Chapter 6
Water Column & Bottom Characteristics

Tiers 1-3 contain active survey and site sampling. Procedures for attaining water column and bottom characteristics are generally the same for each tier. The sampling however, occurs more often over the year. Differences are noted where applicable. Table 6-1 compares the level of effort for each tier. However, agencies will decide which components of each tier will be incorporated into their specific programs, then they will select the level of effort appropriate for their program.

6.1 Salinity, Temperature, Dissolved Oxygen, & pH

Salinity, conductivity, temperature, dissolved oxygen, and pH should be measured at each sampling station using a CTD meter equipped with DO and pH probes. Measurements should be made at 1-m intervals through the water column. In shallow, inshore waters, measurements should be taken at the top, middle, and bottom thirds of the depth. For Tier 3, in some southern waters that undergo significant diel temperature changes, it may be desirable to obtain 24-hour temperature profiles using recording equipment.

6.2 Secchi Depth

Secchi depth is usually measured at the deepest part of the transect or grid. Where the area is classified by depth, Secchi measurements should be made at each station. Readings are obtained with a 40-cm plastic or metal Secchi disk that is either white or is divided into black and white quadrants on a nonstretchable line that is calibrated in decimeters. The disk is lowered into the water until it disappears from view and the depth is recorded. The disk is then slowly raised to the point where it reappears, with the depth being recorded again. The mean of these two measurement is the Secchi depth. Observations are made from the shady side of the boat, without sunglasses, and as close as possible to the water to reduce glare.

6.3 Depth

Depth should be measured at each station using a calibrated depth sounder. Depth can be read off a meter block when sediment sampling by zeroing the block when the sampler is at the water surface. In shallow, inshore waters, a long stick or weighted line calibrated in decimeters may be used.

6.4 Sediment Grain Size

6.4.1 Estimation of "percent fines" (Tier 1)

Analysis of sediment grain size for Tier 1 assessments can be limited to determining the "percent fines" at each station. A rapid wet sieving technique used in Puget Sound (Eaton 1997) can serve as the basis for this characterization. Materials needed for the procedure include:

- standard testing sieve No. 230, 63-µm
- 50-ml plastic beaker (filled to the brim with sediments is about 79-ml)
- 100-ml plastic graduated cylinder
- water bottle(s) with small outlets

Table 6-1. Water Column & Bottom Characteristics. "Addition" refers to added detail or intensities for a parameter initiated in an earlier tier.

Characteristic	Tier	Collection Method	Indicates
Salinity	1 2 3	-measure at each sampling station, CTD meter -continuous or 1-2-m intervals through water column -shallow/inshore -top, middle, bottom thirds of depth	Distribution of flora and fauna
Temperature	1 2	-measure at each station, CTD meter -1-2-m intervals through water column -shallow/inshore -top, middle, bottom thirds of depth	Rate of chemical reactions and biological processes
	3 addition	-some southern waters undergo significant diel changes, it may be desirable to obtain 24-hour temperature profiles	
Dissolved Oxygen	1	-measure at each station, CTD meter w/ DO probe -continuous or 1-2-m intervals through water column -shallow/inshore -top, middle, bottom thirds of depth	Possible reason for modified behavior, reduced abundance & productivity, adverse reproductive effects, and mortality
	2 addition	-measure early in morning at each station at minimum	
	3 addition	-collect along a depth profile from surface to within 1-m of bottom at 1-2-m intervals -in cases of hypoxic site: recording DO meters may be deployed (EMAP - Louisianian Province, Engle et al 1994)	Resource agency may determine need for more detailed information to diagnose sources & causes of impairment
pH	1 2 3	-CTD w/ pH probe -1-2-m intervals -top, middle, bottom thirds of depth	Chemical condition, pollutant input, high concentrations of phytoplankton
Secchi Depth (Turbidity)	1 2 3	-deepest part of transect/grid -if area classified by depth, measure at each station -See Section 6.2 for complete procedure	Reduction of light penetration, deposition of mud and silt, possible contaminated sediment "hot spots"
Depth	1 2 3	-each station w/ calibrated depth sounder -read off meter block when sediment sampling -shallow/inshore waters: long stick or weighted line calibrated in decimals	Depth at sampling station, possible dredging or sediment loading
Nutrients	2 3	-collected w/ bottle samplers or pump -see Section 6.8 for complete procedure	Nutrient loading
Acid Volatile Sulfides	3	-each station during index period, and any other sampling visits through year -once accurate AVS exists for each station, analytes only performed once per year (during index period)	Bottom characteristics, detailed purposes in Section 3.5.4
Water Column Contaminants	3	Choose One: -USEPA's list of Priority Pollutants, Hazardous Substance, or Target Compound/Analytes -same compounds targeted in EMAP-E (Table 3-1) -develop own list (see Section 6.10 for more detail)	Trace distribution of contaminants from a source or to ID potential sources

Table 6-1 (Cont'd). Water Column & Bottom Characteristics. "Addition" refers to added detail or intensities for a parameter initiated in an earlier tier.

Characteristic	Tier	Collection Method	Indicates
Sediment Grain Size	1	-determine "percent fines" at each station, see Section 6.4.1 for complete procedure	Spatial and temporal changes of the benthic habitat, evaluate condition of benthic habitats
	2	-see Section 6.4.2 for complete procedure	Determine extent or recovery from environmental perturbations
	3		Assist in providing early warnings of potential impacts to the estuarine ecosystem
Total Organic Carbon	2	-see Section 6.9 for complete procedure	Provide information regarding sediment organic content (possibly influenced by sewage outfalls)
	3 addition	-measure additional sediment analytes	Examine potential influences of outfalls, ID potential contaminant "hot spots"
RPD Layer Depth	1	-vertical bisection, distance from sediment surface to a noticeable change in color from brownish (oxidizing conditions) to gray (reducing conditions)	Note presence/absence of benthos; learn about life history, taxa abundance, & major taxa biomass distribution; more large, deep dwelling species="healthy" system
Total Volatile Sulfides	1	-deepest section along transect/grid -see Standard Methods (APHA 1992) for sampling & analytical methods	Sediment and carbon content
Sediment Contaminants	1	-conducted at outset of survey	Positive=severe impacts influence spatial sampling design, causal investigations Negative=subsequently collected biological info. essential to ID other, possibly more subtle stresses Provide insight on limiting factors in benthic community
	2	*like TOC, if toxicity tests are initially negative, no need to repeat annually unless biological data from infauna indicate otherwise	
	3	Choose from three approaches: -based on EPA's contaminant lists -NOAA NS&T suite of contaminants (used by EMAP) -targeted list *see Section 6.12 for complete procedure and rationale	

- small stainless or plastic spatula
- stainless butter knife
- hose with nozzle (if running water is available).

Detailed directions for performing this wet-sieving technique are as follows:

Fill a 50-ml plastic beaker to the brim with the sediment to be analyzed. The capacity of the completely filled beaker can be measured using water and the 100-ml graduated cylinder. Clean away any sediment that might adhere to the outside of the beaker. Carefully wash this sediment through a 63-μm standard sieve (USA standard testing sieve No. 230) with stainless steel mesh. The sieve itself is about 9" in diameter with a 2" stainless lip. Be careful not to overflow the sieve with rinsing water. It may be easier to wash half of the sediment through at a time. If running water is available, use a small brass nozzle on the end of the hose with very low water pressure when washing the sediment, otherwise the sediment will need to be washed using the water bottle. If there are occasional large worm tubes or shells, these are discarded and replaced with an approximately equal volume of sediment. The sediment remaining on the sieve is the coarse-grained fraction. This is washed to one side of the sieve, and then carefully placed into the plastic 100-ml graduated cylinder with a stainless steel butter knife, and finally with the small stainless spatula. The water bottle is then used to wash any remaining sediment directly into the graduated cylinder, and to wash down the sides of the cylinder. Let the sediment-water mixture settle in the 100-ml graduated cylinder for approximately 5 minutes until the supernatant water is clear. This may take longer for very fine-grained sediments. Note the volume of the coarse-grained fraction which remains after sieving. This can be divided by the original volume to obtain the percentage of the coarse fraction. The standard usage, however, is for percent fine-grained fraction or "**percent fines**". This is calculated by subtracting the volume of sediment remaining in the cylinder (ml of coarse-grained fraction) from the original volume, and dividing this number (ml of fine-grained) by the original volume to obtain the percent fines.

6.4.2 Sediment Grain Size (Tiers 2 and 3)

Additional grain size data for Tier 2 and Tier 3 assessments should include determination of the size distribution using a standard graded sieve series. This analysis should be performed for a sediment sample collected at each sampling station. In the early years of the assessment program, this analysis should be performed for each sampling period. When an accurate sediment characterization exists for the area of each station, sediment grain size analysis could be performed only annually or biennially (on samples collected in the index period), unless the agency believed that sediment conditions at a site may have changed. This could occur, for example, following a major storm. Buller and McManus (1979) provide a review of the methodological and statistical analysis of sediment samples. If seasonal variations in grain size are exhibited, it is recommended that direct comparisons between samples collected during different seasons be avoided. Studies investigating interannual variation in the percent composition of grain sizes should be conducted during the same season (preferably the same month) each year. Furthermore, it is recommended that grain size be sampled when contaminant concentrations are expected to be at their highest level to evaluate worst-case scenarios.

6.5 RPD Layer Depth

The concept behind using the depth distribution of benthic macroinvertebrates is based on the premise that "healthy" benthic communities in fine sediments in meso- and polyhaline waters consist of relatively large, deep dwelling species; while impaired areas will have fewer of these organisms. The depth distribution of benthic infauna in sediments integrates functional parameters such as life history, taxa abundance, and major taxa biomass distribution.

6.6 Total Volatile Solids

Total volatile solids (TVS) is the Tier 1 indicator for sediment carbon content. TVS should be determined for the deepest station along each transect or grid, based on the assumption that deeper stations will represent sinks for organic carbon in the sediments. Sampling and analytical methods are discussed in Standard Methods (APHA 1992).

6.7 Sediment Contaminant Toxicity

Sediment toxicity testing is a diagnostic indicator for Tier 3. When results are positive for a station, severe impacts at a known locality will influence spatial sampling design and causal investigations. Where toxicity test results are negative throughout the set of stations sampled, subsequently collected biological information is essential to identify other, possibly more subtle stresses on the system.

6.7.1 10-day Static Sediment Toxicity Test with Marine and Estuarine Amphipods

ASTM (1998a) and USEPA (1994b) developed procedures that measure short term adverse effects of potentially contaminated sediment, or of a test material experimentally added to sediment, on marine or estuarine infaunal amphipods during static 10-day exposures for the following species: *Rhepoxynius abronius*, *Eohaustorius estuaris*, *Ampelisca abdita*, *Grandidierella japonica*, and *Leptocheirus plumulosus*. The amphipod *Corophium insidiosum* has also been used in standard testing (Reish and Lemay 1988). Solid phase tests use overlying water in aerated 1-L glass test chambers. Mortality and sublethal effects such as growth, emergence of adults, and inability to bury in clean sediment are determined after exposure of a specific number of amphipods (usually 20) to the test sediment. Response of the amphipods to the test sediment is compared with the response observed in control or reference sediment. The negative control sediment is used to provide a measure of the acceptability of the test by providing evidence of the health and relative quality of the test organisms, the suitability of the overlying water, and test conditions and handling procedures (ASTM 1998b, USEPA 1994b). The reference sediment, which is similar in physical characteristics to the test sediments and typically collected from a similar location, is used as the basis for interpreting data obtained from the test sediments (ASTM 1998b).

The toxicity of field-collected sediments may be assessed by either (a) testing the whole sediment and testing for significant differences in responses between reference or control and test sediment exposed animals or (b) testing dilutions of a test sediment with clean sediment to obtain an LC_{50} or other effect concentration, for survival, reburial success, or growth (ASTM 1998b, Nelson et al. 1993, Swartz et al. 1995).

6.7.2 10-day Static Sediment Toxicity Test with Marine and Estuarine Polychaetous Annelids

Marine or estuarine infaunal polychaetes are used in whole sediment tests during 10-day or 20- to 28-day exposures to determine adverse effects of potentially contaminated sediment, or of a test material added experimentally to sediment. Polychaete species include *Neanthes virens* for the 10-day and *Neanthes arenaceodentata* for the 10-day and 20- to 28-day tests (ASTM 1998c). Other polychaete species that have been used in similar sediment testing include *Capitella capitata*, *Ophrotrocha diadema*, and *Ctenodrilus serratus* (Reish and Lemay 1988). The 10-day test measures effects of contaminated sediment on polychaete survival. The 20- to 28-day test determines effects of contaminated sediment on polychaete survival and growth. If smaller species are used, such as *N. arenaceodentata*, five worms are placed in a 1-L glass test chamber with a minimum sediment depth of 2- to 3-cm and the overlying water is aerated. Either young adults or recently emerged juvenile (2- to 3-weeks post-emergence) worms are used in the 10-day test; only recently emerged (2- to 3-weeks) juveniles are used in the 20- to 28-day test. Survival of worms exposed to the test sediment is compared with the survival in a negative control or reference sediment in either test. If larger species are used, such as *N. virens*, ten worms are placed in a glass aquaria (4- to 37-L) with a minimum sediment depth of 10-cm and the overlying water is aerated.

The percent survival of polychaetes exposed to field-collected sediment is compared to those exposed to a negative control or reference sediment in 10-day tests. Survival and body weight of surviving animals is compared to those exposed to negative control or reference sediment in 20- to 28-day tests. The toxicity of field sediments may also be assessed by testing dilutions of highly toxic test sediments with clean sediments to obtain either an LC_{50} or other effect concentration of the material.

6.7.3 Static Acute Toxicity Tests with Echinoid Embryos

Echinoderm embryos and larval form sea urchins (*Strongylocentrotus purpuratus* and *Strongylocentrotus droebachiensis*) and sand dollars (*Arbacia punctulata*, *Lytechinus pictus*, and *Dendraster excentricus*) have been used in marine sediment interstitial (pore) water tests (ASTM 1998a). Interstitial water from marine sediments is isolated using either *in-situ* peepers (Sarda and Burton 1995, Brumbaugh et al. 1994, Bufflap and Allen 1995), suction in the field (Watson and Frickers 1990), laboratory centrifugation (Ankley et al. 1991, Burgess et al. 1993, Kemble et al. 1994, ASTM 1998b), or sediment squeezing (Long et al. 1990). Embryos are obtained by inducing adults to spawn, using either physical (e.g., electric stimuli) or chemical (injection of potassium chloride) means, and then combining gametes.

Embryos are exposed to the test pore water and controls (culture water) for 48- to 96-hours, depending on the species and test temperature. The test measures the proportion of embryos or larvae that develop into normal pluteus larvae. Pore waters can be tested "whole"; i.e., undiluted, and organism responses expressed in terms of a significant difference between controls and test waters. Alternatively, pore water samples can be diluted with known, clean culture water and the results expressed as an LC_{50} or other

effect concentration with confidence limits.

6.7.4 Toxicity Tests Using Marine Bivalves

Juveniles of the marine bivalve species, *Mulinia lateralis*, have been used in whole sediment tests (Burgess and Morrison 1994). Juveniles are exposed for 7-days to determine adverse effects of potentially contaminated sediment, or of a test material added to sediment. Bivalve responses measured include survival and growth, (total organism dry weight). Ten juvenile bivalves (four weeks old) are placed into six replicate chambers per sediment or treatment. The sediment exposure chambers are prepared by placing approximately 1.0-cm deep sediment into 150-ml dishes, followed by the addition of 100-ml filtered 30-gkg^{-1} seawater. Upon initiation of the test a subsample of organisms are set aside for determination of initial juvenile weights. Bivalve survival in test chambers is compared to survival of bivalves in the negative control or reference sediment. Dry weight of the surviving organisms in test chambers is compared with dry weight of surviving organisms in the reference sediment, and to the dry weight of the subsample set aside at the initiation of the test to determine growth.

Similar to echinoderm testing summarized in Section 6.7.3, bivalve larvae have also been used in sediment pore water and sediment elutriate toxicity tests. Species used include *Crassostrea gigas* and *Mytilus edulis* (PSEP 1995). Bivalve larvae are obtained from laboratory-cultured adult brood stock, which are induced to spawn. Developing embryos are exposed to the pore water or elutriate at 20°C for 48-60-hours using static-test conditions. At test termination, subsamples of the larvae are examined and the percentages of mortality and abnormal survivors are determined and analyzed.

6.8 Nutrients (Tiers 2&3)

Water column samples for nutrient analysis can be collected using bottle samplers such as Kemmerer, Van Dorn, Niskin, or Nansen samplers. A pump may be used as an alternative sampling device. In shallow water less than 2-m depth, a mid-depth sample at each station should be obtained for nutrient analysis. In waters greater than 2-m depth, samples should be collected at each station at 1-m below the surface, 1-m above the bottom, 1-m above the pycnocline, 1-m below the pycnocline, or at mid-depth. Analytical methods for NH_4-N, NO_3-N, NO_2-N, Kjeldahl nitrogen, total N, and total and reactive P; i.e., ortho-P, are presented in APHA (1992) and USEPA (1994c). These nutrient analyses will help identify eutrophication factors affecting biocriteria development, as well as supplement the USEPA's nutrient criteria initiatives so that multiple objectives can be accomplished at once.

6.9 Total Organic Carbon (Tiers 2&3)

In Tier 2, the primary purpose of measuring total organic carbon (TOC) is to provide information regarding sediment organic content, which might be influenced by sewage outfalls containing high organic levels. As noted in Chapter 3, TOC in the sediment is an important analyte for the purpose of evaluating the bioavailability of organic pollutants and metals adsorbed by sediments or contained in sediment porewater. Data on sediment TOC collected in this tier can be used to examine potential influences of outfalls in addition to potential sediment contaminant "hot spots" that can be

assessed in Tier 3 with the measurement of additional sediment analytes.

Standard methods for TOC analysis are presented in APHA (1992). In the early years of the assessment program, TOC analysis should be performed for each station in each sampling period. Once the resource agency is confident that an accurate characterization of sediment TOC exists for each station, the analysis could be performed only once every two or more years (on samples collected in the index period), unless stations that appear to be influenced by organic input (e.g., sewage outfalls) are identified. In this case, TOC analysis should continue to be performed for each sampling period for these stations.

6.10 Water Column Contaminants (Tier 3)

Water column contaminants such as organic compounds (e.g., herbicides, pesticides, hydrocarbons) and metals may be important indicators of sources and causes of impairment to biological assemblages in estuaries and coastal marine waters. Decisions on which chemicals to include in Tier 3 assessments can be difficult. Three approaches to selecting contaminants might be useful. One approach would be to analyze for all chemicals listed on USEPA's Priority Pollutant, Hazardous Substance, or Target Compound/ Analyte Lists. A second approach would be to analyze for the same compounds targeted in the EMAP-Estuaries program (refer to Table 3-1). A third approach would be to develop a targeted list. In this latter approach, the historical information from Tier 0 and subsequent follow-up inquiries of land use in the suspect area could point to common pesticides, herbicides, or industrial products or byproducts that could form the basis of a select list of contaminants to analyze. Sources for this information also include NPDES permit records and discharger toxicity test results. In any case, three replicate water samples should be collected at each sampling station within an appropriate index period and on at least three other visits during the year to capture temporal variations in contaminant concentrations. Historic water contaminant data, plus data collected in this tier, can be used by the state to determine a more limited list of analytes for subsequent years of the assessment and biocriteria program.

The same type of sampling bottle used to collect water samples for nutrient analysis may be used for contaminant samples. USEPA (1992) and APHA (1992) contain detailed information on analytical methods.

6.11 Acid Volatile Sulfides (Tier 3)

Details of the purposes for measuring acid volatile sulfides (AVS) present in bottom sediments are provided in Section 3.5.4. Given the diagnostic intent of a Tier 3 assessment, it is important to include this analyte in determinations of bottom characteristics only if metals are suspected as a cause of biological degradation. Allen et al. (1993) discuss analytical methods for this parameter. AVS measurements should be made on sediment samples collected at each station during an appropriate index period and any other sampling visits made throughout the year. Once the resource agency is confident that an accurate characterization of sediment AVS exists for each station, the analytes should be performed only once per year (on samples collected in the index period).

6.12 Sediment Contaminants

As with water column contaminants, three approaches to selecting analytes could be used: (1) a full scan based on USEPA's contaminant lists; (2) the NOAA National Status and Trends suite of contaminants used by the EMAP program (refer to Table 3-1); or (3) a targeted list.

In this latter approach, the historical information from Tier 0 and subsequent follow-up inquiries of land use in the suspect area could point to common pesticides, herbicides, or industrial products and byproducts that could form the basis of a select list of contaminants to analyze. In addition to sampling organisms for contaminants, sediment samples should be collected from the device used for sampling benthic infauna. The surface sediment (top 2-cm) should be removed from replicate grab samples and composited. During collection, care should be taken to avoid collecting material from the edge of grabs and to use only samples that have undisturbed sediment surfaces. The composite sample should be homogenized, and a subsample taken for measurement of contaminant concentrations. Analytical methods are discussed in APHA (1992) and USEPA (1992).