Appendix CT-6. Selected methods for sampling benthic invertebrate communities

Method: Passive artificial substrates		
Description: Artificial substrate samplers are designed to mimic natural substrates (e.g., gravel, cobble, small spaces) and provide an easily quantified sampling unit. In general, artificial substrate samplers primarily sample the epifaunal community, whereas grab samplers primarily sample the infaunal community. Artificial substrate samplers can provide both qualitative and quantitative samples of benthic macroinvertebrates. Ohio Environmental Protection Agency recommends using of Hester-Dendy artificial substrate samplers in streams and rivers, five samplers exposed for six weeks. **Measured endpoints:** EPT richness and diversity at family and genus level of taxonomic resolution. **References:** OEPA 1989, Johnson 2006, USEPA 2002d	**Advantages:** Mesh artificial substrate samplers are a good alternative to grab samplers when collecting animals for tissue residue analyses. Artificial substrate samplers made of mesh-filled chicken baskets are particularly good at collecting large numbers of animals because of the large number of interstitial spaces. **Disadvantages:** None reported.	**Analyte capability:** Epifaunal populations
Method: Benthic response index (BRI)		
Description: The BRI is the abundance-weighted average tolerance score of organisms occurring in a sample. **Measured endpoints:** Southern California Marine Bays: Reference: <39.96 Low disturbance: 39.96–49.14 Moderate disturbance: 49.15–73.26 High disturbance: >73.26 Polyhaline Central San Francisco Bay: Reference: <22.28 Low disturbance: 22.28–33.37 Moderate disturbance: 33.38–82.08 High disturbance: >82.08 **References:** Smith et al. 2003; California EPA 2008, 2009	**Advantages:** Indices remove much of the subjectivity associated with data interpretation. Indices provide a simple means of communicating complex information to managers, tracking trends over time, and correlating benthic responses with stressor data. **Disadvantages:** Requires development and calibration. Different benthic indices have been used at different times and different places, and results cannot be compared across regions because the various indices have not yet been rigorously compared and intercalibrated. Initial development of each existing benthic index was constrained by data limitations, and they would all benefit from refinement with additional data as well as independent validation. Differences in sampling procedures. Habitat factors such as seasonality and sediment type. Accuracy of identification of benthic organisms of performance of California benthic indices. Indices only one line of evidence in determining causality of impairment. Indices often used in conjunction with multiple lines of evidence.	**Analyte capability:** Species specific

Method: Index of benthic biotic integrity (IBI)		
Description: The IBI identifies community measures that have values outside a reference range. **Measured endpoints:** Southern California Marine Bays: Reference: 0 Low disturbance: 1 Moderate disturbance: 2 High disturbance: 3 or 4 Polyhaline Central San Francisco Bay: Reference: 0 or 1 Low disturbance: 2 Moderate disturbance: 3 High disturbance: 4 **References:** California EPA 2008, 2009	**Advantages:** Indices remove much of the subjectivity associated with data interpretation. Indices provide a simple means of communicating complex information to managers, tracking trends over time, and correlating benthic responses with stressor data. **Disadvantages:** Requires development and calibration. Different benthic indices have been used at different times and different places, and results cannot be compared across regions because the various indices have not yet been rigorously compared and intercalibrated. Initial development of each existing benthic index was constrained by data limitations, and they would all benefit from refinement with additional data as well as independent validation. Differences in sampling procedures. Habitat factors such as seasonality and sediment type not taken into account. Accuracy of identification of benthic organisms of performance of California benthic indices. Indices only one line of evidence in determining causality of impairment. Indices often used in conjunction with multiple lines of evidence.	**Analyte capability:** Species specific

Method: Relative benthic index (RBI)		
Description: The RBI is the weighted sum of (1) several community parameters (total number of species, number of crustacean species, number of crustacean individuals, and number of mollusk species) and abundances of (2) three positive and (3) two negative indicator species. **Measured endpoints:** Southern California Marine Bays: Reference: >0.27 Low disturbance: 0.17–0.27 Moderate disturbance: 0.09–0.16 High disturbance: <0.09 Polyhaline Central San Francisco Bay: Reference: >0.43 Low disturbance: 0.30–0.43 Moderate disturbance: 0.20–0.29 High disturbance: <0.20 **References:** California EPA 2008, 2009	**Advantages:** Indices remove much of the subjectivity associated with data interpretation. Indices provide a simple means of communicating complex information to managers, tracking trends over time, and correlating benthic responses with stressor data. **Disadvantages:** Requires development and calibration. Different benthic indices have been used at different times and different places, and results cannot be compared across regions because the various indices have not yet been rigorously compared and intercalibrated. Initial development of each existing benthic index was constrained by data limitations, and they would all benefit from refinement with additional data as well as independent validation. Differences in sampling procedures. Habitat factors such as seasonality and sediment type not taken into account. Accuracy of identification of benthic organisms of performance of California benthic indices. Indices only one line of evidence in determining causality of impairment. Indices often used in conjunction with multiple lines of evidence.	**Analyte capability:** Species specific

Method: River Invertebrate Prediction and Classification System (RIVPACS)		
Description: The approach compares the assemblage at a site with an expected species composition determined by a multivariate predictive model that is based on species relationships to habitat gradients (originally developed for British freshwater streams and adapted for California's bays and estuaries). **Measured endpoints:** Southern California Marine Bays: Reference: >0.90–<1.10 Low disturbance: 0.75–0.90 or 1.10–1.25 Moderate disturbance: 0.33–0.74 or >1.25 High disturbance: <0.33 Polyhaline Central San Francisco Bay: Reference: >0.68–<1.32 Low disturbance: 0.33–0.68 or 1.32–1.67 Moderate disturbance: 0.16–0.32 or >1.67 High disturbance: <0.16 **References:** Wright, Furse, and Armitage 1993; Van Sickle, Huff, and Hawkins 2006; California EPA 2008, 2009	**Advantages:** Indices remove much of the subjectivity associated with data interpretation. Indices provide a simple means of communicating complex information to managers, tracking trends over time, and correlating benthic responses with stressor data. **Disadvantages:** Requires development and calibration. Different benthic indices have been used at different times and different places, and results cannot be compared across regions because the various indices have not yet been rigorously compared and intercalibrated. Initial development of each existing benthic index was constrained by data limitations, and they would all benefit from refinement with additional data as well as independent validation. Differences in sampling procedures. Habitat factors such as seasonality and sediment type not taken into account. Accuracy of identification of benthic organisms of performance of California benthic indices. Indices only one line of evidence in determining causality of impairment. Indices often used in conjunction with multiple lines of evidence.	**Analyte capability:** Species specific

Method: Integration of benthic community		
Description: The median of all benthic index response categories shall determine the benthic line of evidence category. If the median falls between categories, it shall be rounded up to the next higher category. **Measured endpoints:** Reference: A community composition equivalent to a least affected or unaffected site. Low disturbance: A community that shows some indication of stress but could be within measurement error of unaffected condition. Moderate disturbance: Confident that the community shows evidence of physical, chemical, natural, or anthropogenic stress. High disturbance: The magnitude of stress is high. **References:** California EPA 2008, 2009	**Advantages:** Index performance was evaluated by comparing index assessments of 34 sites to the best professional judgment of nine benthic experts. None of the individual indices performed as well as the average expert in ranking sample condition or evaluating whether benthic assemblages exhibited evidence of disturbance. However, several index combinations outperformed the average expert. When results from both habitats were combined, two four-index combinations and a three-index combination performed best. **Disadvantages:** Requires development and calibration. Different benthic indices have been used at different times and different places, and results cannot be compared across regions because the various indices have not yet been rigorously compared and intercalibrated. Initial development of each existing benthic index was constrained by data limitations, and they would all benefit from refinement with additional data as well as independent validation. Differences in sampling procedures. Habitat factors such as seasonality and sediment type not taken into account. Accuracy of identification of benthic organisms of performance of California benthic indices. Indices only one line of evidence in determining causality of impairment. Indices often used in conjunction with multiple lines of evidence.	**Analyte capability:** Species specific

Method: Rapid bioassessment protocol (RBP)		
Description: Choice of qualitative and/or quantitative protocols (three tiers) for use in streams and rivers. Protocols used to determine whether a stream and associated habitat are supporting a designated aquatic life use, characterize the existence and severity of impairment, and identify the source of impairment. **Measured endpoints:** Macroinvertebrates: Taxa richness, family biotic index, ratio of scrapers, filtering collectors, ratio of EPT and chironomid abundances, % contribution of dominant family, EPT index. Fish: IBI, species richness and composition metrics, trophic composition metrics, fish abundance and condition metrics. **References:** Barbour et al. 1999	**Advantages:** Bioassessment provides indications of cumulative impacts of multiple stressors, not just water quality. Biological community condition reflects both short- and long-term effects, and directly evaluates the condition of the habitat and water resource. Biological data can be interpreted based on regional reference condition where single reference sites are lacking or inadequate. Properly developed methods, metrics, and reference conditions provide a tool that enables a direct measure of the ecological condition of a water body. Once a framework is in place for bioassessment, biological monitoring can be relatively inexpensive and easily performed with standard protocols and consistent training. **Disadvantages:** May be difficult to interpret results in areas impacted by urban/nonpoint contamination or areas impacted by numerous site discharges. Additional chemical and biological (toxicity) testing is usually needed to identify causal agent.	**Analyte capability:** Macro-invertebrate species

Method: Invertebrate community index (ICI)		
Description: ICI is a summary measure of 10 metrics representing aquatic macroinvertebrate community integrity and is evaluated and scored in relation to conditions at relatively undisturbed reference sites In this index, a site can receive a 6, 4, 2, or 0 score depending on how it compares to the specified reference site. **Measured endpoints:** Total number of taxa—species richness and diversity Number of mayfly taxa—pollution-sensitive species; greater proportion of taxa indicates higher environmental quality Number of caddisfly taxa—main component in larger, unimpacted waterways; wide range of pollution tolerances within taxa Number of Dipteran taxa—have wide range of tolerances to pollution; often only organism collected under heavily polluted conditions; greater proportion suggests lower stream quality Percent mayfly composition—easily affected by even minor disturbances; serves as measure of overall levels of stress and disturbance Percent caddisfly distribution—quickly absent under environmental stress; serves as a measure of stream stress Percent tribe Tanytarsini midge composition—often predominant group at minimally impacted sites; pollution tolerances are intermediate Percent other Dipteran and noninsect composition—includes noninsect invertebrates such as worms, isopods, snails, etc.; main community component in poor water-quality conditions; greater abundance indicates lower environmental quality Percent tolerant organisms—tolerant to toxic and organic pollution; greater proportion of organisms indicates lower overall environmental quality Number of qualitative EPT taxa—qualitative measure of habitat diversity and water quality; measures richness of mayfly (*Ephemeroptera*), stonefly (*Plecoptera*), and caddisfly (*Trichoptera*) taxa. **References:** OEPA 1989, USEPA 1992b	**Advantages:** Bioassessment provides indications of cumulative impacts of multiple stressors, not just water quality. Biological community condition reflects both short- and long-term effects and directly evaluates the condition of the habitat and water resource. Biological data can be interpreted based on regional reference condition where single reference sites are lacking or inadequate. **Disadvantages:** Additional chemical and biological (toxicity) testing is usually needed to identify causal agent.	**Analyte capability:** Invertebrates

Method: Macroinvertebrate aggregated index for streams (MAIS)		
Description: MAIS is a rapid bioassessment protocol similar to ICI. MAIS scores are based on macroinvertebrates collected with a prescribed number of kick and dip net sweeps. Organisms are identified to the family (rather than genus) level. Family-level identifications require more training than order level (e.g., EPT, etc.) but can be performed by individuals with an intermediate level of skill. Once macroinvertebrates are collected, identified, and enumerated, an MAIS index score ranging between 0 and 18 is generated from 9 aggregated macroinvertebrate metrics that describe the diversity and abundance of different groups. In the mid-Atlantic highlands, four narrative categories are assigned based on the scores 0–7 = very poor, 8–11 = poor, 12–15 = good, 16–18 = very good. **Measured endpoints:** The nine biological metrics that compose the final MAIS index score: • EPT richness: number of caddisfly, stonefly and mayfly families • *Ephemeroptera*: number of mayfly families • Percent *Ephemeroptera*: % abundance of mayflies • Percent five dominant taxa: five most dominant taxa combined • Simpson Diversity Index: integrates richness and evenness • Modified Hilsenhoff Biotic Index: Integrates abundance and taxa richness; taxa in latter are weighted by pollution tolerance scores • Number of intolerant taxa: number of families with tolerance values of 5 or less (very sensitive) • Percent scrapers: relative abundance of benthos that feed on periphyton • Percent haptobenthos: relative abundance of benthos that require clean, coarse, firm substrates **References:** Johnson 2006	**Advantages:** Bioassessment provides indications of cumulative impacts of multiple stressors, not just water quality. Biological community condition reflects both short- and long-term effects and directly evaluates the condition of the habitat and water resource. Biological data can be interpreted based on regional reference condition where single reference sites are lacking or inadequate. **Disadvantages:** Additional chemical and biological (toxicity) testing is usually needed to identify causal agent.	**Analyte capability:** NA

Method: Benthic infaunal abundance		
Description: This marine-specific method compares the relative abundance of site major taxa to reference-area taxa. A site is considered impacted if (1) the abundance of the Class Crustacea, Class Polychaeta, and Phylum Mollusca in the test sediment is statistically different (t test @ 0.05, www.socialresearchmethods.net/kb/stat_t.php) from the "reference sediment" and (2) the "test sediment" has less than 50% of any one of the major taxa relative to the reference sediment's mean abundance of any one of the major taxa. **Measured endpoints:** Abundance of the following major taxa: Class Crustacea, Class Polychaeta, and Phylum Mollusca. **References:** Washington Administrative Code 173-204	**Advantages:** Bioassessment provides indications of cumulative impacts of multiple stressors, not just water quality. Biological community condition reflects both short- and long-term effects and directly evaluates the condition of the habitat and water resource. Biological data can be interpreted based on regional reference condition where single reference sites are lacking or inadequate. **Disadvantages:** Additional chemical and biological (toxicity) testing is usually needed to identify causal agent.	**Analyte capability:** NA