

**ADEQ
BIOCRITERIA PROGRAM
QUALITY ASSURANCE PROGRAM PLAN**

APPENDIX A:

Lawson, L.L., ed. 2005. Macroinvertebrate Sampling and Analysis Procedures, Section 3, Part A, *in A Manual of Procedures for the Sampling of Surface Waters*. Arizona Department of Environmental Quality, TM05-01. Phoenix, AZ

3.15 Biological Sampling of Wadeable Streams

3.15.1 Macroinvertebrates

Macroinvertebrate sampling is conducted to assess the biological integrity of perennial, wadeable streams. ADEQ has developed bioassessment tools in the form of Indexes of Biological Integrity (IBI) along with habitat evaluations for this purpose. There are two IBIs, the Warm water IBI and Cold water IBI. The procedure for calculating the IBIs is found in Section 3.15.2 and in the Biocriteria Program QAPP (ADEQ, 2001). Guidance for performing habitat evaluations, needed to determine causes and sources of impacts, is presented in Section 3.17. This procedure presents the field methods used for collecting macroinvertebrates, the initial step in conducting a bioassessment.

Equipment required: D-frame net fixed with a 500 micron mesh net (Figure 3.15.1a), forceps, a large bucket, a metal sieve having a 500 μm mesh, a white dissecting tray, 37% formaldehyde, and 99% isopropyl alcohol.

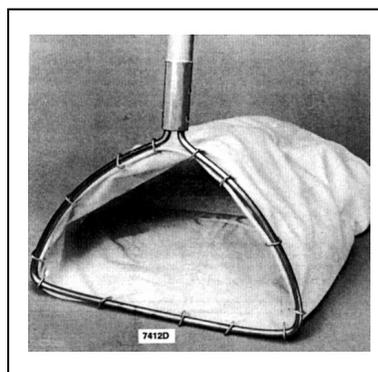


Figure 3.15.1a D-frame net with 500 micron mesh net.

3.15.1.1 Site Selection

The study reach length should be one of the following: 1) 25 times the bankfull width of the stream in wadeable streams, or a minimum of 100 meters in larger streams, or 2) long enough to encompass 2 meander lengths of the stream and multiple riffles in which to produce a composite sample, but not less than 100 feet in length. For a definition of bankfull, see the Rosgen stream type identification section of the habitat assessment protocol, Section 3.17.3. The study reach should be selected to represent typical habitat conditions found in the larger stream segment. The study reach length should begin at the top of a riffle or run and end at the bottom of a riffle or run.

If the reach is to be used as a reference or background reach, the following general criteria must be met:

- ❖ The site must be accessible within a 2-hour walk or 3-4 miles from the nearest four wheel drive road,
- ❖ No known discharges upstream,
- ❖ No major impoundments upstream.
- ❖ No human caused channel alterations at the site; e.g. diversions, dredge and fill projects,

- ❖ At least 0.5 miles downstream of road crossings,
- ❖ The site should be perennial. The indicators for perennial condition are likely to be the presence of fish, univoltine insects, and healthy unstressed riparian plants.
- ❖ The site should be free of local land use impacts,
- ❖ There should be no recorded violations of pH or dissolved oxygen water quality standards, and
- ❖ The Habitat Assessment Index score should be greater than 14 (see Section 3.17.2.6).

3.15.1.2 Sample Collection Information

Biocriteria research efforts have focused on perennial, wadeable streams to date. Sample collection methods have been developed and refined only for this waterbody type at this point. The following sampling conditions and time frames must be met in order to collect macroinvertebrates for ADEQ bioassessment purposes. A stream reach must be:

- ❖ Wadeable
- ❖ Perennial
- ❖ Contain fast-flowing riffle or run habitat
- ❖ Contain heterogeneous substrates
- ❖ Sampled during the spring index period (April-May for warm water streams and May-June for cold water streams).

Wadeable means no deeper than can be safely waded across when collecting samples.

Perennial refers to stream segments which flow continuously throughout the year (excluding effluent dependent waterbodies). **Riffle habitat** refers to the portions of streams where moderate velocities and substrate roughness produce moderately turbulent conditions which break the surface tension of the water and may produce whitewater (Bain and Stevenson, eds. 1999). **Run habitat** refers to segments of streams where there is moderate velocity water, but non-turbulent conditions which do not break the surface tension of the water and do not produce whitewater (Bain and Stevenson, eds.1999).

Heterogeneous substrate means a mixture of particle sizes comprising the stream bottom material that is less than 50% composed by travertine, bedrock or sand. Streams with *homogeneous* substrates, such as bedrock or sand have aquatic communities which exhibit limited taxa richness and loss of structure and function when compared with reference conditions.

The **spring index period** is defined as a period of time following winter runoff in which baseflow conditions will be found in most streams. Baseflow conditions generally are achieved post winter runoff in the desert streams in April-May and in mountain streams in May-June. A period of 4 weeks post-bankfull flood condition is generally required prior to macroinvertebrate sampling, even during the spring index sampling period. Hydrologic conditions are checked in the office prior to a site visit and field conditions are documented on the SEM Field Form for Macroinvertebrate Sample Collection in the field prior to sampling to confirm that sampling is occurring during the correct sample collection conditions.

Macroinvertebrate samples are not collected when the following conditions occur:

- ❖ A bankfull or greater magnitude flow event has occurred within 4 weeks of site visit or when extreme high flow events have occurred, resulting in deep scouring of the streambed and benthic community such that the macroinvertebrate community will not recover within the spring index period.
- ❖ Extended drought conditions have reduced flow from previously perennial condition to pools only or stagnant wetland habitat.
- ❖ Stream substrates are dominated (consisting of >50% of that substrate type) by bedrock, travertine, or sand are considered non-target conditions.

Macroinvertebrate samples should be collected before pebble counts and before any disturbance to the stream channel by investigators. The collection begins at the downstream end of the assessment reach and proceeds upstream.

A macroinvertebrate sample consists of a three-minute timed composite sample from kick samples collected from three riffle habitats within the study reach. The target sampling area is approximately one square meter per each one minute sample. Select 3 or more riffles which represent the variety of substrate sizes, velocities, depths, and habitats found within the reach. Collect one-minute timed samples from each of three habitats or divide the time as needed among the variety of habitats. If three good-sized riffles are not available to be sampled, spread the three minute sample time over whatever riffle/run areas are available.

3.15.1.3 Sample Collection

Once the sampling sites within the reach have been selected, the first sample should be at the lowermost riffle. The procedure is as follows:

1. Fill a bucket half full with stream water.
2. Place the D-frame net on the stream bed in the path of flowing water, and agitate a one square meter area of substrate vigorously for one minute by kicking or hand turning rocks to collect dislodged material. Sample as much variation of the flow and substrate as possible including large and small substrates.
3. Deposit the contents of the net into the bucket. At this point there is no need to pick the net clean.
4. Repeat the sampling procedure for the second and third riffles. After the last riffle, use forceps to remove organisms attached to the D-frame net. Before leaving the site, the D-frame dip net, bucket, and sieve should be rinsed and scrubbed with a brush to dislodge small invertebrates, egg masses, and organic material, so that it is not transferred to the next site.

5. Swirl the contents of the bucket and pour the non-sediment portion into a 500 µm mesh sieve.
6. Add water again to the bucket, swirl and pour the contents into the sieve. Repeat this procedure several times until all insects and organic debris are emptied and only sediment remains.
7. Dump the remaining sediment into a dissecting tray and search the sediment for any remaining organisms, especially cased Trichoptera, snails, and freshwater clams and then discard the remaining sediment.
8. Gently, squeeze the sample to remove excess water from algae laden samples. Using a plastic spoon or hands, gently dispense the sample from the sieve into a wide mouth, one-liter sample jar. Fill the jar half to three-quarters full. If additional sample remains in the sieve, use an extra jar to contain it. Rinse any leftover material in the sieve into a corner and spoon out as much as possible. Check the sieve for any remaining animals. If the entire sample does not fit into one jar, then add the remainder to a second jar. If the sample will not fit into two jars, then field split the sample.
9. To perform a one-half field split, evenly spread the entire sample in a white dissecting tray and divide the sample with your hands into two equal portions. Place one half of the sample into the two sample jars and discard the other half into the stream. Note on the field form that the sample was “field split ½”. A quarter split can be performed if a half-split still provides too much sample material to fit in two jars.
10. Place label(s) (see Section 3.15.3.2) in the jar(s), add enough 99% isopropyl alcohol to cover the sample material by about 1 inch.
11. Seal the jar(s), and affix a second label to outside of the jar(s).

3.15.1.4 Sample Labeling

Each macroinvertebrate sample should have two identification labels penciled on “write-in-the-rain” paper: one placed inside the jar, visible from the outside, and one affixed to the outside of the jar, attached with clear plastic tape. If more than one jar is used for a sample, put jar numbers on all labels (e.g., 1 of 2, 2 of 2). Each tag should have the following information at a minimum:

Waterbody name
Site code number
Habitat sampled (riffle)
Date
ADEQ and collectors' initials

3.15.1.5 Preservation and Storage

After samples have been preserved with 99% isopropyl alcohol, samples should be placed in an ice chest with ice to cool the sample. This prevents overheating and degradation of the sample, and prevents fumes from developing inside truck camper shells.

Samples should be stored in a cool environment and within flammable storage areas in the laboratory prior to shipping to the laboratory.

3.15.1.6 Chain of Custody

To complete the Chain of Custody, samples shall be locked in field trucks when sampling personnel are away from the truck. Sample jars shall be placed in the large, locked flammable cabinet in the equipment storage area of the ADEQ laboratory for storage, prior to shipping. The use of tamper-evident tape on shipping boxes to prevent tampering with samples during shipping is required. A Chain of Custody form will accompany the samples during shipment.

3.15.1.7 Sample Preparation for Shipping to Taxonomy Laboratory

1. Drain sample over a 500 micron sieve and large funnel into a waste container (e.g. 5-gallon isopropanol carboys) under a fume hood and place the sample back into the sample jar along with the correct interior label. Insure that all specimens have been picked off the sieve and placed in sample container, then cap tightly. Rinse and scrub the sieve with a brush prior to draining the next sample.
2. Wrap jars in bubble wrap and place in a heavy duty garbage bag with an absorbent sheet, inside a shipping box. ADEQ uses ADOT approved boxes that are 14x10x10", single-wall construction, edge Crush Test of 32 lbs/inch, gross weight limit of 65 lbs. The bubble wrap prevents shock and deterioration of the sample specimens. Close and seal the garbage bag. Place the Laboratory address on a sheet of paper inside the box, along with an inventory of samples. Seal the box with tamper-proof tape to continue chain of custody while samples are in transit. Mark boxes with "this side up" arrows to further prevent leakage and protect samples.
3. Ship boxes via commercial carrier. No hazardous materials labeling is required. ADEQ has received verbal approval from DOT and UPS to ship decanted macroinvertebrate samples without having to meet hazardous materials shipping requirements because of the very limited quantities of isopropanol alcohol present in the decanted samples. ADEQ marks boxes with "this side up" arrows to further prevent leakage and protect samples. Normal address labels are used for shipping. Enclose Chain of custody form with the samples.

All macroinvertebrate samples from a spring sample event are shipped to the laboratory in July

of each year or as soon as practical after sampling.

The taxonomy laboratory verifies receipt of samples listed on the chain of custody form. The laboratory also refreshes the alcohol preservative.

3.15.1.8 Literature Cited

ADEQ, 2005. Biocriteria Program Quality Assurance Program Plan, revision D. ADEQ, Phoenix, AZ.

3.15.1.9 Macroinvertebrate Taxonomy Laboratory Procedures

The procedures followed at the taxonomic laboratory are not part of the field sampling procedures, however they are presented here for reference purposes. These procedures include sample processing, sorting, taxonomic identification levels, voucher specimens, and general quality control procedures.

Sample processing - Upon receipt of the samples, the laboratory will check and adjust the preservation in each sample, catalog the samples, check the attached inventory for accuracy, and sign the chain of custody papers. The consultant will then notify ADEQ of the receipt of samples, any damaged samples, or discrepancies between the inventory and actual sample labels.

Sample sorting - Samples must be sorted to separate the invertebrates from the sample matrix. The entire sample should be floated in water in a white plastic tray. Large debris is rinsed and removed from the sample until all organic matter and invertebrates are floated off the mineral residue. The mineral residue is then searched for stone-cased caddisflies and molluscs.

Sub-sampling and sorting - Arizona samples typically contain thousands of invertebrates and must be sub-sampled for results to meet a minimum count of 500-600 organisms. A Caton Tray is used to randomly obtain fractions of the total sample from which all the invertebrates are removed and counted. Additional fractions are selected until the 500-600 target level is reached after which the number of squares subsampled are recorded. Terrestrial insects and non-benthic insects (e.g. corixidae, other swimmers, mosquitoes, or surface tension dwellers) should not be included in the count. Additional fractions are examined if one fraction is dominated by a single species. After the target number of specimens has been achieved, the entire unsorted sample is scanned for large or rare taxa, which may aid in identification of smaller instars or may expand the taxa list for that sample. The remaining unsorted sample is re-preserved with 70% isopropanol in individual containers and archived at the laboratory for one year from the date of sample receipt, after which time the laboratory will contact ADEQ prior to disposal.

Sorting - The sorting of invertebrates from the sample matrix shall be performed by trained

technicians, using dissecting scopes with a minimum magnification of 6X. After identifications have been made, the sorted specimens, including the separated Chironomidae, should be archived for one year or incorporated into the reference or voucher specimen set. The laboratory shall keep logs for each sample sorted, the fraction sorted, sample matrix problems, etc. in addition to bench sheets of the taxa identified in each sample.

Sorting efficacy - The laboratory shall check the sample residues to check for a sorting efficacy of 95% or better. A statement of sorting efficacy for the ADEQ batch of samples should be presented in the laboratory report.

Taxonomic identification - Invertebrate identifications shall be performed by a trained and experienced taxonomist. The taxonomy contractor is responsible for obtaining the most accurate, consistently achievable identifications for ADEQ samples, using specialists as needed to obtain identifications to the general taxonomic levels listed in Table 3.15.1.9.

Reference specimens - A set of reference or voucher specimens shall be prepared from the batch of samples each year for incorporation into the reference specimen collection. Several specimens shall be preserved for each new taxon and the best or largest larval instars of other taxa shall be preserved to represent the taxa found that year and to update the historic reference collection at ADEQ. The taxonomist shall make recommendations for archiving any important specimens, if verification of identification by national specialists is required.

Lab Data Reports - Laboratory reports containing taxonomic identifications and counts for all samples for that year shall be submitted to ADEQ in electronic format. The electronic data shall be submitted in ACCESS database format or Excel spreadsheets formatted for database uploading. The Taxonomy Contractor shall perform quality control checks on the electronic data prior to submittal to ADEQ. The data set should contain at a minimum the Station ID, waterbody name and location, habitat, collection date, complete taxa ID from phylum to lowest level ID, raw number of individuals, the portion of sample analyzed including field splits where applicable, and adjusted final counts, which are corrected for sub-sample size and field splits. A copy of the bench sheets used by the taxonomist to develop the raw counts per sample should also be submitted.

Table 3.15.1.9. ADEQ Taxonomic levels of identification for macroinvertebrates.

Invertebrate Group	Level of taxonomy required
Aquatic insects (except the family Chironomidae)	Genus (or species where consistently identifiable)
Chironomidae	Family
Semi-aquatic insects	Family
Arachnida (Mites)	Class
Cladocera, Copepoda, Ostracoda	Class
Amphipoda, Decapoda, Isopoda	Class
Nematoda, Nematomorpha	Phylum
Turbellaria	Class
Annelida	Class
Mollusca	Family or Genus

3.15.2 Arizona Indexes of Biological Integrity

3.15.2.1 Calculating the Arizona Indexes of Biological Integrity

The Arizona Indexes of Biological Integrity can be applied to macroinvertebrate taxonomic data generated by the sample collection procedures provided in this document. All the appropriate sample collection conditions must be met in order to calculate the IBIs for bioassessment purposes (i.e. application of the narrative biocriteria standard). There are currently two Indexes; a cold and a warm water IBI. The following narrative provides the steps needed to calculate these Indexes from taxonomic lists and abundance data generated by taxonomy laboratories from the field collected macroinvertebrate samples.

1. Identify the appropriate reference community using the site elevation.
 - ❖ The warm water community is defined as being located below the 5000 foot elevation.
 - ❖ The cold water community is defined as being located above the 5000 foot elevation.

2. Calculate the macroinvertebrate metric values for the study sample following metric calculation procedures listed Figure 3.15.2.1a. This table lists all the metrics used in both indexes and their definitions. Metrics required for each index are listed in Table 3.15.2.1a.

3. Calculate the metric percent of reference score using either the warm or cold water reference metric threshold values associated with that community type (Tables 3.15.2.1b and 3.15.2.1c)
4. Calculate an average of the percent of reference scores for all metrics to produce the IBI score. Table 3.15.2.1d provides an example of the scoring system for a warm water stream.
5. Determine assessment category for the IBI score from Table 3.15.2.1e.

Figure 3.15.2.1a. Formulas for calculating macroinvertebrate metrics used in the cold water and warm water Indexes of Biological Integrity.

Use the following formula to calculate the metric score (percentage of reference) for sensitive metrics whose values decrease with disturbance. Apply this formula to the following metrics.

$$\text{Metric Score} = (\text{Sample value} / \text{metric threshold value}) * 100$$

1. Total taxa richness
2. Number of Ephemeroptera taxa
3. Number of Tricoptera taxa
4. Number of Diptera taxa
5. Number of intolerant taxa
6. Percent Ephemeroptera
7. Percent Plecoptera
8. Percent scrapers
9. Number of scraper taxa

Apply the following formulas to calculate the metric score (percentage of reference) for tolerant metrics whose values increase with disturbance.

1. Hilsenhoff Biotic Index

$$\text{Metric score} = (10 - \text{Sample value}) / (10 - \text{Metric threshold value}) * 100$$

2. Percent dominant taxon

$$\text{Metric score} = (100 - \text{Sample value}) / (100 - \text{Metric threshold value}) * 100$$

Table 3.15.2.1a Descriptions for the warm water and cold water metrics used in Arizona's IBIs.

Category	Metric	Definition	Expected Response to increasing disturbance
Richness measures	Total number of taxa	Number of different macroinvertebrate taxa	Decrease
	# Ephemeroptera taxa	Number of mayfly taxa	Decrease
	# Trichoptera taxa	Number of caddisfly taxa	Decrease
	# Diptera taxa	Number of true fly larvae.	Decrease
	# Intolerant taxa	Number of taxa having a tolerance value ≤ 3	Decrease
Composition measures	% Dominant taxon	Percent abundance of the single most abundant taxon.	Increase
	% Ephemeroptera	Percent abundance of mayflies, compared to total abundance of the sample	Decrease
	% Plecoptera	Percent abundance of stoneflies, compared to total abundance of the sample	Decrease
Tolerance measure	Hilsenhoff Biotic Index	Abundance-weighted average tolerance of assemblage	Increase
Trophic measures	% Scrapers	Percent abundance of the scraper functional feeding group, compared to total abundance of the sample	Decrease
	# Scraper taxa	Number of taxa in the scraper functional feeding group	Decrease

Table 3.15.2.1b. Reference scoring thresholds for Warm Water metrics, used in the Arizona Warm Water Index of Biological Integrity.

Metric	Metric threshold value
Total taxa	37
Trichoptera taxa	9.0
Ephemeroptera taxa	9.0
Diptera taxa*	10.0
Scraper taxa	7.0
Percent scraper	23.7
Percent Ephemeroptera	70.0
Percent Dominant Taxon	19.1
Hilsenhoff Biotic Index	4.89

* Appropriate taxonomic effort is to genus for insects and to family for midges.

Table 3.15.2.1c. Reference scoring thresholds for Cold Water metrics, used in the Arizona Cold Water Index of Biological Integrity.

Metric	Scoring threshold
Total taxa	38
Diptera taxa*	11
Intolerant taxa	6
Scraper taxa	11
Percent scraper	45.1
Percent Plecoptera	19.1
Hilsenhoff Biotic Index	4.23

* Appropriate taxonomic effort is to

genus for insects and to family for midges.

Table 3.15.2.1d. Example of the ADEQ Warm Water Index of Biological Integrity scoring system; Sycamore Creek near Round Valley bridge (Hwy 87) collected during spring 1995.

Metric	Metric Value	Metric Score (compared to warm water reference scoring threshold)
Total taxa	24	65
Trichoptera taxa	6	67
Ephemeroptera taxa	5	56
Diptera taxa	7	70
Scraper taxa	3	43
Percent scraper	20.3	86
Percent Ephemeroptera	26	37
Percent Dominant Taxon	41	73
Hilsenhoff Biotic Index	5.73	84
Index Score (average of all Metric Scores)		65 Attaining

Table 3.15.2.1e. Assessments based on ADEQ macroinvertebrate IBI scores.

Macroinvertebrate bioassessment result	Index of Biological Integrity Score		Assessment
	Cold water	Warm water	
Greater than the 25th percentile of reference condition	≥ 52	≥ 50	Attaining
Between the 10th and 25th percentile of reference condition	46 – 51	40 – 49	Inconclusive
Less than the 10th percentile of reference condition	≤ 45	≤ 39	Impaired

3.15.3 Diatoms from Natural Substrates

Diatom samples are collected from riffle or run habitats of small to medium sized wadeable perennial streams during the spring index period; April-May for warm water streams and May-June for cold water streams.

The sample is collected from rocks having a flat and somewhat smooth surface. Care must be taken to not disturb the substrate in the area where the sample is to be taken; thus, the sample should be collected after the water sample, but prior to the pebble count and the macro-invertebrate sample. A single sample consists of a 6 cobble composite collection from a variety of riffle/run habitats throughout the study reach. The number of days past the last precipitation or flood event should be documented. Samples should not be taken within 2 weeks of a scouring flood event.

Equipment Required: A nine centimeter square vinyl template, Exacto knife, clean toothbrush, squeeze bottle, 250ml or 500ml sample jars, labels, and isopropanol preservative.

3.15.3.1 Sample Collection and Field Processing

1. Select 3 riffles in the assessment reach. From each riffle, select two relatively flat cobbles which have an obvious biofilm, for a total of six rocks. If there are fewer than 3 riffles in the reach, randomly select six rocks from the available riffle(s). The selected rocks should be in water of less than 1 meter (3 feet) deep. Begin sample collection at the bottom of the reach and move upstream. Try to avoid dislodging substrate when stepping onto riffles.
2. Outline a nine centimeter square area on each rock with the template and Exacto knife. Scrape the outlined area with 30 strokes of an Exacto knife. Make single direction strokes in one direction. Put the scrapings from the knife blade into the sample jar. Rotate the rock 90° and again scrape with 30 strokes in the same direction. Repeat until the rock has been scraped perpendicular to each side of the sample area. Rinse the scraped area of the rock into the sample jar with a squeeze bottle of water. Rinse the knife in the sample jar. Scrub the scraped area of the rock with a toothbrush for 10-15 seconds and rinse the scraped area again with water from the squeeze bottle. Rinse the toothbrush in the sample jar with water from the squeeze bottle. Use a new toothbrush and blade at each site.
3. Repeat the above process for each of the remaining 5 rocks. Composite all rock scrapings into the sample jar.

3.15.3.2 Labeling

Each diatom sample should have two identification labels penciled on “write-in-the-rain” paper: one placed inside the jar, visible from the outside, and one affixed to the outside of the jar, attached with clear plastic tape. If more than one jar is used for a sample, put jar numbers on all labels (e.g., 1 of 2, 2 of 2). Each tag should have the following information at a minimum:

Waterbody name
Site code number
Habitat sampled (riffle)
Date
ADEQ and collectors’ initials

3.15.3.3 Preservation and Storage

1. Preserve the sample with about 10 ml of isopropanol and place in an ice chest with ice to prevent degradation of the sample while in the field. Care must be taken to not entirely submerge the collection jar in ice water.
2. Store the samples in a dark place until shipment to the contract laboratory.

3.15.3.4 Chain of Custody

1. Samples shall be locked in field trucks while sampling personnel are in the field.
2. Sample jars shall be placed in the large, locked flammable proof cabinet in the equipment storage area of the ADEQ laboratory for storage, prior to delivery.
3. A chain-of-custody form will be submitted with the samples at the time of delivery of samples to the laboratory.