Section B2. Sampling Methods Requirements

Field personnel will adhere to recommended SWAMP sample collection protocols or approved and documented alternative protocols, in order to insure the collection of representative, uncontaminated (contaminants not introduced by the sample handling procedure itself) water, sediment, tissue, and biological samples for laboratory analyses. If protocols are revised or altered, the deviations from the standard protocols must be documented.

Appendix D provides a collection of recommended minimum Standard Operating Procedures (SOP's) for Field Sample Collection methods (except for sample collection for Bioassessment and for Benthic Infaunal Community Assessment, which are provided in **Appendix G**), while Field Data Measurement procedures, including probe calibrations and maintenance, are provided in **Appendix E. Appendix D** describes those sampling methods currently being conducted within SWAMP. At the SWAMP Workshop held in July 2001 in Moss Landing, CA, sample collection and processing issues, as well as field data measurement issues, were discussed at length by all SWAMP participants in an attempt to reach consensus on as many points as possible for the use of standardized minimum methods for sample collection/processing activities, as well as field data measurement activities. What was agreed upon for the first several years of the "start-up" of the SWAMP Program was an approach to ***"standardize where possible; document otherwise"***. The need for flexibility to accommodate region-specific sample collection priorities was acknowledged, but the need for striving for moving towards using standard methods to the extent possible was also agreed upon, as practical and appropriate.

Briefly, the key aspects of quality control associated with sample collection for eventual chemical analyses are as follows: 1) field personnel will be thoroughly trained in the proper use of sample collection gear and will be able to distinguish acceptable versus unacceptable water, sediment, or biological specimen samples in accordance with pre-established criteria; 2) field personnel will be thoroughly trained to recognize and avoid potential sources of sample contamination (e.g., engine exhaust, winch wires, deck surfaces, ice used for cooling); 3) sample gear and utensils which come in direct contact with the sample will be made of non-contaminating materials (e.g., glass, high-quality stainless steel and/or Teflon™, according to protocol) and will be thoroughly cleaned between sampling stations according to appropriate cleaning protocol; 4) sample containers will be of the recommended type and will be free of contaminants (i.e., pre-cleaned); and 5) conditions for sample collection, preservation and holding times will be followed.

Corrective Actions for Field Activities

The field sampling staff have primary responsibility for responding to failures in the sampling or measurement systems. Deviations from SWAMP protocols and the SWAMP QAMP are documented in the comment section of field notes. Data problem resolution is discussed in detail

in Section B10 of this document. If monitoring equipment fails, SWAMP personnel will report the problem in the comment section of their field notes and will not record data values for the variables in question. Actions will be taken to replace or repair broken equipment prior to the next field use. No data will be entered into the SWAMP database that were known to be collected with any faulty equipment.

Samples will be collected from four environmental media: water, sediment, tissue, and biota (biological assessment and benthic infaunal community assessment). Sampling of tissue will include methods specific for fish and for deployment/retrieval of mussels, clams, and other bivalves; sampling for biota will include methods for benthic macroinvertebrates and periphyton. For each of these methods described or referenced, it is the combined responsibility of all members of the sampling crew to determine if the performance requirements of the specific sampling method have been met, and to collect an additional sample if required. Summary descriptions of specific sampling methods and requirements are provided below.

COLLECTION OF WATER SAMPLES

Summary of Typical Procedure for Collection of Water Samples for Analyzing Trace Metals, Organics, Conventional Constituents, and for Toxicity Testing

All water samples collected for analyzing trace metals, organics, conventional constituents, and for toxicity testing in water will be collected using clean techniques that minimize sample contamination. Sampling methods will generally conform to EPA "clean" sampling methodology described in *Method 1669: Sampling Ambient Water for Trace Metals* (USEPA 1995a). Specific methods are also documented in **Appendix D**. Samples will generally be collected from shore or in-stream in wadeable waters, or by boat in non-wadeable waters (such as larger rivers, lakes, estuaries, and open coastal waters), in most cases by using a near-surface grab sample, but in those cases where depth-integrated sample collection is desired for water samples, a peristaltic pump and acid-cleaned polyethylene or Teflon™ tubing is used. Grab samples will be collected into appropriate pre-cleaned containers and aliquoted into glass, polyethylene, or Teflon™ sample containers appropriate for the analyses to be performed (see Sample Handling Requirements Tables in Section B3), *or* will be collected directly into the sample containers, if appropriate. Samples to be analyzed for dissolved (filtered) trace metals (including mercury) will be filtered to 0.45 µm in the field using Gelman in-line filtration capsules (in the case of pumped samples) or syringe filters (in the case of grab samples).

After collection, field-collected samples will be stored at 4˚C until arrival at the contract laboratory. Samples to be analyzed for mercury will be preserved at the contract laboratory, immediately on arrival. Samples to be analyzed for other constituents will be preserved in the

lab (in most cases) or field, as appropriate and as described in the SWAMP Sample Handling Summary Tables (Tables 6 and 7, Section B3).

This sample collection method requires that the sample collection tubing, and the sample bottle and lid come into contact only with surfaces known to be clean, or with the water sample. Additionally, mercury samples must have no air bubbles or head space present in the bottle immediately following sample collection. If air is present in the sample container for mercury analyses, additional sample will be aliquoted into the same sample bottle. If the performance requirements for specific samples are not met, the sample will be re-collected. If contamination of the sample container is suspected, a fresh sample container will be used.

Collection of Water Samples for Analyzing Bacteria

Pathogen monitoring in SWAMP will typically include sampling for pathogen indicator organisms (fecal and total coliform bacteria, *E. coli*, and *Enterococcus* bacteria). *Note*: Samplers must wear gloves when collecting any pathogen samples in order to prevent introduced bacterial contamination. In addition, please refer to **Appendix H** (Recommended Minimum Health and Safety Guidance for SWAMP Field Activities), which provides a summary of protective measures that should be employed when sampling areas where there is potential exposure to biohazards (e.g., in some areas with known high levels of bacteria and pathogenic activity). In addition, a detailed protocol specifically dealing with health and safety measures for field and laboratory personnel is in the process of being developed for just such situations. This will be distributed upon completion in the near future.

Samples analyzed for bacteria will be collected as near-surface grab samples. Sampling for bacteria will in most cases be performed according to the sampling procedures detailed for Standard Methods 9221B and 9221E (APHA *et al*. 1998). In brief, the sampling procedures are summarized as follows:

- Sample containers should be cleaned and sterilized using procedures described in Standard Methods 9030 and 9040 (APHA *et al.* 1998). In most cases, these containers are provided by the laboratories conducting the analyses. Alternatively, Whirl-pak bags may also be used, per protocol
- For waters suspected to contain a chlorine residual, sample bottles should contain a small amount of sodium thiosulfate ($Na_2S_2O_3$) sufficient to neutralize bactericidal activity. In most cases, bottles provided by contract laboratories already contain the sodium thiosulfate as a precautionary measure. For water containing high concentrations of copper or zinc, sample bottles should contain sufficient EDTA solution to reduce metal toxicity. *Note*: These conditions are rare in surface waters.

- Sample bottles may be glass or plastic (e.g. polypropylene) with a capacity of at least 100 ml., or again, Whirl-pak bags. After sterilization, sample bottles should be kept closed until they are to be filled.
- When removing caps from sample bottles, be careful to avoid contaminating inner surface of caps or bottles.
- Using aseptic techniques, fill sample bottles (or Whirl-pak bags), leaving sufficient air space to facilitate mixing by shaking. Do not rinse bottles.
- Recap bottles tightly.

If at any time the sampling crew suspects that the sample or sampling container has been contaminated, the sample should be re-collected into a new sample container.

If bacteriological samples are to be used for regulatory compliance purposes, then samples must be kept at 4˚C (dark) and transported to the laboratory so that the analysis begins within 6 hours of collection.

If bacteriological samples are non-regulatory in nature (ie, non-drinking water samples analyzed for non-compliance purposes), after collection, store samples at 4˚C (dark) until analysis, which must begin within 24 hours of collection. The 20th edition of Standard Methods (APHA et al. 1998) recommends analysis of samples as soon as possible, but specifies that non-drinking water samples analyzed for non-compliance purposes may be held for up to 24 hours (below 10˚C) until time of analysis. For this reason, data from these samples should not be used for assessment of regulatory compliance.

Summary of Bioassessment Field Procedures

Bioassessment monitoring includes sampling of benthic invertebrates and periphyton for bioassessment evaluations. The procedure for collecting samples of benthic invertebrates from wadable streams is based on the method detailed in *California Stream Bioassessment Procedures (Habitat Assessment and Biological Sampling)* (CDFG 1996a). Specific procedures are documented in **Appendix G**. Please note that Biological Assessment procedures utilized for RWQCB 6, in the Lahontan Region, are conducted by U.C.'s Sierra-Nevada Aquatic Resources Laboratory (SNARL), and vary in several significant ways from the methods outlined below (number of replicates, level of taxa identified down to, sampling gear mesh sizes, etc. The SNARL procedures for bioassessment are provided in detail in **Appendix G**.

The method used throughout most of the state for SWAMP RWQCB biological assessment (*California Stream Bioassessment Procedures--Habitat Assessment and Biological Sampling;* CDFG 1996a), can be briefly summarized as follows:

1. Reaches for benthic invertebrate sampling are selected after an initial reconnaissance of the section or stream. The overall goal is to select homogenous wadable reaches that best typify a riffle or run condition. Avoid walking in the stream when conducting a reconnaissance survey. Each riffle used for biological assessment must be approached from downstream and no portion of the riffle disturbed until all sampling is complete. Habitat assessment should be conducted after macroinvertebrates have been collected.
2. Fill out a field log sheet for each riffle section. Enter watershed name, station name, sample identification number, date, time and names of crew members.
3. To select a transect, place the measuring tape along the bank of the entire riffle section. Each meter (3 ft) mark represents a possible transect location. Select the transects from all possible meter marks along the measuring tape using the provided table of random numbers. If only one transect is to be sampled, then select one meter mark in the top one-third of the riffle. Record the meter mark in the field log for each transect.
4. Once transects have been selected, benthic macroinvertebrates are collected from several locations along the transect and combine them into one sample. If possible, choose three locations; the two side margins and the center of the stream. If the riffle is not ideal, then make adjustments to accommodate prevailing conditions. When making adjustments, such as increasing or reducing the number of locations for collecting organisms or sampling substrate that is not gravel/cobble, try to sample similar conditions at each reach. Record the number of locations per transect in the field log.
5. Starting from the transect furthest downstream, collect macroinvertebrates with a sampling device appropriate for stream conditions. Appropriate devices for wadable reaches include the D-shaped kick-net, Needham-type kick-screen, Surber bottom samplers, and the Hess bottom sampler. Appropriate devices for non-wadable reaches include Eckman and Ponar dredges, and drift nets. Combine the three collections. Measure and record stream temperature.
6. For wadable reaches, place the combined contents from the transect in a standard size 30 or 35 (0.6 or 0.5 mm, respectively) testing sieve. Large organic material is removed by hand while carefully inspecting for clinging organisms. All remaining material is placed with forceps in a 95% ethanol filled jar. If there is considerable debris in the net, inspect the sample in a white enameled pan and rinse material from the pan through the sieve before placing it in the jar.
7. Using a pencil, record the following information for each sample on a piece of water-proof paper and place in the jar:
    - sample identification number followed by -01, -02 (to identify each transect)
    - collection date and time
    - sampler type

- sample area
- habitat type
- collectors name
- comments

If the sample collection requirements above are not met, the sample will be re-collected, if it is possible to do so without compromising sample quality.

The procedures for collecting biological samples of benthic invertebrates from non-wadable streams generally follow *Methods For Collecting Benthic Invertebrate Samples As Part Of The National Water Quality Assessment Program* (USGS 1993a). Specific procedures and any modifications are documented in **Appendix G.**

COLLECTION OF BED SEDIMENT SAMPLES

NOTE: The summary procedures outlined below for Van veen grab sediment collection are thanks in large part to procedures outlined in "Field Sampling Manual for the San Francisco Bay Regional Monitoring Program for Trace Substances, Version 1, January 1999 (AMS 1999)". The use of this information, with relatively minor modifications, is greatly appreciated.

Collecting sediment samples is problematic. Samples of surficial sediments (top 2-3 cm) for analysis of chemical constituents, and for toxicity testing, must be collected in a manner such that surface layers are not disrupted when removed from the bottom of the sample for processing. Disruption may cause mixing of surficial layers with lower layers in the sample, and may lead to dilution or concentration of the contaminants of concern, depending upon the chemical content of the various layers of sediment.

Bed sediment samples are collected for many RWQCB's for SWAMP, although usually much less frequent. At this point, with the exception of RWQCB 5 and 8, all sediment sample collection is conducted by field staff of DFG and SJSUF/MLML.

The procedure summarized below can be used for collecting bed sediment samples for all types of sediment analyses typically conducted for SWAMP (trace metals, synthetic organic compounds, sediment TOC, and sediment grain size, amongst others). All equipment described is pre-cleaned according to the procedures outlined in specific SOP's (**Appendix D**).

It is critical that sample contamination be avoided during collection. All sampling equipment (i.e., Van Veen grab, compositing containers, and scoops) are composed of a non-contaminating material and are thoroughly cleaned before each use (all scoops are individually pre-cleaned and

bagged at the lab, and a new "pre-cleaned scoop used at each station). Sampling personnel wear polyethylene gloves whenever taking or processing samples to avoid contact contamination. Airborne contamination is avoided by keeping sample containers, sample scoops and compositing container inside bags or coolers with door closed or appropriately covered when not in use.

Sediment Sampling Equipment Preparation

Sediment sampling equipment is prepared in the laboratory by a minimum of four days prior to the start of a cruise. The sediment sampling equipment that is pre-cleaned includes:

- Van Veen Grab (excluding frame and stand)
- Sample scoops (equal to the number of stations where sediment collection is to occur, plus an extra five scoops)
- Compositing container
- Wash bottles

The following procedures are used for cleaning sediment sampling equipment:

1. Soak equipment (fully immersed) for three days in a 0.5 % solution of Alconox™ detergent and deionized water; alternatively, Micro™ detergent may be used.
2. Rinse equipment three times with deionized water and let dry in a clean place.
3. Rinse equipment with 1.0 % solution of hydrochloric acid, followed by a rinse with petroleum ether, followed by another set of three rinses with deionized water. All equipment is then allowed dry in a clean place.

All other equipment is stored in clean Ziploc™ bags until used in the field.

Summary of Wadeable Stream Bed Sediment Sample Collection Process: Using Scoop

The guidelines for obtaining grab samples of bed sediment (using a scoop) at most wadeable stream sites are as follows:

1. Randomly select an area of unconsolidated, recently-deposited fine-grain sediment. Unconsolidated sediments lack a usually visible diatom covering and are very easily penetrated. Typical locations are the side slope or surface of recent slump blocks and the surface of actively accreting point bars on the inside of meander bends. To the extent possible, given the volume of sediment necessary to collect for specific analyses, randomly select a location at least 10 meters from any channel or ditch (if possible), and at least 5 meters from the upland edge of any tidal marsh. If at all possilble, do not select

spots in ponds or channel pans.

2. Insert a cleaned scoop into the sediments to a depth of 2-3 centimeters. Remove sediments from an area in the streambed of approximately 0.1 square meter. The total amount of sediment sampled is proportional to the amount of sediments removed when using the Van Veen grab at non-wadeable sites, or sites where scooping or coring are not possible.

3. Place sediment into a pre-cleaned compositing container. Thoroughly stir (using pre-cleaned polycarbonate stir rod) the combined material into one homogeneous mixture.

4. Place the appropriate amounts of the sediment into pre-cleaned containers with appropriate labels, and place the containers on ice in a cooler, in the dark, for short-term storage at 4°C.

5. To avoid cross-contamination between stations, all utensils, compositing containers must be rinsed between stations with ambient water, then scrubbed thoroughly with Alconox™ or Micro™ detergent, followed successively by one rinse with deionized water, one rinse with 1% HCl, one rinse with methanol, and a final rinse with deionized water. All scoops used to collect sediment are pre-cleaned and bagged at the laboratory, and a "new" pre-cleaned scoop is used for each station.

6. The samples on wet ice in coolers should be checked periodically to ensure that samples are appropriately protected and ice should be added as required. Additionally, coolers containing wet ice should be drained periodically to remove melt water.

7. Grab samples may also be collected using a box core, diver core, or other coring device, and SOP's are available for these procedures; typically in SWAMP to date, the scoop has been sufficient to collect the required bed sediment samples in wadeable stream settings.

Summary of Non-wadeable Bed Sediment Sample Collection Process: Using Young-modified Van Veen Grab

In non-wadeable waters, such as lakes, deeper rivers, reservoirs, estuaries, and open coastal waters, bed sediment samples are collected using a Young-modified Van Veen Grab, following the procedure summarized below:

Sediment sampling is performed using a Young-modified, Van Veen grab with a surface area of 0.1 m^2. The grab is constructed entirely of stainless steel and the jaws and doors are coated with Tefzel™ to improve chemical inertness. A scoop and container used to remove and composite sediments are also constructed of pre-cleaned stainless steel or polycarbonate.

When the vessel reaches a sampling station and the anchor has been deployed, the captain notifies personnel that the vessel is on site and switches on a bilge pump used for rinsing the sampling equipment. Sampling equipment is cleaned at each station in the field using the following methods:

1. Fill the compositing container with ambient water from the raw water pump and add approximately 1/8 cup of Alconox™ or Micro™ detergent to the bucket.

2. Place all sampling scoops into the compositing container and wash thoroughly with the Alconox™ or Micro™ detergent solution. Wash all Tefzel™-coated parts of the Van Veen grab with Alconox™ or Micro™ detergent solution.

3. Completely rinse the grab, compositing container, and sample scoops with ambient water.

4. Rinse the grab, bucket, sample scoops and coring tubes with 1.0 % HCl , followed with a rinse of methanol.

5. Completely rinse the grab, compositing container, and sample scoops with deionized water and let air dry. Cover all cleaned parts with aluminum foil until use.

Two grabs are taken at each site. If sediments at a station are considerably fine, plastic floats may be attached to the grab frame and secured so they do not interfere with grab operation. Likewise, if sediments are considerably coarse, weights are added to the grab frame to assist penetration of sediments. The quality of grab samples is ensured by requiring each sample to satisfy acceptance criteria concerning depth of penetration and disturbance of sediment within grab.

Samples contain only the top 2-3 cm of sediment within the area of the grab jaws. Samples are rejected under the following conditions:

1. There is a rock or shell fragment wedged between the jaws of the grab allowing the sample to wash out.

2. The sample surface is significantly disturbed.

3. The sample is uneven from side to side, indicating that the grab was tilted when it penetrated the sediment.

4. The surface of the sample is in contact with the doors of the grab, indicating over-penetration of the grab and possible loss of material around the doors.

After determining a grab meets acceptance criteria, overlying water is drained off. The remaining

top 2-3 cm of sediment is scooped from each of two (or more if necessary to collect required sediment volume) replicate grabs and mixed in the compositing container to provide a single composite sample from each site. Portions of the composited sample are placed into clean containers provided by each laboratory. In cases where an "archive" sediment sample has been requested by a RWQCB for possible future chemical analysis, a duplicate sediment chemistry sample is collected from the composite for archiving and is labeled as an "archive".

Summary of Recommended Sample Collection Process for Benthic Infauna in Estuarine, Open Coastal, and Non-wadeable Waterbodies*)--currently only RWQCB 8 uses this protocol*

Benthic infauna primarily comprises sedentary, invertebrate organisms that burrow in or live on the surface of sediments. Benthic infaunal communities fluctuate in response to natural and human induced environmental perturbations and therefore can be important indicators of environmental health. For this reason they often are an important component of many ecological monitoring programs. Benthic infauna is sampled with a Ponar grab with a surface area of 0.05 square meters. The grab is equipped with hinged stainless steel mesh lids with rubber flaps to allow flow-through of water during decent and thus minimize disturbance of surface sediments. The rubber flaps close upon retrieval and prevent winnowing of the sample. The Young-modified Van Veen grab may also be used, but is not used by RWQCB 8. The Van Veen grab does not have rubber flaps, and has a larger surface area, although that can be modified.

Sampling procedures will insure that samples are collected from a localized area at each station to reduce uncontrolled temporal and spatial variations. Lead weights are added to or removed from the outside of the grab as appropriate for sediment type to control depth of penetration.

After deployment and retrieval, the grab is placed on a stand for processing. The grab lids are opened and the sample is examined for suitability using the following criteria:

- Complete closure of the grab jaws.
- No evidence of sediment washout through the grab doors.
- An even distribution of the sediment in the grab.
- Minimum disturbance of the sediment surface.
- Minimum overall sediment depth appropriate for the sediment type: 4 cm in coarse sands and gravel, 5 cm in medium sands, 7 cm in fine sands, and 10 cm in silty sands, silts, and clay.

If the sample passes all of the criteria, the grab jaws are opened and the sample is dumped into a five gallon plastic bucket placed beneath the grab stand. Estuary water is used to wash all sediment from the grab and grab stand into the bucket. Care is exercised not to lose sediment by overfilling the bucket. The sample bucket is then moved to a wash table for sample sieving.

When a sample bucket arrives at the sieving station, it is lifted to the sieve table and poured slowly onto the nested sieve screens. The sea water hose with a flow control nozzle is used to slowly wash sediment from the sample bucket onto the sieve screens. The sieving process is aided by keeping sediment in suspension as it reaches the screen. The sample is washed from the sample bucket until the bucket is empty and well rinsed. Sediment is washed through the nested sieve screens by gently running seawater over the top screen. Use of high water pressure damages organisms impinged on the sieve screen mesh.

When all material smaller than 1.0 mm has passed through the top screen, the process is repeated with the finer screen until all material smaller than 0.5 mm has passed through. The material retained on each screen is gently washed into one corner of the screen and with the aid of a canning funnel, washed into separate appropriately labeled sample jars. A wash bottle with seawater is used to rinse any material on the inside screen frame and canning funnel into the sample jar. Any organisms remaining on the screens are carefully removed with forceps and placed in the appropriate sample jars. The sample jars are then capped with dome lids and bands, labeled with indelible ink inside and out, and delivered to the on-board formalin station. Great care is exercised to avoid creating fragments when removing organisms from the sieve screens. The sieve screens are rinsed with high-pressure seawater and scrubbed clean with a stiff-bristle brush between samples.

If the sample contains many shell fragments and/or worm tubes, the sediment sample is added to the top (1.0 mm) screen in stages so that the screen does not become too full. If the bottom screen (0.5 mm) begins to clog with sediment, the field crew ceases adding sample and gently runs the hose nozzle with low flow along the outside bottom of the 0.5 mm screen being careful not to lose sample by allowing water to escape over the top of the sieve. The material retained on a sieve screen is not allowed to fill the sample jar more than half full. In such a case, the material is divided among two or more jars and each jar is labeled as jar 1 of 2, jar 2 of 2, etc., as required.

At the formalin station, each sample jar lid is replaced with screen lids fitted with 0.25 mm Nitex (tm) mesh and the estuary water is decanted from the sample jars through the screen lids. Relaxant (isotonic $MgCl_2$) is added to the sample through the screen lid to a level approximately one-third higher than the sample level. A wash bottle of relaxant is used to wash down the screen lid and sides of the sample jar. The sample jar is recapped with the sample jar lid and gently rotated several times in a tilted position to ensure mixing of the relaxant throughout the sample. The sample is allowed to sit in the relaxant for 15-30 minutes. After this period, the sample jar lid is replaced with a screen lid and the $MgCl_2$ is decanted out of the sample jar in preparation for fixing the sample.

At the formalin station, relaxant is decanted out and fixative (10% buffered formalin in seawater) is added to the sample through the screen lid. Fixative is added to a level approximately one third

higher than the sample level. A wash bottle of fixative is used to wash down the screen lid and sides of the sample jar. The screen lid is removed, 2 or 3 drops of stain (rose bengal solution) are added to the sample and the sample jar is recapped with the sample jar lid. The jar is gently rotated several times in a tilted position to ensure mixing of the fixative and stain with the sample. Safety glasses and nitrile gloves are worn when working with fixative.

While onboard the survey vessel, benthic infauna samples are stored in plastic trays with dividers, then transferred to cardboard cartons with dividers for travel to the laboratory for sample sorting. Benthic infauna samples fixed in formalin are washed in tap water and transferred to 70% ethyl alcohol between 24 and 72 hours after fixation. Samples can then be held indefinitely in 70% ethyl alcohol.

A sample collection log records sample date, station, depth of grab penetration, number of grabs, number of bottles per sample, and any problems encountered.

COLLECTION OF SAMPLES FOR CONTAMINANT ANALYSIS IN TISSUES (FISH, CRABS, BIVALVES, ETC.)

Fish Tissue Collection Procedures for Contaminant Analysis

Fish tissue samples will be collected by DFG and SJSUF contract field crew staff, using protocols detailed in **Appendix D**. Details of the protocols are summarized below.

Collection of fish for analysis of contaminants in tissue may be accomplished by a variety of methods, including hook and line, seines, gill nets, and electroshocking. Species collected will, in most cases, be non-migratory species that are most representative of a given location. Efforts will be made to collect fish of a similar (medium) size for each composite. Fish will be wrapped in trace metal- and organic-free Teflon™ sheets and frozen for transportation to the laboratory. The tissue samples are prepared in the laboratory using non-contaminating techniques in a clean room environment.

Collection, handling and storage of tissue samples will be performed in a manner consistent with other large scale tissue contaminant monitoring programs, such as the Regional Monitoring Program (RMP) protocols (SFEI 1999, SFRWQCB 1995), CALFED DFG protocols, and toxic substances monitoring protocols (see **Appendix D**), to assure the collection of representative, uncontaminated tissue chemistry samples. Field crews must rigorously follow sampling procedures and complete all necessary documentation according to the SOPs.
As a general rule, five fish of medium size or three fish of larger size are collected as composites for analysis. The smallest fish length cannot be any smaller than 75% of the largest fish length. Five fish provides sufficient quantities of tissue for the dissection of 100 grams of fish flesh for

organic and inorganic analysis. The medium size is more desirable to enable similar samples to be collected in succeeding collections.

When only small fish are available, sufficient numbers are collected to provide 100 grams of fish flesh for analysis. If the fish are too small to excise flesh, the whole fish, minus the head, tail, and guts are analyzed as composites.

Fish collected that are too large to fit in our clean bags (>500 mm) are initially dissected in the field. At the dock, the fish are laid out on a clean plastic bag and a large cross section from behind the pectoral fins to the gut is cut with a cleaned bone saw. The bone saw is cleaned (Micro™, DI, methanol) between fish and a new plastic bag is used. The internal organs are not cut into, to prevent contamination. For bat rays, a section of the wing is cut and saved. These sections are wrapped in Teflon™, double bagged and packed in dry ice before transfer to the freezer. During lab dissection, a subsection of the cross section is removed, discarding any tissue exposed by field dissection.

Field data recorded include, but are not limited to site name, sample identification number, site location (GPS), date of collection, time of collection, names of collectors, method of collection, type of sample, water depth, water and atmospheric conditions, fish total lengths (fork lengths where appropriate), photo number and a note of other fish caught.

The fish are then wrapped in cleaned Teflon™ sheets. The wrapped fish are then double-bagged in Ziploc™ bags with the inner bag labeled. The fish are put on dry ice and transported to the laboratory where they are kept frozen until they are processed for chemical analysis.

All samples, once returned to the laboratory for processing, are prepared in a clean room to avoid airborne contamination.

Bivalve Deployment and Retrieval Summary (for bagged bivalve bioaccumulation studies)
Sample collection - mussels and clams

The mussels to be transplanted (*Mytilus californianus*) are collected from Trinidad Head (Humboldt Bay Intensive Survey), Montana de Oro (Diablo Canyon Intensive Survey), and Bodega head (all other statewide transplants). The freshwater clam (*Corbicula fluminea*) source is Lake Isabella or the Sacramento River. Mussel and clam samples are analyzed for background contaminates prior to transplanting (see State Mussel Watch Program staff for more details).

Polyethylene gloves are worn while prying mussels off rocks with stainless steel dive knives. Note: polyethylene gloves should always be worn when handling sample. Mussels of 55mm to 65mm in length are recommended. Fifty mussels are collected for each TM and each SO

sample.

Collected mussels are carried out of collection site in cleaned nylon daypacks. For the collection of resident samples where only one or two samples are being collected the mussels are placed directly into a labeled Ziploc™ or cleaned aluminum foil (SO) and an additional Ziploc™.

Clams (*Corbicula fluminea*) measuring 20 to 30mm are collected by dragging the clam dredge along the bottom of the lake or river. The clams are poured out of the dredge into a 30 gallon plastic bag. 25-50 clams are needed for each TM and each SO sample.

Transplanted sample deployment

With polyethylene gloves, fifty transplant mussels are placed in each 76mm X 13mm polypropylene mesh bag. Each bag represents one TM or one SO sample. A knot is tied at each end of mesh bag and reinforced with a cable tie. On one end another cable tie is placed under the cable tie which will be used to secure the bag to the line for transplant deployment. The mussels in the mesh bag are divided into three groups of approximately equal size and sectioned with two more cable ties.

Once bagged, the mussels are placed in a 30 gallon plastic bag and stored in a cooler (cooled with ice) for no more than 48 hours. The ice is double bagged in Ziploc™ bags to avoid contamination.

If samples are held for longer than 48 hours they are placed in holding tanks with running seawater at the Fish and Game Granite Canyon Lab. Control samples for both SO and TM are also held in the tank.

For freshwater clams: clams (25-50) are placed in 50mm X 7mm polypropylene mesh bags using identical procedures to those used with mussels (section 7.2.1). If clams need to be stored for more than 48 hours, the mesh bags are deployed in Lake San Antonio or another clean source until actual sample deployment.

The mussels are attached to an open water transplant system that consists of a buoy system constructed with a heavy weight anchor (about 100lbs) or screw-in earth anchor, 13mm polypropylene line, and a 30cm diameter subsurface buoy. The sample bags are attached with cable ties to the buoy line about 15 feet below the water surface. In some cases the sample is hung on suspended polypropylene lines about 15 feet below the water surface between pier pilings or other surface structures. Creosote-coated wooden piers are avoided because they are a potential source of contamination. In some cases the mussels are hung below a floating dock. In shallow waters a wooden or PVC stake is hammered into the substrate and the mussel bags are

attached by cable ties to the stake.

The clams are deployed by attaching with cable ties the mesh bag to wooden or PVC stakes hammered into substrate or screw in earth anchors. The bags containing clams are typically deployed 15cm or more off the bottom. In areas of swift water, polypropylene line is also attached to the staked bags and a permanent object (piling, tree or rock).

Transplants are usually deployed for 1-4 months. Ideally mussels are transplanted in early September and retrieved in late December and early January. Clams are usually transplanted in March or April and retrieved in May or June, although this is variable in some cases.

Data is recorded for each site samples are transplanted to or collected from. Data includes, but is not limited to station name, date collected or transplanted, collectors names, water depth, GPS readings, photo, ocean/atmospheric conditions (if appropriate), description of site, and drawing in necessary.

Sample Retrieval

The transplanted or resident and control mussels analyzed for metals are placed into two labeled Ziploc™ polyethylene bags (4mm thickness).

All mussels to be analyzed for organics are placed in an aluminum foil bag. The bags are constructed of two layers of "heavy duty" aluminum foil. Prior to use these bags are cleaned by heating to 500°c or by rinsing in petroleum ether or methanol. The sample is first wrapped in a foil bag, hen placed in two labeled polyethylene Ziploc™ bags. Note: samples should only contact the dull side of the foil.
The bags containing samples are clearly and uniquely identified using a water-proof marking pen or pre-made label. Information items include ID number, station name, depth (if from a multiple sample buoy), program identification, date of collection, species and type of analysis to be performed.

The samples are placed in non-metallic ice chests and frozen using dry ice or regular ice. (Dry ice is used when the collecting trip takes more than two days.) At the lab, samples should be stored at or below -20°c until processed.