involving approximately 46 organizations that will either participate in the field collections, or contribute resources and knowledge towards sampling and processing the data from over 450 sites. For this survey, effort has been dropped from submarine canyons and MPAs. Effort was added to a bioaccumulation survey and a new stratum, brackish estuaries. As in the former surveys, Bight'18 will attempt to quantify the general condition of the benthos and the health of key marine resources in the region. To accomplish this goal, Bight'18 will focus on three objectives: 1) estimate the extent, magnitude, and temporal sediment quality impacts in the SCB; 2) determine the extent, magnitude, and temporal ecological changes in the SCB; and 3) determine the extent and magnitude of bioaccumulation in selected sport/commercial fishes within the SCB.

The Bight'18 summer sampling will be conducted from July 1 through September 30, 2018. The purpose of this document is to provide detailed instructions on trawl and benthic field sampling methods that will be used to conduct this study.

II. OVERVIEW OF FIELD SURVEY

A. Sampling Period

The index period for the Bight'18 study will extend from July 1 to September 30, 2018.

B. Sampling Design

The Bight'18 study will continue to use a probability-based sampling design developed by EPA's Environmental Monitoring & Assessment Program (EMAP) that combines the strengths of systematic and random sampling (Stevens 2004). This Generalized Random Tessellated Stratified (GRTS) sampling design creates a spatially balanced random sampling of resources. Although sites were selected randomly, a systematic component was added to the selection process to minimize clustering of sample sites using a 200-meter radial exclusion zone from other randomly selected site. Some areas had intensified sampling which used smaller hexagonal grids and adjustments were made to their assigned inclusion probabilities to prevent weighting bias. To assess temporal trends, approximately 50% of the Bight'18 samples will be new sites while 25% of the sample sites will be from Bight'98 and 25 % from Bight'03. No Bight'08 or Bight'13 sites will be revisited. See the Bight'18 Sediment Quality Assessment Workplan (2018) for further details.

Bight'18 has identified 11 different strata of stations that will be sampled in this survey. These strata are classified as follows: inner shelf (5-30 m), mid shelf (30-120 m), outer shelf (120-200 m), upper slope (200-500 m), lower slope (500-1000 m), Northern Channel Islands, marinas, ports, bays, estuaries (salinity greater than 27 ppt), and new to the Bight program, brackish estuaries (salinity less than 27 ppt).

C. Indicators of Ecosystem Health

The primary goal of Bight'18 is to provide an assessment of the overall ecosystem condition of the SCB. To accomplish this goal, the following indicators of ecosystem health will be examined:

- Benthic - sediment characteristics, sediment contamination, infaunal assemblages, and sediment toxicity;
- Demersal fish and invertebrate assemblages and gross fish pathology;
- Marine debris (including plastic, lumber, vegetation, glass, etc.).

III. DESCRIPTION OF FIELD TEAMS AND ACTIVITIES

A. Personnel

All field sampling will be conducted by personnel knowledgeable in safe field sampling methodologies (*e.g.*, benthic sampling, trawling, etc.). Teams of field personnel will be on each research vessel participating in the sampling effort. These groups will vary in size depending on which organization is doing the field sampling. The main requirements are that the personnel on board the vessel:

- Have the knowledge and experience necessary for working with different types of sampling devices;
- Have the knowledge and experience necessary for conducting the field collection and analysis of benthic invertebrates and sediments, and trawl-caught demersal fish and megabenthic invertebrates;
- Can troubleshoot problems when they arise.

B. Chain-of-Command

The following chain-of-command is recommended to avoid confusion, identify responsible parties, and ensure that proper sampling protocols and information flow are followed by each organization:

1) The Lead Scientist will be an organization's primary contact regarding all survey and field-related matters;

2) The Boat Captain will not only be responsible for piloting the sampling vessel each day, but will also have the sole authority to cease or continue sampling operations when conditions at sea are judged to be unsafe;

3) The Cruise Leader, designated prior to each sampling day, will be responsible for supervising the scientific crew and sampling operations aboard a sampling vessel. This person will have the final decision on whether to abandon or sample a station, and will be responsible for assuring the quality of the data. At the end of each sampling day, this person will make sure that all field data and samples are delivered to the appropriate processing personnel in a timely manner. Cruise Leaders are not required to be the same person from field day to field day;

4) Significant changes to the established logistical plan that are outside of the jurisdiction of the Lead Scientist will be communicated to the Regional Monitoring Coordinator (Karen McLaughlin), or the Project Manager (Ken Schiff) before any change is implemented. The teams will accept technical direction from no other authority. All changes to the sampling plan that occur during the field surveys must be documented;

5) All technical matters, such as questions regarding station locations, major sampling

schedules changes, etc., will be discussed between the Regional Monitoring Coordinator and the Lead Scientist, **AS SOON AS POSSIBLE, to address and resolve issues. Specific sampling (how-to) and equipment issues should be addressed by the chairs of the Field Sampling/Logistics Committee. The Toxicity Committee chair or her designee may request delays of field teams to accommodate overloaded laboratories and minimize holding time issues;**

6) On the day of a field audit, the auditor and Cruise Leader will discuss any procedural and/or taxonomic issues observed during field operations. Additional concerns may be communicated to the Lead Scientist by the Lead Field QA/QC Auditor. The Lead Scientist will be expected to take the appropriate action to correct the situation as soon as possible.

C. Station Assignments

The study area of the Southern California Bight will be divided among the participating organizations according to the level of effort contributed by each. The number of stations to be sampled by each organization, with associated lab effort are summarized in Table 1. See Bight'18 Sediment Quality Assessment (SQA) Workplan for details on contributed lab effort. Maps and coordinates of the stations to be sampled by each organization are provided in Appendices A and B, respectively. Lab assignments by stations numbers are found on Appendix C. An interactive map to zoom into select areas is available at:

https://gis.sccwrp.org/arcgis/apps/webappviewer/index.html?id=8476b5a6697b47c090352879fcccbe3c

TABLE 1. Number of stations (by sample type) to be sampled by organizations participating in the Bight'18 study, summer 2018.

					Sediment Toxicity	
Field Organization	Trawl Sites	Grab Sites	Benthic Infauna	Sediment Chemistry	*Eohaustorius*	*Mytilus*
CLAEMD	26	32	32	32	19	15
LACSD	20	34	34	34	2	0
OCSD	20	34	34	34	14	6
CSD	17	43	43	43	25	0
Oxnard/ABC	29	29	29	29	9	5
NOAA/SCCWRP	0	15	15	15	15	0
Power Plants/MBC	10	27	27	27	18	15
POLA/POLB	0	3	3	3	3	3
RHMP	17	75	75	75	75	75
SD Stormwater	0	16	16	16	16	16
SDC Stormwater	0	3	3	3	3	3
OC Public Works	0	15	15	15	15	15
LAC Public Works	0	5	5	5	5	5

RMC	0	22	22	22	22	22
SDC/San Luis Rey	0	1	1	1	1	1
RCFC&WCD	0	2	2	2	2	2
US Navy	0	16	16	16	16	16
Dominguez Channel WS	0	2	2	2	2	2
Unassigned	0	7	0	0	0	0
Totals	139	381	374	374	262	201

ORGANIZATION CODES

CLAEMD	City of Los Angeles, Environmental Monitoring Division
Dominguez Channel Watershed	Dominguez Channel Watershed Management Group
LACSD	Los Angeles County Sanitation Districts
OCSD	Orange County Sanitation Districts
OXNARD/ABC	City of Oxnard contracting Aquatic Bioassay and Consulting
NOAA/SCCWRP	National Oceanic Atmospheric Administration partnering with Southern California Coastal Water Research Project
Power Plants/MBC	Power Plants contracting MBC Applied Environmental Sciences
POLA/POLB	Port of Los Angeles and Port of Long Beach
RCFC&WCD	Riverside County Flood Control and Water Conservation District
RHMP	Regional Harbor Monitoring Program
RMC	Greater Los Angeles and Long Beach Harbor Waters Regional Monitoring Coalition
SD Stormwater	City of San Diego Stormwater Group
SD County Stormwater	San Diego County Stormwater Group and Municipal Co-permittees
OC Public Works	Orange County Public Works
LAC Public Works	Los Angeles County Department of Public Works
SDC/San Luis Rey	San Diego County and the San Luis Rey watershed group
US Navy	U.S. Navy

D. Equipment

All groups or organizations involved in the sampling program will provide their own research vessel, crew, Van Veen grab, otter trawl, and any other equipment necessary to complete the sampling assignment. A list of equipment used during the survey and characteristics of each vessel are provided in Appendix D and E, respectively.

Grab Sampler

Each organization will have a minimum of two modified Van Veen grab samplers for offshore stations. Grab specifications are given in Section 8. In addition, organizations sampling brackish estuaries will have a minimum of two plastic corers and extension poles. Core construction information is found in Appendix L.

Trawl Nets

Each organization will have a sufficient number of 7.6 m (headrope) trawl nets and sets of otter boards (doors) available. Net and door specifications are given in Section 9.

Mobile Phones

Mobile phones are required to facilitate communication between the Cruise Leader on the sampling vessels and land based Bight'18 project personnel. Vessel mobile telephone numbers are listed in Appendix E.

E. Weekly Communications

Representatives from each participating organization will be required to provide SCCWRP with weekly, if not more frequent schedules, of proposed sampling activities prior to conducting operations in the field. A calendar (http://data.sccwrp.org/calendar/index.php) has been set up with an instruction button for schedule entry and edits (changes). This notification will include targeted sample types (sediment, trawl, etc.), and station(s) where sampling is expected to occur. The calendar is not setup for a range of dates, so give expected site visits for any given date. Up-to-date information is critical for toxicology lab sample coordination. The toxicology lab sample coordinator may contact Lead Scientists regarding delays if laboratories are overwhelmed. Field QA/QC Auditors will also use this information to schedule when they can conduct field audits for a particular organization. Prior to a QA/QC audit, the auditor will contact a Lead Scientist to verify that their proposed schedule is still in place.

Each organization will at a minimum also be required to make weekly electronic submissions of - station occupation and event information (*i.e.*, grab and trawl) regarding success and failures sampling sites. This information will be used to verify that each field team is accurately and completely sampling each station, and track the overall progress of the project.

F. Important Telephone Numbers

The names and phone numbers of appropriate personnel and emergency services are listed in

Section 13 and Appendix J. If an individual cannot be reached at the listed number, the caller should contact SCCWRP, where an attempt will be made to provide an alternate means by which the individual can be reached.

IV. SAFETY

Sample collection at sea is inherently hazardous and this danger is greatly compounded in bad weather. Thus, the safety of the crews and equipment is of paramount importance throughout the project. Each person working onboard a vessel during the project should take personal responsibility for their own safety. Bight 18 organizers strongly encourage field sampling teams to closely monitor weather conditions while out sampling in the field and always secure any equipment on deck. The Lead Scientist should ensure all crew members/biologists are aware of the task at hand for the day and are comfortable using sampling equipment.

Many accidents at sea are preventable. Safety awareness by the Boat Captain and all crew members is the greatest single factor that will reduce accidents at sea. Each field crew should follow all established rules and provisions within their respective organization's safety program. Sampling should be canceled or postponed during hazardous weather conditions. The final decision shall be made by the Boat Captain, who is responsible for the safety of everyone onboard. As with any field program, the priority is the safety of the people onboard, followed by the safety of the equipment, and then the recovery of the data.

V. QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES

A. Protocol Calibration/Quality Assurance Procedures

The Bight'18 survey will be conducted cooperatively by a number of organizations which routinely monitor the marine environment according to established protocols. It is important to the success of the Bight'18 study that comparable data are collected by each organization. This SQA Field Operations Manual will provide information on how field operations will be conducted to meet this requirement. The Lead Scientists and Boat Captains will be instructed on the field procedures to be followed during the survey and they, in turn, will instruct their field personnel on the proper procedures for the survey.

The Lead Scientist of each organization is responsible for distributing the Bight'18 SQA Field Operations Manual to all field personnel and ensuring that their staff understands and uses the protocols detailed in the manual.

Lead Scientist/Boat Captain Protocol Orientation Meeting

Lead Scientists and Boat Captains of all organizations participating in the survey are required to attend the protocol calibration meeting scheduled for June 21, 2018. The goals and objectives of the project will be discussed at this meeting, as will the responsibilities of the Bight'18 field personnel. Each Lead Scientist participating will be provided with a Bight'18 SQA Workplan and SQA Field Operations Manual. Participants will be instructed on field procedures. The discussion will also include instruction on proper data entry into the field computers and on field data forms. The meeting will emphasize decision-making procedures for determining station and/or sample acceptability, and the conditions that must be met before a station is abandoned. Lines of communication within the project and QA/QC activities occurring on the boat during the survey will also be discussed.

Scientific Team Training

The Lead Scientist from each organization will be responsible for ensuring that their field personnel have been trained properly on all field methods and procedures that will be used during the survey. It will be their responsibility to review the SQA documents (Workplan and Field Operations Manual) with their field crews, and to make sure that each person understands that these procedures must be followed during the survey. Personnel that cannot perform a required operation will not participate in conducting that operation.

Benthic Sampling (See Section 8)

The participation of several different vessels and field sampling teams in Bight'18 requires that uniform procedures be followed in the field to ensure high quality samples and consistent data. All field personnel will be provided with and are expected to have a working knowledge of the Bight'18 SQA Field Operations Manual. The Lead Scientist of each organization will provide the necessary training to ensure their staff fully understands and uses the protocols as detailed in the manual. All participants are expected to understand and properly carry out all steps in the collection, screening, relaxation, and fixation of infaunal samples. They must also understand the

techniques related to the collection and handling of sediment chemistry and toxicity samples.

Field audits will be conducted to ascertain an organization's field sampling capability and their adherence to standard Bight'18 protocols. These audits will be conducted by Field QA/QC Auditors. A QA/QC audit will be completed for each organization, when possible, with priorities going to those who are new to the regional survey or have undergone a significant turnover in personnel since previous surveys. Pre-survey audits are acceptable for organizations that use Bight survey protocols as their normal monitoring procedure. Field QA/QC Auditors can request additional field audits at any time and the subject organization is obligated to arrange and allow access to field crews.

The goal of the Bight'18 survey is to collect the full range of predesignated samples at all sites. The Measurement Quality Objective (MQO) of 90% which had been established for completeness for the collection of samples in earlier surveys will apply to the current effort. This completeness goal was established to derive the maximum statistical power of the sampling design and was not set at 100% in recognition that some sites will be difficult, if not impossible to sample. Nevertheless, field crews are expected to strive to collect samples at 100% of the stations.

Trawl Sampling (See Section 9)

Demersal fish and invertebrate assemblage data (species identification, enumeration, biomass, and length) are greatly influenced by the collection methods. Therefore, strict adherence to prescribed sampling protocols is critical. Fish catches are influenced by gear type, deployment, towing speed, tow duration, and method of retrieval. All organizations collecting samples in the field must use standard nets and follow standard trawling procedures to ensure comparable samples are collected. Field personnel will be provided with and are expected to have a working knowledge of the Bight'18 SQA Field Operations Manual. The Lead Scientist of each organization will provide the necessary training to ensure their staff fully understands and uses the protocols as detailed in the manual.

Several QA/QC activities will help to ensure the quality of the trawl survey data. These include intercalibration cruises, checks of equipment, sample processing, and taxonomic identification. Trawl equipment, deployment, and sample processing protocols will be checked during audits. The Field QA/QC Auditors will ensure that the methods used are those prescribed in the Field Operations Manual.

Pre-survey audits will be conducted, when possible, for those organizations who have been consistent participants in past surveys, who have adopted Bight protocols in their normal operations and who have not undergone a significant turnover in field personnel since the last survey. These audits will permit the Field QA/AC Auditors more time to evaluate field teams with less project-related experience and re-visit as necessary.

Prior to initiating the field checks, each organization will submit complete inventories and dimensions of their field equipment to SCCWRP. That information will be forwarded to auditing teams to assist in their QA/QC evaluations on adherence to procedures and protocols outlined in the field manual. Audit data will be recorded on a Field QA/QC Checklist (Appendix I). Any significant deviations will be noted and reported to the crew and the organization. If left

uncorrected, that data could be flagged for QA/QC deficiencies.

During a field audit, the Field QA/QC Auditor will inventory equipment and ensure that an organization conducts trawling operations in the manner outlined in the manual, and that the appropriate information is recorded on a data sheets (Appendix I QAQC Audit Form). The Auditor will make sure that: 1) the appropriate processing equipment is onboard a vessel; 2) the scales are calibrated at the start of the day; 3) the net is rigged properly; 4) the trawl is deployed and retrieved properly; 5) the catch is properly processed; 6) the appropriate data are recorded and 7) that the pressure-temperature sensor has been used to record trawl bottom time. The Lead Scientist will be notified of the field audit results so that any problems can be addressed and corrected.

Lead Bight'18 fish and invertebrate taxonomists will be designated prior to the sampling period. In addition, each organization will identify a Lead fish and invertebrate taxonomists for their respective agency. These individuals must have the required expertise in field identification of trawl-caught fishes and/or invertebrates of coastal southern California in depths ranging between 5-500 m. They will be responsible for providing accurate identifications of species collected during the survey and will complete/oversee a review of the voucher collections before they are shipped to SCCWRP.

While it is expected that the lead taxonomists of each organization will have a wide range of knowledge of the common trawl caught species, it is not expected that all persons making field identifications will know every species. *It is, therefore, very important to avoid guessing when finalizing any identification*. An error made in the identification of an organism may result in an irretrievable error in the database because most of the organisms that are identified in the field are returned to the sea. If there is any question regarding the identity of a specimen, that specimen shall be returned to the laboratory for final identification. Once the final identity of any specimen has been ascertained in the organization's laboratory, that change will be made on either the trawl fish, or the invertebrate species sheets by crossing out the original name (do not erase the original name) and writing the correct name. Conversely, if it has been determined that a species cannot be identified at the organization's laboratory, the specimen will be sent to SCCWRP for identification.

Three QA/QC activities will help to ensure accurate taxonomic identification of fishes and invertebrates by providing training and intercalibration among organizations:

1) Prior to the survey, a list of recommended taxonomic identification aids will be distributed to participating organizations. Lists of trawl-caught fish and invertebrate species for southern California will also be distributed. A reference collection of voucher specimens of species collected during former Bight surveys is available at the Natural History Museum of Los Angeles County for individuals wishing to see species likely to be encountered in Bight'18. In addition, it is recommended (but not required) that field taxonomists attend Southern California Association of Marine Invertebrate Taxonomists (SCAMIT) and Southern California Association of Ichthyological Taxonomists and Ecologists (SCAITE) meetings and the pre-survey information transfer meetings given at SCCWRP on the identification of expected trawl species;

2) Lead Taxonomists from every field sampling organization will be required to participate in at least one pre-survey intercalibration cruise to ensure that identifications of commonly occurring species are standardized;

3) Lead Taxonomists from each organization will also be required to participate in another pre-survey intercalibration exercise meant to assess the probability of taxonomic error in the field. In this exercise, a bucket of fish and a bucket of invertebrates will be passed among all participating organizations prior to the survey. The taxonomists will identify specimens of representative trawl-caught species in each bucket to the lowest taxon possible. A numbered tag will be attached to each organism so that the identifications can be checked against the correct specimens. This exercise will focus on identification errors. Correct identifications or "Return for Further Identification" (FID) are acceptable. FID indicates that the specimen would have been returned to the laboratory (where additional information or expertise can be found) for final identification. Organizations with more than 10% misidentifications (fish and invertebrates separately) will redo the exercise with a different bucket of organisms. If an organization cannot meet this requirement on the second or third attempt, a qualified taxonomist from another organization must be on board when trawl sampling is conducted.

Measurement Quality Objectives (MQOs) for the trawl fish and invertebrate sampling effort are defined in terms of accuracy, precision, and completeness. Acceptability criteria have been established for trawl sample collections. The goal of the Bight'18 trawl survey is to collect samples at all designated trawl stations to identify all of the organisms correctly, and to obtain accurate counts, measurements, and weights on all species. However, the MQOs will be set at lower values in recognition of the realities of field sampling. Because some stations may occur on rocky bottom, the MQOs for the study completeness objective for trawl sample collection will be 90%. Of the samples collected, 100% will be processed, identified, counted, measured, and weighed. Accuracy and precision expectations for the crew performance are 90% for identification, counting, lengths, and biomass (± 0.2 kg) and 95% for anomalies.

VI. FIELD COMPUTER

A. General Requirements

A field computer will be used whenever possible to collect station occupation/visual observation data during the Bight'18 survey. Sampling organizations have the discretion to use their own field computer system. If a field computer cannot be used, all required sampling event information must be recorded on Bight'18 field data sheets and subsequently loaded into Microsoft Excel data files for submission to the Bight'18 Information Manager. Data submission formats and standards are described in the Bight'18 Information Management (IM) Manual and general Bight'18 field web portal or landing page, http://bight-sccwrp.opendata.arcgis.com/pages/bight-2018-field. The EXCEL templates can be downloaded from the SCCWRP web site under Data/DataTools/Bight18FieldDataTemplates.

Bight'18 Field Data System Version 1.0

For those organizations not using their own computer system, a new field data acquisition application has been developed by SCCWRP. It is based on an ESRI system with a completely new look and feel. Available options can be found at http://bight-sccwrp.opendata.arcgis.com/pages/bight-2018-field. This system facilitates the collection of all the required station occupation and field sampling event information (*e.g.*, grab and trawl sampling events). SCCWRPs new system has been designed to be used on Android tablet/phones, iPad/iPhone, and PC laptop computers. The app should be intuitive (instruction sheet can be sent via email). Use of the Bight'18 Field Data System is optional during the survey. Survey123 app can be downloaded from ESRI and the SCCWRP portal must be added to the app to download survey dataforms.

- Runs in Android, iOS, and Windows environments;
- Employs drop-down lists or radial button selections of acceptable values in many entry fields, which reduce entry time and assures accuracy and compliance with Bight'18 data standards;
- Data is stored locally as a Postgres database that links the web portal. The data file can be exported for field organizations' internal use;
- Capable of being used as simple data entry system for information collected at sea on paper field sampling data sheets, or may be used as a primary data collection tool.

Bight'18 Field Data Submissions

Web portal. The preferred method of data submission is through SCCWRP's online data submission page. This page can be found at: http://bight-sccwrp.opendata.arcgis.com/pages/bight-2018-field. The system requires that files be submitted as Microsoft Excel spreadsheets with specific tab names and field names (see table structures in the Bight'18 SQA IM Manual or instructions on the web portal). No csv files will be accepted. Specific questions regarding how-to instructions for data entry should be directed to the Trawl Committee.

Web portal data checker. A Python-base program checks for appropriate parameter ranges, required fields, valid values from constrained look-up lists, and proper formatting/adherence to

Standard Data Transfer Protocols (SDTPs) described in Bight'18 SQA IM Manual or on the web portal. Spelling, punctuation, and proper formatting are extremely important. For example, improper capital letter, additional characters (*i.e.*, spaces, underscores), character data in numerical fields, inputted values into fields constrained by a list, or omitting fields that require a value will generate an error that needs fixing. In addition, there may be QA calculations done on the data to look for outliers which generate warnings but meet IM checks.

VII. SAMPLING LOGISTICS

A. Navigation

Accurate location of sampling sites is crucial to the success of the Bight'18 survey. Station maps and coordinates (latitude and longitude) are provided in Appendices A and B. Vessel positioning will be determined by means of a Differential Global Positioning System (DGPS) or Wide Area Augmentation System (WAAS). If, during a field-sampling day, the differential signal is interrupted or lost, sampling may continue using standard GPS. If a vessel with an integrated GPS is not available to work within the four types of inner coastal strata, using a hand-held device is an acceptable substitute.

B. Sampling Schedule

The benthic and trawl surveys may begin July 1, 2018. All field work may be completed in the order that each organization sees fit, as long as the survey is completed by September 30, 2018.

All grab samples will be collected between sunrise and sunset, except for sediment chemistry and sediment toxicity; those samples may be collected anytime throughout the 24-hour period. Otter trawl samples must be collected between one hour after sunrise and one hour before sunset.

C. Station Types

Stations located within the eleven strata will be sampled during the survey. These strata are classified as follows: Inner Shelf (5-30 m), mid-shelf (30-120 m), outer shelf (120-200 m), upper slope (200-500 m), lower slope (500-1000 m), Northern Channel Islands, marinas, ports, bays, estuaries, and brackish estuaries.

The project sampling station/stratum information is listed in Appendix B. If relocating a station moves the station into a different sampling stratum, the station will be abandoned and a new replacement site within your region will be assigned. Note in the comments section of the field data sheet the reason for abandonment.

D. Site Acceptability Criteria

The location of each sampling site will be designated in advance as a set of coordinates (latitude and longitude). Upon arrival at the site, the depth will be determined by fathometer and recorded prior to sampling, as well as a validation coordinate. This will be regarded as the target depth for all subsequent sampling at the site during the survey and will be used for determining site acceptability. While all sites are single points defined by latitude and longitude, occupation within a specified distance (*i.e.*, the radius limit) of the target coordinates will be considered acceptable. This radius limit will be 100 m for all sites except those within the island strata. The radius limit at the Northern Channel Islands will be 200 m because of the known extent of rocky bottoms in the area.

Sampling may not be possible at some sites for a variety of reasons (*e.g.*, kelp beds, rocky bottom, falling outside depth range of stratum, otherwise obstructed or unapproachable, etc.) Sites may be abandoned if they fail to meet site acceptability criteria, or if samples at the site fail to meet

sample acceptance criteria. The criteria and process guiding this assessment are described below and summarized as a decision tree in Figure 1 (benthic sites) and Figure 2 (trawl sites).

1) Occupy the target coordinates as closely as possible.

2) If occupation is not possible within the radius limit due to physical obstructions (*e.g.*, harbor facilities), or access prohibitions (*e.g.,* harbor security closures), or if the site target coordinates fall on land, abandon the site and record the reason for abandonment in the field computer or on a field data sheet. Sites with temporary obstructions (*e.g.*, moored vessel) should be revisited and sampled when the area has been vacated. If the station cannot be sampled due to an extended period of occupation, note the justification on the data sheet and abandon the site.

3) For benthic sites, if occupation is possible but the target coordinates lie over unsuitable substrate or the site is physically obstructed (*e.g.*, dock, vessel, rocky reef or kelp bed, is beyond the depth limits of the survey, is beyond the capability of a sampling vessel, etc.) as determined by visual observation and fathometer survey, attempt to find an acceptable occupation within the radius limit and record target depth. If unsuccessful, check at least one other site. If an acceptable occupation is not possible, abandon the site and record the reason for abandonment on the field computer, or on a field data sheet. If intermittent success is achieved, a minimum of 9 attempts at stations <500 m is required before abandoning the site. The Cruise Leader can choose to continue sampling beyond the minimum limit if it is decided the effort is warranted.

4) For trawl sites, occupy the station location and record the depth before conducting a pre-trawl fathometer survey. The survey should then be conducted to determine if the site can be sampled within the radius limits. If that survey identifies unacceptable substrate or if the site is deemed otherwise unsuitable for trawling by the Cruise Leader, the site should be abandoned.

5) If an acceptable occupation is possible, proceed with sampling.

6) Sample acceptance criteria are described for benthic sampling in Section 8 and for trawling in Section 9 and are summarized in the decision tree Figures 1 and 2.

E. Site Rejection Strategy

A sampling site may be rejected if any of the following occurs:

1) If the location places the site on land or in an obviously unsuitable location.

2) If the site exceeds or falls below the depth boundaries defined by the strata (*e.g.*, inner shelf 5-30 m, mid shelf 30-120 m, outer shelf 120-200 m, upper slope 200-500 m, lower slope 500-1000 m). Another depth related rejection strategy for grabs and trawls are changes of +/-10 % to the established station occupation depth. Safety related rejection strategies are depths less than 6 m in coastal ocean, 3 m in embayment, and 1 m in estuaries (main channels). The brackish estuary stratum has no limit set because

sites could be wadable so field crews must decide on safety concerns during sampling.

3) Two strata have salinity criteria: estuaries and brackish estuaries. Salinity is measured near the sediment/water interface. The definition for brackish estuaries is 0 – 27 ppt. The definition for estuaries is > 27 ppt. If salinity measurements do not meet these criteria, the site is abandoned. Note salinity calculated from conductivity is unitless, ppt and psu can be used interchangeably.

4) For benthic sites <500 m, if suitable substrate cannot be found after three grabs at the nominal location, and up to three attempts at a second and third location, the station will be abandoned. For benthic sites >500 m, 3 attempts at 2 locations for a total of 6 unsuccessful attempts. Field crews have the option to attempt more grabs. Adequately record the reason(s) for abandonment in the field computer, or on the field data sheet.

5) For trawl sites, if the fathometer survey identifies unsuitable substrate at three locations within the radius limit, if any equipment is lost or damaged, or if the site is deemed unsuitable by the Cruise Leader, the site will be abandoned completely. Adequately record the reason(s) for abandonment in the field computer, or on the field data sheet.

F. California Department of Fish and Wildlife Scientific Collecting Permits

Each organization is responsible for procuring their own permits. Prior to collecting fish and invertebrate specimens in the field, each organization must fax or email a copy of the Notification of Intent to Collect for Scientific Purposes form to the Marine Region (Monterey, CA) office of the California Department of Fish and Wildlife (CDFW) a minimum or 24 hours (business day only) prior to any collection activity.

This form can be found at https://www.wildlife.ca.gov/Licensing/Scientific-Collecting.

Individual permit holders and their permit must be onboard during sampling, and it must be presented to any CDFW warden, or personnel who request to see it. In the case of entity permits, the permit holder does not need to be present, but the permit (or reasonable facsimile) must be onboard the vessel. The phone and fax numbers of the local offices of the CDFW are listed in the next section.

G. Contact Information

It is recommended that all groups conducting field work in harbors, ports, and marinas contact local security prior to attempting fieldwork in the area. Prior experience suggests that you contact the security several days prior to the work through their central numbers, then again on the day of operations, through dispatch if possible. Have an idea of where you will be working and when, and note the names and date on which you called the security agency. If you fax in information, have a copy with you in the field, and always have your collecting permit – security may never have seen one before, but it does help to be able to show a permit for the activities.

In the Port of LA, call the Wharfinger and Port Police. In the Port of Long Beach, call the Harbor Police and leave a message with the City Police. The Port Pilots have been included to notify

them of trawling operations and check traffic planning. It is very important in the Ports to notify the United States Coast Guard (USGS) Waterway Management of sampling plans, since the USCG is likely to be first to respond if you are reported.

It is also recommended that the USCG be informed of <u>all</u> nearshore sampling activity. USCG permission is needed to enter some security areas before sampling. Navy or Marine permission may also be needed.

MONTEREY

Dept. of Fish and Wildlife Marine Region	831- 649-2870	831-649-2894 (fax)

OXNARD/VENTURA/SANTA BARBARA

Dept. of Fish and Wildlife LA Region	562-342-7100	562-342-7139fax

US Coast Guard

Channel Islands Coast Guard	805-985-9822

Santa Barbara Harbor

Santa Barbara Harbor Patrol	805-564-5530

VENTURA HARBOR

Ventura Harbor Patrol	805-642-8538	805-642-8618 0600-0200hr
VHF radio channel 16 Ventura Lifeguards	805-648-3321	

Channel Islands Harbor

Channel Islands Harbor Patrol	805-382-3007	and	805-382-3001
Emergency line:	805-382-3000		
VHF radio channel:	16, 12 and 73		

Channel Islands Coast Guard	805-985-9822

Port Hueneme

Oxnard Harbor District	805-488-3677
Navy	805-982-4711

Mugu Lagoon

Pt. Mugu Security Dispatcher	805-989-7907	

SANTA MONICA/LOS ANGELES PORT/LONG BEACH PORT/ORANGE COUNTY

Dept. of Fish and Wildlife LA Region	562 342-7100	562-342-7139fax
USCG Waterway Management		
USCG LA Region	310-521-3860	310-521-3869fax
VTS	Channel 14	
In POLA/POLB Bridge to Bridge	Channel 13	
Santa Monica Bay Area		
Redondo Beach Harbor Patrol	310-318-0632	310-374-2286fax
Marina Del Rey Harbor Patrol	310-823-7762	
Manhattan/Hermosa Beach Lifeguards	310-372-2166	310-372-6902fax
Redondo Lifeguards	310-372-2162	
Los Angeles Harbor/POLA		
Los Angeles Wharfinger	310-732-3810	310-521-8917fax
LA Port Police	310-732-3491	310-831-3689fax
Los Angeles Pilot	310-732-3805	310-519-9189fax
notify and monitor on 73		
Los Angeles City Lifeguards	213-485-5162	
Marine Exchange	310-519-3134	310-241-0300fax
Long Beach Harbor/POLB		
Long Beach Wharfinger	562-590-4180	562-901-1731fax
Long Beach Police Dept. (leave msg if no ans.)	562-570-7182 msg	
LB Harbor Patrol Dispatch (On-Water)	562-283-7820	562-436-5590fax
Long Beach Pilots - Field office	562-432-0664	562-432-3597fax
notify and monitor on 12 and/or 74		
Long Beach Pilots - Main Office	562-435-5435	
ask for Capt. Strong or Capt. Jacobson		
City of Long Beach Police Dispatch	562-435-6711	
(San Gabriel River work notification)		

Long Beach Downtown Marina/Alamitos Bay

Long Beach Marine Patrol		
Non-emergency patrol dispatch	562-435-6711	562-570-3249fax
Administration	562-570-3245	0700-1700hr
E-Mail:	marinepatrol@longbeach.gov	

Orange County Harbors

Orange County Sheriff's Harbor Patrol Division

Sunset / Huntington Harbor	714-840-5222	
Newport Harbor	949-723-1002	
Dana Point Harbor	949-248-2222	
Seal Beach Lifeguards	562-431-3567	562-598-8560fax
Huntington Beach Lifeguards	714-536-1454	714-536-0074fax

SAN DIEGO REGION

Dept. of Fish and Wildlife

SD Region	858-467-4201	858-467-4299fax

US Coast Guard

USCG San Diego Region	619-683-6495

SONGS Area

SONGS Security Zone extends 1 nautical mile radius. See below.
Need authorization from SD USCG Captain of the Port to enter, transit, or anchor. Only SONGS Security can initiate the request for authorization.

Pendleton Area Marine Activity Exclusion Zones

Two restricted navigation areas have been established offshore of Camp Pendleton for military training and activities. The area between the downcoast mouth of the Santa Margarita River and the upcoast edge of the Oceanside Harbor breakwater, is a restricted area that extends 1,800 m offshore. Any activity in this restricted area that may endanger underwater installments such as anchoring, fishing, or trawling is prohibited at all times. Traffic may cross the area if the vessel maintains a direct route without delay. A second restricted area occurs north of the Santa Margarita River for most of the length of Camp Pendleton. This is a military exercise area, which cautions mariners of activity between 0600 and 2400 hrs

Oceanside Harbor

Harbor Police	760-435-4050

Mission Bay

Mission Bay Harbor Patrol	619-531-2000
Lifeguard Business Office	619-221-8899
Mission Bay Harbor Unit	619-221-8985

San Diego Bay

San Diego Bay Harbor Police	619-686-6272
Navy Base San Diego (NBSD) Patrol Operations	619-556-1442
Deputy Chief of Police	619-556-6662
Security Officer	619-556-6954

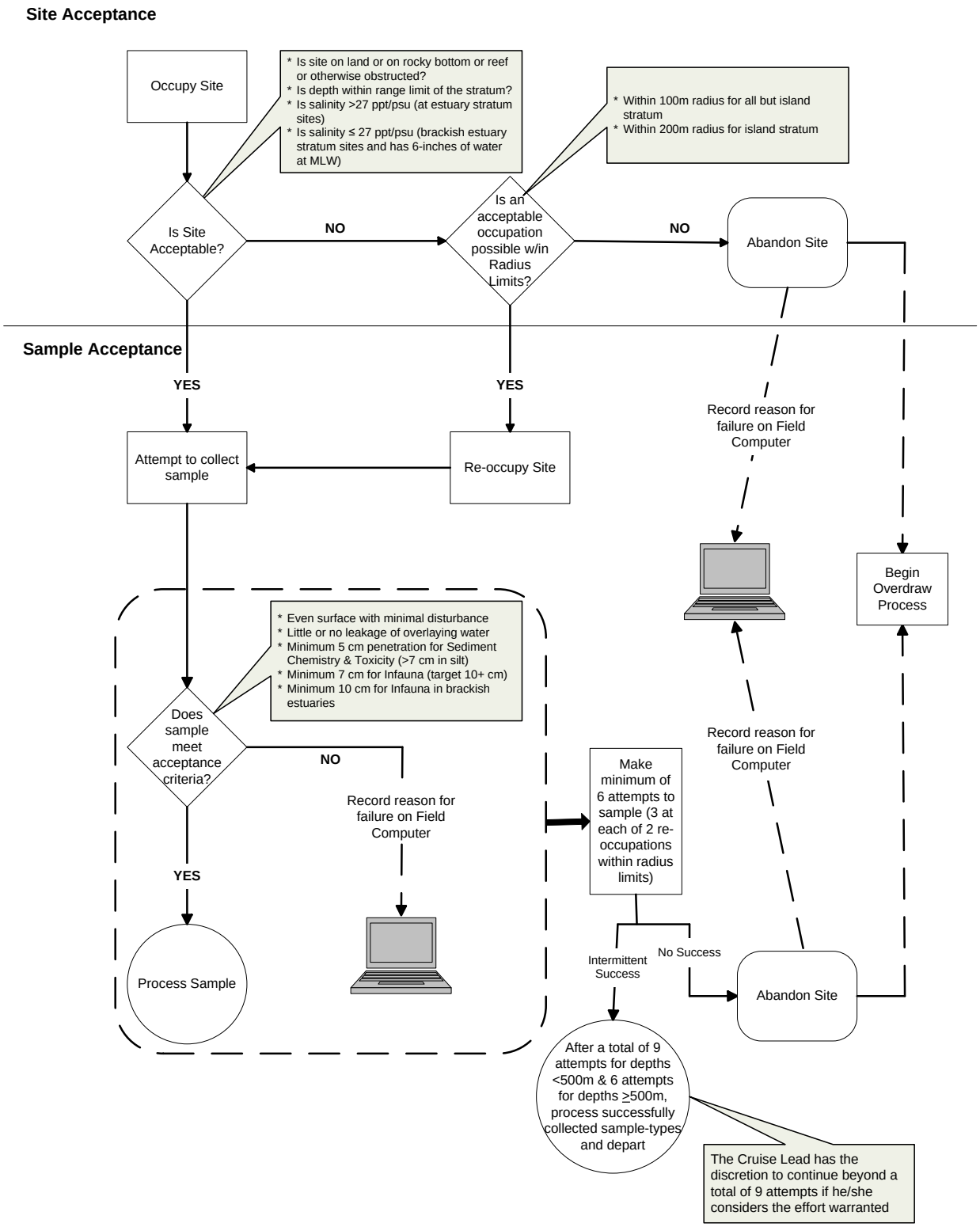

Figure 1. Benthic sampling site and sample acceptance process

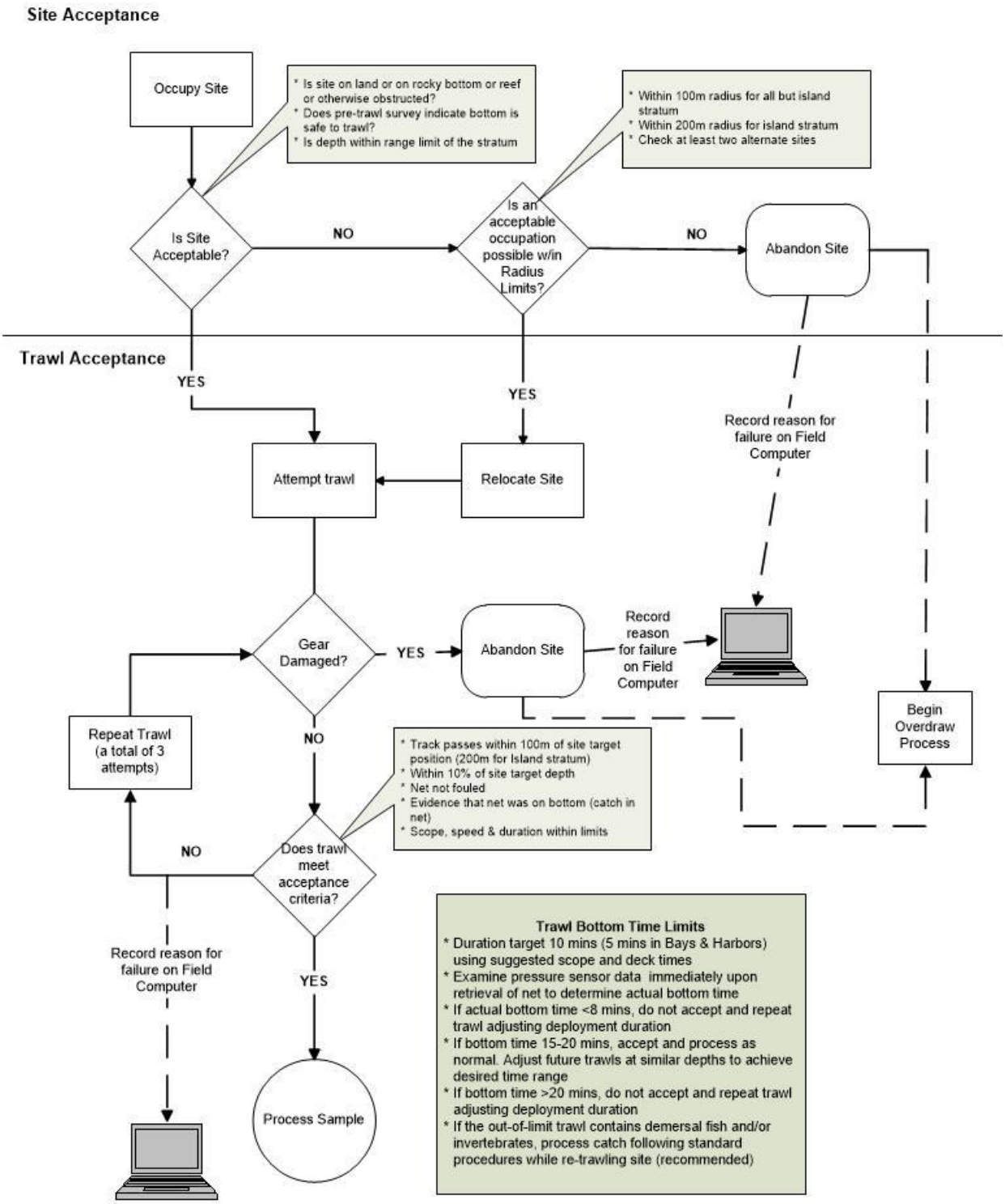

Figure 2. Trawl sampling site and sample acceptance process

VIII. BENTHIC SAMPLING

A. Purpose

The purpose of benthic sampling is to obtain data on localized community structure of infaunal invertebrate assemblages, the surrounding sediment chemistry characteristics and contaminant load from specific sampling sites. The pooled information is useful in determining not only the distribution, abundance and diversity of infaunal organisms, but also whether the observed community patterns have been influenced by environmental and/or anthropogenic perturbations. Cleaning procedures between sites are minimal because of potential contaminant and toxicity introduction from detergents currently on the market. Use best professional judgement if sites exhibit oily residue or other potential cross-contamination issues. Follow established EPA or SQO procedures.

A new brackish estuary stratum will be sampled as part of this survey. Benthic sediments from this stratum will continue to be collected using either a Van Veen grab, Ponar grab, or 4-inch plastic cores. All biology or infauna samples must be collected using a 4-inch core (Appendix L for construction details) and screened using a 0.5 mm sieve size. Chemistry samples can be collected using a light weight stainless-steel Petite Ponar, plastic core, or chemistry scoop. See chemistry sections for details.

B. Sampling Effort

A total of 381 benthic stations will be sampled during the survey. Table 1 and Appendices A and B provide information on the total number of stations and the parameters that will be sampled by each participating organization.

C. Sediment Samplers

Van Veen Grab. A 0.1 m^2 modified Van Veen grab will be used to collect sediment samples (optional in brackish estuaries) for physical, chemical, and infaunal analyses (Stubbs et al. 1987). This device is manufactured by a few vendors, which include the University of Washington, Jon Carr, and others. The grab may be constructed of galvanized, stainless, or Teflon-coated steel. All surfaces of the grab must be clean and free of rust. Either single or tandem Van Veen grabs are acceptable.

Petite Ponar. If a Petite Ponar is used for chemistry in the **brackish estuary** stratum, it must be stainless-steel because sediment touching the sides may get sampled. It is a miniature size of a Van Veen with small surface area and maximum penetrations of 7-8 cm with little or no room to scoop sediment from retractable doors. The inner surface of this small grab must be clean, free of residual sediment, and rust free.

Plastic Push Core. A 4-inch plastic core has been designated as the standard sample device for benthic infauna sampling in the **brackish estuary** stratum. It can be used for chemistry

sampling in the **brackish estuary stratum only**. The construction SOP can be found in Appendix L. The diameter of the core is standardized to the inner diameter (ID) of the tube. Biological samples must have a penetration depth on 10 cm.

D. Salinity Measurement at Estuary Sites

Water samples should be taken at or near the bottom (near the sediment/water interface). Use a Niskin sampler, other water sampling devices, or overlying water from the grab. It is recommended that a salinity meter be used to measure salinity in Parts Per Thousand (ppt) units. A conductivity meter (uS/cm) can be used, but temperature (°C) must also be recorded and the values converted to Practical Salinity Units (psu) through a formula in Standard Methods (1999). Allow the temperature to stabilize before recording values. Follow the steps outlined below:

1. Determine an expected reference Kcl conductivity (C) for the measured temperature (t)
   $C\ (Kcl) = d_0t^3 + d_1t^2 + d_2t + d_3$
   Where: d_0= -0.0267243, d_1= 4.6636947, d_2= 861.3027640, d_3= 29035.1640851
2. Determine the conductivity ratio from measured conductivity divided by reference C (Kcl)
   R = C (sample) / C (Kcl)
3. Determine Delta S for a reference temperature of 15°C
   $Delta\ S = ((t - 15 / (1 + 0.0162\ (t - 15))) (b_0 + b_1R^{1/2} + b_2R + b_3R^{3/2} + b_4R^2 + b_5R^{5/2})$
   Where: b_0= 0.0005, b_1= -0.0056, b_2= -0.0066, b_3= -0.0375, b_4= 0.0636, b_5= -0.0144
4. Determine Salinity for the measured temperature
   $S = a_0 + a_1R^{1/2} + a_2R + a_3R^{3/2} + a_4R^2 + a_5R^{5/2} + Delta\ S$
   Where: a_0= 0.0080, a_1= -0.1692, a_2= 25.3851, a_3= 14.0941, a_4= -7.0261, a_5= 2.7081

*Note: ppt/psu are historical unit references for salinity because calculations using a conductivity cell are unitless.

E. Special Brackish Estuary Sampling

Site requirement is 6-inches or more of estuary water at Mean Lower Low tide. If the site is on land or less than 6-inches of water, move to nearest main channel or deeper area but staying within 100 m of the assigned site. It is recommended that field teams do reconnaissance at the site close to MLLW, less than or equal to 0.5 ft on tide charts, and measure salinity near sediment interface to determine site acceptability (salinity 0-27 ppt). Unacceptable sites get abandoned and new overdraw site is assigned. If site is acceptable, field teams can revisit the site anytime afterwards to complete sampling. Infauna biological sampling will standardize on a 4-inch PVC push cores with minimum 10 cm penetration (Appendix L for construction SOP). Attach a pole extender to the core in intermediate water depths (Appendix L for construction SOP). Organizations can use a Van Veen in deep water but must subsample with 4-inch core. Take **two** core samples at each station for infauna and composite them into a single sample. Infauna samples will be screened using 0.5 mm sieve.

Chemistry sampling can use the 4-inch push core. Push the core 5 cm into sediment and dump into clean pan to remove overlying water then scoop into a Teflon bag. A Petite Ponar grab can also be used for chemistry. Ensure a minimum penetration of 5 cm, dump contents into clean pan

to remove overlying water than scoop into a Teflon bag.

F. Grab Sampling Procedures

Van Veen Grab

Prior to deployment, the grab is cocked with the safety key in place. The grab is then hoisted over the side, the safety key is removed. The grab is lowered at up to 2 m/sec until it is approximately 5 m above the bottom, then lowered at 1 m/sec to minimize the effects of bow wave disturbance of the surface sediment. In water depths greater than 300 m, the rate of deployment may have to be reduced to <1 m/sec to avoid "kiting" of the grab and/or premature tripping in the water column. After bottom contact has been made (indicated by slack in the winch wire), the tension on the wire is slowly increased, causing the lever arms to close the grab. Once the grab is back on board, the top doors are opened for inspection.

While a radius limit of 100 m (200 m for island strata) has been established for site occupancy, once sampling has begun, the Cruise Leader will ensure that the vessel is maintained on station with as much precision as conditions allow. Because analytical results from separate grab samples will be used to characterize the benthic community biointegrity, contaminant load and, in many cases, toxicity of the sediment, each successive grab must be collected as close as possible to the others.

***Brackish Estuary Sampling*.** A Van Veen may be used in brackish estuary sites. Biological samples must have penetration depths greater than 10 cm. A core must be inserted into the grab. Open the top doors and insert the plastic core. The core must be tapered to fit the tapered bottom of a Van Veen. Seal the rubber top on the core so no air enters through the top. A vacuum is created as someone removes the core from the grab. Cover the bottom if needed to retain the contents of the core. Two core samples will be taken from the Van Veen for infauna at each site.

A Petite Ponar is like a Van Veen grab, just a small version with low surface area and penetration capability. The jaws stay open with a spring-loaded pin that pops off once it hits the bottom and tension is relieved. Lowering is the same as described for the Van Veen above. If the Ponar has a sliding door, the opening is too small for standard scoops. Ensure a 5 cm penetration depth for sediment and dump into a clean pan to remove residual water.

The Push Core can be used in intermediate depths with an extension pole (Appendix L) or stand-alone in wadable sites. Insert the core into the sediment to the desired depth. Seal the rubber top on the core so no air enters through the top. The extension pole has centimeter marking as a guide and a one-way valve to seal the top. A vacuum is created as someone removes the core from the grab. Cover the bottom if needed to retain the contents of the core. Dump into a clean pan to remove residual water. For biological samples, dump directly into a 0.5 mm screening sieve. Two core samples will be taken for infauna at each site.

G. Priority of Grab Sampling

The priority of sampling at offshore sites are 1) infauna, 2) sediment chemistry, grain size, special studies and 3) sediment toxicity. Assigned sites may not have these three sample types. If it is impossible to obtain all assigned sample types required at a station, those samples successfully collected shall be processed and retained. The field crew has the discretion to return and complete sampling or abandon the site. Embayment sites that require both sediment chemistry and toxicity samples must collect sufficient sample for homogenization and distribution (up to 8 L). Only those samples meeting the sample acceptance criteria and sample volume requirements are considered to be successfully sampled.

H. Criteria for Acceptable Grab Samples

Site acceptance criteria and procedures are described in Section 7. Both site and sample acceptance criteria are summarized as a decision tree in Figure 1.

Once a site has been successfully occupied, grab sampling may still prove impossible or very difficult. Different sediment types (*e.g.*, cobble, gravel, well-sorted sands, etc.) and localities (*e.g.*, canyons, slopes, and rocky areas) may be difficult to sample. Sediments containing rocks often create the most common problem by preventing complete closure of the grab and allowing sediment to wash out during retrieval. The randomized sampling design may cause some of the Bight'18 sampling sites to occur on these difficult sediment types or localities. Therefore, if after three consecutive unsuccessful grab attempts at a site and up to three more consecutive unsuccessful attempts at two other locations (9 total attempts within the radius limit and +/-10% of the depth of the target site), the station should be abandoned, and the reason noted in the field computer or on a data sheet. Note: *if any grab was unsuccessful due to the result of mechanical (early closures, chain fouling, flipped grab, etc.) versus natural causes, it will not be included in the failure total and sampling should continue.*

If sampling success at a station is inconsistent, sites >500 m may be abandoned after a minimum of six (6) attempts at two locations. In this case, only the successfully (complete) collected sample types should be processed and retained.

These are the minimum efforts justifying site abandonment. Sampling failures due to operational error (*e.g.*, premature tripping) do not count towards this minimal effort. The Cruise Leader has the discretion to make a greater or lesser effort if he/she feels that it is warranted or equipment safety is a concern. The reason for site abandonment must be documented in the field computer or on the field data sheets.

Upon retrieval of the grab, the acceptability of the sample must be determined. Acceptability is based upon two characteristics of the sample: sample condition and depth of penetration. Sample condition is judged using criteria for surface disturbance, leakage, canting, and washing (Figure 3).

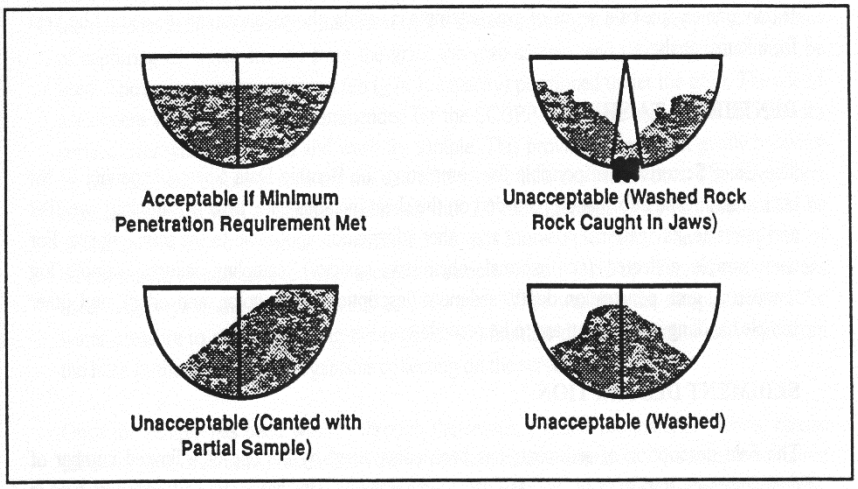

Figure 3. Examples of acceptable and unacceptable grab sample condition (from Tetra Tech 1986).

An acceptable sample condition is characterized by an even surface with minimal disturbance and little or no leakage of the overlying water. Heavily canted samples are unacceptable. Samples with a large amount of "humping" along the midline of the grab, which indicates washing of the sample during retrieval, are also unacceptable. While some humping will be evident in samples taken from firm sediment where penetration has been poor, this can be due to the closing action of the grab and is not necessarily evidence of unacceptable washing.

If the sample condition is acceptable, the overlying water is drained off and the depth of penetration determined by insertion of a plastic (rather than metal) ruler vertically along the grab midline and measuring to the nearest 0.5 cm. Sediment penetration depth for all grabs must be at least 5 cm. Penetration depths of 7-10+ cm should be obtained in silt (fine sand to clay) and whenever possible, infaunal samples should be a minimum of 7 cm but target 10+ cm. In habitats where sediments are unusually soft (*e.g.,* some estuary muds), it may be necessary to remove the lead weights to prevent over-topping the grab.

Extra caution should be taken to drain the overlying water from the grabs for chemistry and toxicity samples. It is recommended that a siphon be employed for these grabs to avoid disturbance and loss of the surface sediments. The overlying water in grabs intended for infaunal samples must be retained. Drain by slightly opening the jaws of the grab and allowing the water to run off into a tube or container for screening with the sediments (see Sample Processing below).

If both sample condition and penetration are acceptable in the first grab (*i.e.*, infauna) of offshore sites, sampling at the station will proceed with the collection of chemistry and then sediment toxicity samples from successive grabs. At embayment sites, sufficient volume must be collected to homogenize chemistry and toxicity samples in a Teflon bag before distribution. **It is required that all the grabs taken at a station be of similar sediment type (except at embayment sites) and depth penetration.**

I. Benthic Sampling Event Data

The Cruise Leader is responsible for collecting all the required information associated with each station occupation and each grab sampling event. While the Field Computer is the preferred method of collecting these data, paper data forms may be used (Appendix F). The required station occupation information includes:

- Station ID
- Date
- Time of day
- Agency code
- Collection Type
- Vessel name
- System used for navigation
- Weather and sea conditions
- Occupation Latitude and Longitude
- Target depth
- Salinity (at sites in the Estuary and Brackish Estuary strata)
- Station fail code (if site is abandoned)
- Comments

The required grab event information includes:

- Station ID
- Grab event number
- Gear
- Time of day for event (when grab on bottom)
- Latitude and Longitude at time of event (when grab on bottom)
- Depth of water (when grab hits bottom)
- Distance from station target location (when grab on bottom)
- Fail code (if sample fails to meet sample acceptance criteria, see Field Sheets or Information Management Plan for codes)
- Penetration
- Sediment composition (type)
- Sediment odor
- Sediment color
- Presence of shell hash (categorical: none, low 1-25%, medium 26-50%, high > 50%)
- Presence of Debris (Yes/No)
- Sample types produced from sediment grab

J. Sediment Description

The field description of sediments is required following measurement of penetration depth. The sediment should be characterized as being coarse sand, fine sand, silt, clay, gravel, or of a mixed

type. The presence of petroleum tar and shell hash should also be recorded. Obvious odors, such as hydrogen sulfide (the odor of rotten eggs), petroleum, other odors, or a lack of noticeable odors should be recorded. General sediment colors (*i.e.*, black, green, brown, red, olive, or gray) should also be recorded.

K. Sample Processing

Benthic Infaunal Samples

After the sample description has been completed, the sediment sample intended for biological analysis is washed from the grab and screened. Raw water used to wash the samples is to be filtered in some fashion to prevent the accidental introduction of surface-water organisms. Thoroughly wash the sediment from the grab and transfer it to a sediment-washing table (screen box, etc.) for screening. An alternative sieving method for small vessels without wash water would involve semi-submerging the sieve overboard and swirling it in the water (taking care to prevent the loss of grab organisms and/or the introduction of surface water organisms) until the sediment washes away.

In any estuary strata, the necessity of sampling from small craft may not permit onboard screening of the sediment. In these cases, the samples may be screened and processed on land at a screening station temporarily established near the sampling location. To assure that the sample does not deteriorate, such "off-site" screening must be completed as soon as possible and no longer than 90 minutes after sample collection.

All the water drained from the grab and used to wash the grab must be captured and subsequently processed through screening. Typically, a tub ($\geq$70 L capacity) is positioned under the grab. The use of a sediment-washing table is recommended, but not required. The table is useful in that it provides a flat, smooth surface over which to spread and wash the sample, thereby providing a means of gently breaking up the sediment before it runs off the end of the table into the screen box. The screening box must be equipped with a stainless-steel mesh with 1.0-mm openings (0.5-mm for brackish estuary samples). Wire diameter should be similar to that found in the U.S. Standard 1.00 mm Sieve (*i.e.*, 0.58 mm for brackish waters). The surface area of the screen should be adequate to easily accept the sample without build-up. Typical surface areas used in surveys in the Bight are 1500 to 2100 cm^2. While washing the sample, control the water pressure to avoid damaging the organisms. Minimize direct application of water from the hose to the material and organisms collecting on the screen.

Once the sample has been washed through the screen, transfer the material (debris, coarse sediment, and organisms) retained on the screen to a sample container. Label the sample container with an external label containing the agency code, station name, gear type, "split number" (*i.e.*, 1 of 1, 2 of 3, etc.), collection date, and preservation method (see Appendix F for labeling example). An internal label bearing the same information is placed inside the infaunal samples. This label can be written in pencil or laser printed ink on 100% rag paper or other museum quality paper (*e.g.*, Resistall) suitable for wet labels. The sample container must have a screw-cap closure and be sufficiently large to accommodate the sample material with a headspace of at least 30% of the container volume. A sample may be split between two or more containers. However, each

container must have external and internal labels (as described above) with the appropriate "split number" clearly marked. Field crews should have a broad range of sample container sizes available to them, with none less than 16 oz (0.47 L) capacity.

Gently remove the material retained on the screen, taking care to avoid damaging the organisms. The sample container should be filled to approximately 50 to 70% of capacity with screened material. After the bulk of material has been transferred to the container, closely examine the screen for any organisms caught in the mesh. Remove any organisms with forceps and add them to the sample container. Thoroughly wash the screen box and scrub the mesh before the next sample is screened.

All infaunal samples will be treated with a relaxant solution for approximately 30 minutes prior to fixation. Either an Epsom salts ($MgSO_4$) solution or a propylene phenoxytol solution (formulations below) may be used for this purpose. Relaxant solutions may be used as the diluent water for the fixative, or may be decanted after exposure and replaced with 10% buffered formalin. If it is used as diluent water, fill the sample container to 75 to 80% of its volume, close the container and invert it several times to distribute the solution. Leave the sample in the relaxant for 30 minutes. After 30 minutes, top off the container with enough sodium borate buffered formaldehyde to achieve a 10% formalin solution. Close the container, once again, and invert it several times to assure mixing. Store the sample for return to the laboratory.

If the relaxant solution is not used as the diluent water, the relaxant must be removed from the sample container and replaced with 10% buffered formalin. After the 30 minutes of treatment, decant the relaxant from the sample through a screen with a mesh size of 1.0 mm or less (0.5 mm or less for brackish waters). Ensure that all animals are removed from the screen and placed in the sample container. Fill the container with sodium borate buffered 10% formalin rather than undiluted formaldehyde, then close the container, invert it several times and store it for return to the laboratory.

Relaxant and fixative stock solution alternatives are as follows:

1)	Epsom salts relaxant solution:	1.5 kg Epsom salts ($MgSO_4$ @ $7H_2O$) per 20 L of freshwater.
2)	Propylene phenoxytol solution:	30 ml propylene phenoxytol to 20 L of seawater.
3)	Buffered formalin solution:	50 g sodium borate ($Na_2B_4O_7$) per liter of formalin.
4)	Buffered 10% formalin solution:	1 part buffered formalin to 9 parts fresh or salt water.

In some instances, samples may be preserved for DNA analysis. Use of a relaxant is not recommended because it could interfere with the DNA analysis. Decant any liquid from the sample through a screen with a mesh size of 1.0 mm or less. Ensure that all animals are removed

from the screen and placed in the sample container. Fill the container with 95% Ethanol (ETOH), then close the container, invert it several times and store it for return to the laboratory. In laboratory, remove the old ETOH and replace with fresh 95% ETOH. **Do not use 70% ETOH for DNA specimens**. Be aware that ETOH removes most inks and archival pens are fussy to use in the field, so a No. 2 pencil is preferred for writing on internal labels.

Natural History Museum of Los Angeles County still has very poor DNA coverage for infauna invertebrates. If specimens are small, ideally 3-5 individuals of any species will elucidate the diversity at a specific locality (*i.e.*, stations). For species complexes and troublesome taxa, specimens from multiple localities are extremely useful for quickly discerning relevant distinguishing morphological characters. If specimens are large, specimen photo-documentation and a preserved piece of a tentacle or arm will suffice. Small specimens should be double-vialed (inner vial contains specimens, outer vial contains label data, shell vials stoppered with only 100% cotton -- as specified in the benthic lab manual). The museum only accepts completely identified animals. It will be the taxonomic identification laboratories or associated sampling organizations responsibility to transport specimens to the museum. The museum will need a copy of CDFW field collection permit.

Special Sampling for Meiofaunal Assemblages: There are two methods for sampling, see Appendix M for details. The preferred method uses a 5-7 cm plastic core (2 or 3 inch) pushed approximately 15 cm depth into the sediment and carefully removed. PVC core tubes should be subsequently extruded and the **top 10cm fraction** of sediment sliced off and placed directly into a plastic bag (Whirl-Pak or Ziploc bag). Do not transfer the portion of the core that has come into direct contact with the extruder (*e.g.*, bottom ~2cm) in order to avoid cross-contamination of samples. In wadable areas, cores should be collected from relatively flat patches of sediment, where possible. Before inserting core into the sediment, visually inspect the surrounding area and avoid coring on top of large invertebrates, burrows, bioturbation mounds, or biological/artificial debris (*e.g.*, piles of shells, rocks or plastic).

The second alternative involves scooping the top 2 cm from the sides of the grab into Whirl-Pak or Ziploc bag. The alternative method collects non-quantitative samples. While acceptable, the secondary method is the preferable option rather than losing a site altogether. Data from as many Bight samples sites as possible are needed in order to accurately quantify meiofaunal species diversity and geographic patterns within the Southern California Bight.

Bight sampling location and sample code (*e.g.*, replicate number) should be written clearly in Sharpie marker on the outside of each plastic bag. Indicate any deviations from standard coring on the outside of the plastic bag (*e.g.*, scooping surface sediment as opposed to coring with a PVC tube).

Collected samples should be kept cool on ice or placed on dry ice to immediately freeze the sample upon collection. **Slow freezing, as in a -20° C freezer, damages the DNA** of the meiofauna and must be avoided. Frozen/chilled samples should be transferred into -80°C storage as soon as possible after collection.

PVC core tubes, slicing plates, and extruder should be washed thoroughly in seawater between each replicate and between sample sites, to avoid cross-contamination.

L. Sediment Chemistry

General Sediment Chemistry Samples from Offshore Sites

Following collection of benthic infauna, the next grab(s) will be taken for sediment chemistry samples. More than one grab may be necessary to meet the sample volume requirements of this sample type. If a second grab is necessary, the sediment from each grab will be distributed evenly among the individual sample containers. Sediment samples will be collected using the top **2 cm** of the undisturbed surface material **at the offshore** sites (inshore, mid, outer shelf areas). Sediment will be collected using a stainless-steel scoop (a plastic scoop is acceptable for TOC and grain size samples). Scoops will be washed with seawater and rinsed with de-ionized (DI) water between stations. Use of a new scoop with each sample is also acceptable. Sediment in contact with or within 1 cm of the metal sides of the grab will be avoided to prevent sample contamination. Wearing Nitrile powder-free gloves during sediment sampling is recommended.

Embayment sampling. Following collection of benthic infauna with a Van Veen grab, the next grabs will be taken for both sediment chemistry and toxicology. More than one grab will be necessary to meet the sample volume requirements of this sample type. The sediment from each grab will be collected from using the top **5 cm** of the undisturbed surface material **at the inner coastal stations** (bays, harbors, marinas, and estuaries). Sediment will be collected using a stainless-steel scoop. Scoops will be washed with seawater to remove residual sediment and rinsed with de-ionized (DI) water between stations. Use of a new scoop with each sample is also acceptable. Sediment in contact with or within 1 cm of the metal sides of the grab will be avoided to prevent sample contamination. Depending on site analysis, the total minimum volume may be 8 liters of sediment (2L for chemistry, 3L for amphipod test, 3L for mussel test). If samples contain excessive shell hash or other debris, additional sample volume is recommended. Carefully remove large pieces of debris (*e.g.*, eelgrass, trash, rocks, shells) without touching the sediment with your gloved hand. Wearing Nitrile powder-free gloves during sediment sampling is recommended.

Sediment is scooped into a single Teflon bag placed within a pre-labeled food-grade polypropylene bag, lining a 3 to 5-gallon bucket. The double lining provides extra support and protection from contamination should there be accidental tearing of the inner bag. Massage, knead and squeeze the bag for at least 3-5 minutes with your hands while holding the top of the bag closed in a twisted fashion (move material around to homogenize the sediment), taking care to not tear the bag or squeeze sediment out of the top of the bags. A two-person team may be needed. Homogenization should result in a uniform color and texture throughout. In the unlikely event that the inner Teflon bag tears before chemistry samples are taken, a new bag and additional grabs are necessary to start the process again.

Once sediment is fully homogenized, use a stainless-steel scoop to transfer the sediment to the chemistry sample jars. No proportioning is necessary for chemistry jars. If chemistry samples are to be frozen, leave enough headspace for expansion. The remaining sediment is for toxicity testing. If two toxicology labs are processing the sediment, fill an additional Teflon bag with half the remaining sediment (3 L) or use three HDPE 1-liter sample containers for mussel testing (field

team's choice). Zip tie the inner bag closed, then zip tie the outer pre-labeled bag. A waterproof label should also be securely attached to the zip tie in addition to the labelling on the outer plastic bag itself. Place the zip tied Teflon bag in a third outer polypropylene or Zip Lock bag for extra protection (optional), and place directly on ice in a cooler. Toxicology sample cannot be frozen. If Teflon bag tears after chemistry samples are taken, but retained in the outer plastic bags, sediment is acceptable for toxicology. Place an additional bag over contents for extra protection.

Brackish estuary sampling follows the same compositing procedure outlined for estuaries except the sampling equipment may differ. If a Van Veen is used, follow the procedure outlined above.

If a Petite Ponar is used, sediment touching the side of the grab is used. Ensure the grab is free of residual sediment in the laboratory by scrubbing the inside with soap and water using a brush, then thoroughly rinsing with tap water, followed by deionized (DI) water and placing grab in a plastic bag to prevent contamination. In the field, in-between stations, scrub the inside of the grab with a brush to remove residual sediment and thoroughly rinse with ambient seawater, then place in bag again. On site, rinse grab with ambient seawater before use. Take the grab sample and dump the contents into a clean tray or container. An aluminum tray is recommended because sediment has aluminum concentrations in the percent range and highly unlikely to add significant contamination to the sample. Next choice would be stainless steel. Clean the tray following the same procedure outlined for the Petite Ponar. A completely full Ponar grab has approximately 7-8 cm of sediment. Pour off overlying water from tray. Scoop the top 5 cm of sediment from the tray into a Teflon lined bucket. Repeat the process until the required sediment volume is obtained for the site. Follow the homogenization procedure outlined for estuaries to distribute samples for chemistry and toxicology.

If a plastic push core is used, sediment touching the side of the core is used. Ensure the core is thoroughly cleaned in the laboratory after construction. All plastic and rubber items including core, scoops and trays should be washed in the laboratory with hot soap and water, rinsed with tap water, rinsed with DI water, followed by a methanol rinse and bagged for the field. In the field, in-between stations, scrub the inside of the core with a brush to remove residual sediment and thoroughly rinse with ambient seawater, followed by a methanol rinse then place in bag again. On site, rinse grab with ambient seawater before use. Take the core sample, pushing it to the 5-cm mark and dump the contents into a clean tray (pour off overlying water from tray). Scoop the top 5 cm of sediment from the tray into a Teflon lined bucket. Repeat the process until the required sediment volume is obtained for the site. Follow the homogenization procedure outlined for estuaries to distribute samples for chemistry and toxicology.

If site is shallow enough to wade into the water, a stainless-steel scoop can be used directly. Ensure the sediment being scooped is undisturbed by wading action or foot prints. Follow the cleaning procedures outlined for scoops. Pour off any residual overlying water if necessary. Scoop the top 5 cm of sediment into a Teflon lined bucket. Repeat the process until the required sediment volume is obtained for the site. Follow the homogenization procedure outlined for estuaries to distribute samples for chemistry and toxicology.

The following container types, samples sizes, and storage requirements will be used with the analytical laboratory supplying all sample containers for all parameters (see Appendix G for summary sediment chemistry guide).

1) **Sediment Grain Size**-- Using a stainless steel or plastic scoop, approximately 100 g of sediment material will be collected at each station. The sample shall be placed in a 4-oz (118 mL) plastic container, filling it 80% full, and taking care to leave an air space at the top. Samples should be stored at approximately 4 °C by placing them on wet ice or in a refrigerator until returned to the laboratory. **Do not freeze these samples**. They should be returned to the analytical laboratory within a week of sampling.

2) **Total Organic Carbon/Nitrogen**-- Using a stainless-steel scoop, approximately 200 g of sediment material will be collected at each station. The sample shall be placed in an 8-oz (~250 mL) amber glass container with a Teflon-lined lid filling it 80% full, and taking care to leave an air space at the top. Samples should be stored at < 4 °C by placing them on wet ice or in a refrigerator, but must be frozen within 24 hours. If frozen, they should be returned to the laboratory within a week; if not, they should be returned to the analytical laboratory within 24 hours.

3) **Trace Metals**-- Using a stainless-steel or plastic scoop, approximately 200 g of surface sediment will be collected at each station. The sample shall be placed in an 8-oz (~250 mL) amber glass container with a Teflon-lined lid, filling it 80% full, and taking care to leave an air space at the top. Samples should be stored at < 4 °C by placing them on wet ice or in a refrigerator but must be frozen within 24 hours. If frozen, they should be returned to the laboratory within a week; if not, they should be returned to the analytical laboratory within 24 hours.

4) **Trace Organics (PCBs, CHCs)** -- Using a stainless-steel scoop, approximately 100 g of sediment material will be collected at each station. The sample shall be placed in a 4-oz (~125 mL) amber glass container with a Teflon-lined lid, filling it 80% full, and taking care to leave an air space at the top. Samples should be stored at < 4 °C by placing them on wet ice or in a refrigerator but must be frozen within 24 hours. If frozen, they should be returned to the laboratory within a week. If not frozen, they should be returned to the analytical laboratory within 24 hours.

5) **Trace Organics (PAHs)** -- Using a stainless-steel scoop, approximately 100 g of sediment material will be collected at each station. The sample shall be placed in a 4-oz (~125 mL) amber glass container with a Teflon-lined lid, filling it 80% full, and taking care to leave an air space at the top. Samples should be stored at < 4 °C by placing them on wet ice or in a refrigerator but must be frozen within 24 hours. If frozen, they should be returned to the laboratory within a week. If not frozen, they should be returned to the analytical laboratory within 24 hours.

6) **Pyrethroid Pesticides--** Using a stainless-steel scoop, approximately 200 g of sediment material will be collected at each station. The sample shall be placed in an 8-oz (~250 mL) amber glass container with a Teflon-lined lid, filling it 80% full, and taking care to leave an air space at the top. Samples should be stored at < 4 °C by placing them on wet ice or in a refrigerator but must be frozen within 24

hours. If frozen, they should be returned to the laboratory within a week. If not frozen, they should be returned to the analytical laboratory within 24 hours.

7) **PBDE--** Using a stainless-steel scoop, approximately 200 g of sediment material will be collected at each station. The sample shall be placed in an 8-oz (~250 mL) amber glass container with a Teflon-lined lid, filling it 80% full, and taking care to leave an air space at the top. Samples should be stored at < 4 °C by placing them on wet ice or in a refrigerator but must be frozen within 24 hours. If frozen, they should be returned to the laboratory within a week. If not frozen, they should be returned to the analytical laboratory within 24 hours.

8) **Fipronils**-- Using a stainless-steel scoop, approximately 200 g of sediment material will be collected at each station. The sample shall be placed in an 8-oz (~250 mL) amber glass container with a Teflon-lined lid, filling it 80% full, and taking care to leave an air space at the top. Samples should be stored at < 4 °C by placing them on wet ice or in a refrigerator but must be frozen within 24 hours. If frozen, they should be returned to the laboratory within a week. If not frozen, they should be returned to the analytical laboratory within 24 hours.

9) **Special Study: Domoic Acid (DA)** – These samples will only be collected at the inner, mid, and outer shelf stations. Using a stainless-steel scoop, approximately 200 g of sediment material will be collected at each station. The sample shall be placed in an 8-oz (~250 mL) amber glass container with a Teflon-lined lid, filling it 80% full, and taking care to leave an air space at the top. Samples should be stored at < 4 °C by placing them on wet ice or in a refrigerator but must be frozen within 24 hours. If frozen, they should be returned to the laboratory within a week. If not frozen, they should be returned to the analytical laboratory within 24 hours.

10) **Cell Assays** -- Using a stainless-steel scoop, approximately 200 g of sediment material will be collected at each station. The sample shall be placed in a 8-oz (~250 mL) amber glass container with a Teflon-lined lid, filling it 80% full, and taking care to leave an air space at the top. Samples should be stored at < 4 °C by placing them on wet ice or in a refrigerator but must be frozen within 24 hours. If frozen, they should be returned to SCCWRP as soon as possible. If not frozen, they should be returned to SCCWRP within 24 hours.

If any samples need to be transported to another organization for processing, they should be packed appropriately (blue ice or dry ice) and shipped to SCCWRP via overnight express, or a local carrier. Check with carrier for shipment restrictions.

Labeling of sample containers will be the responsibility of the field sampling crew. The following minimum information will be required on each sample label: 1) station number; 2) sampling date; 3) agency code; and 4) parameter.

Samples that will be analyzed by the organization conducting the field collection will be returned to their laboratory by the field crew. Unless specifically instructed otherwise, samples to be

analyzed by other laboratories will generally be transported to SCCWRP for later distribution. It is recommended that SCCWRP (Darrin Greenstein, 714/755-3224) be contacted prior to delivery of samples so that arrangements can be made to transfer custody. A **completed chain of custody form** must accompany all shipments of samples. Allow time for verification of the chain of custody. If samples are shipped by carrier, a copy of the chain of custody form is to be sent to SCCWRP for tracking purposes.

M. Toxicology

General Sediment Toxicity Samples

Following the collection of sediment chemistry samples in the offshore strata (inner, mid, outer shelfs), grabs will be taken for sediment toxicity analysis. More than one grab may be necessary to meet the sample volume requirements of this sample type. If a second grab is necessary, the sediment from each grab will be distributed evenly among the individual sample containers. Sediments will not be homogenized in the field for offshore sites located on the inner, mid, and outer shelfs.

Sediment samples will be collected by scooping the top 2 cm of the undisturbed surface material from offshore. At the very minimum, the scoop will be washed with sample water and rinsed with de-ionized (DI) water between stations**.** Use of a new scoop with each sample is also acceptable. Sediment within 1 cm of the metal sides of the grab will be avoided to prevent sample contamination. A Teflon bag or High-density polyethylene (HDPE) containers will be used for collection of sediment toxicity samples. The toxicity laboratories performing the required analysis will provide sample containers. Labeling of sample containers will be the responsibility of the field sampling crews and the following minimum information will be required on each sample label: 1) station number; 2) sampling date; 3) parameter; and 4) split (if required).

Special Embayment and Estuary Sediment Samples for Chemistry/Toxicology. Following the collection of benthic infauna samples, sediment grabs will be taken for combined toxicity/chemistry analysis. Multiple grabs are necessary to meet the sample volume requirements. The total recommended minimum volume is 2L for chemistry and 6L for toxicity (3 L for *Eohaustorius* and 3 L of *Mytilus*). If samples contain excessive shell hash or other debris, additional sample volume is recommended. Scoops will be washed with seawater and rinsed with de-ionized (DI) water between stations. Use of a new scoop with each sample is also acceptable. Sediment in contact with or within 1 cm of the metal sides of the grab will be avoided to prevent sample contamination. The toxicity laboratories performing the required analysis will provide sample Teflon bags and HPDE containers.

Use a single Teflon bag placed within a pre-labeled food-grade polypropylene bag, lining a 3 to 5-gallon bucket. The double lining provides extra support and protection from contamination should there be accidental tearing of the inner bag. Scoop the top 5 cm of sediment from the Van Veen grab sampler using a stainless-steel scoop and place in the double-lined bucket. Repeat from all grab samples until required volume is met (8 L). Carefully remove large pieces of debris (*e.g.*, eelgrass, trash, rocks, shells) without touching the sediment with your gloved hand. Knot or zip tie both bags sequentially, inner bag first than outer bag. Knead and squeeze the bag with your

hands, moving material around to homogenize the sediment, taking care to not tear bag or squeeze sediment out of the top of the bags. Homogenization should result in a uniform color and texture throughout.

Once sediment is fully homogenized, use a stainless-steel scoop (a plastic scoop is acceptable for grain size and trace metal samples) to transfer the sediment to the chemistry sample jars. See chemistry sample section above for container size and preservation methods. If samples are to be frozen, leaving enough headspace for expansion.

Once the chemistry containers are filled, the remaining sediment in the Teflon bag will be for toxicity analysis. If two toxicology labs are processing the sediment, fill another Teflon bag with half the remaining sample (3 L) or three HDPE 1-liter sample containers (field team choice) completely for the mussel test using a stainless-steel or plastic scoop and the remaining bagged sediment goes to the other lab. Zip tie the inner bag closed, then zip tie the outer pre-labeled bag. A waterproof label should also be securely attached to the zip tie in addition to the labelling on the outer plastic bag itself. Place the zip tied Teflon bag in a third outer polypropylene or Ziploc bag for extra protection (optional), and place toxicity samples directly on ice in a cooler for toxicity testing.

In the unlikely event that the inner Teflon bag tears before chemistry samples are taken, a new bag and additional grabs are necessary to start the process again. If Teflon bag tears after chemistry samples are taken, but retained in the outer plastic bags, sediment is acceptable for toxicology. Place an additional bag over contents for extra protection.

Sample volume requirements for sediment toxicity samples are:

- Three liters per species; 6 liters total for two species testing (*e.g.*, *Eohaustorius, Mytilus*). Teflon bags or 1.0 L high-density polyethylene (HDPE) containers with Teflon-lined lids are the container choices. If insufficient sample volume is available after nine (9) additional grab attempts, a minimum of 2.5 liters per species (5.0 liters total) will satisfy the sampling requirement. Each labeled container should then be refrigerated, or placed on wet ice. **Do not freeze these samples.** Samples may be held in the field, or laboratory, on wet ice, or in a refrigerator at 4^{o} C, for no more than three days before transport to the designated toxicity laboratories. The inter-laboratory transport time will not exceed 24 hours. Upon arrival at the analytical laboratory, the samples will continue to be stored at 4^{o} C. Chain of custody procedures and holding times should be followed throughout the sampling and analysis procedures.

Labeling of sample containers will be the responsibility of the field sampling crews with the following minimum information required on each sample label: 1) station number; 2) sampling date; 3) agency code; 4) parameter; and 5) container number (if needed).

Samples to be analyzed by the organization conducting the collection will be returned to their laboratory by the field crew. Samples to be analyzed by other laboratories will be transported to SCCWRP for later distribution. It is recommended that SCCWRP (Darrin Greenstein, 714-755-3224) be contacted prior to delivery of samples so that arrangements can be made to transfer

custody. A **completed chain of custody form** must accompany all shipments of samples. Allow time for verification of the chain of custody. If samples are shipped by carrier, a copy of the chain of custody form is to be sent to SCCWRP for tracking purposes.

N. Special Studies

Special Studies

Cell Assays: At select sites, additional sediment samples will be collected for cell assay testing. Please contact Alvina Mehinto (alvinam@sccwrp.org) for details, sampling containers, and transfer instructions. The minimum information required on each sample label is station number, sampling date, agency code, and parameter. Samples must be frozen within 24 hours and stored at -20°C until transported to SCCWRP for distribution. A **completed chain of custody form** must accompany all sample shipments to SCCWRP. See B'18 Sediment Quality Assessment Workplan for further details.

Domoic Acid Concern: At many sites, additional sediment samples will be collected for Domoic Acid compounds. An additional container will be provided by the field crew. See above chemistry section for sampling instructions. The minimum information required on each sample label is station number, sampling date, agency code, and parameter. Samples must be frozen within 24 hours and stored at -20°C until transported to SCCWRP for distribution. Samples are to be transported to SCCWRP for distribution. A **completed chain of custody form** must accompany all sample shipments to SCCWRP. See B'18 Sediment Quality Assessment Workplan for further details.

Meiofauna studies: At select sites, additional mud samples will be collected for university studies. See above infauna section for sampling instructions and Appendix M. The minimum information required on each sample label is station number, sampling date, agency code, and parameter. Samples should be stored cool on wet ice or in a refrigerator. Samples immediately frozen on dry ice should stay on dry ice or placed in a -80 freezer. Samples are to be transported to SCCWRP for distribution. A **completed chain of custody form** must accompany all sample shipments to SCCWRP. See B'18 Sediment Quality Assessment Workplan for further details.

IX. TRAWL SAMPLING

A. Purpose

The purpose of trawl sampling is to obtain data on the distribution, abundance, biomass, diversity, and disease prevalence of demersal fish and invertebrate assemblages. It is also used to collect fish and invertebrates for tissue contaminant analysis. This information is useful in characterizing possible anthropogenic effects on demersal fish and invertebrate populations. Mearns and Allen (1978) provide a comprehensive description of how small otter trawls should be designed and used for conducting biological surveys in coastal waters.

B. Sampling Effort

A total of **139** trawl stations will be sampled during the survey. Information regarding trawl stations and the corresponding parameters that will be measured at each of these sites are listed in Table 1 and Appendix A, respectively.

C. Otter Trawl Specifications

A semi-balloon otter trawl (Figure 4) will be used to collect epibenthic invertebrates and demersal fish. Net dimensions are as follows: 7.6-m headrope (25 ft); 8.8-m footrope (29 ft); 3.8-cm (1.5 in) body mesh; and a 1.3-cm cod-end mesh (0.5 in). This net will have 22.9-m (75 ft) long bridles made of 1.0-1.6 cm (3/8 to 5/8 in) diameter rope (*e.g.*, Samson braid). Typical otter boards (doors) will have a width of 76 cm (30 in), height of 50 cm (20 in), and a suggested weight of 16 kg (35 lb) (Figure 5). Slight deviations (< 10%) from the dimensions are acceptable. The recommended door chains should be 5-mm (3/16 in) in diameter and should have the following numbers of links: front top -- 12; front bottom -- 11; back top -- 17; back bottom -- 16. The actual specifications of how any trawl door is set up may depend on the manufacturer of the otter trawl, but the user of the equipment should be sure to follow the factory recommended set-up procedures to ensure that the net fishes appropriately in the field.

The Bight'18 survey will require two additions to the trawl specifications: 1) non-crushable floats are required for any nets used to trawl deeper than 200 m; and 2) pressure-temperature (PT) sensors (withstand 500 m depths) will be attached to one of the trawl doors to measure water temperature, depth, and time of the individual trawls. Data collected by these sensors will be downloaded to a computer so that data regarding bottom time and depth of the trawls can be monitored in the field and analyzed after the survey has been completed. Time synchronizing between multiple computers can be problematic, so record the time offset between field data tablets/computers and computers used to download PT data in the datasheet comments field.

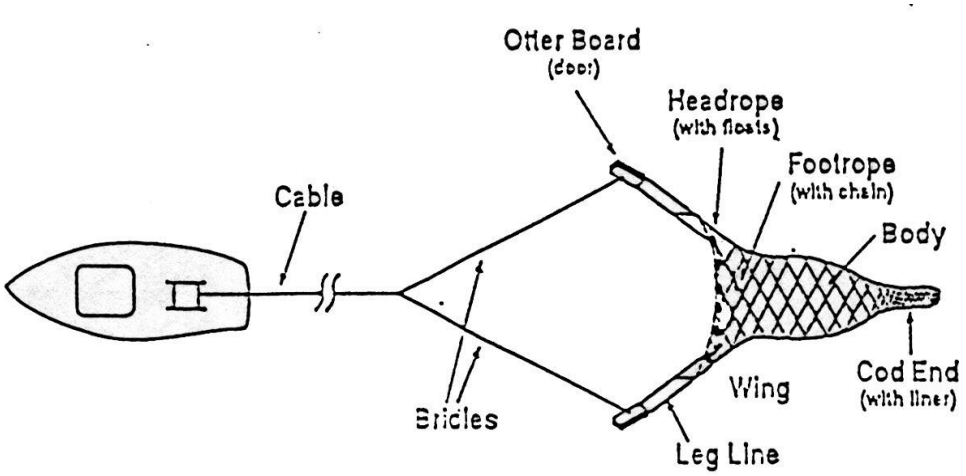

Figure 4. Semi-**balloon otter trawl recommended for marine receiving-water monitoring programs in southern California (modified from Mearns and Allen, 1978)**

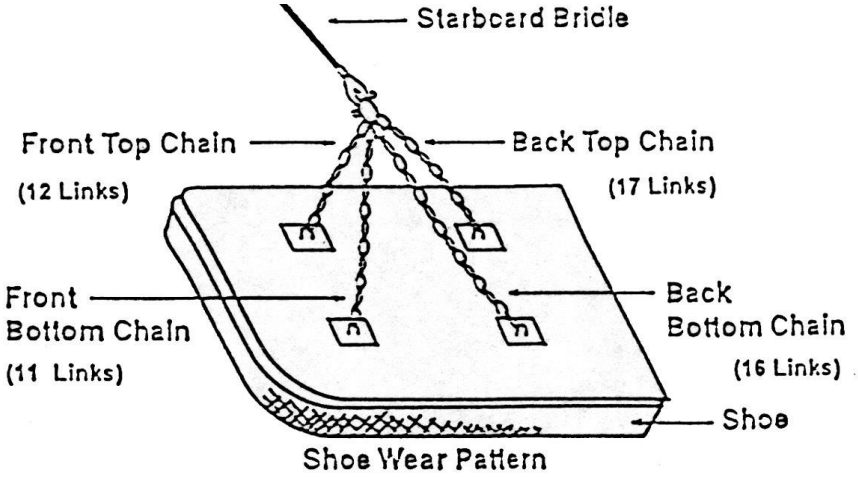

Figure 5. **View of an otter board of a semi-balloon otter trawl with recommended numbers of chain (5-mm or 3/16 in. diameter) links (modified from Mearns and Allen, 1978)**

D. Trawl Data Flow and Responsibilities

The collection of trawl data (identifications, measurements, etc.) is largely a field activity for which there is little opportunity to clarify or correct errors. Therefore, it is important that the field personnel appreciate the ultimate fate of the data records they are creating and assure that their field records support subsequent steps in the data creation process. For example, specimens

collected as vouchers or as FID specimens, must be labeled under the same name as recorded on the field data sheet. This allows these specimens to be unambiguously associated with the data records for purposes of data QC or revision.

In addition, each organization conducting trawling must complete all stages of sample analysis (lab IDs, voucher confirmation, data sheet revisions, etc.) prior to submitting data and voucher specimens to the project for further review. The flow of data from the trawl to final data set and the parties responsible for completion of each stage is summarized in Figure 2.

E. Trawl Data Log

If for any reason the Field Computer stops functioning, the field crew will be responsible for keeping a manual trawl data log (Appendix F). The information recorded in the log includes water depth, length of tow wire used, times and coordinates (latitude and longitude) for net on the bottom and the end of the trawl (beginning of trawl retrieval). Similar information for when the net was deployed (net over) and when the net was retrieved (net on deck) may also be recorded. Any anomalous conditions, such as rocky substrate, debris in the catch, and/or a torn net should also be recorded in the log.

F. Net Preparation

The trawl components should be properly prepared prior to trawling so that the trawl can be deployed in an orderly and safe manner upon arrival at a station. Nets should be inspected for holes prior to deployment and repaired as needed. The net should be laid out and stacked on the stern of the vessel in the same configuration that it will be deployed: cod-end to the stern, floats up, and footrope down. The trawl net should be checked to make sure that the cod-end is tied correctly, the doors should be connected properly to the leg lines, and the bridles should be securely fastened to the doors and to the tow wire.

G. Station Occupation

Every effort should be taken to ensure that any trawl track passes the station coordinates at a distance of no greater than 100 m, and that the trawl course varies no more than +/- 10% of the target depth (Figure 2). The trawl track can be plotted prior to sampling so that a successive series of waypoint locations along the track can be obtained. These coordinates can then be entered into the navigation system and then retrieved at the time of sampling to ensure that the vessel maintains its course along the trawl track.

H. Pre-Trawl Survey

After recording the depth at the assigned station, a pre-trawl survey of the trawl course will be conducted to determine site acceptability and whether uncharted features such as reefs, wrecks, etc., could obstruct the trawl and potentially damage equipment. Trawl gear can be lost if it becomes snagged on obstructions and replacement of nets can be costly. The trawl track should be evaluated by the Cruise Leader using a fathometer and following the expected course along the isobath.

If the first run indicates that a particular site is unacceptable, another survey will be conducted within 100 m of the original location and within +/-10% of the original depth. If this attempt is unsuccessful, a third attempt will be conducted at a different location using the same protocols (100 m of the original location, and +/-10% of original depth). The site will be abandoned after three unsuccessful attempts (Figure 2).

H. Trawling

Trawls will be towed along, rather than across, isobaths. While the vessel is underway the net and doors are placed in the water. It is important that the floats skim the surface and that the net is not entangled (*e.g.*, crossed leg lines, bunched or hooked portions of the net) prior to paying out the bridles. This small step could mean the difference between a successful or unsuccessful trawl. The bridles should be paid out by personnel on either side of the net, to avoid becoming entangled in the rigging during deployment.

Use of the proper scope (*i.e.*, length of hydrowire paid out versus the water depth) is important for successful trawls. After the net touches the bottom, a sufficient length of hydrowire (towing wire) should be paid out to ensure that the net is pulled from a horizontal rather than a vertical position. Insufficient scope will prevent the net from consistently fishing the bottom and will result in a no-catch, or a short-catch situation. In general, the required scope declines with increasing depth because the additional weight of the hydrowire enhances the horizontal component of the towing forces (Table 2, Appendix H).

Table 2. Recommended scope and length of wire for trawling and estimated times for trawl performance at different depths in the Southern California Bight (expanded table in Appendix H).

Station Depth (m)	Depth/Wire Scope[1]	Wire (m)	Winch[2] Time (min)	Initial Wire[3] Depth (m)	Minutes To Bot Lag[4]	Minutes Off Bot Lag[5]	10 Min Trawl Est Deck Time (min)
50	5.0	252	6.12	50.7	-0.05	2.20	7.75
100	4.1	410	9.97	82.5	1.33	2.91	8.42
150	3.6	545	13.25	109.6	3.06	3.62	9.44
200	3.3	668	16.22	134.2	4.99	4.33	10.67
250	3.1	781	18.97	157.0	7.06	5.04	12.02
300	3.0	888	21.56	178.4	9.23	5.75	13.48
350	2.8	989	24.03	198.8	11.47	6.46	15.02
400	2.7	1,086	26.39	218.4	13.78	7.17	16.62
450	2.6	1,180	28.67	237.2	16.15	7.87	18.27
500	2.5	1,271	30.87	255.5	18.56	8.58	19.97

[1] Power function was 16.139219 * (Station Depth $^{-0.297449384}$) based on method protocol.

[2] Average agency winch rate was 41.16 m/min.

[3] Average descent rate was 8.3 m/min. Average lag on bottom decent rate changed +1.6 times.

[4] Used: (Station Depth – Wire Depth) / (Avg Descent Rate * Avg Change Rate Factor).

[5] Used: regression formula: 1.4903252151 + (0.0141874591*Station Depth)) based on Lag Off vs. Depth data.

These scopes are for 1.0 cm (0.38 in) hydrowire. These scopes will have to be adjusted accordingly when using a different diameter of hydrowire.

Trawling is conducted at a speed-over-ground of 1.0 m/sec (or 1.5 to 2.0 kt) and the net is generally towed for 10 minutes (see Table 2 for modifications), measured on deck from start of trawl to end of trawl (*i.e.*, lock down of winch to start of retrieval). *All vessels will maintain speed while retrieving the net*. In confined areas (*e.g.*, bays and harbors), the trawl duration may be reduced to 5 min, or a distance over ground of 225-300 m. While 10 minutes on the bottom is the nominal target time for each trawl, a working range of 8-15 minutes as determined by the PT sensor is acceptable. Upon completion of each trawl the PT sensor data will be downloaded immediately to determine the actual on-bottom duration. If bottom time is less than 8 minutes or greater than 20 minutes, the trawl is repeated. If the bottom time falls between 15-20 minutes, crews must adjust subsequent deployment durations, as necessary, to fall as close to10 minutes as possible. If there are demersal fish and invertebrates in trawls falling under 8 minutes or greater than 20 minutes, the catch can be processed (field crew's discretion) while the station is being re-trawled. An error code is provided for the data sheets to indicate that the data are from a failed trawl, outside the on-bottom time limits, and additional comments should indicate why a re-trawl was needed. This allows rare and unusual species to be documented while not compromising the study design.

All PT sensor information will be retained electronically and submitted with the other data types at the end of the project.

J. Criteria for Accepting a Trawl

At the end of the prescribed trawl time, the net is retrieved and brought onboard the vessel. The cod-end is then opened and the catch deposited into a tub or holding tank. The catch is subsequently released to the scientific crew for processing. If the trawl is retrieved with little or no catch in the cod-end, its acceptability will be evaluated according to whether the trawl was conducted properly. The criteria used to evaluate the success of any trawl include making sure that proper depth, scope, speed, and distance (or duration) were maintained, whether the net was fouled (net tangled), and whether the catch shows evidence that it was on the bottom (*e.g.*, rocks, benthic invertebrates, benthic fish) (Figure 2). If any trawl procedures were not followed, if the net was fouled or torn (the tear must be sufficient to allow escapement), or if there was no evidence of contact with the bottom (PT sensor), the trawl will be considered unacceptable and the site will be re-trawled. When evaluating the situation to decide whether to abandon or re-trawl a station, the Cruise Leader should keep in mind that the goal is to collect the best sample possible.

If a retrieved net has been irreparably torn during a trawl, the station will be abandoned. If the trawl hangs up on the bottom, the site can be resampled or abandoned at the discretion of the Cruise Leader. If re-trawling that station proves unsuccessful after another two attempts, the site will be abandoned (Figure 2).

K. Special Case: High Density (*e.g.,* Red Crabs) Species Incidence

If at the end of the prescribed trawl time, the net is so full of a species (*e.g., Pleuroncodes planipes*) that it cannot be brought onboard normally or the species is falling out of the net on retrieval, the site may be abandoned temporarily. These occurrences are generalized to certain areas and depths. These species can move, so revisiting the site several weeks later may have

different results. Field organizations revisiting these sites may want to test the area with a 1-minute trawl. The Cruise Leader has the discretion to abandon the site if abundances remain significant from a 1-minute trawl. Field organizations worried that new sites within the same general area could experience high density abundances may use a 1-minute test trawl for evaluation purposes. If high densities are present, the site can be temporarily abandoned. The protocol is to quantify a standard 10-minute trawl. One-minute evaluation trawls are not to be quantified for Bight'18. A Cruise Leader has the discretion to work-up the trawl but must qualify the event as a failed trawl.

To process these high-density catches, follow the standard procedures listed in the subsequent sections. An optional procedure can be used for invertebrate species. It was specifically designed for Red Crabs. At the beginning of the day obtain the weight of a wet empty trawl net (net tare weight). When a high-density trawl is obtained record the weight of the total catch (total net and catch weight) and subtract the net tare weight then record the result as the catch weight in the comments section of the trawl invertebrate data sheet. Sort through the entire catch and place the high-density species into multiple bins or buckets for weighing, counting, and anomaly quantification. Using the aliquot datasheet, weigh out 1 kg of high-density invertebrates (minus the tare) and count the number of individuals comprising the weight. Record the numbers on the aliquot datasheet (*e.g.*, 1kg = X#). Batch weight any remaining (not high-density species) invertebrates, fish, and debris separately and record on the aliquot datasheet. Subtract the weights of the remaining invertebrates/fish/debris from the catch weight and multiply that weight (*i.e.*, the weight of the high-density species) by number of individuals comprising 1 kg of the high-density species. Begin processing the debris, fish, and remaining invertebrates as listed below.

L. Sample Processing

Sorting

The trawl catch will be sorted on deck into containers. The catch may initially be rough sorted into major categories (*e.g.*, urchins, shrimp, other invertebrates, flatfishes, rockfishes, other fishes). The categories used are not important, but it is more efficient to sort into rough categories before identifying organisms to species. Trawl debris should also be sorted into containers for processing. Objects, including organisms, less than 1 cm in largest dimension, should not be included for quantification.

Trawl Debris

Debris, anthropogenic or otherwise, collected during any trawl will be quantified by recording the specific types of material and their quantities on the Trawl Debris Form (Appendix F). If possible, debris should be quantified by direct enumeration and recorded on the form.

Identification

The goal is to provide species-level identifications for all fish and invertebrates captured in the trawl. Most, if not all, of the trawl-caught organisms should be identifiable to species in the field using the recommended taxonomic keys and field guides. Species of fish and invertebrates that cannot be reliably identified to species in the field should be returned to the laboratory for further

identification. In these instances, it is better that the field crew recognize their taxonomic limitations, record "FID" (further identification required) on the field sheet, and include descriptions or photographs of any attributes that may later aid in the identification of that specimen.

Under no circumstances should an organism be discarded if the identity is in question.

When the "FID" organisms have finally been identified, the correct identity of the species should be recorded on the original data sheet. If the laboratory identity differs from that recorded in the field, the original name should be crossed out with a single line only; do not erase the original name. If a specimen cannot be identified by the sampling organization, it will be sent to SCCWRP for further analysis.

Although all fish and invertebrates collected during Bight'18 should be identified to the lowest possible taxon (either in the field or in the laboratory), only certain trawl-caught animals meeting very specific criteria will need to be identified to that level. There are likely to be infaunal and pelagic species that will be taken incidental to the trawl catch. These need not be processed or documented. Only epibenthic invertebrates and fish greater than 1 cm in the largest dimension must be recorded on the datasheet. Fouling colonial and pelagic invertebrates will not need to be enumerated. Recently extruded juvenile fish (e.g., from live bearing Sea Perch) or shark egg sacs will be recorded separately from the adults. Common Cymothoidae fish parasites will be recorded on the trawl invertebrate datasheet as present and given the name "Cymothoidae". Cruise leaders have the discretion to keep separate records of animals for organizational database purposes. Post-survey data analysis will identify all species which do not meet the megabenthic invertebrate and demersal fish definition and flag the data in the final database records. These data will be excluded in the final report but remain in the final database records.

A recommended list of field guides and taxonomic aids for identifying fish and invertebrates will be distributed to participating organizations prior to the survey. The most basic and comprehensive guides for fish are Miller and Lea (1972) and Eschmeyer et al. (1983). Allen (1977) provides information for identifying juvenile rockfishes (*Sebastes* spp.), while Orr et al. (2000) and Love et al. (2002) provide keys to larger rockfishes. Kramer et al. (1995) provides information for identifying flatfishes. Generally, there are no widely comprehensive guides to the epibenthic invertebrates.

Either common or scientific names of fish may be used in the field, however, in the case of invertebrates, only scientific names are permissible. Use standard common and scientific names of fishes and scientific names of invertebrates given in a list of expected or trawl-caught species of fishes and invertebrates in southern California that have been distributed to organizations prior to the survey. For species not in these lists, use only standard common and scientific names of fishes given in Page et al. (American Fisheries Society publication 2013), and scientific names of invertebrates from the SCAMIT edition 12 list of benthic macro- and mega-invertebrates. Remember, data submissions must have current scientific names.

For every species caught, each organization will provide at least one representative of that species to the Bight'18 voucher collection (see Voucher Collection).

Each organization should have a kit containing a variety of tools which will aid in field identification. The kit should include forceps (small with sharp points and large with blunt points), a hand lens, dividers or calipers, dissecting needles, scalpels with scalpel blades, probes, and plastic rulers (marked in millimeters).

Diversity Index Exclude Column

The fish and invertebrate datasheets include a "diversity index exclude" column. A "Yes" response represents the taxonomist's recommendation that the taxon should be excluded from counts of the number of taxa reported in the sample. It only pertains to organisms not identified to species-level (e.g., class/order/family/genus). Three conditions must co-exist for the reported taxon to be excluded: (1) identification is not to species-level; (2) the reported taxon is represented at the station by other members of its same taxon group identified to a lower level (e.g. species); (3) the taxonomist cannot determine if the reported taxon is distinct from other members of same taxon group identified at the same station. It is necessary that the taxonomists make this evaluation during sample analysis (i.e., by annotation of the field sheet). It cannot be effectively applied after sample analysis as there is no way of determining later whether the third criterion for use was met. **Example**: The final identification of a specimen is "Virgulariidae". There is not enough information for the taxonomist to determine whether the specimen might be "*Virgularia agassizii*", which was also found in the same sample. The "Virgulariidae" record is given an Exclude =" Yes" on the datasheet.

Length Measurement

All fish species will be measured using measuring boards, a meter stick, or a tape measure for very large specimens. Lengths of invertebrate species will not be measured. A measuring board typically consists of either a flat or trough shaped board with a part of a meter stick running down the middle. A smaller board (cross board) is attached across the zero-end of the meter stick. Centimeter size-classes can be marked along the side of the measuring board with the number of the size class marked next to the appropriate centimeter. Measuring boards should be checked periodically for accuracy (+/- 1 mm).

When measuring a fish, the head should be pushed gently against the cross member at the zero-end of the measuring board. Standard length in bony fishes is obtained by measuring from the anterior tip of the head to the posterior end of the caudal peduncle, located slightly anterior of the externally visible origin of the caudal fin rays. Bending the tail laterally upwards and noting the point of sharp flexure can most closely approximate where standard length is measured (Figure 6). Total length will be measured for all cartilaginous fishes and some bony fishes (*e.g.*, eel-like fish). Wingspan will be measured in addition to total length for stingrays and whip tailed rays because the tips of their tails are frequently broken off (Figure 7).

The length of all fish specimens will be reported in size classes of 1 cm intervals (Mearns and Allen 1978). The first centimeter size class (size class number 1) extends from >0 to 1.0 cm; size class 2 extends from >1.0 to 2.0 cm, and so forth (Figure 8).

All species will be recorded on either the Demersal Fish Identification Form or the Epibenthic Invertebrate Identification Form (Appendix F). If using a field app to record data, ensure a

hardcopy is available in case of power failure. For fish species with 10 or fewer individuals, each size class measurement will be recorded on the Demersal Fish Identification Form (Appendix F), separated by commas. For species with more than 10 individuals, the species identifications and totals are listed on the data sheet, but the individual sizes are tallied on a separate Demersal Fish Size-Class Form (Appendix F).

An attempt should be made to size-class all fish. For the rare occasions when size classing is not possible (*e.g.*, a huge catch of a single species), a subsample of at least 250 individuals should be measured. This subsample should contain size classes which are proportional distributed to represent the overall catch for that particular species (see Appendix F for more details). When this occurs, the reason should be noted on the data sheet. All anomalies must be individually noted by their size class.

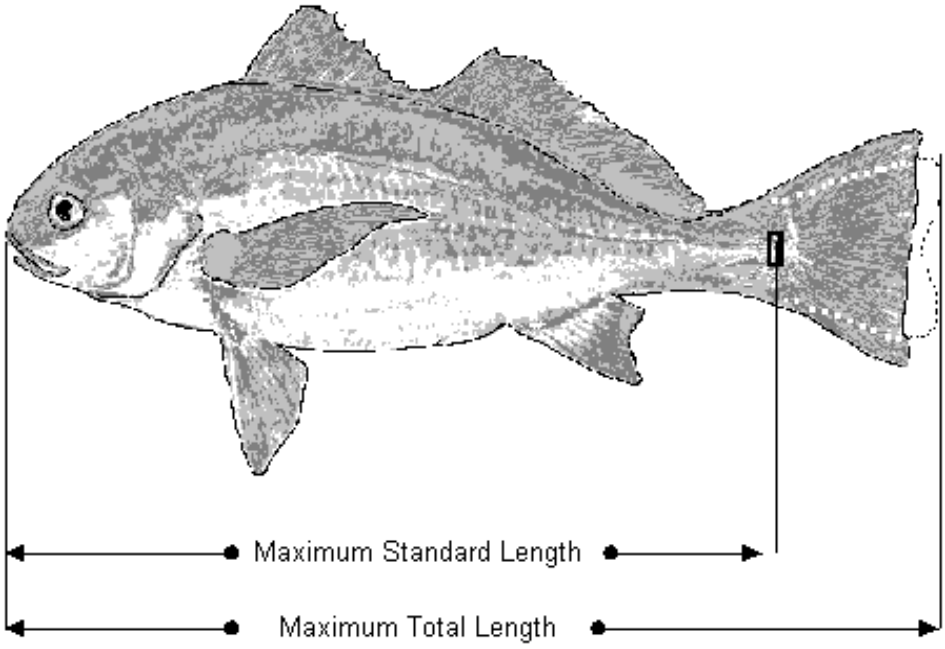

Figure 6. Endpoints for Standard Length (SL) and Total Length (TL) for bony fish.

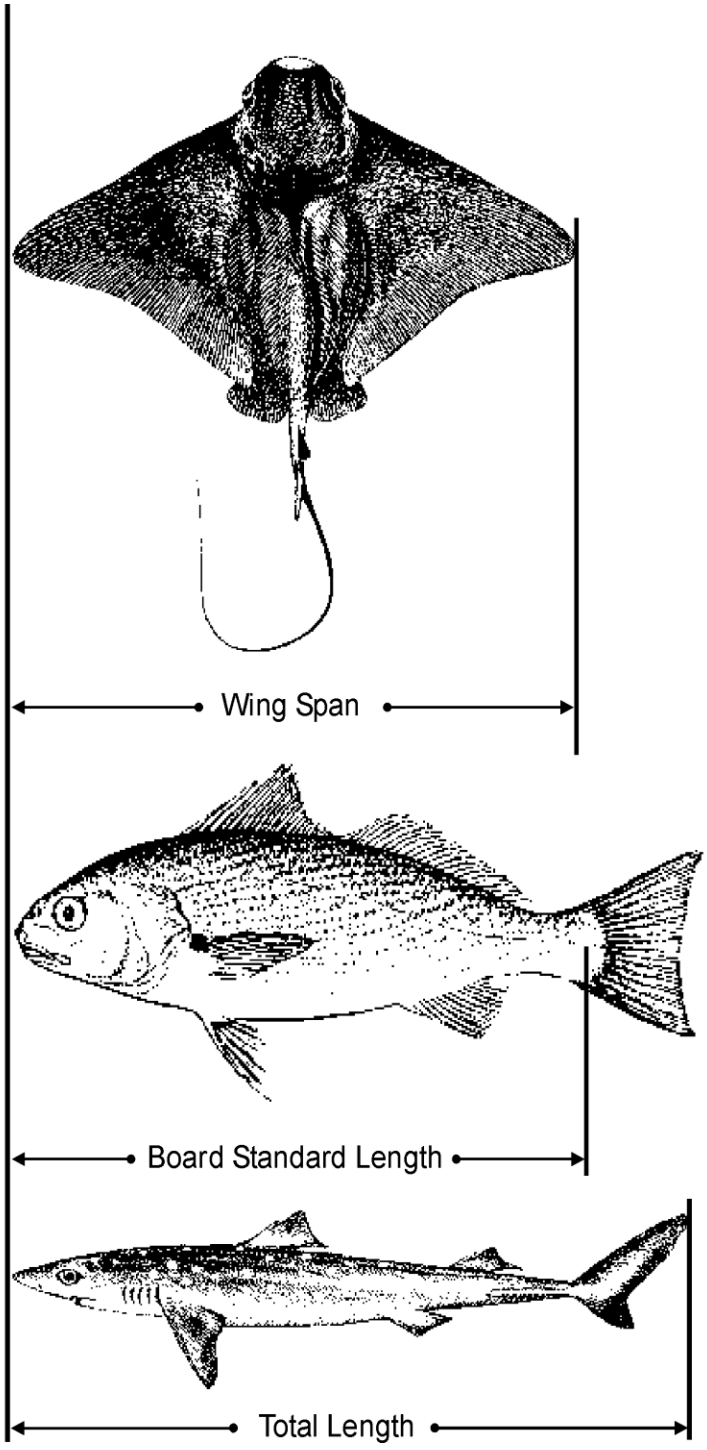

Figure 7. Endpoints for Wingspan (WS), Standard Length (SL), and Total Length for measuring the length of bony and cartilaginous fishes.

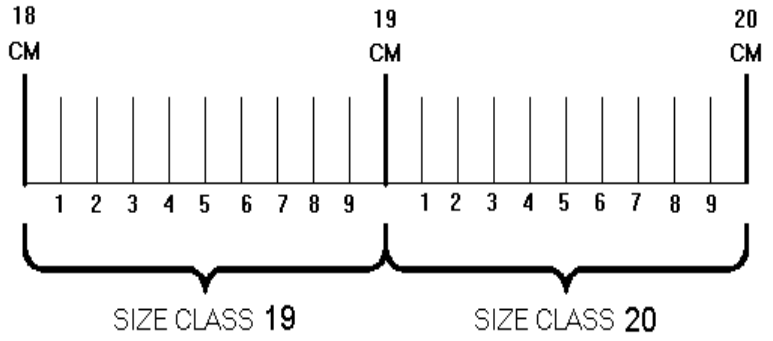

Figure 8. Relationship of centimeter size classes to millimeter values using centimeter and millimeter marks on a meter stick where size class 20 is defined as 19.1 to 20.0 mm.

Weighing

Weight data collected from fish and invertebrate species will be used to estimate the total biomass of the catch and for each species where practical. Each organization should have a range of spring scales capable of weighing to the nearest 0.1 kg. The scales should be calibrated at the start of each trawling day using a standard set of at least three weights which cover the low, mid and high ranges of the scale. Weighing will be done using a pre-weighed tare bucket, or another suitable container (*e.g.*, plastic net bags). If a tare bucket is used, the bottom should have many holes drilled through it to allow any excess liquid to drain off before the weight is recorded. Tare buckets should be washed periodically to remove the accumulated slime.

The total biomass of each species will be measured with a spring scale. Species with a biomass greater than 0.1 kg will be recorded to the nearest 0.1 kg. The tare container weight will be subtracted from the gross weight (species plus tare container) to give the weight of the species (net weight). Tare and gross weight can be recorded on the data sheet but are not required. Small species weighing less than 0.1 kg will be recorded as <0.1 kg. These weights will assist in calculating the total biomass of the catch.

Large organisms may be weighed individually. Individual weights of smaller specimens may also be collected using a range of scales capable of weighing to the nearest 0.1 g.

Enumeration

Fish and invertebrates are normally enumerated after identification. The total number of each fish and invertebrate species should be recorded on their respective identification form. When catches of single fish exceeds 10 individuals, those counts will also be recorded on a Demersal Fish Size-Class Form. If a particularly abundant species (250+) is encountered, the aliquot method of enumeration can be employed (at the discretion of the Cruise Leader).

Aliquots

A generalized aliquot method is commonly used to subsample large catches of fish and

invertebrate species. Begin by selecting a representative subsample of the catch by counting a minimum of 250 specimens from the catch and weigh the subsample to the nearest 0.1 kg. Next, weigh the remaining specimens and then divide that weight by the aliquot weight. Multiply that by the number of individuals in the aliquot to arrive at an estimate of the total number of individuals.

An alternative method for invertebrates can also be used. Add an unknown number of animals to a bucket until a weight of 1 kg is reached. Determine the number of animals it took to achieve the 1 kg weight. Weigh the remaining specimens, and then divide that weight by the aliquot weight. Multiply that weight by the number of individuals in the aliquot to arrive at an estimate of the total number of individuals.

The aliquot method has some inherent biases that the field crew must guard against.

1) The size class distribution of the individuals in the subsample should be representative of the specimens from which the aliquot was taken. Very large or small individuals could bias the weight so they should be enumerated separately.
2) Choose a spring scale where the weights fall within the mid to upper range of the spring scale being used. This prevents the inherent inaccuracy of the spring scale at the low end from being multiplied throughout the entire biomass calculation.
3) **Do not overlook anomalies** when processing aliquots. The number of anomalies should be recorded in the aliquot comments section of the data sheet and transcribed to station species list. For fish, include size class information. **This anomaly information needs to be included with the data submittal**.

Examination for Gross Pathology

During the identification and measurement procedures, all fish and invertebrates will be examined for gross pathology. This entails a scan of an individual organism for anomalies and noting the type of pathology (by abbreviation) next to the length of organisms (for fish) during measurement on the appropriate data sheet. The following anomalies will be noted for fish:

1) fin and tail erosion
2) tumors
3) leeches (Hirudinea)
4) monogeneans
5) other external parasites (*e.g.*, copepods, isopods)
6) eye parasites (*i.e., Phrixocephalus)*
7) color anomalies (ambicoloration, albinism) (Mearns and Haaker 1973)
8) skeletal deformities (Valentine 1975)
9) lesions
10) other anomalies

An observation should be noted next to the individual length on the Demersal Fish Identification Form (Appendix F) and described in the comments section. However, when recording anomalies in the database, a separate record should be used for fish of the same species and size class with and without anomalies. For example, if five *Citharichthys sordidus* of size class 10 were collected

at a given site and only one had an eye parasite, then two records would be needed. One record would record four *C. sordidus* of size class 10 with no anomalies, and the other would record one *C. sordidus* of size class 10 with an eye parasite (see Bight'18 Information Management Plan for more detailed information and anomaly codes pertaining to multiply occurrences on an individual).

For invertebrates, anomalies will be noted and counted as in the fish example in the Epibenthic Invertebrate Identification Form (Appendix F). Invertebrate anomalies are largely restricted to external parasites and include the following: surface-dwelling parasites; copepod parasites; other large, surface-dwelling molluscan, crustacean (barnacles), or turbellarian parasites; burn-spot disease (decapods); echinoderm wasting disease (asteroids and echinoids). Copepod parasites of the gills, which are hidden from external view and generally too small for field identification, are excluded from the anomaly category. Larger surface-dwelling molluscan, crustacean, or turbellarian parasites are included. In cases where decapods are infested with parasitic barnacles, the presence is recorded as an anomaly. Although the primary parasite is internal, it is reflected in an external brood sac visible on the body surface. The presence of species using the exoskeleton of decapods as substrate for growth is not considered parasitic. Burn-spot disease in decapods should be counted as one anomaly per infected member of the catch, not by counting individual burn-spots on each carapace. Similarly, in echinoderm wasting disease as seen in asteroids and echinoids, each infected echinoderm should be counted as one anomaly.

For fish, anomalies will be noted next to their associated length measure or tally on the Trawl Fish Species Sheet or Size Class Sheet (Appendix F). Fin erosion can be found on the dorsal, anal, and caudal fins of flatfishes, and on the lower caudal fin and pelvic fins of bilaterally symmetrical fishes. Tail erosion occurs on the top and bottom of the caudal fin or along the entire posterior caudal fin of bilaterally symmetrical fishes. Tumors can be smooth and rounded (angioepithelial nodule) or furrowed (epidermal papilloma). Leeches are small worm-like animals that often occur on the body of some flatfishes. Monogeneans look like scales that are moving. Externally obvious copepod parasites occur on the eye, fins, or body of fish. Ambicoloration is often found on the blind side of flatfish (Figure 9). Skeletal deformities include crooked backs, snub noses, or bent fin rays. Lesions include sores that do not appear to be caused by net damage, often black in color. Note that common Cymothoidae gill parasites are not to be marked as a parasite if seen on a fish.

Remember to associate an anomaly incidence with an individual, not an entire size class grouping or an entire group of identified species.

Retain representative examples of fish and invertebrates exhibiting each new instance of disease or parasite. These vouchers should be returned to SCCWRP.

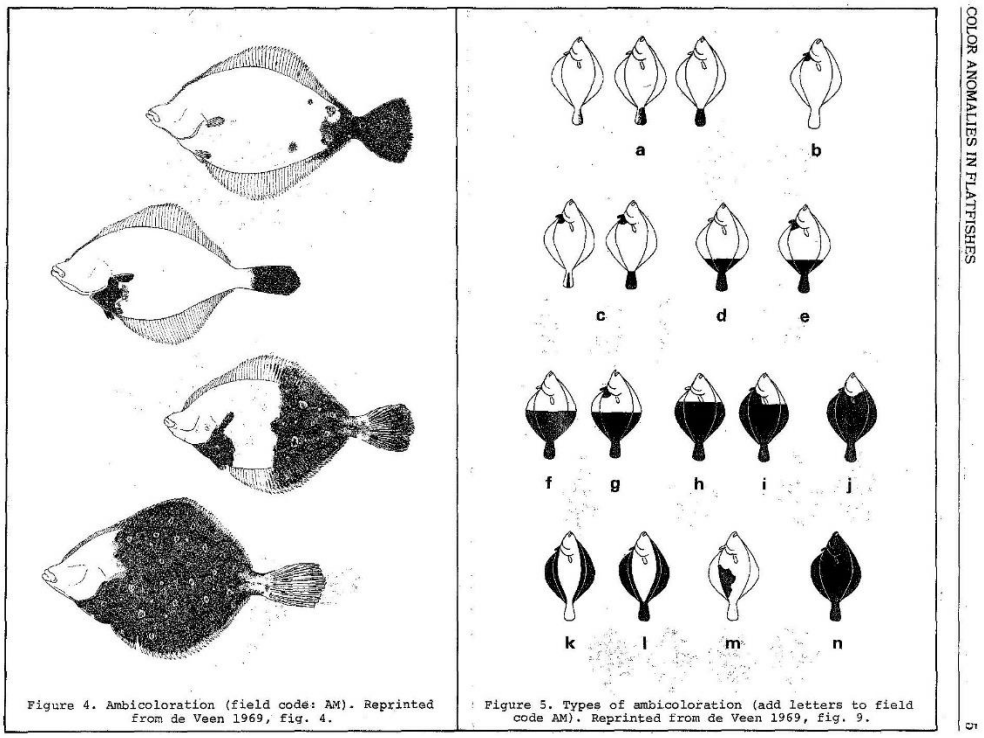

Figure 9. Examples of ambicoloration (Mearns and Haaker 1973)

Process Stage Monitoring

Accidental omissions can occasionally be made if a bucket of organisms is not processed. One method to avoid this problem is attaching a colored rubber tag (made of a square with a slit in one side) to the handle of each bucket to indicate a stage of processing. For instance, different tags can represent that the bucket is ready for identification, measurement, weighing, preservation, or discarding. As the bucket progresses to the next stage, the current tag can be pulled off and a new tag can be added. This procedure is not necessary for small catches but may be helpful when catches are large. Another method uses tags with commonly caught species names that can be temporarily attached to buckets to facilitate sorting and processing. The field crew has discretion to use whatever agency specific method they choose to stop accidental omissions.

Safe Handling of Organisms

Field personnel are likely to encounter a variety of organisms that are potentially harmful. California Scorpionfish (*Scorpaena guttata*) have venomous fin spines that can cause severe pain. This species should be handled with leather gloves and/or pliers. Hot water should be applied to any puncture wound inflicted by this fish; heat is useful in breaking down the protein in the venom.

Several species of rockfishes and the Spotted Ratfish (*Hydrolagus colliei*) also have mildly venomous spines which can cause a burning sensation. The Round Stingray (*Urobatis halleri*), the California Butterfly Ray (*Gymnura marmorata*), and the Bat Ray (*Myliobatis californica*) all

have venomous spines on their tails. The wound should be immersed in hot water to break down the protein in the venom.

The Pacific Electric Ray (*Torpedo californica*) can emit a very strong electric shock. If you must handle this species, wear rubber gloves and hold it by the tail. **Do not grasp the disk with both hands!**

Pacific Angel Shark (*Squatina californica*), Spiny Dogfish (*Squalus acanthias*), Spotted Ratfish, Midshipman (Portichthys), and California Halibut (*Paralichthys californicus*) are some of the encountered fishes with sharp teeth that can result in painful bites if they are not handled properly.

Care must also be taken in handling the Blue Leg Mantis Shrimp (*Hemisquilla californiensis*). This species is capable of severely cutting a person with its raptorial appendages. Care should also be taken in handling any of the large crabs and octopus.

Preservation of Specimens

Voucher specimens, DNA specimens/samples (optional), incompletely identified fish and invertebrate specimens, and those with diseases that require further examination should be returned to the laboratory. Fish and invertebrate specimens may be preserved or documented for QC or identification purposes in one of four ways:

1) fixing in buffered formalin-seawater;
2) 95% ethanol (ETOH) for **DNA specimens only**; Do not use denatured ETOH;
3) freezing;
4) photographing.

Most specimens should be fixed in buffered formalin-seawater UNLESS (1) they are destined for DNA barcoding or (2) are absolutely too large for preservation in the field.

The preferred method for preserving small specimens is to fix them in 10% buffered formalin-seawater. Buffered formalin is made by mixing 50 g Na_2B4O_7 (sodium borate) per liter of formaldehyde or 5 g per liter of 10% formalin. The body cavities of fish greater than 6 cm in length should be slit with a scalpel on the right (for most bilaterally symmetrical fish), the blind side (for flatfish), or ventral side (for dorsoventrally flattened fish, such as rays) before the specimen is placed in formalin. The slit allows preservative to enter the body cavity and preserve the internal organs. **Note that by convention, bilaterally symmetrical fish are photographed or drawn with their heads facing left and dissections or gut cavity incisions are conducted only on the right side of the fish.**

1) Fish and invertebrates will be placed in plastic bags or plastic jars and fixed in 10% buffered formalin-seawater. Fish should be inserted tail-first into jars so that they can be removed easily without destroying the fin rays or spines.

2) Fish should remain in formalin for no more than a week before being transferred to a freshwater bath. It is recommended that fish specimens soak in freshwater for at least two days. The water should be replaced at least once during that period. The fish should then

be transferred to a solution of 70% ethanol for long-term preservation.

3) Trawl-caught invertebrates will also be fixed in 10% buffered formalin-seawater and preserved in 70% ethanol.

4) Very large specimens can be placed in plastic bags and frozen on dry ice if excessively large quantities of formalin would be required to fix the specimen in the field. These can then be thawed and fixed in the laboratory with a 10% buffered formalin solution. If possible, large specimens with tumors, fin erosion, or lesions should be fixed in the field with formalin rather than frozen. **Do not freeze specimens that can otherwise be preserved in the field in formalin-seawater.**

5) Small invertebrates (*e.g.*, nudibranchs) may be kept cold in seawater and returned alive to the lab for identification. Color photographs of these specimens are strongly recommended.

Photography of recently caught specimens can be useful in documenting color patterns that can be used in subsequent field identifications. It is, therefore, recommended that whenever possible a specimen be photo-documented. However, only large specimens of fish and invertebrates can be officially photo-vouchered in the field. The photograph should show the overall appearance of the specimen, and additional photos for any important identifying features. If characters necessary for the identification of a species cannot be seen in the photograph, the photograph will not be accepted as a voucher. Colorful fishes may also be photographed in addition to providing a preserved specimen to aid in identification of the voucher. Photographs of unidentified rockfishes, in particular juveniles, should be taken as soon as possible after capture because their color, which is an important taxonomic character, fades during preservation.

Bilaterally symmetrical fish and dorsoventrally flattened fish (skates, rays) should be photographed facing left. Flatfish should be photographed with the eyed side up. The left-eyed species should be photographed facing to the left and the right-eyed species should face to the right (**Note: To prevent upside-down photos, t**he gill cover slit should be oriented towards the bottom profile of the body). If an anomaly or important character occurs on the opposite side of the recommended profile, a photo should <u>also</u> be taken of the afflicted side. All specimens should be photographed on a light background with a ruler alongside and a label giving date, station number, and species in large bold letters. Notes should be made of character states that can aid in identification (*e.g.*, counts of fin rays, gill rakers, and scales).

Specimens preserved for further identification must be noted on the field data sheet. Note whether the organism is fixed (formalin or ethanol), frozen, or photographed. A photograph log should be kept during the survey, documenting species name, the frame number, the date, and the station location of each photograph. Voucher specimens will not be submitted to SCCWRP until they have been transferred to alcohol, numbered, and an associated inventory list presented.

M. Voucher Collection

Participating organizations will provide at least one representative of each new species collected for the Bight'18 voucher collection. This collection will document and verify trawl diversity and

the types of diseases or anomalies found in the examinations for gross pathology. Voucher specimens should be preserved in an appropriate manner and clearly internally labeled with identity, collection date, site name, site location, and depth (Appendix F). It represents the final QAQC check for taxonomic identification. Field crews are responsible for creating, maintaining, and checking a species list for specimens collected as vouchers. These specimens are to remain with the Bight'18 collection and cannot be used for their own organizations collection.

The Bight'18 voucher collection of trawl organisms will be temporarily housed at SCCWRP. Submit an electronic voucher inventory list with the specimens on an Excel spreadsheet (*i.e.*, Organization code, Specimen Number, Scientific Name, Common Name, Station ID, Collection Date, Collection Depth, and Preservation Method). Clearly number the outside of the voucher containers so it matches the inventory list. The invertebrate collection and selected rare/unusual fishes will later be transferred to the Natural History Museum of Los Angeles County. The collection will be taxonomically validated at SCCWRP by members of SCAMIT (invertebrates) and SCAITE (fish). The Bight program encourages new and existing participants to continue developing an organizational voucher collection for their future needs if accessing museum collections is not a good alternate. Fishes not transferred to the museum will be delivered to the collecting organization for disposal.

N. DNA Barcoding Specimens

DNA barcoding specimens are optional for B'18 and at the discretion of field crews and their organizations. If more than one specimen of a newly encountered species is taken, a second specimen (tissue clips are acceptable substitutes) can be retained for the future DNA analysis. Each of these specimens/samples will be preserved in 95% ethanol (not denatured ethanol or isopropyl alcohol). For large specimens, or if only one individual of a species is collected, the whole specimen will be photo vouchered or retained for the voucher collection and a snip of a fin will be retained in 95% ethanol. Priority should be with whole animals for DNA analysis because of potential mucus contamination from other trawl-caught species. Store DNA samples individually or in a bucket of 95% ethanol and away from formalinized voucher specimens. Upon returning to the laboratory, transfer specimens to fresh 95% ethanol. DNA specimens will be transferred to SCCWRP in clean glass jars with fresh 95% ethanol. Label specimens appropriately (see Appendix F for examples of inside and outside labels) so specimen can be tracked back to its voucher counterpart and database record. Distinguish DNA specimens with a color dot on the outside of the container for post-survey voucher validation with coded numbering (*e.g.*, D-1, DNA-1, etc.).

At present, the museum still has poor DNA coverage for any invertebrate. If specimens are small, ideally 3-5 individuals of any species will elucidate the diversity at a specific locality (*i.e.*, stations). For species complexes and troublesome taxa, specimens from multiple localities are extremely useful for quickly discerning relevant distinguishing morphological characters. If specimens are large, specimen photo-documentation and a preserved piece of a tentacle or arm will suffice. For fish, the museum is only interested in new, rare, or unusual specimens.

O. FID Specimens

Specimens requiring further identification should be reexamined in the lab by the same

organization and the data corrected as appropriate on the field data sheet. Do not submit FID specimens to SCCWRP unless the identifications cannot be reliably resolved in-house by staff taxonomists. Any unresolved FIDs SCCWRP does receive will be identified at the time the vouchers are validated. FID data will be returned to the responsible organization so the data sheets can be revised and the database submissions corrected.

P. Quality Assurance/Quality Control Procedures

In addition to the pre-survey QA protocols, the following QC measures will check the accuracy of taxonomic identifications and counts made during the survey:

1) Measurement Quality Objectives (MQO) for trawl-caught organisms are as follows:

   Identification- 90%,
   Enumeration- 90%
   Length- 90%
   Biomass- 90%
   Gross pathology- 95%

2) External QA/QC field audits of each field group will be conducted during Bight'18 to ensure that trawling is being carried out per project protocols and that the specimens are being processed properly. Taxonomic identifications will be checked during at least one visit to each vessel by Field QA/QC Auditors. They will observe species identification by each organization in the field and record the data on a Taxonomy QA/QC Data Sheet (Appendix I). Their duties include rechecking the identifications of at least 25% of the species collected during the day and noting any problems with the identification of pathologies. The Lead Scientist will be informed of any problems and the field personnel will be instructed regarding the appropriate identifications as needed. Each vessel will be expected to have appropriate taxonomic identification aids during the survey. The trawl committee recommends that data from organizations that fail their external audit be flagged in the database for possible exclusion from the Bight'18 Trawl report.

3) The Cruise Leader for the field team will perform QC field audits on a minimum 10% of their assigned trawl sites. For example, if 21 sites are assigned then 3 QC audits are performed or if 4 sites are assigned then 1 QC audit. The audit is performed on fellow team members conducting trawling operations. The Cruise Leader will predetermine the QC stations. Per QC audit, two species of fish and invertebrates will be internally audited. Whenever possible, the species selected for auditing should have a minimum of 10 individuals (greater is recommended). After normal processing, the crew will retain these species. The Cruise Leader (or designee) will reprocess the same specimens with the results recorded on a QA/QC data sheet (Appendix I) and then compare with the original results. If obvious discrepancies occur, the Cruise Leader is responsible for re-training and oversight as the specimens are reprocessed, in addition to oversight at subsequent trawl sites. Species selected for QC processing should change throughout the project. If low

abundances of invertebrate and fish (<10 individuals) occur, QC audits can run into subsequent trawl sites until complete.

4) Taxonomic QC voucher checks. A voucher specimen of each species collected by each organization will be preserved and returned to SCCWRP during the survey (see Voucher Collection above). The identification of these specimens will be checked by qualified taxonomists (*i.e.*, SCAMIT, SCAITE) following the survey to further ensure that identifications were made correctly. Anomalies will also be verified. Errors will be corrected in the data.

Lead Scientists, Cruise Leaders, and Lead Taxonomists are responsible for training their staff on methods described within this SQA Field Operations Manual. A check list of internal QA/QC activities (*e.g.*, fishboard accuracy check, scale calibration, oversight of measuring and weighing techniques, anomaly checks, datasheet review, etc.) is recommended.

Q. Special Studies

Bioaccumulation in Seafood: California Department of Fish and Wildlife (CDFW) will collect all the samples and ship them frozen to SCCWRP for storage. These special samples will be wrapped in foil, placed in a plastic bag, and labeled (externally). The minimum information required on each sample label is an area description, zone number, sampling date, and species code. A **completed chain of custody form** must accompany all sample shipments to SCCWRP. They will be distributed later for dissection and analysis. See B'18 Sediment Quality Assessment Workplan for further details.

California Halibut Essential Fish Information Study: The California Department of Fish and Wildlife (CDFW) is evaluating the California Halibut fisheries in the SCB. The goal is to get fishery independent assessment information on sub-legal fish from shallow water strata during the Bight survey. Fish sacrificed for bioaccumulation are to be retained after dissections. Fish in poor condition and unlikely to survive during release should be retained. In addition, between Oceanside and Los Angeles, CDFW aids/scientist or equipment will be available for tagging fish or processing tagged fish. All Halibut will be processed or tagged and immediately released. Information needed include tag number, location, and size. Biologists from CDFW have requested that each **vessel** can retain up to 40 sublegal or "short" California Halibut during the Bight survey. These fish will be delivered to SCCWRP for storage. All retained fish are to be sent the CDFW Monterey laboratory (project NCCFRMP). These special samples will be bagged, labeled (internally and externally), and frozen. The minimum information required on each sample label is station number, sampling date, agency code, study and species. A **completed chain of custody form** must accompany all sample shipments to SCCWRP. See B'18 Sediment Quality Assessment Workplan for further details.

X. LABELING AND SHIPPING OF SAMPLES AND FIELD DATA SHEETS

A. Sample Labels/Tracking

Each sample will be identified and tracked by the station, parameter, date sampled, and split number if required. Individual log numbers may be used at the discretion of the sampling organization. Sample log numbers will be handled by SCCWRP for the samples shipped to SCCWRP that are not run by the organization that collected them in the field.

B. Labels

Labels will be printed by the organization responsible for field sampling prior to the survey and will include, at a minimum, the station number, parameter, date, and split (*i.e.*, 1 of 1, 2 of 3, etc.). Dates will be reported as day/month/year. External labels should be affixed with clear postal tape to the outside of the container. Internal labels for biological samples must us archival paper. Use 100% cotton rag (*e.g.*, Resistall, available from University Products) which can be both laser printed and written on with No. 2 pencil. These labels are put directly into the container with its specimen. Ethanol removes most inks and archival pens are fussy to use in the field – hence No. 2 pencil is preferred. Plasticized label paper is not suitable for wet collections of any kind.

C. Field Data Sheets

If a field computer data system is not being used during any part of the Bight'18 sampling, data sheets and cruise logs will be retained by the sampling organization until sampling has been completed. Retain trawl data sheets until all species identifications are complete. Species identified in the laboratory must be added to these data sheets and verified within the laboratory.

Upon completion of all laboratory identifications, the good quality hardcopies of original field data sheets, photographs, and collection permit are to be retained by the sampling organization. Submit all field data electronically to the SCCWRP web portal (data checker). Submit good quality PDFs or hardcopies of all field data to SCCWRP as soon as the data sheets have completed internal QA/QC review by sampling organizations. Ensure all handwritten comments from pencil are visible and clear in the PDF or hardcopy. SCCWRP may request the originals if sampling organization submit poor PDF copies or ask for additional electronic copies which clearly highlight problematic text.

D. Shipping of Samples

All benthic infauna, sediment chemistry, and toxicity samples not analyzed by the field sampling organization's laboratory will be shipped to SCCWRP within the prescribed holding time. All shipping of samples will be the responsibility of the field sampling organizations. See Appendix K for detailed SCCWRP shipping information. Check regulations for shipping hazardous materials.

Voucher collections will be taken or shipped to SCCWRP after an organization has completed

proper specimen preservation, transfers to specimen jars, internal taxonomic identification, and inventory list.

E. Chain of Custody Forms

Chain of custody forms (Appendix F) are to be filled out at the end of each sampling day detailing the transfer of samples from the vessel crew to the laboratory, or to delivery personnel. A form is to be filled out for each set of samples that will be transferred to a specific location. The sample and container type is to be included on the form to identify the samples being transferred. This form is to be signed by the crew member transferring the samples and the laboratory staff member receiving them. A copy of the form is to be kept and the original form with signatures will accompany the samples. If samples are shipped by carrier, a copy of the chain of custody form is to be sent to SCCWRP for tracking purposes.

XI. CONTINGENCY PLANS

A. Purpose

Any field program can be affected by factors outside the control of the sampling crews. Weather, equipment failure, errors in designating station locations, and accidents can all prevent the field crews from obtaining samples at one or more stations. Contingency plans made in advance of the survey can greatly facilitate decision-making in the field. It is the responsibility of the Cruise Leader to make most of these decisions in the field, based on the protocol described below. If there is any question regarding which protocol to follow, the Field Coordinator should be notified immediately.

B. Adverse Weather Conditions

If the weather conditions deteriorate during any sampling day, it is ultimately the responsibility of the Boat Captain to determine if the conditions are sufficiently bad to prevent further sampling. The Cruise Leader in consultation with the Boat Captain should evaluate all alternatives, such as changing the sampling plan to more protected areas or returning to the prescribed schedule when the weather improves. Every attempt should be made to avoid wasting the entire day. However, **the safety of the crew is priority number one.**

C. Station Inaccessibility

Stations can be inaccessible because 1) they were incorrectly positioned on land, 2) located in water too shallow for the boat, or 3) they cannot be sampled for unforeseen circumstances. If it cannot be sampled, the sampling site will be moved to a location within 100 m horizontal distance from the original site, staying within +/-10% of the depth of the original site. If it still cannot be sampled, the station will be abandoned. For most Bight'18 strata (shelf, slope, Northern Channel Islands), no station should be sampled in less than 6 m or more than 1000 m. In bays and harbors, the safety margin is 3 meters. In estuaries with greater than 27 ppt salinity, 1 m is the safety margin using shallow draft vessels. Estuary samples should only be collected within subtidal portions of the channel. In brackish estuaries with potential wadable sites, it is the judgement of the field team as to safety and accessibility of the site.

D. Lost Gear

Lost gear can potentially have a significant effect on the sampling program. Equipment can be expensive and replacements may not be obtained in a timely manner. Crews should take every precaution against the loss of gear by properly tightening shackles and other connectors.

If important gear is lost, notify the Boat Captain immediately, so he can record the position using the vessel's navigation system. If possible, deploy a buoy at that exact location so relocation is made easier. Attempt to recover the equipment for a reasonable amount of time. If unsuccessful, use spare equipment (when available) or continue sampling without that particular equipment. Notify the Regional Monitoring Coordinator as soon as possible when equipment is lost.

XII. WASTE DISPOSAL

Proper disposal of all waste is an important component of field activities. At no time will any waste be disposed of improperly. The proper methods of waste disposal are outlined below:

A. Routine Garbage

Regular garbage (paper towels, paper cups, etc.) is placed in trash containers on board the boats. It can then be disposed on land in public receptacles or recycled.

B. Detergent Washes

Biodegradable detergents are not to be used for routine cleaning of any sampling equipment during Bight'18.

C. Chemicals

Acetone, formalin, and other hazardous materials should be disposed of by following all appropriate hazardous materials regulations. They should never be disposed of at sea.

D. Fish Waste

After each trawl catch has been processed completely, the remaining catch should be disposed of at sea. Use discretion when discarding the catch. For sampling conducted nearshore or in bays and harbors, return only live fish and invertebrates to the area where trawling occurred. All of the remaining fish should be disposed of offshore. Under no circumstances should fish be given to the public.

XIII. BIGHT'18 PROGRAM ORGANIZATION

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XIV. LITERATURE CITED

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See Associated PDF for Appendices