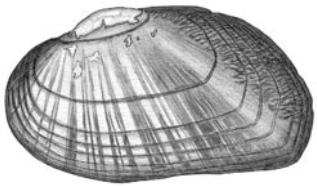
7. Macroinvertebrates

We do not recommend a specific macroinvertebrate method in this Monitoring Guide because of the inherent complexity of conducting statistically valid macroinvertebrate assessments. We recommend that the user consult with professionals in their region who have the expertise necessary to design a macroinvertebrate monitoring plan appropriate for the stream barrier removal project. Appendix D provides an in-depth discussion of planning macroinvertebrate monitoring for stream barrier removal projects. Table 8 provides a summary of macroinvertebrate monitoring protocols used by different Gulf of Maine jurisdictions.



Brook floater

8. Fish Passage Assessment

We do not recommend a specific fish-monitoring method in this Monitoring Guide because fish monitoring should be managed by trained fisheries experts and must be tailored to the project site and target species. We recommend consulting with experts in the region with the necessary jurisdiction to design and implement fish monitoring for barrier removal projects.

Fish ladders sometimes are not effective at enabling fish to move past dams. After a dam is removed, monitoring can reveal if more fish are traveling up and down the river.

Table 7. Fish-monitoring methods that may be recommended by local fisheries experts.

Method	Technique	References
Visual	Human visual identification and counts of fish at specific locations.	Nelson, 2006; Stevenson et al., 1999
Simple presence/absence	Electrofishing is a commonly used and inexpensive technique to assess the presence or absence of fish species above and below a barrier.	Reynolds, 1997
Video	Pre-positioned video camera recording fish at specific locations.	Bowen, 2006
Passive Integrated Transponder (PIT tags)	Fish are captured and are inserted with a Passive Integrated Transponder (PIT tag). Fish injected with this tag can be automatically recognized by strategically located detecting/recording devices.	Bruyndoncx, 2002
Mark and recapture	Fish are captured and are fin clipped and/or have an external fish tag attached; employs nets, traps, or electrofishing.	Nielson, 1992; Parker, 1990
Telemetry	Fish are captured and tagged with electronic transmitters. Transmitters can be applied to fish internally or externally. Fish movements are subsequently determined by locating fish/transmitters using mobile and/or fixed telemetry receivers.	Amlaner and MacDonald, 1980; Baras, and Philipart, 1996; Burnham et al., 1987; Cheeseman and Mitson, 1982; Finkenzeller, 2000; Lucas and Baras, 2001; Moore and Russell, 2000; Pincock, and Voegeli, 1990; Priede and Swift, 1992; Sibert and Neilson, 2001; Spedicato et al., 2005; Winter, 1983; Winter, 1996; Zydlewski et al., 2006

Table 8. Relevant state and provincial protocols for monitoring macroinvertebrates.

	Recommended Collection Method	Index Period	Recommended Sampling Processing Protocol	Identification Level	Recommended Assessment Approach	Protocol Reference
New Hampshire	Artificial substrates (wire mesh, rock-filled baskets); 6-8 week deployment in riffles or at base of riffles	Fall	Standardized gridded tray with sample evenly distributed across tray; 1/4 of grids randomly selected for complete sorting with 100 individual minimum subsample; additional 1/4 of grids incrementally selected for complete sorting until 100 individual subsample satisfied	Genus (if possible), except for chironomids (sub-family), ribbon worms (phylum Nemertea), nematodes (phylum Nemata), and oligochaetes (sub-class)	Multimetric index: average score of 7 metrics; index range 0 (poorest condition) to 100 (best condition); biological condition threshold established via minimally impacted reference sites	Neils, D. 2007. New Hampshire Department of Environmental Services (NHDES) Protocols for Collection, Identification and Enumeration of Aquatic Macroinvertebates for Computation of a Benthic Index of Biotic Integrity (B-IBI). Concord, NH: NHDES.
Maine	Artificial substrates (wire mesh, nylon bags, or cone-shaped containers filled with standard-sized stones); 24- to 32-day incubation; rock containing device	Fall	Full sample sort using shallow sorting pan(s) unless >500 individuals; if >500 individuals, then 100 individual subsample permissible using air-generated suspension of individuals in Imhoff-type settling cone	Genus or species for all individuals (if possible)	Multivariate linear discriminate model to predict probabilities of membership to predetermined water quality classes	Davies, S.P. and L. Tsomides. 2002. Methods for Biological Sampling and Analysis of Maine's Rivers and Streams. DEP LW0387-B2002. Augusta, ME: Department of Environmental Protection.
Massachusetts	Kick nets (500µm); 10 area delineated sampling efforts composited for a single sample within 100m reach; 2 m^2 total area; samples collected from areas with coarse substrates OR artificial substrates (rock baskets, Hester-Dendy multiplate samples in deep water or soft bottom habitats or as directed)	Fall	Standardized gridded tray with sample evenly distributed across tray; individual grids randomly selected and sorted until 100 individual subsample requirement satisfied; large and rare organisms sorted for entire sample OR full sort of entire sample for artificial substrates where quantitative sample is desired	Genus or species for all individuals (if possible)	Multimetric index following RBP II or III (Plafkin et al., 1989); index range 0 (poorest) to 54 (best) and compared to applicable reference sites	Nuzzo, R.M. 2003. Standard Operating Procedures: Water Quality Monitoring in Streams Using Aquatic Macroinvertebrates. Massachusetts Department of Environmental Protection, Division of Watershed Management.
Canada Ecological Monitoring & Assessment Network	Several noted depending on water body and habitat types; kick net (400µm) suggested as technique for wading-depth streams; multihabitat collection effort; one to many 3-minute collection efforts	Late fall or early spring	Several noted; subsampling at the 100, 200, or 300 individual target level suggested; gridded trays, flotation, or elutiation can be used to obtain representative subsample	Genus or species for all individuals (if possible)	None recommended; several options referenced	Rosenberg, D.M., I.J. Davies, D.G. Cobb, and A.P. Wiens. 1997. Protocols for Measuring Biodiversity: Benthic Macroinvertebrates in Fresh Waters. Winnipeg, Canada: Canada Department of Fisheries and Oceans, Freshwater Institute.
Canada Canadian Aquatic Biomonitoring Network	Kick net (400µm); multihabitat	None indicated	Even distribution of entire sample across gridded tray; 300 individual subsample target	Family	Multivariate discriminate model using Observed: Expected species occurrence ratios to determine similarity to a "reference" condition (1=identical; 0=no similarity)	Reynoldson, T.B., C. Logan, T. Pascoe, and S.P. Thompson. CABIN (Canadian Aquatic Biomonitoring Network): Invertebrate Biomonitoring Field and Laboratory Manual. Environment Canada, National Water Research Institute.

APPENDIX D: MACROINVERTEBRATE MONITORING GUIDANCE

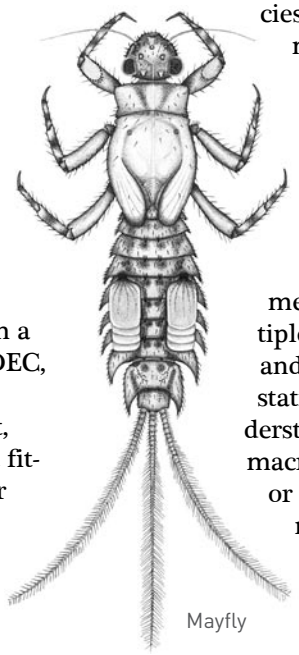
Planning Macroinvertebrate Monitoring at Stream Barrier Removal Projects

Given their utility as indicator organisms, macroinvertebrates are frequently used to document the responses of the aquatic community following barrier removal. The sections below describe the important components necessary in planning macroinvertebrate monitoring to assess aquatic community health and document shifts in community composition.

We advise that macroinvertebrate sampling be conducted in close coordination with the project's regulatory authority. Because of the inherent complexity of conducting statistically valid macroinvertebrate assessments, we encourage practitioners to use protocols recognized by state, provincial, or federal authorities.

Equipment

Several types of sampling equipment can be used to collect macroinvertebrates from wading-depth streams. Devices range from a Surber sampler to artificial substrates. While each sampling device has its benefits, the most commonly used and cost-effective sampling device currently employed is the dip net. Standard collection techniques call for the frame to be fitted with a 500µm mesh net (Lazorchak et al., 1998; VTDEC, 2006; Barbour et al., 1999) attached to a long wooden pole. Along with the rectangular net, often referred to as a kick-net, a sieve bucket fitted with 500µm mesh, and several 1- to 4-liter plastic sample containers complete the basic elements necessary to collect a representative macroinvertebrate sample.



Mayfly

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Design

As for all scientific studies, considerable time and effort should be spent prior to any fieldwork to determine what questions are to be answered through the collection of data. Once determined, careful study design must be employed so that sufficient data are collected in an accurate manner. For studies associated with barrier removal projects, documentation of the changes in macroinvertebrate community composition, abundance, or overall biomass may be of interest. In all cases, an understanding and accounting of the natural sources of variation (error) must be completed in order to draw correct conclusions. The basic sources of error that are manifested in all sampling efforts include collection techniques, laboratory processing, and spatial and temporal heterogeneity in macroinvertebrate populations. A good study design will minimize, or at least account for, each of the potential sources of error. In reality, minimizing error sources means the selection of appropriate field techniques, collection of an adequate number of samples, careful adherence to standardize operating procedures in the field and laboratory, and the use of well-developed biological indices.

With respect to biological indices, a section below focuses on regionally developed macroinverterbate indices that are widely applicable to the detection of pollution sources, including nutrient enrichment, toxic inputs, and flow modifications. In general, these indices have been developed by resource agencies and use a network of reference or minimally disturbed sites to establish acceptable conditions in overall macroinvertebrate community composition. Benefits of using regionally developed indices include the direct comparison of sample results to index thresholds, known estimates of natural variation in undisturbed macroinvertebrate community composition, use of metrics known to be most responsive to multiple pollution sources, and predetermined field and laboratory techniques. In most cases, the statistical properties of these indices are well understood and will allow for the determination of macroinvertebrate community health as above or below an established threshold and/or placement into one of many narrative categories (i.e., poor, fair, good) with a known level of certainty.

However, regional biological indices are, in most cases, not specific to barrier removal projects and have drawbacks that should be considered based on the study's questions of interest. For example, if macroinvertebrate community biomass or area of colonizable habitat is of interest, alternative measures will be required. In cases where previously developed indices are not applicable, one must decide what community measures are most representative of the questions being asked, how to obtain the necessary data, and what comparisons will best assist in determining if significant changes have occurred. In cases where established indices are not applicable, the greatest

limiting factor frequently will be the establishment of thresholds for detecting change. In other words, if data collected at a site is presumed to be impacted by an existing barrier, how can that be determined if the macroinvertebrate community differs from a site where no barrier exists? While it is beyond the scope of this document to develop a detailed discussion of study designs and the limitations of data usage, the before-after-control-impact (BACI) study design provides a basic framework to begin answering such questions. Under a BACI study design, samples are collected at sites where target conditions are presumed to exist before and after a barrier removal. These are considered control sites. Concurrently, samples are collected at sites presumed to be impacted by the barrier. These are considered impact sites. Ultimately, differences between control and impacted sites are compared before and after the barrier improvement or removal event to determine if significant changes have occurred. The ability to detect significant differences is a function of the number of samples collected and the quality of data.

Other study designs are possible. All parties involved in the project should be consulted to determine how best to design the macroinvertebrate monitoring efforts. The use of regional biological indices offers the most cost effective and least labor intensive approach in determining overall changes in community condition, but may be limited in terms of the specific questions that can be answered.

Areas of Sample Collection

Macroinvertebrate samples can be collected from several macrohabitat types, such as riffles, pools, stream banks, or a combination of habitat types. Sample collection from each specific habitat type requires careful consideration of available collection techniques. Current collection techniques include two main approaches: single- or multi-habitat sampling. Single-habitat sampling is used by several states and usually includes the collection of samples at the "single, most productive" area within a selected stream reach (Barbour et al., 1999). Macroinvertebrate production generally is maximized in riffle habitats leading to the common terminology of "riffle-kick" for single-habitat samples (VTDEC, 2004). Single-habitat sampling techniques employ multiple, individual, timed sampling efforts in one or many riffles within the study reach. Individual timed sampling efforts generally range from 3 to 5 in number and are grouped together for a representative sample of the macroinvertebrate community.

More recently, some U.S. and Canadian macroinvertebrate sampling protocols have promoted the use of multi-habitat collection techniques (Lazorchak et al., 1998; Rosenberg et al., 1997) as a more complete representation of the resident community. Multi-habitat sampling techniques include the collection of macroinvertebrates from a variety of habitats in approximate proportion to the habitat types observed within the study reach. Points of collection may be randomly selected or placed along predetermined transects. Multiple, individual, timed sampling efforts are used to standardize collection techniques and are variable in number depending on the sampling protocol. As with single-habitat collection techniques, individual timed sampling efforts are grouped together to approximate the macroinvertebrate community within the study reach.

Sampling Timing and Frequency

Most macroinvertebrate collection protocols have an established index period that standardizes a window of time (weeks) during which samples should be collected. Since many aquatic macroinvertebrates have regular development and emergence patterns, the establishment of a standardized collection window minimizes the amount of observed natural variation in community composition. Based on known life cycle patterns, macroinvertebrate sampling for riverine systems in northeastern North America occurs primarily from September through November (USEPA 2002). Alternative sampling times are possible but should be considered with respect to organism developmental patterns, climatic conditions, and the protocols advocated by the applicable regulatory authority.

Site-specific Considerations

The sampling methods described herein are applicable to wading-depth sections of riverine systems. Wading-depth streams can be defined as first through fourth order streams ranging in watershed size from approximately 2 to >200 km^2 (0.77 to >77 mi^2). However, from a practical standpoint, wading-depth can be defined as any section of river where water depth is less than thigh high. Conditions prior to barrier removal often preclude wading-depth sampling techniques. In these cases, alternative macroinvertebrate sampling procedures must be employed. See Blocksom and Flotemersch (2005) for comparison of several non-wading-depth methods.

Sample Processing

Macroinvertebrate sample processing consists of two main phases: sorting and identification. In the sorting phase, organisms are separated from the sample de-

bris. Identification generally takes place following the sorting phase and requires varying levels of expertise depending on the desired level of taxonomic specificity.

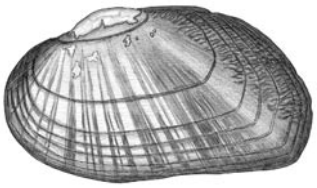
Sample Sorting

Because most whole samples contain more organisms and/or debris than can be processed, the sorting phase usually includes a sub-sampling method. Sub-sampling usually involves the homogenization of all sample contents in a single shallow pan followed by an objective process for selecting a pre-determined fraction of the sample. Currently, the most common method of sub-sampling uses the separation of a fixed-count target number of organisms from a predetermined fraction of the whole sample (Barbour et al., 1999; Lazorchak et al., 1998; Rosenberg et al., 1997). The sample fraction generally is defined by randomly selecting a minimum number of standardized areas (grids) identified by a template overlain upon the entire sample.

Debate still exists over the proportion of the whole sample that must be processed and number of organisms retained for identification and enumeration (Courtemanch, 1996; Barbour and Gerritsen, 1996; Vinson and Hawkins, 1996). A common target is the removal of organisms from enough full grids to meet a 300-individual fixed count target (VTDEC, 2006; Barbour et al., 1999). Doberstein et al. (2000) demonstrated that the results of samples processed using fixed counts of less than 300 individuals differed significantly from whole sample counts of the same sample and that sub-sample counts of up to 1,000 individuals incrementally increased the similarity to whole sample results. Thus, fixed sub-sample count targets are often based on resource availability and may vary among protocols. For this reason, one should consult the protocols advocated by the relevant resource agency before selecting a fixed count target and general sorting procedures. In all cases and regardless of the target, the fraction of the sample processed may differ among samples based on stream productivity. Therefore, a record must be kept for each sample so estimated whole sample results can be standardized.

Identification

The recommended level of taxonomic identification (i.e., family, genus, species) can be highly variable. Several protocols call for the lowest practical level (Barbour et al., 1999; VTDEC, 2006), but researchers have differing opinions as to what taxonomic level is most appropriate (Bailey et al., 2001; Lenat and Resh, 2001; Hawkins et al., 2000; Reynoldson et al., 1997). The academic reasons (i.e., geographic location, ecological diversity, evaluation tool) to select one level of taxonomic specificity over another must be considered in concert with the required level of expertise necessary to achieve the desired results. Highly trained taxonomic experts and expensive equipment generally are required to identify aquatic macroinvertebrates to genus and species levels. In contrast, an experienced field biologist may be able to identify insects to the family level with the naked eye. Thus, one must consider resource availability when deciding on a prescribed taxonomic identification level.



Brook floater

Drawing © Ethan Nedeau / Biodrawversity

Regardless of taxonomic level of resolution chosen, the protocol must provide detailed identification directions to the people responsible for sample processing. Some groups of macroinvertebrates (i.e., chironomids, nematodes) require additional taxonomic expertise and steps for identification. A less specific identification endpoint is common for these groups. Correct identification serves as the foundation for building the final dataset. Therefore, it is critical that this phase of sample processing be performed in a consistent manner to produce accurate results.

Because of the debate regarding recommended sorting processes and identification levels, specific sample processing protocols are not included herein. If well-tested and widely accepted field and laboratory protocols are selected, evidence suggests that differences between methods can be small. However, it is important they meet minimal performance measures (Herbst and Silldorff, 2006). In Canada and the United States, national protocols exist and should be consulted for further guidance (Barbour et al., 1999; Lazorchak et al., 1998; Rosenberg et al., 1997). Ideally, state, provincial, or federal protocols will be available to guide sample processing.

QA/QC

After sample processing is complete, it is important to verify the results. A common practice for determining the quality of the results is to re-process a minimum of 10% of the samples. A rigorous quality assurance program should test the effectiveness of the sorting and identification phases. As recommended above, it is best to follow the QA/QC procedures advocated by the appropriate regulatory authority. The goals are to document that the reported results are repeatable and that minimal variation can be attributed to the process-

ing methods. As an example, the following is a generic QA/QC procedure:

1. For previously sorted grids, have a second qualified individual re-examine each grid. If less than 95% of the individuals or 95% of the taxa were not removed in the original sort then the sample fails to meet the QA/QC requirements.

2. From a previously identified and enumerated sort, have a second qualified taxonomist re-identify and enumerate all individuals. If 5% or greater of the individuals are misidentified or incorrectly counted, then the sample fails to meet the QA/QC requirements.

3. Individual samples that fail by either (1) or (2) must be reprocessed and adequately justified. An overall sample failure of greater than 2% requires reprocessing for the entire lot of samples.

Use of Resulting Data

In contrast to chemical samples where individual parameter results are compared to their respective thresholds, results from macroinvertebrate samples initially are more complex. With multiple species and individual abundances for each species, long lists of scientific names must be translated into an understandable format. Contemporary efforts to understandably convey taxonomic composition and abundance information include two approaches. First, the multimetric approach relies on the differential tolerances, ecological roles and strategies, and overall composition of the macroinvertebrate taxa found in the sample. Multiple individual measures that are most important in describing community condition are aggregated together to produce a single index of biologic health. This multimetric approach is well documented and has been widely advocated for bioassessments (Karr and Chu, 1999; Barbour et al., 1995; Gerritsen, 1995). Alternatively, the multivariate approach uses detailed statistical estimates of community similarity to establish expected community compositions at minimally disturbed sites. Once these expectations are established, test sites are compared to the minimally disturbed sites to determine the difference in community composition. An observed (test site) to expected (reference expectation) (O/E) ratio is used as the measure of community health (or taxonomic loss). Ratios near 1 indicate minimal taxonomic loss while lower ratios indicate divergence of test sites from expectations. Originally developed in Great Britain and Australia, the multivariate approach has gained acceptance in North America (Reynoldson et al., 1997; Hawkins et al., 2000).

Regardless of the approach used, both techniques provide defensible alternatives to collapse taxonomic lists and respective abundances into understandable and similar assessment outcomes (Herbst and Silldorff, 2006). Prior to any sampling, protocols for collection and processing must be selected that are consistent with the approach and evaluation tool that will be used to assess the status of the macroinvertebrate community. In most cases, the appropriate regulatory authority should be contacted to suggest a recommended index that is locally applicable. In addition, the suggested index may have one or more threshold levels to assist in estimating biological condition and completing formal assessments for water quality reporting requirements.

Documentation

Integral to the success of all components of macroinvertebrate sampling is the maintenance and documentation of the associated data. Given the wide variety of potential sampling methods, laboratory protocols, and data summary approaches, a detailed record must be kept of all data elements. The primary data elements for sampling techniques are sampling device (including net mesh size, if applicable); type(s) of habitat sampled; approximate area sampled; number and approximate length of individual sampling efforts (i.e., five one-minute kicknet efforts); length of incubation (if artificial substrates are used); extent to which individual sampling efforts are grouped together; and the number of replicates. Laboratory processing data elements should include subsample fraction (percent of whole sample sorted); target number of individuals (i.e., 300 individual minimum); number of individuals per taxon; current scientific nomenclature for each taxon (with reference to naming organization); stage of development (larvae, pupa, adult); and QA/QC results for overall sample lot processing. In addition, laboratory metadata should include subsampling procedure (e.g., grid, number of cells, aeration); keys used to identify major taxonomic groups; and target level of identification for major taxonomic groups (i.e., family, genus, species). Data summary approach elements should include final metric and index results for each replicate/sample and reference to applicable index. The referenced index should detail the computation of individual metrics and the final index score, as well as the distribution of index scores for the reference condition and the method for threshold establishment. The ideal data storage vehicle is a relational database that allows for the efficient and long-term storage of large quantities of data in a consistent manner.