Chapter 9
Tier 2

Tier 2 assessment is considered a routine biological survey that incorporates two or more field visits per year to capture variations due to seasonal differences. Tier 2 comprises increased sampling effort and additional assemblages compared to Tier 1. It includes two or more biological assemblages (benthos, fish, macrophytes, phytoplankton, or epibenthos), in 2 or more visits per year, in addition to more detailed characterization of the water column and bottom (Chapter 5). State agencies can modify this schedule to accommodate their program objectives.

This level is sufficient for identification of appropriate habitat classes and determination of the reference condition for development of biological criteria. Data collected in Tier 2, which incorporates both Tier 1 and Tier 0, should permit the state to confidently develop biocriteria and apply them to identify problem areas. This assessment level enables:

- Establishment of the biocriteria "benchmarks" for decision-making about impaired areas; including identification of priorities;
- Identification of trophic state based on chlorophyll *a* and water column nutrient measurements;
- Detection of impairment of benthic macroinvertebrate, fish, or epibenthos assemblages, and evaluation of potential causes of the impairment;
- Measurement of extent of macrophyte coverage;
- Identification of phytoplankton taxa responsible for blooms.

A Tier 2 assessment should allow identification of multiple probable causes of impairment, given an adequate number and placement of sampling stations. This includes point sources, and nearfield and farfield nonpoint sources. Preliminary management plans in response to the biocriteria information can be developed. Table 9-1 gives an overview of the components, data collection, methods, and indicators for Tier 2.

9.1 Benthos

Sampling and analysis of benthic infauna in Tier 2 is intended to provide a level of assessment consistent with routine benthic macroinvertebrate surveys presently conducted by states in estuaries and coastal marine waters. As with Tier 1, this assemblage, and the methods presented, will be most appropriate for soft sediments. For sites with hard bottom substrates, other biological assemblages (e.g., fish, macrophytes, phytoplankton) could be selected to provide information on the biological condition of the target waters.

The sampling strategy for Tier 2 entails a minimum of two field collection visits, one of which should occur within the chosen index period. Organisms are identified to genus and species to determine major taxa and the presence of indicator organisms. Water column and bottom characteristics are also measured to evaluate the status of physicochemical conditions.

Table 9-1. Tier 2 Assessment. Requires two or more field visits, one of which should occur within chosen index period. In addition to requirements from Tiers 0 & 1.

Component	Data Collection	Indicator of	Uses
Biological Assemblages			
Benthic Infauna	*3 replicate grabs (or as determined by testing; see Section 5.2.6 *identify major taxa and indicator spp. of each grab to genus and species		-Establishment of biocriteria "benchmarks" for decision-making about impaired areas, including identification of priorities -Identification of *trophic* state based on chlorophyll *a* and water column nutrient measurements -Detection of impairment of benthic macroinvertebrate, fish, or epibenthos assemblages and evaluation of potential causes of impairment -Measurement of extent of macrophyte coverage -Identification of phytoplankton taxa responsible for blooms
Fish	*3 or more replicates *biomass by species		
Macrophytes	*area *maximum depth *identify taxa and measure wet weight of 2-3 samples per transect	Habitat impairment	
Phytoplankton	*identify dominant species, including "nuisance" taxa, on a seasonal basis		
Epibenthos (developmental)	*mid-summer or growing season average at genus and species level *calculate sensitivity metric		
Water Column Characteristics			
	*nutrients: NH_4, NO_3,NO_2, Kjeldahl N, total and reactive P		
Bottom Characteristics			
	*grain size measurements *TOC	Organic enrichment	

9.1.1 Sampling Procedure

Primary objectives of Tier 2 benthic infaunal sampling are to evaluate potential impairment to this assemblage and to generate the data necessary to develop biocriteria. This Tier, unlike Tier 1, incorporates multiple sampling visits to allow a basic discrimination of seasonal differences in the benthic infaunal macroinvertebrate assemblage. The recommended sediment sampling procedure involves collecting three replicate grab samples at each station using a Smith-McIntyre or Young grab. Sampling gear should be selected to maximize compatibility with historic data. The sediment sample is vertically bisected using a sheet metal partition. The RPD layer depth is noted and measured, if present, as the distance from the sediment surface to a noticeable change in color from brownish (oxidizing conditions) to gray

or black (reducing conditions). The sample should be wet sieved through a sieve mesh size determined to be appropriate for the region (Section 6.3.2). For cost and effort savings, an appropriate diameter subcore (2.5- or 5-cm) can be taken from each of the four quadrants of the intact core. These subcores should be compared to organism counts taken from full cores to establish the baseline relationship between the two. Organisms and sediment fractions should be placed in tagged and labeled sample jars with a 10% solution of magnesium chloride or magnesium sulfate to narcotize the animals. After at least 30-minutes, concentrated formaldehyde with rose bengal dye can be added to the jars to make a 10% solution of formaldehyde by volume. The sediment/organism material should never exceed half the container volume to ensure adequate mixing and fixation of the sample. For preservation, the samples should be transferred to 70% ethanol (APHA 1992).

9.1.2 Index Period

Benthic infaunal macroinvertebrates are sampled once during an appropriate index period, the timing of which is driven by the goals of the Tier 2 assessment and regional considerations. At least one other sampling visit is made outside the index period to capture basic seasonal differences in the assemblages. The timing of this visit(s) will depend on the specific goals of the assessment.

9.1.3 Analysis

Organisms in each sample are identified to genus and species. Metrics selected by the state can then be calculated to assess the condition of the assemblage. Metric values can then be used to help develop biocriteria against which the condition of the macroinvertebrate assemblage is evaluated.

9.2 Fish

Tier 2 assessment of the fish assemblage is intended to provide data sufficient to evaluate impairment and to develop biocriteria. Fish sampling in Tier 2 can include shallow water, pelagic, and demersal fish communities (Carmichael et al. 1992, Eaton and Dinnell 1994, Guillen 1994).

9.2.1 Sampling Procedure

See Section 8.2.1 for full procedure on sampling fish.

9.2.2 Sample Processing

See Section 8.2.2 for full procedure on sample processing.

9.2.3 Analysis

Based on the enumerated species list, metrics selected by the state can be calculated to evaluate potential impairment to the fish assemblage and to develop biocriteria for this assemblage.

9.3 Macrophytes

Tier 2 assessment of macrophytes is intended to provide sufficient data to assess impairment to the macrophyte assemblage as a significant habitat variable and potential element of biocriteria. Because of its importance as habitat for other assemblages, procedures for Tier 2 assessment of macrophytes are considerably more involved than for Tier 1.

9.3.1 Sampling Procedure

The extent of coverage and distribution of macrophytes should be determined

from aerial photographs. Existing aerial photographs are inexpensive; however, they may not be sufficiently recent to depict present macrophyte distribution in the water body. If new aerial photographs are determined to be needed, states should recognize that overflights can be expensive and complicated; often requiring assistance from firms specializing in aerial photography. Factors to consider when planning new overflights include: tidal stage; weather conditions; time of day; and water turbidity (USEPA 1992). Ferguson and Wood (1994) and Orth et al. (1993) describe details of planning aerial overflights, obtaining imagery, photointerpretation, and preparation of macrophyte distribution maps.

A key aspect of interpreting aerial photographs is the performance of ground surveys that serve to confirm the existence of macrophyte beds identified in the photographs, as well as beds that may not be visible in the photos (Orth et al. 1993). Transects can be plotted across macrophyte beds in the various salinity zones within an estuary or within the sampling strata used for marine waters. At each station on the transect a 1-m^2 quadrat can be used for the purpose of measuring percent cover and collecting macrophyte samples for taxonomic identification and measurement of wet weight (USEPA 1992). Depth at the channel-ward or seaward edge of macrophyte extent should be recorded.

9.3.2 Index Period

Aquatic macrophytes should be sampled once during an appropriate index period, preferably during the time of year when they would be expected to be most dense and extensive. Other sampling periods should be selected based on the specific goals of the Tier 2 assessment, perhaps to measure seasonal periods of stress or diminishment of important nursery or food areas.

9.3.3 Analysis

Percent cover and area may be derived from analysis of aerial photographs. The maximum depth of occurrence is a good indicator of water quality. Taxonomic identification from the field trips will allow development of a species list.

9.4 Phytoplankton

9.4.1 Sampling Procedure

Phytoplankton standing stock is estimated by chlorophyll *a* measurements. One approach might be three replicate samples collected at each station at one-half the Secchi depth using a Kemmerer or Van Dorn sampler. Another approach would collect a depth-integrated sample through the entire photic portion of the water column. Chlorophyll *a* is determined using a fluorometer or spectrophotometer as discussed in APHA (1992). The presence of any phytoplankton blooms should be noted. In addition to chlorophyll *a* measurements, samples from each station should be preserved for subsequent analysis to identify the dominant taxa and those taxa that might be responsible for observed blooms (USEPA 1992).

9.4.2 Index Period

Phytoplankton populations can vary rapidly over space in response to tides and currents, and over time in response to ambient temperature and nutrient inputs. For Tier 2, phytoplankton should be sampled at least once during an index period (usually summer) and at least once outside that index period.

9.4.3 Analysis

Chlorophyll *a* measurements can be used to estimate phytoplankton standing stock. Assuming that chlorophyll *a* is about 1.5% of the ash-free dry weight of algae, algae biomass can be estimated by multiplying the chlorophyll *a* content by a factor of 67 (APHA 1992). This information can be used in concert with the identification of dominant taxa and "nuisance" taxa to assess the overall condition of the phytoplankton assemblage.

9.5 Epibenthos (Developmental)

Although its use as an indicator of estuarine and coastal marine biological condition is considered to be under development, epibenthos could be selected as one of the biological assemblages for a Tier 2 assessment and has potential as an element of biological criteria consistent with fish and benthic invertebrates.

9.5.1 Sampling Procedure

Farrell (1993a, b) describes the use of a beam trawl to collect epibenthos. A beam trawl is a conical-shaped net, open at the large end, which is towed over the substrate surface. The net is kept open by attaching each end of it to a rigid pole or beam. This beam replaces the doors of an otter trawl and forward movement of the boat is not required to keep the net open. The net is constructed in two parts. The body is nylon bolting cloth (50 openings/cm^2), tapering to a plankton net fitted with a removable container. An effective swath width of 1.25-m has been tested in Florida waters (Farrell 1993a, b). In wadeable water, a D-frame net could be used to collect epibenthos, or the beam trawl could be pulled by hand. A relatively short tow length of the beam trawl (4-m, effectively sampling 5-m^2 of bottom) in estuaries may be beneficial for reducing the sample size and detrital bulk. If a D-frame net is used, at least an equivalent area should be sampled. In offshore waters, it may be necessary to increase the tow length due to reduced organism densities. Small otter trawls or an epibenthic sled sampler can also be used.

9.5.2 Index Period

Epibenthos should be sampled once, preferably during an appropriate index period. For many temperate areas of the country, this is probably mid-summer. Other sampling periods should be selected based on the specific goal of the Tier 2 assessment.

9.5.3 Analysis

The samples should be identified to genus and species. The Farrell Index (described in Chapter 13 - Case Studies, as modified to reflect tolerance values of taxa in the area sampled) should be calculated to provide an assessment of the condition of the assemblage in response to organic pollutants and eutrophication. Other metrics could be calculated based on the specific taxa present.