5.0 DESCRIPTION OF METHODS

The purpose of this chapter is to provide brief descriptions on the sample collection and analytical methods used in Status and Trends Monitoring component of the Regional Monitoring Program for Trace Substances (RMP). Water, sediment and bivalve tissue samples are collected and analyzed for trace elements and trace organics. Conventional water and sediment quality parameters are also measured, and aquatic and sediment toxicity testing conducted. Information on sampling methods and analytical procedures for RMP pilot and special studies and fish contamination monitoring will be provided in separate technical reports available on the RMP Reports and Publications page at http://www.sfei.org/rmp/reports.htm.

In addition, several other documents containing more thorough discussions of methods and procedures are available:

(1) ***Field Sampling Manual for the Regional Monitoring Program for Trace Substances*** provides standard operating procedures for sampling of water, sediment, and bivalve tissue (http://www.sfei.org/rmp/documentation/fom/FOM2001.pdf).

(2) ***Quality Assurance Project Plan for the Regional Monitoring Program for Trace Substances*** describes the quality assurance and quality control (QA/QC) protocols and requirements for RMP field sampling and laboratory analyses (http://www.sfei.org/rmp/reports/2001_QAPP/2001_QAPP_v2.PDF).

(3) ***Standard Operating Procedures*** for each analytical laboratory are on file at SFEI and maintained by the RMP QA Officer, Dr. Donald Yee (donald@sfei.org).

5.1 SAMPLE COLLECTION METHODS

5.1.1 Water Sampling

One function of the RMP is to evaluate if water quality objectives are met in the Estuary. Therefore, the sampling and analytical methods must be able to detect, and wherever possible quantify, substances below these levels. In order to attain the low detection levels used in the RMP, ultra-clean sampling methods are used in all sampling procedures (Flegal and Stukas 1987; EPA 1995).

Water samples are collected approximately one meter below the water surface using peristaltic and gear-driven pumps. The sampling intake ports for both the trace organic and trace element samplers are attached to aluminum poles that are oriented up-current from the vessel and upwind from equipment and personnel. The vessel is anchored and the engines turned off before the sampling begins. Total and dissolved fractions of Estuary water are collected for trace element analyses. Particulate and dissolved fractions are collected for trace organics.

Collection of Samples for Trace Organics

Background

The RMP used a polyurethane foam plug sampler to collect water for trace organics analyses during the first four years of the Program (Risebrough et al. 1976; de Lappe et al. 1980, 1983) and phased in a new, modified, commercially available resin extraction sampler in 1996, beginning with side-by-side comparisons of both sampling systems. XAD (cross-linked

amberlite dininyl benzene) resins have been used throughout the world to measure synthetic organic contaminants in both water and air (Infante et al. 1993). The sampler comparisons were continued in 1997, and results from both years are presented in the *RMP 1997 Annual Report* (SFEI 1999).

Since the 1997 monitoring year, an Axys Infiltrex system (Axys Environmental Systems, Ltd., Sidney, B.C.) has been used to collect all RMP water samples for analysis of trace organic pollutants. It consists of a constant-flow, gear-driven positive displacement pump, 1/2 inch Teflon® tubing, 1 µm glass fiber cartridge particulate filter, and two parallel Teflon® columns filled with XAD-2 resin with a particle size range of 300-900 µm. Amberlite XAD-2 resin is a macroreticular, styrene-divinyl benzene copolymer, nonionic bead, and each bead is an agglomeration of microspheres. This sponge-like structure offers excellent physical and chemical stability. The discrete pores allow rapid mass transfer of analytes, and the mesh size ensures very little, if any, back pressure during use. The hydrophobic nature of the resin leads to excellent capability of concentrating hydrophobic contaminants.

Collection of Particulate and Dissolved Fractions

To remove large debris that may interfere with sample collection, the sample water is first passed through a coarse screen before the Teflon® intake line. Particles greater than 140 µm are removed by a second inline pre-filter. The water then passes through the pump head and a pressure gauge, before it goes through a four-inch diameter, wound glass fiber filters (1 µm). Flow may be redirected without interruption to a second installed filter if the first filter becomes clogged. Material retained on the glass fiber filter (or filters) is designated the particulate fraction. After passing through the filter, the water is split and routed through two Teflon® columns, packed with 85 mL of XAD-2 resin. Two filters are used simultaneously to increase the flow to approximately 1.3 L/min. The compounds adsorbed to the XAD resin are classified as the dissolved fraction. Lastly, the water passes through a flow meter and out the exit tube, where the extracted water volume is verified by filling 20 L carboys.

Collection of Field Blanks for Trace Organics

Field blanks are taken for both the resin columns and the glass fiber filters. The two column blanks are collected by leaving both ends of a column open while the filled sample columns are being loaded into the sampler. Similarly, the two glass fiber filter blanks are collected by exposing a filter to the air while loading the sample filters into the cartridges. The field blanks receive the same analytical treatment in the laboratory as the field samples.

Collection of Samples for Trace Metals

Collection of Total and Dissolved Fractions

For trace metals, water samples are collected using a peristaltic pump system equipped with C-Flex tubing in the pump head. Sample containers are filled on deck on the windward side of the ship to minimize contamination from shipboard sources (Flegal and Stukas 1987). Filtered (dissolved fraction) water samples are obtained by placing an acid-cleaned polypropylene filter cartridge (Micron Separations, Inc., 0.45 µm pore size) on the outlet of the pumping system. Unfiltered (total) water samples are pumped directly into acid-cleaned containers. Prior to collecting water, several liters of water are pumped through the system, and sample bottles are rinsed five times with site water before filling. The bottles are always handled with polyethylene-gloved “clean hands”. The sample tubing and fittings are acid-cleaned polyethylene or Teflon®,

and the inlets and outlets are kept covered except during actual sampling. Samples are acidified within two weeks in a Class 100 trace metal clean laboratory.

Collection of Field Blanks for Trace Metals
During the collection of one sample, a pre-cleaned bottle filled with a diluted acid is opened and exposed to the air. Field blanks are collected during the sampling of both the total (unfiltered) and dissolved (filtered) fractions and receive the same analytical treatment in the laboratory as the field samples.

Collection of Water Quality Samples
Samples for conventional water quality parameters are collected using the same apparatus as for trace metals. However, containers are rinsed only three times, and the "clean hands" procedure is unnecessary.

Collection of Aquatic Bioassay Samples
Water samples are collected for toxicity tests using the same pumping apparatus as for the collection of the trace organic samples, however they are not filtered. Five gallons of water are collected and placed in ice chests for transfer at the end of each cruise day to the testing laboratory. Two field blanks are collected each cruise by filtering (0.45 μm) water known to be non-toxic from the Bodega Marine Laboratory.

5.1.2 Sediment Sampling
Sediment sampling is conducted using a Young-modified Van Veen grab with a surface area of 0.1 m^2. The grab is made of stainless steel, and the jaws and doors are coated with Dykon® (formerly known as Kynar®) to achieve chemical inertness. All scoops, buckets, and stirrers used to collect and homogenize sediments are also constructed of Teflon® or stainless steel coated with Dykon®. Sediment sampling equipment is thoroughly cleaned prior to each sampling event. In order to further minimize sample contamination, personnel handling samples wear gloves.

If the 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 the sediments are considerably coarse, weights are added to the grab frame to assist penetration of the sediments. To ensure the quality of the sediment samples, each grab must satisfy several criteria in order to be accepted: complete closure, no evidence of sediment washout through the doors, even distribution of sediment in the grab, minimum disturbance of the sediment surface, and minimum overall sediment depth appropriate for the sediment type.

Collection of Sediment Samples
Two grabs are taken at each site, and sediment sub-samples are removed for toxicity tests and pore water analysis. Overlying water is drained off an accepted grab, and using pre-cleaned glass cores, three 5 cm deep cores are taken from each side of the grab. Cores collected for analysis of pore water are centrifuged on-board the vessel. Part of the supernatant is then used for analysis of ammonia and pH, which is performed on-board the vessel, and part is preserved for analysis of sulfides in the laboratory.

The remaining top 5 cm of sediment is scooped from each of two replicate grabs and mixed in a compositing bucket to provide a single composite sample for each site. Between sample grabs, the compositing bucket is covered with aluminum foil to prevent airborne contamination. After two sediment samples have been placed into the compositing bucket, the bucket is taken into the

ship's cabin and thoroughly mixed to obtain a uniform, homogeneous mixture. Aliquots are subsequently split for each analytical laboratory and for archive samples.

Collection of Benthic Infauna Samples

At stations where benthic sampling is conducted, a Ponar grab sampler is used to collect one acceptable benthic sample prior to using the Van Veen grab for collecting sediment chemistry samples. After benthic samples have been collected, the Ponar grab is replaced on the winch wire with the Van Veen grab for collection of chemistry samples. An accepted grab is placed on a stand designed with a stainless steel funnel directed to a sample bucket. The grab jaws are then opened, and the sediment is dumped into a five-gallon plastic bucket.

The sample is then moved to a wash table for sieving through two screens stacked on top of each other. The top screen retains animals in its 1 mm mesh, and the lower screen has smaller 0.5 mm mesh. The material retained in each screen is gently washed into separate, labeled sample jars. A wash bottle with seawater is used to rinse any material on the inside of the screen frame and funnel into the sample jar. Any organisms remaining on the screens are carefully picked off with forceps and placed in the appropriate sample jars. Jars are taken to the formalin station where seawater is decanted from the sample jars with a 0.25 mm Nitex mesh. Relcant (isotonic $MgCl_2$) is added to the sample through the mesh to a level approximately one third higher than the sample level. The sample is allowed to sit in the relaxant for 15 to 30 minutes. The relaxant is then decanted, and 10% buffered formalin is added to the sample through the screen lid. As a final step, two to three drops of stain (rose bengal solution) are added to the sample for ease of organism identification.

Collection of Intact Sediment Cores for Toxicity Sampling

Intact sediment cores were collected for Sediment-Water Interface (SWI) toxicity testing from the grab sampler by pressing polycarbonate core tubes 5 cm into the sediment, sealing the bottom of the cores for removal from the sampler with a gloved hand, and removing the cores. Cores were quickly capped, the polyethylene caps were dried, tightly sealed with Parafilm® to prevent leakage, then stored upright on ice for transport. Intact samples were stored for less than 4 days prior to initiation of the experiments (Anderson et al. 2001).

5.1.3 Bivalve Tissue Sampling

Bivalve Collection

Bioaccumulation is evaluated by collecting oysters (*Crassostrea gigas)* and mussels (*Mytilus californianus)* from uncontaminated "background" sites of known chemistry and deploying these bivalves at 12 locations in the Estuary for approximately 100 days. Resident clams (*Corbicula fluminea)* are also collected from one site on the Sacramento River and another site on the San Joaquin River. Bivalves are deployed once each year during the dry season, usually in June. Since the RMP sites encompass a range of salinities, the species of bivalves used at each site depends on the expected salinities in the area and the known tolerances of the organisms.

Mussels (*Mytilus californianus*) are collected from Bodega Head and stored in running seawater at the Bodega Marine Laboratory until deployment at the stations west of Carquinez Strait, which are expected to have the highest salinities. *Mytilus californianus* will survive short-term exposure to salinities as low as 5 ppt (Bayne 1976).

Oysters (*Crassostrea gigas*) are obtained from Tomales Bay Oyster Company (Marshall, California) and deployed at moderate-salinity sites closest to Carquinez Strait and in the extreme South Bay. *Crassostrea gigas* tolerates salinities as low as 2 ppt. To minimize the effects of high, short-term flows of freshwater on the transplanted bivalves, bivalves are deployed near the bottom, where density gradients tend to maintain higher salinities. All bivalves are kept on ice after collection and deployed within 24-48 hours.

Since a viable reference population of *Corbicula fluminea*, which tolerates salinities from 0 ppt to perhaps 10 ppt (Foe and Knight 1986), can no longer be found in sufficient numbers, resident freshwater clams are now collected from near the historical deployment sites in the Sacramento River and San Joaquin Rivers. The Grizzly Bay sample station was discontinued due to the lack of transplantable "clean" clams and a reliable resident population at the site. Resident clams are collected using a clam dredge approximately two feet wide by three feet long and 50 pounds in weight. The dredge is deployed from a boat and is dragged along the bottom. When brought to the surface, the clams are placed into a clean plastic container and packaged for analysis.

Deployment of Transplanted Bivalves

Depending on the salinity at a site, oysters or mussels (150 and 160, respectively) are randomly allocated and placed into nylon mesh bags (five for oysters and four for mussels) for deployment. Within each species, animals of approximately the same shell length are used (49-81 mm for mussels and 71-149 mm for oysters). The same number is also used for the travel blank (time zero) samples, which are analyzed for tissue condition before deployment.

At each site, a line runs from the bottom of the fixed structure out to the bivalve mooring, which consists of a large screw (earth anchor) that is threaded into the bottom and is associated with pilings or other permanent structures. A large subsurface buoy is attached to the earth anchor by a 1-2 meter-long line. The bivalves are in enclosures (mesh bags or cages) attached to the buoy line, which keeps the bivalves off the bottom to prevent smothering. In one hundred and fifty individual deployments, loss of a mooring has occurred on only two occasions, probably due to being ripped out by a vessel anchor. Mooring installation, bivalve deployment, maintenance, and retrieval are all accomplished by SCUBA divers.

Maintenance of Transplanted Bivalves

The deployed samples are checked approximately 50 days after deployment to ensure consistent exposure. Moorings and enclosures are checked for damage and repaired if necessary, and fouling organisms are removed.

Retrieval of Transplanted Bivalves

Upon retrieval, the bivalve enclosures are placed into polyethylene bags and taken to the surface. On the vessel, the number of dead organisms is recorded. Twenty percent of the live organisms are allocated for condition measurement, and the remainder is equally split for analyses of trace metal and organic compounds. Bivalves used for trace organic analyses are rinsed with reagent grade water to remove extraneous material, shucked using a stainless steel knife (acid-rinsed), and homogenized (until liquefied) in a combusted mason jar using a Tissumizer or Polytron blender. Bivalves used in trace element analyses are shucked with stainless steel knives, and the gonads are removed. The remaining tissue is rinsed with ultrapure water and placed in acid-cleaned, plasticcoated, glass jars. The sample is then homogenized (until liquefied) using a Brinkmann homogenizer equipped with a titanium blade.

Based on findings by Stephenson (1992) during the RMP Pilot Program, bivalve guts are not depurated before homogenization for tissue analyses, although the gonads are removed from organisms for trace metal analyses. With the exception of lead and selenium, no significant differences exist in trace metal concentrations between mussels depurated for 48 hours in clean Granite Canyon seawater before homogenization and undepurated mussels. However, sediment in bivalve guts may contribute to the total tissue concentration for trace organic contaminants.

5.2 ANALYTICAL METHODS

5.2.1 Analysis of Water and Sediment Quality

Conventional Water Quality Parameters

Dissolved nutrients in samples are analyzed using the Lachat QuikChem 800 System Nutrient Autoanalyzer (Ranger and Diamond, 1994). The QuickChem methods used are: 31-114-27-1 for silicates, 31-107-06-1 for ammonia, 31-107-04-1 for nitrate/nitrite, and 31-115-01-3 for phosphate. Chlorophyll and phaeophytin are measured using a fluorometric technique with filtered material from 200 mL samples (Parsons et al., 1984). Shipboard measurements for temperature, salinity, pH, and dissolved oxygen content are made using a hand-held Solomat 520 C multi-functional chemistry and water quality monitor. Dissolved organic carbon (DOC) is measured using high-temperature catalytic oxidation with a platinum catalyst (Fitzwater and Martin 1993). Total suspended solids (TSS) are determined using method 2540D in Standard Methods for the Examination of Water and Wastewater (APHA 1992).

Sediment Quality Parameters

Sediment size fractions are determined with a grain-size analyzer based on x-ray transmission (Sedigraph 5100). Total organic carbon is analyzed according to the standard method for the Coulometrics CM 150 Analyzer made by UIC Inc.,which determines light tranmitted through a cell containing the carbon dioxide evolved from a combusted sample. Sulfide analysis in sediment porewater is performed using a combination of the methylene blue and iodimetric methods from Fonselius (1985) and Standard Methods (APHA 1998).

Conductivity, Temperature, and Depth (CTD) Casts

CTD casts are taken at each site during water and sediment sampling. A Sea-Bird SBE19 CTD probe is used to measure water quality parameters at depths throughout the water column. At each site, the CTD is lowered to approximately one meter below the water surface and allowed to equilibrate to ambient temperature for 3 minutes. The CTD is then lowered to the bottom at approximately 0.15 meters per second and raised. However, only data from the down cast are kept. Data are downloaded onboard the ship and processed in the laboratory using software supplied by Sea-Bird.

The CTD probe measures temperature, conductivity, pressure, dissolved oxygen, and backscatter at a sampling rate of two scans per second. These data are compiled and averaged into 0.25 m depth bins during processing. At this time, salinity (based on conductivity measurements), depth (based on pressure), and depth are also calculated. Although the CTD data are not included in the *2001 RMP Monitoring Results*, SFEI maintains these data in its database.

5.2.2 Trace Elements

Analysis of Water Samples

In water, total and dissolved (0.45 µm filtered) concentrations of mercury, arsenic, selenium, cadmium, copper, nickel, lead, silver, and zinc are measured. Mercury, arsenic, and selenium samples are obtained from the same field sample. The mercury sub-samples are photo-oxidized by addition of bromium chloride and quantified using a cold-vapor atomic fluorescence technique. Arsenic and selenium are analyzed by hydride-generation atomic absorption with cryogenic trap preconcentration, based on a method described in Liang et al. (1994) and Cercelius et al. (1986). The remaining trace elements in water are measured using an APDC/DDDC organic extraction and preconcentration method (Bruland et al. 1985; Flegal et al. 1991) with quantification by graphite furnace atomic absorption spectrometry (GFAAS).

The laboratory reports cadmium, copper, nickel, lead, silver, and zinc concentrations in weight/water weight units (µg/kg). For use in this report, these values are reported as µg/L, without taking into account the difference in density between Estuary water and distilled water because this difference is much less than the precision of the data, which is on the order of 10%. In some instances, reported dissolved metal concentrations are higher than total (ostensibly including dissolved and particulate fractions) metal concentrations. This is due to expected analytical variation, which is proportionally larger at concentrations near the detection limits. Such results should be interpreted as no difference between dissolved and total concentrations, or that the total fraction of metals is in the dissolved phase.

Analysis of Sediment Samples

Sediments are digested in nitric/hydrochloric acids to obtain "near-total" concentrations of trace metals. Extracts are analyzed for silver by GFAAS and for aluminum, cadmium, copper, iron, manganese, nickel, lead, and zinc by inductively coupled plasma atomic emission spectrometry (ICP-AES) with cyclonic nebulization. The method chosen for RMP sediment analysis is comparable to standard EPA procedures (Tetra Tech 1986) but does not decompose the silicate matrix of the sediment. Because of this, any element tightly bound as a naturally occurring silicate may not be fully recovered.

Analysis of Bivalve Tissue Samples

Bivalve tissue samples are homogenized and then digested with *aqua regia* to obtain near-total concentrations of trace elements. Digestion techniques are similar to the California State Mussel Watch Program (e.g., Flegal et al. 1981; Smith et al. 1986) and consistent with the RMP Pilot Program (Stephenson 1992). Sample aliquots are extracted with methylene chloride using a Tissumizer. Extracts are then concentrated and purified by various chromatographic techniques prior to instrumental analyses.

The trace metals are quantified by ICP-AES or ICP-MS. Selenium is quantified by hydride generation coupled with atomic absorption spectroscopy. Arsenic is analyzed by Method 200.9 (stabilized temperature platform graphite furnace atomic absorption, STP- GFAA) (EPA 1994a). Butyltins are measured following NOAA Status and Trends Mussel Watch Project methods (NOAA 1993). This technique involves extracting the sample with hexane and the chelating agent tropolone and measuring the butyltin residues by capillary gas chromatography. Concentrations are expressed in total tin per gram of tissue dry weight.

5.2.3 Trace Organics

Analysis of Water Samples

Two parallel resin columns and one glass fiber filter cartridge (or two at sites with high suspended solids) contain the organic compounds extracted from water at each site. The glass fiber cartridges are extracted by elution in methanol and methylene chloride. Each of the resin columns and glass filters are spiked with extraction surrogates. Surrogates for electron capture detector (ECD) analyses (PCBs and organochlorine pesticides) consisted of PCB 207 and Polychloronitorobenzene (PCNB). The surrogate for mass spectral detector (MSD) analyses was deuterated acenaphthalene. XAD columns are eluted by reverse flow with methanol and methylene chloride. The extracts from each pair of columns for a site are combined prior to Florisil fractionation.

The extracts are subjected to Florisil column chromatography, resulting in three fractions: F1, with PCB/aliphatic compounds; F2, the pesticide/aromatic fraction; and F3, which contains diazinon and other polar pesticides. Chlorinated hydrocarbons (CH), the PCBs and organochlorine pesticides, are analyzed on a Hewlett Packard 6890 capillary gas chromatograph utilizing electron capture detectors (GC/ECD). A single 2 μL splitless injection is directed onto two 60 m x 0.25 mm columns of different polarity (DB-17 and DB-5) using a y-splitter to provide two-dimensional confirmation of each analyte. The quantitation internal standards utilized for the CH analysis are dibromo-octafluorobiphenyl (DOB) for F1, and PCB 209 for F2 and F3. Analyte concentrations are corrected for surrogate losses prior to reporting. PAHs are quantified in the F-2 fraction by analysis on a Hewlett-Packard 6890 capillary gas chromatograph equipped with a 5971A mass spectral detector (GC/MS). A 2 μL splitless injection is separated on a DB-5 column and analyzed in a selected ion monitoring (SIM) mode. The quantitation internal standard utilized for the PAH analysis is hexamethyl benzene (HMB). DOB is used as an internal standard for diazinon.

Analysis of Sediment Samples

Sediment samples are analyzed based on the methods followed by NOAA's Status and Trends Program. Samples are extracted according to EPA Method 3545 (acclerated solvent extraction) using elevated temperature (100 ºC) and pressure (1500-2000 psi) to achieve analyte recoveries equivalent to those from Soxhlet extraction, using less solvent and taking significantly less time. This extraction procedure is applicable to the extraction of all trace organic compounds of interest to the RMP. Surrogate standards are added prior to extraction to account for methodological analyte losses. ECD surrogates consist of DOB, PCB 103, and PCB 198. The extract is concentrated and purified using silica/alumina column chromatography to remove matrix interferences. Internal standard solutions are tetrachloro-m-xylene (TCMX) and dibutyl-chlorendate (DBC). Chlorinated hydrocarbons are quantified in sediment extracts via high-resolution capillary gas chromatography using GC/ECD. Dual-column confirmation on 30-m long, 0.25-mm internal diameter fused silica capillary columns of different polarity (DB-5 and DB-17) is conducted.

Analysis of Bivalve Tissue Samples

Tissue samples are homogenized and macerated, and the eluate is dried with sodium sulfate, concentrated, and purified using a combination of EPA Method 3611 alumina column purification and EPA Method 3630 silica gel purification to remove matrix interferences. PAHs and their alkylated homologues in tissue extracts are quantified by GC/MS in the SIM mode with a temperature-programmable gas chromatograph with a 30-m long, 0.32-mm internal diameter fused silica capillary column, with DB-5MS bonded phase. Surrogates for PAHs consist of naphthalene-d8, acenaphthene-d10, phenanthrene-d10, chrysene-d12, and perylene-d12. PCBs and Pesticides in tissue are quantified via high-resolution capillary gas chromatography using

GC/ECD. Dual-column confirmation on 30-m long, 0.25-mm internal diameter fused silica capillary columns of different polarity (DB-5 and DB-17) is also conducted on tissue samples.

5.2.4 Toxicity Testing

Aquatic Bioassays

Water column toxicity is evaluated using a seven-day growth test, based on EPA test method 1007, with the estuarine mysid *Americamysis bahia* (formerly *Mysidopsis bahia*). The mysid survival test consists of exposing 7-day old juveniles to different concentrations of Estuary water in a static system during the period of egg development. Salinity adjustments are made for Estuary water from sampling stations with salinities below the test species' optimal ranges. Reference toxicant tests with potassium dichromate are performed for mysid tests. These tests are used to determine if the responses of the test organisms are relatively consistent over time.

The salinities of the ambient samples and the control/diluent (Evian spring water) are adjusted to 5 ppt using artificial sea salts (Tropic Marin). The test concentrations are 100%, 50%, and control, each with eight replicates and 20 larvae per replicate. Waste, dead larvae, excess food, and 80% of the test water are siphoned from the test chambers daily, and general water chemistry parameters of dissolved oxygen, pH, and salinity are recorded before and after each water change.

Sediment Bioassays

The RMP uses three sediment bioassays: (1) a ten-day acute mortality test, where the estuarine amphipod *Eohaustorius estuarius* is exposed to whole sediment using ASTM method E 1367 (ASTM 1992), (2) a sediment elutriate test, where larval bivalves (*Mytilus spp*.) are exposed to the material dissolved from whole sediment in a water extract using ASTM method E 724-89 (ASTM 1991) and percent normally developed larvae measured as the endpoint, and (3) sediment-water interface (SWI) test, where *Mytilus galloprovincialis* larvae are exposed to SWI for 48 hours and percent normally developed larvae measured as the endpoint.

Solid-phase samples were prepared as described in the amphipod protocol (EPA 1994b). Sediment was re-homogenized in the sample jar with a polypropylene spoon and then distributed to form a layer 2 cm deep in each of five one-liter replicate beakers. Overlying water was added to the test containers, and sediment and overlying water were allowed to equilibrate overnight before the amphipods were added.

Elutriate solutions are prepared by adding 50 g of sediment to 200 mL of Granite Canyon seawater or freshwater in a clean 250 mL borosilicate glass jar with a Teflon-lined lid (1:4 volume to volume ratio; EPA and ACOE 1991). The elutriate mixture was shaken vigorously for 10 seconds and allowed to settle for 24 hours (Tetra Tech 1986) before being pipetted into replicate containers for testing.

The SWI exposures were conducted with intact sediment core samples taken with minimal disturbance from the Van Veen grab sampler. Test containers consisted of a polycarbonate tube with a 25 µm screened bottom, which was placed within 1 cm of the surface of an intact sediment core (Anderson et al. 1996). Overlying seawater was poured into the intact core tubes and allowed to equilibrate for 24 hours prior to initiation of the toxicity tests. Five replicate cores were tested per station, with a sixth core used for interstitial sulfide and ammonia measurements at the termination of the test. Screen tubes were gently added to the cores 2 hours prior to

inoculation of embryos (Anderson et al. 2001). After inserting the screen tube into the equilibrated cores, each tube was inoculated with approximately 134 bivalve embryos. The laboratory control consisted of Yaquina Bay amphipod home sediment from Northwestern Aquatic Scienes. SWI exposures were conducted simultaneously with elutriate exposures. The SWI test was terminated by removing the screen from the core tube and rinsing larvae into a 20 mL scintillation vial for preservation with formalin.

5.2.5 Bivalve Condition and Survival

Analysis of contaminant concentrations is conducted on a subset of the transplanted bivalves (composites contain 40-60 individual bivalves from each site) prior to deployment in Estuary locations (T-0) and after deployment. The differences between pre- and post-deployment concentrations allow determination of contaminant uptake during the period of deployment. Transplanted bivalves (T-1) are also collected from the T-0 collection sites at the end of the deployment period to obtain information on uptake variables affecting wild populations during the deployment period

The condition of bivalves is a measure of their general health following exposure to Estuary water in the 90-100 day deployments. Measurements are made on subsamples of specimens before deployment and on the deployed specimens following exposure. Dry weight (without the shell) and the volume of the shell cavity of each bivalve is measured. Bivalve tissue is removed from the specimens and dried at 60 ºC in an oven for 48 hours before weighing. Shell cavity volume is calculated by subtracting shell volume of water displaced by a whole live bivalve from the volume of water displaced by the shell alone. The condition index is calculated by taking the ratio of tissue dry weight and the shell cavity volume. Survival during deployment is also measured.

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