Macroinvertebrate Sampling & Analysis Procedures

1. Site selection

   a. The study reach length should be 25 times the bankfull width of the stream in wadeable streams, or a minimum of 100 meters in large rivers. For a definition of bankfull, see the Rosgen stream type identification section of the habitat assessment protocol. This reach length is long enough to encompass 2 meander lengths of the stream and multiple riffles in which to produce a composite sample. The study reach should be selected to represent typical habitat conditions in the larger stream segment. Begin the study reach at the top of a riffle and end it at the bottom of a riffle or run.

   b. I f the reach is to be used as a reference reach, the following criteria must be met:

      i. Accessible (within a 2-hour walk or 3-4 mi from nearest 4WD road)
      ii. No known discharges upstream
      iii. No major impoundments upstream
      iv. No human caused channel alterations at the site (eg. diversions, dredge and fill projects)
      v. >0.5 mi downstream of road crossings
      vi. Site should be perennial (indicators: fish, univoltine insects, riparian indicators, and flows continuously throughout the year)
      vii. Site should be free of local land use impacts
      viii. No recorded violations of pH or dissolved oxygen water quality standards
      ix. Habitat Asssessment Index score > 14 (for definition of index, see the Habitat Assessment protocol)

2. Sample collection information

   a. Macroinvertebrate samples are collected from riffle 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).

   b. Required Equipment includes a D-frame net fixed with a 500 micron mesh net (Figure 8), forceps, a large bucket, a metal sieve having a 500 mm mesh, and a white dissecting tray.

   c. The macroinvertebrate sample should be collected after the water and algal samples have been collected, before the pebble count, and before any disturbance to the stream channel by investigators.

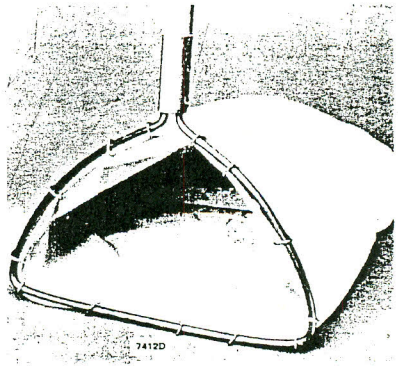

Figure 8. D-frame net

The collection begins at the bottom of the assessment reach and proceeds upstream.

d. A macroinvertebrate sample consists of a three-minute timed composite sample from kick samples collected from three riffle habitats within the study reach. 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.

e. Do not collect macroinvertebrate samples when the following conditions occur:
   i. Bankfull flows within 4 weeks of the sample date
   ii. Reach is >50% bedrock or travertine dominated
   iii. Reach is a cienega, wetland, or is sand dominated with edge vegetation only
   iv. Reach is ephemeral or intermittently flowing
   v. Flow is too deep or fast to safely wade

3. Sample collection and field processing procedure for Riffle/Run Habitat

a. Begin at the lowermost riffle of the riffles selected for sampling and fill a bucket half full with stream water.

b. Place the D-frame net on the stream bed in the path of flowing water, and agitate the 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 and attempt to sample large and small substrates.

c. Deposit the contents of the net into the bucket. At this point there is no need to pick the net clean.

d. 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.

e. Swirl the contents of the bucket and pour the non-sediment portion into a 500 μm mesh sieve. 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.

f. Dump the remaining sediment into a dissecting tray and search the sediment for any remaining organisms, especially cased Trichoptera, and then discard the remaining sediment.

g. 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; use an extra jar if necessary to contain the sample. 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.

h. 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; 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.

i. Place the label(s) in the jar(s), add enough 99% isopropyl alcohol to cover the sample material by about 1 inch, then add approximately one capful of 37% formaldehyde.

j. Seal the jar(s), and affix a second "write-in-the-rain" label to outside of the jar(s) with clear plastic tape.

4. Labeling

Each macroinvertebrate sample should have two identification labels penciled (or permanent ink) on "write-in-the-rain" paper: one placed inside the jar, visible on 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:

a. Waterbody name
b. Site code number
c. Habitat sampled (riffle)
d. Date (spelled out, e.g., April 1, 1992)
e. ADEQ and collectors' initials

5. Preservation & storage

a. As indicated in section 3i, macroinvertebrate samples in 1-liter jars are preserved with 99% isopropanol and a capful of 37% formaldehyde. Samples should be placed in an ice chest with ice to cool the sample, prevent overheating and degradation of the sample, and to prevent fumes from developing inside truck camper shells, which would cause a toxic exposure to isopropanol and formalin.

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

6. Chain of Custody

a. Samples shall be locked in field trucks while sampling personnel are in the field.
b. 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.
c. Use tamper-evident tape on shipping boxes to prevent tampering with samples during shipping.
d. A chain of custody form will accompany the samples during shipment.

7. Shipping procedures

a. Drain sample over a 500 micron sieve and large funnel into a waste container (we reuse 5-gallon isopropanol carboys) under a fume hood, then place sample back into sample jar along with the correct interior label. Be sure to pick any specimens off the sieve and place in sample container, then cap tightly. Rinse sieve and scrub with a brush prior to draining the next sample.

b. Pack jars in 2-3" of vermiculite within a heavy duty garbage bag within 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. After placing the jars into the box, pack additional vermiculite to about the halfway mark around the jars. The vermiculite is absorbent and 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.

c. Ship boxes via commercial carrier. ADEQ received verbal approval from DOT and UPS to ship <u>decanted</u> macroinvertebrate samples without having to meet hazardous materials shipping requirements because of the very limited quantities of isopropanol and formalin present in the decanted samples. No hazardous materials labeling is required. 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 samples.

d. All macroinvertebrate samples from a spring sample event are shipped to the laboratory in July of each year.

8. General lab procedures

a. Sample receipt - Upon receipt of 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 lab will then notify ADEQ of receipt of samples, any damaged samples, or discrepancies between the inventory and actual sample labels.

b. Sample Processing - 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. Then the mineral residue is searched for stone-cased caddisflies and molluscs.

c. Sub-sampling - Arizona samples typically contain thousands of invertebrates and must be sub-sampled to result in a minimum of 500-600 organisms. A Caton Tray should be 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 and the number of squares subsampled are recorded. Terrestrial insects and non-benthic insects (eg. corixidae, other swimmers, mosquitos or surface tension dwellers) should not be included in the count. Examine additional fractions if one fraction is dominated by a single species. After the target number of specimens is 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 shall be re-preserved with 70% isopropanol in individual containers and archived at the laboratory for one year from the date of sample receipt, at which time the laboratory will contact ADEQ prior to disposal.

d. 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.

e. 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.

f. 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 below and to be consistent with identifications presented in the attached "ADEQ

Macroinvertebrate Master Taxa List" (Table 1, Appendix A).

Table 1. 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 level only
Semi-aquatic insects	Family
Arachnida (Mites)	Genus
Cladocera, Copepoda, Ostracoda	Class
Amphipoda, Decapoda, Isopoda	Class
Nematoda, Nematomorpha	Phylum
Turbellaria	Class
Annelida	Class
Mollusca	Family or Genus

g. Reference Collection and Storage - A set of reference or voucher specimens shall be prepared from the batch of samples each year for incorporation into ADEQ's 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 Contractor shall make recommendations for archiving any important specimens, if verification of identification by national specialists is required.

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

i. Quality Control Tasks - ADEQ may conduct Laboratory audits as needed. ADEQ may periodically request the laboratory to examine quality control reconstituted samples or voucher specimens from another lab and produce a short letter regarding the

accuracy of identifications.

9. Quality Control field and lab procedures

a. Cleaning field equipment - 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, prior to leaving the site.

b. Biological field and laboratory quality control procedures and performance characteristics are listed in Table 2.

Table 2. Biological field and laboratory quality control procedures and performance characteristics.

Procedure	Performance Characteristic	Description
Sampling device	Precision - repeatability in a habitat	The D-frame dip net is a good choice for use in Arizona streams, as it can be used in riffle habitats with virtually all substrate sizes. The precision of sampling with this net is repeatable because a timed sampling effort is used which applies across different stream substrate types.
	Bias - exclusion of certain taxa (mesh size)	The D-frame sampler is outfitted with a 500μ mesh size net opening, which retains organisms of a consistent size for identification.
	Interferences - matrix/physical limitations	Excess filamentous algae can foul a sample, but it is considered part of the organic matter of a sample and is packaged with the biological sample.
Sampling method	Precision - variable metrics or measures among replicate samples at a site	Measurement error is quantified by replicate sampling at 10% of our sampling sites each year. Samples are processed and analyzed separately and their metrics and IBI score compared to obtain a measure of the method precision. This is an estimate of the precision of the entire method which includes variability due to small-scale spatial variability within a site, operator consistency and bias, and laboratory consistency.
	Bias - exclusion of certain taxa or habitats	Riffle only, 500μ mesh size
	Performance range - limitations in certain habitats or substrates	Riffle only, sample edge vegetation for sandy substrates riffles
	Interferences - high river flows, training of personnel	Sampling not performed during high flows for safety reasons. The method has only been tested on a limited basis for large river sampling.
Field Sample Processing	Bias - efficiency of locating small organisms in sample transfer	The sieve is carefully rinsed after straining a sample. The sieve is washed prior to leaving a sample site.

	Performance range - sample preservation and holding time	Sample preserved with isopropanol and capful of formalin for better preservation in Arizona heat. Formalin also allows longer holding time.
	Interferences - Weather conditions	Sample taking maybe performed during light rains and slightly elevated flows, but not during bankfull or greater flows.
	Accuracy - of sample transfer process and labeling	There is a standard format for sample labels which includes stream name, site id, date, habitat sampled, collector info, whether sample was field split and # of jars in sample.
Laboratory sample processing	Precision - split samples	Duplicate samples are collected at the rate of 10% of the total # of samples during each year's index period. This is a test of the labs ability to create consistent IDs.
	Bias - sorting certain taxonomic groups or organism size	Large specimens are removed first from the sample. All organisms, regardless of size are sorted for ID from each 1/32 section of the sample.
	Interferences - distractions, equipment	Field and lab equipment, such as sieves and nets, are thoroughly washed between sites and samples.
	Accuracy - sorting method, lab equipment	Caton Tray used for consistent method of sorting samples, especially where thousands of insects per sample are found.
Taxonomic enumeration	Precision - split samples	The similarity of duplicate samples is verified using the Arizona warm water and cold water IBI's, rather than the individual taxonomic identifications.
	Bias - counts and identifications for certain taxonomic groups	Our taxonomist offers 500 counts of insects per sample, which exceeds the number of specimens counted by many other states. Where a particular taxa is dominant in the sample, that taxon is not included in the 500 count. Our laboratory has used a number of nationally recognized specialists to provide confirmed identifications of specimens for our reference/voucher collection.
	Interferences - appropriateness of taxonomic keys	List of taxonomic keys used by our laboratory is included in our SOP's.
	Sensitivity - level of taxonomy related to type of stressor	Our standard taxonomic effort (identifications to genus in most cases, with midges at family level) is generally used for all samples. Identification of Chironomidae to genus can be done on an as-needed basis for samples/sites found to be impaired.
	Accuracy - identification and counts	Use of nationally recognized specialists to create the Arizona reference collection, by which all other samples are identified.

10. Data analysis - Calculating the Arizona Indexes of Biological Integrity

To calculate the warm water and cold water indexes of biological integrity for appropriate macroinvertebrate samples, follow the following steps. It is not appropriate to apply these indexes to samples from intermittent or effluent dominated streams, nor to samples collected in bedrock, travertine or sand dominated or wetland habitats.

a. Identify the appropriate reference community using the site elevation (warm water community <5000'; cold water community >5000').

b. Calculate the macroinvertebrate metric values required for either the cold or warm water index following metric calculation procedures listed Figure 1. Table 3 lists all the metrics used in both indexes and their definitions. Metrics required for each index are listed in Tables 4 and 5.

c. Calculate the metric percent of reference score using either the warm or cold water reference metric threshold values associated with the appropriate community type (Tables 4 and 5).

d. Calculate an average of the percent of reference scores for all metrics to produce the IBI score. An example of the scoring system is provided in Table 6.

e. Determine assessment category for IBI score from Table 7. Interpretive descriptions of the categories Exceptional, Good, Fair and Poor are provided in Table 8.

11. Bioassessment requirements for receiving water bioassessments in NPDES permits - In addition to previously mentioned bioassessment protocols, the following apply to permittees:

a. Bioassessment should occur concurrently with ambient water monitoring

b. A bioassessment survey plan containing sample dates, locations of background and study sites, sampling personnel and qualifications, name and location of contract laboratory, biological and habitat sampling protocols and method of analysis, should be completed and submitted to ADEQ by December 31st each year.

c. ADEQ sampling and analysis protocols should be followed as closely as possible, using the most updated Quality Assurance Program Plan.

d. Laboratory protocols should follow ADEQ recommendations in Table 2 above.

e. The bioassessment report should be submitted to ADEQ for review. The report should contain: an executive summary, introduction, study area descripton including maps and photos, methods, results and discussion, literature cited, and appendices with complete taxa lists and copies of completed field forms for each site. The results and discussion section should cover a physical characterization of the sites, a habitat assessment, water quality, fish & wildlife, macroinvertebrates, and long term trends at the study sites.

f. Macroinvertebrate analyses should contain: a list of taxa and abundances, the calculated warm or cold water IBI score, the benthic habitat score, and graphs indicating a comparison of reference and study site IBI scores for the current year, changes in the reference and study IBI scores over a permit period and changes in the reference and study site habitat scores or habitat values over the permit period.

g. The first bioassessment shall be subject to a quality assurance review to be conducted by ADEQ. The voucher specimens from the laboratory should be submitted to ADEQ for a quality control review of the taxonomic identifications by the ADEQ contract taxonomist. Major revisions should be incorporated into the final bioassessment report.

Table 3. 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

Figure 1. Formulas for calculating macroinvertebrate metrics used in the cold water and warm water Indexes of Biological Integrity.

Metric Calculations

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

Metric Score = (Sample value / metric threshold value) * 100

1. Total taxa richness
2. Number of ephemeroptera taxa
3. Number of trichoptera 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

Metric score = (10 - Sample value) / (10 - Metric threshold value) * 100

2. Percent dominant taxon

Metric score = (100 - Sample value) / (100 - Metric threshold value) *100

Table 4. Reference scoring thresholds for warm water metrics, used in the Arizona warm water Index of Biological Integrity.

Metric	Taxonomy ID Level	Metric threshold value
Total taxa	Split-Level ID	34
Trichoptera taxa	Split-Level ID	8.0
Ephemeroptera taxa	Split-Level ID	7.0
Diptera taxa	Split-Level ID	9.0
Scraper taxa	Split-Level ID	7.0
Percent scraper	Split-Level ID	25.1
Percent Ephemeroptera	Split-Level ID	70.8
Percent Dominant Taxon	Split-Level ID	20.9
Hilsenhoff Biotic Index	Split-Level ID	4.9

Table 5. Reference scoring thresholds for cold water metrics, used in the Arizona cold water Index of Biological Integrity.

Metric	Taxonomy ID Level	Scoring threshold
Total taxa	Split-Level ID	23
Diptera taxa	Split-Level ID	5.0
Intolerant taxa	Split-Level ID	4.0
Scraper taxa	Split-Level ID	5.0
Percent scraper	Split-Level ID	8.8
Percent Plecoptera	Split-Level ID	1.3
Hilsenhoff Biotic Index	Split-Level ID	5.5

Table 6. 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	71
Trichoptera taxa	6	75
Ephemeroptera taxa	5	71
Diptera taxa	7	78
Scraper taxa	3	43
Percent scraper	20.3	81
Percent Ephemeroptera	26	37
Percent Dominant Taxon	41	75
Hilsenhoff Biotic Index	5.73	84
Index Score (avg. of all scores)		68 Good

Table 7. Index of Biological Integrity (IBI) assessment categories and ranges of values for cold water and warm water waterbody types in Arizona.

Waterbody type	Exceptional	Good	Fair/Impaired	Poor/Very Impaired
Warm water	73 - 100	53 - 72	27 - 52	0 - 26
Cold water	97 - 100	88 - 96	44 - 87	0 - 43

* Exceptional and good are attaining A&W use.
* Fair and Poor are not attaining A&W use.

Table 8. Interpretive descriptions of ADEQ bioassessment categories.

Assessment Category	Description	Attainment Status
Exceptional	Exceptionally high species richness, sensitive species abundant, community tolerance low	Attains A&W use
Good	High species richness, sensitive species present, community tolerance moderate	Attains A&W use
Fair/Impaired	Declining species richness, sensitive species absent or in low abundance, community tolerance high	Does not attain A&W use
Poor/Very impaired	Very low species richness, only most tolerant species present, community tolerance very high and community organization lacking	Does not attain A&W use