Chapter 10
Tier 3

Tier 3 is the most rigorous of the assessment tiers. It includes more detailed assessment procedures that allow monitoring agencies to focus on specific water and sediment quality problems in estuarine or coastal marine waters. Tier 3 is intended to provide definitive information needed to act on biocriteria and to measure potential success or failure of the management effort. It allows states to conduct a detailed diagnosis of the sources and causes of impairment to biological assemblages and the physicochemical environment and to monitor their response to subsequent mitigation actions. However, the Tier 3 approach can be customized to accommodate specific state program objectives. Table 10-1 gives an overview of the components, data collection methods, and indicators for Tier 3.

Tier 3 assessments include multiple sampling visits per year (four or more) that occur within each season including the index period. Data collected in Tier 3, which includes information compiled in Tier 0 desktop screening and comprises the information collected in Tiers 1 and 2, involves sampling and measurement of three or more biological assemblages (benthos, fish macrophytes, phytoplankton, zooplankton, or epibenthos), in addition to more detailed characterization of the water column and bottom. A Tier 3 assessment enables:

- Identification of nutrient state based on chlorophyll *a* and water column nutrient measurements;
- Detection of impairment of benthos, fish, macrophytes, phytoplankton, zooplankton, epibenthos, or paleoenvironmental systems;
- Diagnosis of specific sources and causes of impairment;
- Measurement of extent of macrophyte coverage;
- Identification of phytoplankton taxa responsible for blooms;
- Evaluation of seasonal dynamics of biological assemblages;
- Detailed monitoring of sites requiring management initiatives to meet the biocriteria;
- Inferences of past conditions as a site-specific reference.

10.1 Benthos

Sampling and analysis of benthic infaunal macroinvertebrates in Tier 3 is intended to provide a diagnostic level of assessment. This assemblage, and the methods presented, will be most appropriate for soft sediment. For sites with hard bottom substrates, other biological assemblages (e.g., fish, macrophytes, phytoplankton, zooplankton) could be selected to provide information on the biological condition of the target waters.

The sampling strategy for Tier 3 entails a minimum of four field collection visits per year, one of which should occur within the chosen index period. The remaining visits should occur throughout the year to allow evaluation

Table 10-1. Tier 3 Assessment. Requires four or more field visits, one of which should occur within the chosen index period. In addition to requirements from Tiers 0-2.

Component	Data Collection	Indicator of	Uses
Biological Assemblages			
Benthic Infauna	*determine biomass *calculate multiple metrics		-Identification of *nutrient* state based on chlorophyll *a* & water column nutrient measurements -Detection of impairment of benthos, fish, macrophytes, phytoplankton, zooplankton, epibenthos, or paleoenvironmental systems -Diagnosis of specific sources & causes of impairment -Measurement of extent of macrophyte coverage -Identification of phytoplankton taxa responsible for blooms -Evaluation of seasonal dynamics of biological assemblages -Detailed monitoring of sites requiring management initiatives to meet the biocriteria -Integrate conditions over broad spatial scales
Fish	*5 or more replicates *histopathology on representative subsample of catch	Fishing pressure, disease	
Macrophytes	*stem counts *biomass *record pathology	Toxicity, habitat impairment, disease	
Phytoplankton	*full community characterization to species		
Paleoenvironmental Systems (developmental)	*2 or 3 cores from basin (one-time sample)	Past conditions	
Epibenthos (developmental)	See Tier 2		
Zooplankton (developmental)	*identify to species	Water quality impairment, DO stress	
Water Column Characteristics			
	*pesticides, herbicides *metals	pesticides/ herbicides, metals	
Bottom Characteristics			
	*AVS *sediment contaminants (organics, metals)		

of seasonal differences in the benthic macroinvertebrate assemblages. Organisms are identified to genus and species. Water column and bottom characteristics are also measured to evaluate the status of physicochemical conditions.

10.1.1 Sampling Procedure

Primary objectives of Tier 3 benthic infaunal sampling are to evaluate potential impairment to this assemblage, to develop and refine biocriteria, to diagnose causes and sources of observed impairment, and to evaluate seasonal changes in the benthic infauna. This tier includes more frequent sampling (a minimum of four times per year) than either Tiers 1 or 2 to allow detailed discrimination of seasonality of benthic abundance. See Section 8.1.1 for full detail on sampling procedures.

10.1.2 Index Period

Benthic infaunal macroinvertebrates are sampled once or twice during an appropriate index period, the timing of which is driven by the goals of the Tier 3 assessment and regional considerations. At least two or three other sampling visits are made throughout the remaining portion of the year to capture more detailed seasonal differences in benthos than would be possible in a Tier 2 assessment. Data collected in a previous Tier 2 assessment, or historic benthic infaunal macroinvertebrate data, can be used to determine the timing and frequency of non-index period sampling.

10.1.3 Analysis

Organisms in each sample are identified to genus and species. If desired, and resources are available, ash-free dry weight, at least to the family level, may be measured to determine the viability of biomass-based metrics to the overall assessment. Other metrics should be selected by the resource management agency as appropriate based on historic data, data collected and metrics used in preceding tiers, and regional considerations.

10.2 Fish

Tier 3 assessment of the fish assemblage is intended to allow evaluation of impairment, to develop and refine biocriteria, to diagnose causes and sources of impairment, and to evaluate seasonal differences in the assemblage. Fish sampling in this tier can include shallow-water, pelagic, and demersal fish communities (Carmichael et al. 1992, Eaton and Dinnell 1994, Guillen 1994).

10.2.1 Sampling Procedure

See Section 8.2.1 for full description of fish sampling procedures.

10.2.2 Sample Processing

See Section 8.2.2 for full description of fish sample processing.

10.2.3 Analysis

Based on the enumerated species list, metrics selected by the state can be calculated to evaluate potential impairment to the fish assemblage, to develop or refine biocriteria, to examine seasonal dynamics of the assemblage, or to diagnose sources and causes of impairment.

10.3 Macrophytes

Tier 3 assessment of macrophytes is intended to provide sufficient data to

assess impairment to the macrophyte assemblage, to develop or refine biocriteria, or to diagnose sources and causes of impairment.

10.3.1 Sampling Procedure

See Section 8.3.1 for full description of macrophyte sampling procedures.

10.3.2 Index Period

See Section 8.3.2 for full description of the macrophyte index period.

10.3.3 Analysis

Percent cover and area may be derived from analysis of aerial photographs. Taxonomic identification from the field trips will allow development of a species list. Stem counts made within quadrats along each sampling transect in addition to biomass determination will provide more detailed information on assemblage condition. Detailed pathology observations should be made; they can be used to evaluate potential causes of impairment.

10.4 Phytoplankton

10.4.1 Sampling Procedure

See Section 9.4.1 for a full description of the phytoplankton sampling procedure.

10.4.2 Index Period

Phytoplankton should be sampled at least once during an appropriate index period and a minimum of three other times per year to capture seasonal changes in the composition and abundance of the assemblage. Following review of data collected from historical data or through any of the assessment tiers described here, the resource management agency may determine that a higher frequency of sampling is needed to characterize the phytoplankton assemblage based on its potential for rapid spatial and temporal variation.

10.4.3 Analysis

See Section 9.4.3 for a full description of phytoplankton analysis.

10.5 Epibenthos (Developmental)

As in Tier 2, even though epibenthos is currently under development as a biological indicator, it can still be useful in the Tier 3 assessment.

10.5.1 Sampling Procedure

See Section 9.5.1 for a full description of the epibenthos sampling procedure.

10.5.2 Index Period

See Section 9.5.2 for a full description of the epibenthos index period.

10.5.3 Analysis

See Section 9.5.3 for a full description of epibenthos analysis.

10.6 Zooplankton (Developmental)

Zooplankton are an important link between phytoplankton in estuaries and coastal marine waters and higher consumers. States might choose to include this developmental assemblage as part of a Tier 3 assessment.

10.6.1 Sampling Procedure

Three replicate vertical tows using a 118-µm mesh net, 30-cm in diameter should be made at each sampling location. The tow should be vertically integrated; that is, starting from 0.5-m from the bottom to the surface, with a retrieval rate of 0.5- to 1-ms^{-1}. Collected organisms should be anesthetized with carbonated water and preserved in 4% formalin. For long-term storage after fixing, specimens should be transferred to 70% ethanol. APHA (1992) describes procedures for concentrating the samples and preparing them for examination.

10.6.2 Index Period

Zooplankton should be sampled once during an appropriate index period and a minimum of three other times during the year to capture seasonal variation in taxonomic composition and abundance.

10.6.3 Analysis

Samples should be identified to the lowest practical taxonomic level, preferably genus and species. Subsampling may be required to achieve reasonable numbers of organisms for identification.

10.7 Paleoenvironmental Systems (Developmental)

Developmental assessment of paleoenvironmental systems is intended to provide site-specific reference by showing past conditions. Several groups of organisms leave remains in the bottom sediments. Some of the remains are resistant to decay and become a permanent biological record of life in the estuary. By comparing past biota with present-day biota, past environmental conditions can be inferred. Several groups of organisms have been used: diatoms, foraminifera, and dinoflagellate cysts. Of these, diatom frustules and foraminifera have been used most often, and most successfully, to infer past conditions. A sample of the top 1- to 2-cm of sediment contains a representative sample of diatoms from the most recent 1- to 5-years. If the sediments remain undisturbed, then remains preserved in the sediments are integrators of estuarine history (Charles et al. 1994, Dixit et al. 1992). Because of the developmental nature of this indicator, states or agencies wishing to use paleoenvironmental reconstruction should contact one of the laboratories engaged in this research for further information. The methods described here are intended to give a brief overview of the field, but should not be used to plan a monitoring program.

10.7.1 Sampling Procedure

Cores are generally taken with standard gravity corers, such as the K-B, Phleger, or Piston. The chosen corer should retrieve a core deep enough to sample sediments from the earliest desired time period, with minimal edge disturbance. Core length thus depends on time period and sedimentation rate. Core samples are extruded from the corer and subsectioned immediately after collection. Sections 1-cm thick are removed from the core at intervals according to the time resolution desired. These sections are removed from the core using an apparatus described by Glew (1988) then are bagged, labeled and identified using a permanent ink pen. The bags prepared from a single core sample

are placed in a sealed container for storage and transport. Samples are kept at 4°C until shipment.

10.7.2 Sample Processing

Once in the laboratory, the sections are dried and weighed. Foraminifera and diatoms are processed so as to digest organic matter and preserve carbonate (foraminifera) or silica (diatoms), following the standard methods of Krom and Berner (1983) and EMAP (USEPA 1994e). An aliquot of frustules or tests is mounted for optical and/or scanning electron microscopy for identification. Dinoflagellate cysts are subjected to a standard pollen analysis involving the digestion of minerals in cold HCl, followed by warm HF (adapted from Barss and Williams 1973). They are processed on a 10 μm sieve. Samples for counting and identification are not random, but systematic.

Transects are taken on microscope slides, counting and identifying all target taxa encountered. A count of 300 or more is necessary for meaningful analysis of percentage data, but lower counts are still valid if results are reported on a concentration basis. In some depositional systems it is not feasible to count 300 dinoflagellate cysts, but the data is still informative.

Charcoal is seen in pollen analysis and dinoflagellate cyst preparations. The larger sieve size used for foraminifera would exclude most charcoal particles, thus make this material unsuitable for charcoal studies.

10.7.3 Analysis

Standard dating methods use either carbon-14, pollen, ^{137}Cs, or ^{210}Pb (Dixit 1992, Cooper and Brush 1991, Dale et al. 1999, Alve 1991). Additional time points can be established from traces of known historic events (charcoal from large-scale fires, radioisotopes from atmospheric testing and the Chernobyl accident). Known responses of indicator taxa or biogeochemical indicators (e.g., biogenic silica) are used to infer past environmental conditions of an estuary. This allows for the assessment of current environmental conditions based on those of the past.

Quantitative paleoenvironmental reconstruction in estuaries requires the development of a data set that associates current conditions with current surficial diatom, dinoflagellate, or foraminifera assemblages. Present-day associations are used to infer past conditions based on fossil assemblages in deeper sediment layers. Quantitative prediction is usually done in two steps: development of predictive models (calibration or transfer functions), followed by use of the models to infer environmental variables from fossil assemblages (Charles and Smol 1994). Quantitative reconstruction has not yet been widely developed for estuaries.