Development of a Comprehensive State Monitoring and Assessment Program for Wetlands in Massachusetts

Appendix P

Assessment of Wetland Communities: Macroinvertebrate Analysis

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Assessment of Wetland Communities: Invertebrate Identification

I. 2008 Invertebrate Samples

In 2008, invertebrates were sampled from 72 sites in the Chicopee watershed in Massachusetts. This field study was part of the program to develop a site-level assessment method for forested wetlands and the calibration of the CAPS landscape assessment method that will be used to assess and monitor the condition of MA wetlands.

Several sampling techniques were used to sample the aquatic and terrestrial invertebrate community in forested wetlands. Emergence traps were set at 35 sites, stovepipe samples were collected from 35 sites, and pitfall traps were set at 68 sites. Not all sites were sampled using the same technique due to some problems encountered with the design of the emergence traps, the late start in the season (lack of standing water to collect stovepipe samples), and a few instances where access was denied upon the return visit to a site.

Invertebrate Orders

The emergence trap and pitfall samples were initially sorted to Order. No subsampling technique was used. The stovepipe samples have not been sorted. We will conduct a subsampling analysis to determine the best procedure to use for the stovepipe samples which may include fixed counts, large-rare taxa searches, fixed area, or fixed volume approaches.

Four emergence traps were set at each plot. Upon collection, the samples were composited. The total number of specimens collected in the emergence traps for 35 sites was 2,777. A total of 14 Orders (Table 1) were collected (Collembola was treated as an Order). The most abundant Orders were Diptera (1659), Isoptera (511, note 1 plot contained 382 specimens), Acari (488, note 1 plot contained 479 specimens), Hymenoptera (26), Hemiptera (24), and Araneae (18).

Eight pitfall traps were set at each plot. The traps were kept separate to enable an analysis of sampling intensity. Due to heavy precipitation in July, many traps were flooded. In total 225 individual traps (across all sites) were flooded, 282 traps were in good condition and 22 were partially flooded. 253 samples have been sorted to Order. Nine classes of invertebrates (Table 1) and 28 Orders (did not sort Bivalves to Order) were collected. The total number of specimens from the pitfall traps that have been sorted is 20,367. The most abundant Orders were Collembola (10,243), Acari (2,292), Hemiptera (1,286), Hymenoptera (1,273), Diptera (1,741), Araneae (1709), and Coleoptera (1,130).

The following Orders have been selected to have species level identification work contracted: Araneae, Diptera, Hymenoptera, Hemiptera, Coleoptera, Collembola (Table 2). These Orders were selected because of their abundance, their relationship to changes in land use and water quality, and resources available for identification work. Individual

species will be analyzed to elucidate any dose-dependent relationships that may exist with the stressors modeled in CAPS.

Specimen identifications will facilitate development of Indices of Biotic Integrity (IBIs). These IBIs will be incorporated into a Site Level Assessment Method (SLAM) for forested wetlands. The IBIs will also be used to calibrate the CAPS landscape-based models for assessing ecological integrity in wetland and aquatic ecosystems.

Invertebrate Sample Identification

Invertebrate specimens collected in the 2008 and 2009 field seasons will be sent to taxonomic experts for identification (see Table 2). Identifications will be to the species level whenever possible (depending on the availability of suitable keys, life stage and condition of specimens). Specimens that cannot be identified to species will be identified to the lowest taxonomic level possible. Any individual specimens not identified to species will be returned to UMass and stored for potential further identification work.

General Laboratory protocols

Specimens will be preserved in denatured 70% ethanol and kept in glass vials. A label (Fig. 1) with the following information will be inserted into each vial: plot ID, the date the sample was collected, identifier ID, the taxonomic ID, and number of individuals in the vial. A separate label will be placed on the outside of the vial with the plot ID and taxonomic ID. The vials will be organized by plot number in numerical order.

Figure 1. Sample Label

Plot ID:	Date of sample collection:
Identifier ID:	
Taxon: #:	

Invertebrates will be identified using microscopes and taxonomic keys. In some cases identification to species will require research papers specific to genus, or in some cases, individual species.

Taxonomic References:

Adult Dipterans (provided by John Tipping, Lotic, Inc.)

Carpenter, S.J. and W.J. Lacasse. 1955. Mosquitoes of North America north of Mexico. University of California press, Berkeley and Los angeles, CA.

Crampton, G.C. 1942. Guide to the Insects of Connecticut. Part VI. The Diptera or true flies of Connecticut. Bull. Conn. St. geol. Nat. Hist. Surv. 64:10-165.

Jamnback, H.A. 1965. The *Culicoides* of New York State (Diptera:Ceratopogonidae). Bull. N.Y. St. Mus. 399:1-154.

McAlpine, J.F., B.V. Peterson, G.E. Shewell, H.J. Teskey, J.R. Vockeroth and D.M. Wood (coords). 1981. Manual of Neartic Diptera. Vol. 1. Research Branch, Agriculture Canada, Monograph 27. 674 pp.

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Merritt, R.W., K.W. Cummins and M.B. Berg (Eds.) 2008. An Introduction to the Aquatic Insects of North America, 4th Ed. Kendall Hunt. 1158 pp.

Shaw, F.R. and E.G. Fisher. 1952. Guide to the Insects of Connecticut. Part VI. The Diptera or true flies of Connecticut. Family Fungivoridae (Mycetophilidae). Bull Conn. St. geol. nat. Hist. Surv. 80:177-250.

Stojanovich, C.J. 1961. Illustrated key to common mosquitoes of northeastern United States. Atlanta, GA.

Wiederholm. T.A. (ed.) 1989. Chironomidae of the holarctic region. Keys and diagnoses. Part 3. Adult Males. Ent. Scand. Suppl. 34:1-524.

Hemiptera and Hymenoptera (provided by Eric Eaton)

Borror, Donald J. and Richard E. White. 1970. A Field Guide to the Insects North of Mexico. Boston: Houghton Mifflin Co. 256 pp.

Caldwell, John S. 1938. The Jumping Plant-Lice of Ohio. Columbus: Ohio Biological Survey Bulletin 34 (Vol. VI, No. 5).

Eaton, Eric R. and Kenn Kaufman. 2007. Kaufman Field Guide to Insects of North America. Boston: Houghton Mifflin Co. 392 pp.

Goulet, Henri and John T. Huber. 1993. Hymenoptera of the World: An Identification Guide to Families. Ottawa: Agriculture Canada. 668 pp.

Johnson, Dorothy M. 1935. Leafhoppers of Ohio: Subfamily Typhlocybinae. Columbus: Ohio Biological Survey Bulletin 31 (Vol. VI, No. 2).

Marshall, Stephen A. 2006. Insects: Their Natural History & Diversity. Buffalo: Firefly Books (U.S.). 732 pp.

Osborn, Herbert. 1940. The Membracidae of Ohio. Columbus: Ohio Biological Survey Bulletin 37 (Vol. VII, No. 2).

--- 1938. The Fulgoridae of Ohio. Columbus: Ohio Biological Survey Bulletin 35 (Vol. VI, No. 6).

--- 1928. The Leafhoppers of Ohio. Columbus: Ohio Biological Survey Bulletin 14 (Vol. III, No. 4).

Slater, James A. and Richard Baranowski. 1978. How to Know the True Bugs. Dubuque, IA: Wm. C. Brown Publishers. 256 pp.

Triplehorn, Charles A. and Norman F. Johnson. 2005. Borror and Delong's Introduction to the Study of Insects (7th Ed.). Belmont, CA: Thomson Brooks/Cole. 864 pp.

Coleoptera (provided by Don Chandler)

Downie and Arnett (1996), "The beetles of Northeastern North America," 2 volumes, 1721 pages.

Araneae (provided by Pierre Paquin)

Paquin, P. and N. Dupérré. 2003. Guide d¹identification des Araignées (Araneae) du Québec. Fabreries, Supplément 11. 251 pages.

Ubick, D., P. Paquin, P.E. Cushing and V. Roth (eds.) 2005. Spiders of North America: an identification manual. American Arachnological Society. 377 pages.

Collembola

Merritt, R.W., K.W. Cummins and M.B. Berg (Eds.) 2008. An Introduction to the Aquatic Insects of North America, 4th Ed. Kendall Hunt. 1158 pp.

Quality Control and Assurance

The sample information will be recorded on data sheets to be entered into an Access database. Taxonomist will record the plot ID of the sample and the identity and counts of individuals in the sample. The data entered into the database will be double checked by a reviewer for mistakes. When possible ten percent of the samples will be verified by an expert taxonomist (see Table 2). The samples that are sent out for validation will be recorded. Once the validated sample identifications are returned corrections (if any) will be made to the data sheets and entered into the database. Corrections will be labeled on the data sheets with an asterisk. Verified specimens will be stored in a reference collection.

Data Analysis

The overarching goal of the data analysis is to determine whether CAPS IEI and the component ecological integrity metrics (e.g., habitat loss, connectedness, etc.) are related to observed ecological conditions, and to further quantify the magnitude and nature of those relationships. To accomplish this goal, we will use a variety of statistical methods including principally quantile regression (Cade et al. 1999) and a custom analytical method based on the method of indicator species analysis (Dufrene and Legendre 1997). The data input for both analytical methods will be a list of the sample points and the corresponding values for each of the CAPS metrics and a suite of variables representing the presence or standardized abundance of each species or group of species and/or one or more derived biotic indices (e.g., Simpson's diversity index). For more information on data analysis see section 2.4 Analytical Method in the QAPP.

Table 1. 2008 Taxonomic List for Emergence and Pitfall Samples

Emergence traps: 35 sites/composite samples		
Class	**Total**	**Notes**
Insecta	10	
Arachnida	3	
Entognatha	1	Collembola has recently been updated to class but we treated it as an Order, Enognatha was the previous class that Collembola was placed
Order	**Total**	**Notes**
Diptera	1659	selected to contract for species ID
Isoptera	511	*1 plot contained 382 isopterans that were covered in acari
Acari	488	subclass/ *1 plot contained 479
Hymenoptera	26	selected to contract for species ID
Hemiptera	24	selected to contract for species ID
Araneae	18	selected to contract for species ID
Collembola	14	selected to contract for species ID
Coleoptera	13	selected to contract for species ID
Ephemeroptera	7	
Opiliones	7	
Trichoptera	5	
Lepidoptera	3	
Thysanoptera	1	
Psocoptera	1	
Total number	**2777**	
Pitfall traps	**253 samples**	*not complete, 46 more samples to process
Class	**Total**	
Insecta	13	
Arachnida	4	
Diplopoda	3	
Gastropoda	1	

Chilopoda	2	
Malacostraca	2	
Bivalvia	1	not classified to order
Maxillopoda	1	1 subclass
Entognatha	1	
Orders	**Total**	
Collembola	10243	selected to contract for species ID
Diptera	1741	selected to contract for species ID
Coleoptera	1130	selected to contract for species ID
Hymenoptera	1273	selected to contract for species ID
Hemiptera	1286	selected to contract for species ID
Isoptera	2	
Trichoptera	11	
Lepidoptera	40	
Ephemeroptera	0	
Thysanoptera	43	
Psocoptera	47	
Orthoptera	134	
Mecoptera	3	
Plecoptera	1	
Pseudoscorpiones	14	
Opiliones	25	
Araneae	1709	selected to contract for species ID
Acari	2292	selected to contract for species ID, but have not identified a taxonomist
Isopoda	46	
Pulmonata	84	
Copepoda	11	
Amphipoda	1	
Bivalvia*	4	not sorted to Order
Julida	142	
Polydesmida	35	
Chordeumatida	1	
Lithobiomorpha	4	
Geophilomorpha	1	
Unknown	44	
Total number	**20367**	

Table 2. List of Orders that will be contracted for identification

Order		Number	ID level	Justification	Taxonomist	QA/QC, 10% verification	Cost
Araneae		PT:1709	Family for juveniles, genus/species depending on condition of sample	Respond to changes in plant and invertebrate community	Pierre Paquin Cave and Endangered Invertebrate Research Laboratory, SWCA Environmental	Nadine Duperre	$500.00

					Consultants, Austin Texas		
Diptera		ET: 1676 PT: 1741 Total: 3417	Species, if possible	Abundant, response water quality and land use	John Tipping, Lotic Inc., Environmental Consultants		$0.70 per specimen
Hymenoptera		ET: 27 PT:1273 Total: 1300	Species, if possible	Abundant, response to land use	Eric Eaton Author, Kaufman Field Guide to Insects of North America	Entomologists at the University of Arizona, will depend on taxa	$1.00 per specimen
Hemiptera		ET: 24 PT:1286 Total: 1310	Species, if possible	Abundant, response to land use	Eric Eaton Author, Kaufman Field Guide to Insects of North America	Entomologists at the University of Arizona, will depend on taxa	$1.00 per specimen
Coleoptera		ET: 13 PT: 1130 Total: 1143	Species/ morphospecies	Abundant, response to land use, habitat frag, changes in insect community	Don Chandler Curator UNH Insect and Arachnid Collections	Christopher Majka, Nova Scotia Museum	$1.50 per specimen
Collembola (Any aquatics)		PT:10243	Family/genus, species if possible	Abundant, response to soil disturbance	Sean Werle Umass Amherst Adjunct Faculty Curator of Invertebrates for the Umass Natural History Collections		$25 per hour

II. Sub-sampling Analysis for 2009 Stovepipe Samples

A simulated fixed-count subsampling analysis will be conducted on a subset of aquatic macroinvertebrate stovepipe samples to determine the tradeoff between sample size and precision in the estimate of IBI. This will facilitate the selection of a fixed-count subsampling methodology to apply to the rest of the samples. The samples from 2009, collected in the Millers and Concord Watersheds, will be analyzed. Samples collected from the low and high range of IEI (for each watershed) will be prioritized first (the total number of samples processed will be determined by available resources).

Field Collection

Macroinvertebrates were collected using a stovepipe sampler (5 gallon plastic bucket with the bottom cut off). Collections were made in two locations dispersed within the 30 m radius plot where surface water and/or wet depressions were present. The minimum distance between samples was 3 m.

The stovepipe sampler was pushed firmly into the substrate (few cm deep) and held in place. The water was agitated by the surveyor using their hand for 10 seconds to dislodge organisms from the substrate and vegetation. If surface water (>2 cm) was present five sweeps within the sampler were taken with a 500 micron mesh hand net (10.5x12.5 cm).

After each sweep, all material was transferred into a 32 oz collecting jar. The net was inspected for any clinging organisms and, if found, were added to the sample. The jar was filled halfway with sample material and additional jars were used when necessary. Containers were filled with 95% ethanol.

For wet depressions (with little or no standing water,<2cm) three, one-hand leaf litter grab samples from within the stovepipe were collected. Grabs were distributed evenly throughout the stovepipe area. These were preserved as previously described for the dipnet samples.

Upon return to the lab, each sample was strained using a #35 soil sieve to remove as much silt as possible and placed back into the sample container. 70% ethanol was added for preservation.

Laboratory protocols

Macroinvertebrate identification will be conducted by Lotic Inc. John Tipping is the Senior Entomologist at Lotic, and will serve as the project lead and sole point-of-contact.

The samples from 2009, collected in the Millers and Concord Watersheds, will be analyzed. The two samples taken at each plot will be composited for analysis. The sample will be poured into white trays, multiple if necessary, and water may be added to reduce the concentration of fine particulate matter. The samples will not remain in water for longer than 8 hours. The entire sample will be sorted.

Macroinvertebrates will be identified using state-of-the-art stereo microscopes and the most recently published taxonomic references. Chironomidae are cleared by immersion in a 10% solution of room temperature KOH for 24-48 hours. Once cleared the specimens are neutralized in 5% glacial acetic acid, rinsed in distilled water and slide mounted in a solution of CMC-10. Once the mountant has dried, the coverslips are ringed with clear nail polish. Oligochaeta are mounted in polyvinyl lactophenol. All slide material is identified with a compound microscope. Slides are labeled with the plot ID and date collected.

Organisms will be identified to genus/species unless the condition of the organism or lack of workable keys prevents it. All identified organisms will be retained in the original sample containers, with the exception of the voucher collection. Identifications will be recorded in an excel datasheet.

Quality Assurance/Quality Control

Internal Taxonomic Quality Assurance (Lotic Inc. QA/QC)

All sample specimens will be identified to genus or species as allowable by specimen condition and maturity. 10% of the samples identified by each taxonomist will be set aside for re-identification by another qualified taxonomist. If taxonomic agreement (as

determined with the Bray-Curtis Index of Similarity) is less than 95%, the taxonomists will discuss the differences, identify where errors were made, and take corrective action.

As a routine component of Lotic QA/QC protocols, a voucher collection is assembled of at least three specimens (when possible) of every taxon identified for each project. This collection will be retained by Lotic until requested by the client or permanently archived to resolve any taxonomic issues. Each vial in the voucher collection will be labeled with the taxon name, sample ID, sample date, taxonomist, and any other relevant sample information.

External Taxonomic Quality Control

Lotic maintains professional contacts with numerous research taxonomists and systematists for taxonomic verification of unusual or rare specimens. Any uncertain unusual taxa will be sent to one or more of these outside experts for verification. All samples subject to QA/QC procedures, along with the results of those procedures, will be recorded in the QA/QC logbook.

Data Entry and Reporting Quality Assurance

After data entry in Microsoft Excel format, a qualified taxonomist will check 25% of the completed data sheets against the original bench sheets. If no errors are found, the check is complete. If any errors are found, then all data sheets are checked against the database and all errors are rectified. Any necessary corrections will be noted in the QA/QC logbook.

Statistical analysis

Subsampling will be simulated using a computer program following a similar procedure conducted by Doberstein et al. (2000). Individuals will be randomly sampled by the computer program, without replacement, to simulate the fixed-count method. For each site, subsamples will be drawn from the original sample (whole sample count) using a range of sizes (counts). For each subsample size (e.g. 100, 200, 300, etc.) 500 replicates will be generated. The distribution of IBI for a given sample size will be created using the replicate samples. The distribution of IBI values will be plotted against sample size and examined to determine the tradeoff between sample size and precision in IBI estimates.

References Cited

Doberstein, C.P, Karr, J.R., and Conquenst, L.L. 2000. The effect of fixed-count subsampling on macroinvertebrate biomonitoring in small streams. Freshwater Biology 44:335-371.

Table 2. List of Orders that will be contracted for identification

Order	Number	ID level	Justification	Taxonomist	QA/QC, 10% verification	Cost
Araneae	PT:1709	Family for juveniles, genus/species depending on condition of sample	Respond to changes in plant and invertebrate community	Pierre Paquin Cave and Endangered Invertebrate Research Laboratory, SWCA Environmental Consultants, Austin Texas	Nadine Duperre	$500.00
Diptera	ET: 1676 PT: 1741 Total: 3417	Species, if possible	Abundant, response water quality and land use	John Tipping, Lotic Inc., Environmental Consultants		$0.70 per specimen
Hymenoptera	ET: 27 PT:1273 Total: 1300	Species, if possible	Abundant, response to land use	Eric Eaton Author, Kaufman Field Guide to Insects of North America	Entomologists at the University of Arizona, will depend on taxa	$1.00 per specimen
Hemiptera	ET: 24 PT:1286 Total: 1310	Species, if possible	Abundant, response to land use	Eric Eaton Author, Kaufman Field Guide to Insects of North America	Entomologists at the University of Arizona, will depend on taxa	$1.00 per specimen
Coleoptera	ET: 13 PT: 1130 Total: 1143	Species/ morphospecies	Abundant, response to land use, habitat frag, changes in insect community	Don Chandler Curator UNH Insect and Arachnid Collections	Christopher Majka, Nova Scotia Museum	$1.50 per specimen
Collembola (Any aquatics)	PT:10243	Family/genus, species if possible	Abundant, response to soil disturbance	Sean Werle Umass Amherst Adjunct Faculty Curator of Invertebrates for the Umass Natural History Collections		$25 per hour

III. Identification of Earthworm Samples

Introduction

Earthworms were collected using several techniques including liquid mustard extraction, flip and search, and soil pits. Samples collected in forested uplands and wetlands from 2007-2009 will be sent to The Great Lakes Worm Watch (GLWW) lab at the University of Minnesota Duluth for identification. Cindy Hale, the GLWW program director, will oversee sample identification. Species level identification will be conducted when possible given the condition and life stage of the specimen (e.g. juveniles only to genus).

Specimen Identification

Laboratory procedures for sample identification will follow protocols of the GLWW program. The principal taxonomic reference that will be used is Hale (2007). Specimen ID's will be entered into an excel spreadsheet.

Great Lakes Worm Watch Lab Protocols

1. The Identification Process for Field Samples
   - 1.1. Start by removing the Earthworm samples from the vial and placing them in the large Petri dish
   - 1.2. Sometimes it helps to pre-sort the Earthworms on a general basis in the large Petri dish if there are a lot. You can separate them based on pigmented vs. non-pigmented, clitellum present vs. no clitellum, and/or big vs. small body sizes (see picture)
   - 1.3. Take the Earthworms out of the large petri dish and look at each one under the microscope carefully. Use the dichotomous key in the book (pages 30-31) to identify genus and species.
     - 1.3.1. Note – It is important to keep the Earthworm samples moist, so be sure to give the Earthworms in all the petri dishes and under the microscope a squirt of water regularly.
   - 1.4. If you are having trouble identifying an Earthworm from the red book, there is another helpful identifying book, titled "Earthworms" (by R.W. Sims and B.M. Gerard), and a 3-ring binder with helpful information and different pictures that may help in the identifying process.
   - 1.5. Once identified, place Earthworms in the labeled Petri dishes, one species per dish, adults separated from juveniles (this will make it easier for measuring them).
   - 1.6. Measure each Earthworm using the ruler, gently stretching the Earthworm sample with the tweezers so it is straight and accurately measured.
   - 1.7. Stick the measured and recorded Earthworms back in the vial they came from, and place the vial in the completed stack.
   - 1.8. Things to keep in mind when identifying:
     - 1.8.1. If the Earthworm is an adult (A) if it has a clitellum, it is a juvenile (J) if it does not. If a clitellum is present, but it is not fully formed, it is considered an aclitellate adult (AC).
     - 1.8.2. Preserved Earthworm specimens in the lab usually have a light grey to white appearance if unpigmented, and a dark grey to reddish appearance if pigmented.
     - 1.8.3. Be careful when counting segments, the segments on some Earthworms have creases part way through the middle so be sure not to mistake those creases for more segments.

1.8.4. The Lumbricus genus has a tanylobic mouth, pigmentation, and closely paired setae, so look for these characteristics in pigmented specimens. Count the segments to identify the species.

1.8.5. The Apporectodea species can be difficult to identify, pay close attention to the TP, GT, and Clitellum shape and don't hesitate to ask for a second opinion.

2. Record the data

2.1. Record the data for each sample of each sample point on the data collection sheet (an example sheet should be on the clipboard)

2.2. Write the genus name as a proper noun on the datasheet, and the species name in all lowercase (ex. *Lumbricus terrestris*) and write neatly. Only write the genus name for juveniles, unless otherwise stated in the book.

2.3. Next, record the length in the length column. To save space, if there are multiples of a certain length, they can be combined into one multiplication expression (for example, if you have 8 Earthworms that are 25mm, you can write 25mm X 7 on the sheet).

Quality Assurance/Quality Control

Voucher specimens will be set-aside for the first of each unique species identified and verified by another lab technician. In addition, another lab technician will verify 10% of the identified specimens.

Data Analysis

The overarching goal of the data analysis is to determine whether CAPS IEI and the component ecological integrity metrics (e.g., habitat loss, connectedness, etc.) are related to observed ecological conditions, and to further quantify the magnitude and nature of those relationships. To accomplish this goal, we will use a variety of statistical methods including principally quantile regression (Cade et al. 1999) and a custom analytical method based on the method of indicator species analysis (Dufrene and Legendre 1997). The data input for both analytical methods will be a list of the sample points and the corresponding values for each of the CAPS metrics and a suite of variables representing the presence or standardized abundance of each species or group of species and/or one or more derived biotic indices (e.g., Simpson's diversity index). For more information on data analysis see section 2.4 Analytical Method in the QAPP.

References:

Hale, Cindy. 2007. Earthworms of the Great Lakes. 36 pages. Kollath and Stensaas, Duluth, MN.