STANDARD OPERATING PROCEDURE
for the
BENTHIC MACROINVERTEBRATE LABORATORY

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Prepared by

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TABLE OF CONTENTS

Appendices

Introduction

The Wallace & Pancher Inc. (WPI) laboratory has developed standard operating procedures (SOP) to process benthic macroinvertebrate samples in the laboratory for the purpose of measuring the taxonomic composition and abundance of benthic macroinvertebrates found in freshwaters. These SOP's are based on several standard laboratory practices, including those used by the U.S. Environmental Protection Agency (USEPA), and the Pennsylvania Department of Environmental Protection (PADEP) Bureau of Mining and Reclamation.

In an effort to promote consistent bioassessments of water quality, the USEPA has produced Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers (Barbour et al, 1999) for use in collecting and processing benthic macroinvertebrate samples from streams and rivers. The WPI "Standard RBP Methodology" and "Modified RBP Methodology" benthic macroinvertebrate laboratory SOP's borrow heavily from the USEPA protocols, but incorporate modifications based on WPI's experience. This SOP describes the 200-count sub-sampling method and the "entire sample" sorting method used by the benthic macroinvertebrate laboratory of WPI to process benthic macroinvertebrate samples.

In 2005, the PADEP Bureau of Mining and Reclamation developed the "*Appendix A and Appendix B*" sampling methodologies based on the aforementioned USEPA methodology. However, these protocols involve sampling a variety of habitat types as opposed to sampling only the most productive habitat. This SOP describes the 200-count sub-sampling method used in the USEPA Appendix B methodology.

Sample Processing

All samples, except *Appendix A Methodology*, will undergo three stages of laboratory processing:

1. receipt of samples from the field
2. sample rinsing and set-up, extraction/sorting of benthic macroinvertebrates, and sample cleanup
3. taxonomic identification and enumeration of benthic macroinvertebrates

Recommended quality control (QC) measures will accompany each of these stages in order to meet or exceed both the PADEP and the USEPA quality assurance (QA) standards.

Laboratory equipment and supplies needed for the receipt of samples from the field, initial rinse, and sample set-up, extraction, sorting, and cleanup are listed in Appendix A.

Receipt of samples from the field & sorting procedures

Benthic macroinvertebrate samples will be delivered by WPI field teams or will arrive by a mail delivery service. When samples arrive via non-WPI personnel, a Chain of Custody form (Appendix B) must be: 1) completed and signed by WPI laboratory personnel, 2) returned to the party of origin with a copy, 3) signed by the party of origin, and 4) returned to the WPI laboratory. Completed, signed, and returned Chain of Custody forms will be stored in an "all-projects" Chain of Custody binder in the benthic macroinvertebrate laboratory.

All samples arriving by WPI field teams must be recorded upon arrival from the field to the laboratory to verify sample arrival and condition using a Sample Log In form (Appendix C). Missing or damaged samples must be reported to the laboratory supervisor immediately. Samples will be carefully tracked within the lab throughout the project using the site name and collection date recorded on the Sample Log In form. The Sample Log In form will be stored in a project-specific binder with other forms for the same project. Laboratory personnel must keep accurate records of the location and status of all samples at all times by logging bottles into the EXCEL database and by recording sample progress on the Laboratory Log Sheet (Appendix D) for the corresponding project. After completion of a specific project, its Laboratory Log Sheet will be stored in the Laboratory Log Sheet binder on the shelves in the laboratory.

Samples should arrive at the laboratory in pre-labeled, plastic screw-top jars, preserved in 85% isopropyl alcohol. These samples will be stored at room temperature in their designated cardboard boxes on numbered shelving units in the sample storage room until sorted.

Refer to the dry erase board (project board) in the macroinvertebrate lab for the prioritized list of samples to be sorted and due dates for the macroinvertebrate data. The project board will provide the shelf unit number(s), shelf number(s), and box number(s) of samples to be sorted. The project board will be updated weekly as project priorities frequently change.

Appendix A samples will arrive from WPI field teams in pre-labeled, 60ml, plastic, screw-top bottles. After being recorded on sample sign in sheets, *Appendix A* sample bottles should be numbered chronologically on the bottle lid using black Sharpie. The bottles should be cataloged into the electronic EXCEL database immediately and stored in a box with the appropriate label. No further processing is required for the *Appendix A* methodology. For sorting instructions for *Standard RBP Methodology, Modified RBP Methodology,* and *Appendix B Methodology,* see Appendix E.

Taxonomic identification and enumeration

This procedure is to be used to facilitate identification and enumeration of benthic macroinvertebrate organisms from each sample. Recommended USEPA quality control (QC) methods will be used in order to meet or exceed the USEPA quality assurance (QA) standards. The taxonomists must have training and experience in the identification of freshwater benthic macroinvertebrates or must undergo a rigorous QC of each sample by the senior taxonomist in the lab.

It is important that the WPI taxonomists maintain contact with other taxonomists through professional societies and other interactions, and keep up with the pertinent literature because taxonomy and species identifications change over time.

1. Laboratory equipment and supplies

Laboratory equipment and supplies needed for the identification and enumeration of benthic macroinvertebrate samples are listed in Appendix F. These supplies and equipment are numerous and some will require occasional repair or replacement. Therefore, the laboratory administrator will maintain a list of items that need service, replacement, or acquisition in order to continue and improve the standard of processing.

2. Taxonomic level of effort

The objective of species identification and enumeration is to accurately identify all organisms sorted from a sample to the lowest possible taxonomic category according to the clients needs and to accurately count the number of organisms in each taxon. Specimens will be identified to the genus level whenever possible. Due to various taxonomic difficulties, certain groups will not be identified to genus (Appendix G).

3. Taxonomic literature

Identifications will be based on current published taxonomic references.

A list of technical literature used in completing the identifications for each macroinvertebrate group has been compiled by the laboratory administrator and is included in Appendix H.

4. Reference collection

The laboratory will prepare a reference collection consisting of the best available specimen(s) in each genus. When a reference specimen is removed from a sample and placed in the reference collection, the taxonomist will document the removal on the identification sheet. The senior taxonomist will finalize a completed and organized reference collection and associated reference specimen list for the laboratory. The reference collection is discussed further in the Quality Assurance and Quality Control Section.

5. Taxonomic Identifications and Enumeration

Specimens will be identified and enumerated from visual inspection in 75% isopropyl alcohol using a high quality dissecting stereomicroscope and will be stored in alcohol filled bottles.

Sample processing for identification and enumeration begins by retrieving the sample bottle for a particular site. Obtain a Benthic Macroinvertebrate Sample ID Form (Appendix I). Record the sample label information on the data sheet. Pour the specimens from the sample bottle into a small plastic Petri dish. Using 75% isopropyl alcohol, rinse the bottle into the Petri dish. Examine the bottle and its lid under the dissecting microscope for specimens. Add more isopropyl alcohol to the tray if needed to cover the specimens.

Begin by viewing the sample under the stereomicroscope using taxonomic keys and other supportive taxonomic literature to identify the specimens. Record on the original taxonomic data sheet: the family and genus identifications; counts of larvae, pupae, and adults as appropriate for the taxonomic group; and comments if any. Upon complete identification of the organisms in the sample, place the completed form and the sample into the designated box on the appropriate shelf. The data from the sheet will be entered into the appropriate EXCEL spreadsheet by laboratory employees, the sheet will be scanned into the appropriate laboratory folder, and the completed Benthic Macroinvertebrate Sample ID Sheet will be stored in a project-specific, three-ring binder containing only Benthic Macroinvertebrate Sample ID Sheets.

After identification and enumeration, return the identified specimens to the labeled 60ml bottle, fill with 75% isopropyl alcohol, and replace the cap tightly. Place the bottle in the designated box according to the information listed on the project board.

When organism fragments are encountered, count only the heads; posterior body fragments will not be counted. Do not count empty snail or clam shells. Larval and pupal exuviae (shed skins) will not be counted, nor will fully emerged, aerial adults (Ephemeroptera, Odonata, Plecoptera, Megaloptera, Neuroptera, Hymenoptera, Diptera, Trichoptera, Lepidoptera), or any terrestrial organisms.

If the taxonomic target level cannot be achieved due to immature, damaged, or pupal specimens this should be noted in the comments section of the data sheet.

Data entry and management

Data from the Benthic Macroinvertebrate Sample Identification Form will be entered into an MS EXCEL database developed by WPI. One laboratory employee or enters the data from the bench sheets, and a Quality Control officer checks all records for accurate entry. The Quality Control officer will report gross mistakes or consistent errors to both the data entry person and also the database manager who will take corrective action. Each Form will then be scanned into the appropriate laboratory folder. The "hard copy" will be filed in a project-specific, three-ring binder containing only Benthic Macroinvertebrate Sample ID Sheets.

Quality Assurance and Quality Control

Responsibility and personnel qualifications

All laboratory personnel will receive basic instruction and evaluation in the sample processing procedure by experienced laboratory staff. A Quality Control (QC) officer must be present when samples are processed by an inexperienced individual (one who has not achieved a >90% sorting efficiency) or when QC checks are needed for 10% of the samples sorted by an experienced sorter (one who has achieved a >90% sorting efficiency). The qualifications of a QC officer include consistent achievement >90% sorting efficiency, taxonomic knowledge of benthic macroinvertebrates, and North American Benthological Society certifications as available.

The roles and responsibilities of the QC Officer are described below.

- Provide oversight of daily operations and sample processing.
- Verify the completeness of every Benthic Macroinvertebrate Sample Sort sheet to ensure information is correctly entered into the header.
- Check sorted grids of all inexperienced laboratory personnel (those who have not achieved a >90% sorting efficiency) for missed organisms and record the number of missed organisms in the appropriate blank on the Benthic Macroinvertebrate Sample Sort sheet.
- Quarterly checks of an experienced individual's samples.

- Ensure that 10% of the samples from each project sorted receive QC.
- Determine the sorting efficiency for each checked sample and sorter. Record the sorter's sorting efficiency on the bench sheet.
- Ensure that QC is maintained during all laboratory sorting and sub-sampling procedures. This includes double-checking work as it is completed and providing written documentation to ensure that our standards are met or exceeded.

A QC check must be performed by qualified laboratory scientists (QC Officers) who are experienced in sorting benthic macroinvertebrate samples, as it is often difficult to detect organisms in benthic samples due to inexperience, the presence of detritus, and different substrate types. These QC checks must be performed immediately following the sorting of each sample to be checked.

Quality control (QC) procedures are used to ensure that the data consists of <10% total error for the extraction of benthic macroinvertebrates from samples and <10% total error for the identification and the enumeration of the extracted organisms.

QC of sample set-up, extraction, sorting, and cleanup

The Quality Control procedure for monitoring taxonomic sample set-up, extraction, and sorting of benthic macroinvertebrates uses a re-sort method to identify unacceptable (>10%) levels of error in the data, and implements corrective actions that decrease the data error to acceptable levels (<10%). Each project received in the laboratory will receive QC on at least 10% of its samples.

- An experienced QC Officer will check all sorted grids from the first five samples processed by a sorter to ensure that each meets the >90% sorting efficiency. Qualification will only occur when a sorter achieves >90% sorting efficiency for five samples consecutively.
- The QC Officer will calculate percent sorting efficiency (PSE) for each sample as follows: PSE = A / (A +B) x 100 where A = the number of organisms found by the primary sorter and B = the number of organisms missed by the primary sorter and found during the QC check. If the sorting efficiency for each of these five consecutive samples is >90% for a particular individual, this individual is considered "experienced".
- In the event that an individual fails to achieve >90% sorting efficiency, they will be required to sort an additional five samples and their sorting efficiency will continue to be monitored until they become "experienced".
- After individuals qualify, their sorted samples will be checked quarterly.
- If an "experienced" individual fails to maintain a >90% sorting efficiency as determined by QC checks, QC checks will be performed on every grid of five consecutive samples until a >90% sorting efficiency is achieved on all five.

QC of taxonomic identification and enumeration

Every scientist will achieve and maintain at least 90% accuracy rating in macroinvertebrate identification.

- Each scientist will have each sample site bottle that he/she identified to genus level reviewed in its entirety by the laboratory administrator or a senior taxonomist until he/she achieves 90% accuracy rating to the family and genus levels.
- At this point the will be considered "experienced".
- Each "experienced" scientist in the lab will perform a 10% sample exchange weekly and Percent Difference in Enumeration and Percent Taxonomic Disagreement will be calculated for each sample.
    - Every Friday afternoon, each "experienced" scientist in the laboratory will randomly select 10% of his/her samples identified for the week. The selected samples will be given to another "experienced" scientist in the lab for identification and enumeration to be completed as the first task on Monday morning of the following week.
    - Percent Difference in Enumeration (PDE) will be calculated for each sample as follows: (n1 – n2) / (n1 + n2) x 100, where n1 is the number of specimens counted in a sample by the first scientist and n2 is the second scientist. The purpose is to find the samples where counts differ and determine the reason for the miscounts. The PDE for each sample checked will be entered into an EXCEL database and kept indefinitely. The goal of the WPI Laboratory is for each scientist to achieve 90% agreement in enumeration for each sample identified.
    - Percent Taxonomic Disagreement (PTD) will be calculated for each sample as follows: 1 – (number of agreements / N) x 100, where N is the total number of specimens in the larger of the two counts. Agreements are determinant upon the targeted level of identification. For example, if family is the target, and one taxonomist provides a name for a specimen at the species level and the other leaves the name at the family level, it would constitute an agreement. However, if genus is the target, and one taxonomist identifies at the genus level, and the other identifies it at the family level, it would not be scored as an agreement. The PTD for each sample checked will be entered into an EXCEL database and kept indefinitely. The goal of the WPI Laboratory is for each scientist to agree 90% of the time in family and genus level identification.
- Each "experienced" scientist will also have 2-3 sample site bottles that he/she identified to genus level reviewed by the laboratory administrator or a senior taxonomist at least four times a year (Quarterly Annual Macroinvertebrate Review).
- If a scientist is not in the lab for two consecutive weeks, the first week he/she returns to the lab, every sample bottle will be spot checked by the laboratory administrator or a senior taxonomist to determine as to what level the scientist is identifying. If the scientist is below 90% accuracy, the previous steps will be repeated until he/she is again determined to be "experienced".

- The Macroinvertebrate Identification Quarterly Quality Assurance Sheet is found in Appendix J. This sheet evaluates a scientist's macroinvertebrate identification skills to family and genus levels by determining the percentage of macros correct to each level. At the bottom of the Q/A Sheet is the scoring system used during the scientists' annual review.
- If the scientist does not achieve at least 90% accuracy during his/her Quarterly Macroinvertebrate Review, problem areas will be reviewed and any additional bottles he/she ID's will be reviewed by the laboratory administrator until he/she reaches the necessary accuracy ratings.
- If a scientist is unable to consistently perform at the designated standards, he/she will be instructed on an individual basis. If the scientist fails to improve to the designated standards, a written disciplinary report will go into his/her employee file.
- If after individual instruction, the scientist does not improve, he/she will no longer be permitted to identify macroinvertebrates in the lab until he/she demonstrates an ability to adhere to the set standards. If identification of macroinvertebrates is over 25% of the scientists' job description, that scientist may be terminated.
- A reference collection will be established and stored in designated cabinets in the benthic macroinvertebrate laboratory. The collection will consist of representative specimens of each species to the lowest identifiable taxon (i.e., genus, family). In addition, species new to the laboratory's reference collection will be sent to recognized experts for taxonomic verification. The verified specimens will then be added to the collection. All specimens in the reference collection will be preserved in 75% isopropyl alcohol in glass vials with labels made of waterproof paper printed with a laser printer. Reference specimens will be organized within major taxonomic groups. The laboratory administrator will maintain the collection log including the organism name, the location of the reference specimen, the status of the specimen if it has been loaned to outside experts, and information about confirmation by outside experts.
- At least one scientist in the laboratory will obtain North American Benthological Society (NABS) certification in Ephemeroptera, Plecoptera, Trichoptera.

The laboratory administrator will provide the tools necessary for the scientists to achieve the required accuracy ratings.

- Each scientist may research new equipment and provide information to the laboratory administrator for review and recommendation at any time throughout the year. (This includes: books, microscopes, cold lamps, forceps, dishes, etc.)
- The macroinvertebrate lab will maintain membership in the North American Benthological Society (NABS) and scientists will have the opportunity to review literature from the Society.
- Individual scientists may be offered the opportunity to participate in NABS or some other professional organization and may be offered the opportunity to take the NABS certification test in a category of his/her choice that is relevant to the laboratory.

Quality Control for *Appendix A Methodology*

Every WPI field scientist will achieve and maintain at least 95% accuracy rating in macroinvertebrate identification to the family level. The laboratory staff will assume the responsibility for training new field scientists in the identification of macroinvertebrates in preparation for the *Appendix A methodology*.

- The laboratory administrator will maintain 15 bottles (numbered 1-15) with various macroinvertebrates previously identified by a senior taxonomist or the laboratory administrator. An answer key will be maintained for each bottle.
- Each bottle will contain: four Plecoptera, Ephemeroptera, Trichoptera, Diptera, and Coleoptera; three Odonata, Molluska, and Crustacea; and one Megaloptera.

- Prior to each field season, each field scientist will be required to schedule time in the laboratory for quality control purposes.
- Each field scientist will be assigned two sample bottles from the 15 bottle collection and will be required to identify the macroinvertebrates in each bottle to the family level. The scientist will record his/her answers on the Macroinvertebrate Identification for *Appendix A Methodology* Quality Control Sheet found in Appendix K.
- During the identification process, the scientists will be permitted to use any literature they would carry with them in the field, and either a hand lens or the magnifying lamp found in the lab.
- After the scientist has submitted their answer sheet to the laboratory administrator, they may view the insects from their samples under a stereo microscope.
- The laboratory administrator will compare each answer sheet with the corresponding answer key and calculate the percent of identification accuracy for the two bottles collectively.
- If a scientist achieves 95% accuracy, he/she is permitted to perform *Appendix A* field work. The laboratory administrator will inform their immediate supervisor.
- If the scientist does not achieve at least 95% during his/her testing, problem areas will be reviewed. The scientist will be provided with materials to review and a second test will be scheduled.
- When the scientist feels he/she is prepared, they may attempt the test a second time by identifying two new bottles assigned by the laboratory administrator.
- If a scientist is unable to consistently perform at the 95% standard, he/she will be instructed on an individual basis. If the scientist fails to improve to the designated standards, a written disciplinary report will go into his/her employee file.
- If after individual instruction, the scientist does not improve, he/she will not be permitted to perform *Appendix A* methodology until he/she demonstrates an ability to adhere to the set standards. If *Appendix A* methodology is over 25% of the scientists' job description, that scientist may be terminated.

Laboratory Supply Ordering Procedure

It is the responsibility of the laboratory administrator to maintain a well-stocked laboratory. It is the responsibility of the laboratory staff to inform the laboratory administrator of supplies needed.

To order laboratory supplies:

- Obtain a Laboratory Supply Order Form, (Appendix L) from the administrative binder on the shelf in the laboratory.
- On page 1, complete the date, place your name as the "requestor" and sign, complete the "need by" date only if you need the item by a certain date, if not, place "N/A" in that space.
- If the item is one that is frequently ordered, refer to the WPI catalog sheet attached to the front of the folder for the WPI catalog number, place that number in the "WPI Catalog number" space, and enter the quantity needed.
- If the item has not been previously ordered, enter as much information on page 2 as is known about the needed item including a detailed description, the size, color, etc. Also include supplier's name, address, phone number, website, email, etc. If the item must come from a specific supplier, please specify not to substitute and the reason.
- Place the completed form in the "In Box" on the desk in the laboratory.

The laboratory administrator will submit a Purchase Order Form (Appendix M) to the appropriate supervisor for approval and will then submit the approved request to the secretary for ordering.

The laboratory manager will track the items using the Laboratory Supply Tracking Form (Appendix N).

Laboratory Sample Identification Requests

WPI staff members who need samples processed by the macroinvertebrate laboratory should obtain a Benthic Macroinvertebrate Sample ID Request Form (Appendix O) and complete the required fields on the top half of the form. After completing the form, it should be submitted to the project manager who will verify the need for the data and assign it a priority level according to project deadlines. The project manager will sign the request and submit it to the laboratory administrator who will document the date the form was received in the lab and determine if the macroinvertebrate data will be completed by the date requested. After data is compiled, an email will be sent to the requestor verifying completion of the samples and giving notification of the location of the file in the laboratory folder. The request form will be placed in the requestor's mailbox and after the requestor has removed the data from the folder, he/she should sign the bottom of the form and place it in the laboratory administrator's mailbox. Upon receipt of the completed form, the laboratory administrator will delete the file from the laboratory folder. Therefore, it is imperative that the form is not signed and returned to the lab prior to retrieving the data from the appropriate folder.

Laboratory Safety

It is the policy of Wallace & Pancher, Inc. to protect the safety and health of each part-time or full-time employee. The laboratory supervisor is responsible for describing safety and health procedures, providing job training, and hazard elimination practices.

Safe laboratory practices must be followed at all times.

- Emergency exits are clearly marked and emergency exit routes are posted throughout the lab.
- Emergency exits must be kept clear of debris and walkways kept clear.
- Fire extinguishers must be easily accessible with no clutter obstructing them.
- Each employee should know the locations of emergency exits, first aid kits, and fire extinguishers.
- Each employee should know communication procedures in the case of an emergency.
- Fire extinguishers must be checked monthly.
- All cabinet and desk drawers must be kept closed when not in use.
- Laboratory chemicals must be disposed of properly. Isopropyl alcohol may be disposed of in the sink in the rinsing room. Water must run for 2-3 minutes to flush and dilute the isopropyl alcohol. The City of Hermitage Waste Water Treatment Facility is to be notified if a quantity of alcohol over 1 gallon is to be disposed of at one time.
- All containers containing isopropyl alcohol should be clearly marked.
- All containers containing chemicals other than alcohol should be clearly marked.
- Materials Safety Data Sheets for all chemicals used in the laboratory are readily available in the administrative binder kept on the shelf in the laboratory.
- Slop buckets and waste cans should be emptied daily.
- Work areas must be kept clean and neat.
- Laboratory ventilation must be activated when using sorting/ID procedures containing isopropyl alcohol.
- Wallace & Pancher, Inc. employees are required to participate in safety committee activities and support safety committee membership.

Safety hazards must be reported to the laboratory supervisor immediately. A safety report may be completed by any laboratory employee who witnesses a safety violation. The Safety reports can be found in the administrative binder on the shelf in the laboratory. Upon completion, the report is to be given to the laboratory supervisor. If the laboratory employee does not feel comfortable giving the report to the laboratory supervisor, the report may be given to any safety committee member. A

list of safety committee members is maintained in the folder with the Safety Reports. No job in the laboratory is so important that we cannot take time to do it safely.

A laboratory safety checklist is attached in Appendix P.

A Safety Report is attached in Appendix Q.

General Laboratory Policies

The WPI laboratory equipment list is attached in Appendix R. Each scientist is responsible for the equipment he/she is using for the day. Any problems with equipment should be reported to the laboratory administrator as soon as possible. If any accidents involve damage to equipment, an incident report (Appendix S) must be filed with the laboratory administrator.

Clients often enter the upstairs office for appointments and many times may be brought into the laboratory for various reasons. Maintain a professional working atmosphere in the lab. Listening to the radio at an acceptable volume and quite conversations are appropriate. Excessive noise, inappropriate music or radio shows will not be tolerated. Out of respect for others in the building, exhaust fans must be used when sorting.

Prior to leaving the lab for the day, please consider the following:

- All equipment must be turned “off”
- Microscopes should be wiped clean and covered
- All “slop buckets” must be emptied outside
- Stations should be wiped clean
- Any equipment used during the day should be cleaned and returned to its proper storage place.
- Fans, heaters, and lights must be turned “off”.

APPENDIX A

Materials List
For Receipt, Initial Rinse, Set-Up, Sub-Sampling, and Sorting of Benthic Macroinvertebrate Samples

Materials List for receipt, initial rinse, setup, subsampling, & sorting of samples

Attachment 1 – Materials List for receipt, initial rinse, setup, subsampling, and sorting of samples
Attachment 2 - Benthic Macroinvertebrate Chain-of-Custody form if needed (one per project)
Attachment 3 - Benthic Macroinvertebrate Sample Log-in form (one per project)
Attachment 4 - Benthic Macroinvertebrate Sample Sort Sheet (one per sample)
Storage shelves and boxes for unsorted, sorted, and identified samples
5-gallon plastic storage/dispense container for isopropyl alcohol
Isopropyl alcohol 75% by volume
U.S. standard soil sieve # 35 (500 µm)
2-gallon plastic round buckets
Large forceps
24 grid porcelain pans used as macroinvertebrate sub-subsampling devices
2” diameter pipe fittings
Random number generators (bottle caps numbered 1-24)
Dropping pipettes
White plastic teaspoons
Scoops constructed of dowel rods and mesh netting
Exacto knives
Fine point jewelers forceps (#5)
Fine point dissecting probes (probe handles with #000, #1, #3, and #7 insect pins)
Plastic wide-mouth pint jars
Plastic wash bottles (500ml)
Plastic squirt bottles (500ml or smaller)
Plastic petri dishes with lid
Glass vials (1 dram) with caps
Magnified ring lights (3X)
Preprinted sample-jar labels
Calculators
Clear packing tape
Pencils
Permanent felt-tip markers
Highlighters
Post-it notepads
3-ring binders (1.5 inch, 2 inch, 3 inch)
Binder dividers
Materials Safety Data Sheets (MSDS) for all chemicals used in the lab
Paper towels
Dishtowels
Hand soap
Dish soap
All-purpose spray cleaner

APPENDIX B

Benthic Macroinvertebrate Chain-of-Custody Form

Wallace & Pancher, Inc. Laboratory
Chain of Custody Form

Business: _____
Name:_____
Address: _____
_____
_____
Phone: _____

Contents:	Total Number of:
_____ Macroinvertebrates	_____ jars
_____ Fish	_____ vials
	_____ other

Page _____ of _____

Wallace & Pancher, Inc. Laboratory
1085 South Hermitage Road
Hermitage, PA 16148
Lab Phone: 724-981-0155 x110
Fax: 724-981-0156

Project	Sample ID	Collection Date	# of Bottles	Comments	WPI Rec'd	Date Rec'd	Date Shipped

Comments: _____

WPI Personnel Packed By: _____
Date Packed: _____
Shipped By: _____
Shipping Method: _____

Post Processing
Recipient: Please complete and fax this form back to Wallace & Pancher, Inc. Laboratory at 724-981-0156
Recipient Signature: _____
Date Received: _____

APPENDIX C

Benthic Macroinvertebrate Sample Log-In Form

Wallace & Pancher, Inc. Laboratory
Sample Log In Form

Team Members: _____ Date: _____

Mine/District or Project Name: _____

#	Sample ID	Collection Date	# of Bottles	Methodology	Submitted By (Initials)	Rec'd	Received By (Initials)
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
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25							
26							
27							
28							
29							
30							
31							
32							

Methodology Codes: SRBP - Standard RBP Methodology MRBP - Modified RBP Methodology
APPB - Appendix B Methodology APPA - Appendix A Methodology

APPENDIX D

Laboratory Sample Log Sheet

Project Name
Season
Methodology

Date	Site	Sort Date	Sorted By	Hours	ID Date	ID'd By	Hours	Macro Data Entered By	Date	Bio Score #1 (Initials & Score)	Date	Bio Score #2 (Initials & Score)	Date

APPENDIX E

Standard and Modified RBP Methodologies
and
Appendix B Methodology

Standard RBP Methodology **sample rinsing and set-up, sorting, and cleanup**

This procedure functions to provide samples that are free of the field-applied isopropyl alcohol to laboratory personnel who will subsample and sort the samples in the benthic macroinvertebrate laboratory.

Rinsing and set-up:

1. Obtain a sample from the appropriate box on the designated shelf.

2. Record the sample label information, date of sample rinse and sorting, and the initials of the person rinsing and sorting the sample on the laboratory log sheet for the designated project and on the Benthic Macroinvertebrate Sample Sort sheet (Appendix T). (Keep track of the number of hours spent sorting the sample as this will be recorded on the log sheet as well.)

3. Pour and spread the sample evenly onto a U.S. standard soil sieve #35 (500µm) held above a waste bucket in the sink. Use large forceps if necessary to empty the sample container and spread the sample over the sieve. Drain the alcohol solution into the bucket and gently rinse the sample with water, draining the rinse water into the bucket until the water runs clear. Rinse the sample bottle through the sieve into the bucket to collect any residue remaining in the sample jar. Place used sample jars and lids into the "needs washed" bottle bin in the lab.

4. Remove the rinsed sample from the sieve to the gridded sorting tray, back rinse the sieve to collect all sample residues into the tray and add enough water to the tray to cover the sample.

5. If the sample contains a low amount (one jar) of inorganic substrate such as sand or gravel, proceed in the following manner. Transfer the sample material to the gridded pan and spread the contents evenly across the pan. If the amount of leaf litter or other detritus material exceeds that which fills the gridded pan, then divide the sample among two or more gridded pans.

6. If the sample contains a large amount (two or more jars) of inorganic substrate such as sand or gravel, carefully empty each sample jar into its own gridded pan.

7. Backwash the sieve to prevent cross-contamination of samples.

8. After the sediment from rinsing the sample has settled to the bottom of the waste bucket in the sink, dump the water from the top of the bucket through the sieve

into the drain, reserving the sediment at the bottom of the bucket. Add water to the bucket and dump the bucket and sediment behind the building in the gravel area designated for samples.

9. Transport rinsed samples to the benthic macroinvertebrate laboratory to begin the sub-sampling procedure.

Quantitative samples are sub-sampled to a randomized fixed count of 200 +/-20% benthic macroinvertebrate specimens per sample using standard EPA laboratory sorting methods or sorted whole if the target number of 200 is not reached. All sub-sampling is done using the 24-grid white porcelain sorting trays. If a sample is spread between two or more sorting pans, repeat each of the following steps on each pan, combining the organisms from each gridded pan into one porcelain sorting pan.

Sorting:

1. Remove large objects (sticks, stones, empty clam shells) and carefully inspect them under the 3X magnifying ring light for organisms. Return organisms, if found, to the sample in the gridded pan and place the large objects into a residue bucket. Gently spread the sample material over the bottom of the gridded pan as evenly as possible. Move the sample material into the corners of the gridded screen using forceps. Gently vibrate or shake the pan to help spread the sample.

2. Use a random number generator (numbered bottle caps) to select four grids (sub-sample) from the gridded tray. The goal is to randomly select at least four of the grids from the 24 grids on the gridded screen in an effort to ensure that the sub-sample material is representative of the overall sample. Record the total number of randomly chosen grids on the Benthic Macroinvertebrate Sample Sort Sheet (Attachment 4).

3. Place the plastic dividing frame (pipe fitting) over the sample at the approximate locations of the grids selected for processing based on the numbers marked on the gridded tray.

4. Remove or extract the sample material within the pipe fitting (step 3) from each of the four grids using a white plastic teaspoon, mesh scoop, and/or forceps. Place the extracted sample material in a white bowl or white porcelain pan and add water to cover the sample material. Rinse the extraction tools and the inside surface of the pipe fitting over the bowl or pan and inspect these tools and the grid for any remaining organisms using the 3X magnifying ring light.

Use the following rules when dealing with organisms that lie on the line between two grids:

- An organism belongs to the grid containing its head.
- If it is not possible to determine the location of the head (i.e., for worms), the organism is considered to be in the grid containing most of its body
- If the head of an organism lies on the line between two grids, all organisms on the top border of a grid and those on the right border of a grid belong in that grid, and are picked with that grid.

5. Set the gridded tray aside until the macroinvertebrates have been sorted and counted from the previously extracted grid material.

6. If the number of organisms within the first four grids appears to be lower than the sample target count (200 +/-20%), proceed in the following manner:
    - • If after sorting and counting all the organisms from the first four grids the organism count is greater than 140, put aside the organisms for that count and randomly choose a new grid following step 2.
    - • If four complete grids have yielded fewer than 30 organisms, group the next four randomly chosen grids and sort and count the target organisms. If the organism count from the first eight grids is lower than 60, extract the entire remaining sample from the gridded pan and sort and count the benthic macroinvertebrates from it. Write in the "Number of Grids Picked" section of the Benthic Macroinvertebrate Sample Sort sheet that all grids were combined for sorting. If the organism count from the first six grids is higher than 120, resume random selection of individual grids until the target count (200 +/-20%) is reached. If it is uncertain whether a sample should be processed whole, it is at the discretion of the laboratory supervisor to determine whether a sample can be processed whole or by individual grids.

7. If the number of organisms in any four grids appears to exceed the target count (200 +20%), spread the sorted organisms in a second gridded pan and randomly choose individual grids, picking the organisms from each grid until the expected target count (200 +20%) is reached. Once a grid is chosen, the entire grid must be picked. Document in the "General Comments" section of the Benthic Macroinvertebrate Sample Sort sheet that this procedure was used.

8. Obtain a 60ml bottle from the bottle storage area and label it with the appropriate sample label from the project clipboard in the laboratory. Add approximately 45ml of 75% Isopropyl Alcohol.

9. Slowly search the entire pan in a systematic pattern to locate all identifiable benthic macroinvertebrates. First search the base of the pan and then search focusing on the surface of the water, looking for surface floating organisms. Remove organisms and place in the appropriately labeled 60ml bottle keeping a tally of the number of organisms placed in the bottle. Record the total number of organisms removed from the sample in the appropriate space on the Benthic Macroinvertebrate Sample Sort Sheet.

10. Do not remove or count: empty snail or bivalve shells; empty caddisfly cases; fragments such as legs, antennae, gills, wings, or headless bodies; round worms (Nematoda); microcrustacea (copepods, ostracods, branchiopods); eggs; or winged adult aquatic insects (except Coleoptera). Search inside empty snail and bivalve shells and caddisfly cases for the presence of smaller target organisms. Also, search inside aquatic plant stems and leaves for small invertebrates such as diptera larvae and pupae that mine such tissues. Insects thought to be terrestrial should be verified as such by a taxonomist at sorting time or placed in the labeled bottle for later verification but should not be counted. For segmented worms (Oligochaeta) remove and count only whole bodies and fragments that include a rounded end that could be a head or tail end. Count Oligochaeta end fragments as 1/2 counts (two ends equals one count). Count a whole worm as one count. If unsure as to whether any specimen should be counted, place the organism in the labeled bottle without counting it (the final identity and count will be made by a taxonomist).

11. Record the date each sample was sorted in the appropriate space on the Benthic Macroinvertebrate Sample Sort sheet. Keep a record of the amount of time spent sorting each sample and record it in the appropriate space on the Benthic Macroinvertebrate Sample Sort sheet. Record the total number of grids chosen in the appropriate space on the Benthic Macroinvertebrate Sample Sort Sheet.

12. Prior to discarding the remaining un-sorted sample, briefly look through the sample and record the presence of any large or obviously abundant organisms in the appropriate space on the Benthic Macroinvertebrate Sample Sort Sheet.

13. Place the completed Benthic Macroinvertebrate Sample Sort sheet in the project-specific binder with the Benthic Macroinvertebrate Sample Log-in form.

14. Record the date sorted, initials of the person sorting, and the number of hours spent sorting the sample on the laboratory log sheet on the clipboard in the lab.

Sample cleanup:

- Clean the gridded pan using dish soap or all-purpose cleaner and a rag if needed and then backwash the screen.

- Thoroughly wash and rinse all sorting equipment used including pipe-fittings, scoops, bowls, etc.

- Thoroughly wipe the laboratory table using all purpose cleaner and paper towels.

Modified RBP Methodology sample rinsing and set-up, sorting, and cleanup

Rinsing and set-up:

1. Obtain the three sample bottles corresponding to the designated site from the appropriate box on the designated shelf.

2. Record the sample label information, date of sample rinse and sorting, and the initials of the person rinsing and sorting the sample on the laboratory log sheet for the designated project. (Keep track of the number of hours spent sorting the sample as this will be recorded on the log sheet as well.)

3. Process each sample bottle (1 of 3, 2 of 3, and 3 of 3) separately repeating the next steps for each bottle from each site. Pour and spread the contents of the sample evenly onto a U.S. standard soil sieve #35 (500µm) held above a waste bucket in the sink. Use large forceps if necessary to empty the sample container and spread the sample over the sieve. Drain the alcohol solution into the bucket and gently rinse the sample with water, draining the rinse water into the bucket until the water runs clear. Rinse the sample bottle through the sieve into the bucket to collect any residue remaining in the sample jar. Place used sample jars and lids into the "needs washed" bottle bin in the lab.

4. Remove the rinsed sample from the sieve to a sorting tray, back rinse the sieve to collect all sample residues into the tray and add enough water to the tray to cover the sample.

5. Backwash the sieve to prevent cross-contamination of samples.

6. After the sediment from the sample has settled to the bottom of the bucket, dump the water from the top of the bucket into the drain, reserving the sediment at the bottom of the bucket. Add water to the bucket and dump the bucket and sediment behind the building in the gravel area designated for samples.

7. Transport rinsed samples to the benthic macroinvertebrate laboratory to begin the sorting procedure.

Samples are sorted whole, removing every insect from each sample.

Sorting:

1. Remove large objects (sticks, stones, empty clam shells) and carefully inspect them under the 3X magnifying ring light for organisms. Return organisms if found to the sample in the gridded pan and place the large objects into a residue bucket.

2. Obtain a 60ml bottle and label it with the appropriate sample labels found on the project clip board in the lab. Add approximately 45ml of 75% Isopropyl Alcohol.

3. Slowly search the entire pan in a systematic pattern to locate all identifiable benthic macroinvertebrates. First search the base of the pan and then search focusing on the surface of the water, looking for surface floating organisms. Remove organisms and place in the appropriately labeled 60ml bottle keeping a tally of the number of organisms placed in the bottle. Record the total number of organisms removed from the sample in the appropriate space on the Benthic Macroinvertebrate Sample Sort Sheet.

4. Do not remove or count: empty snail or bivalve shells; empty caddisfly cases; fragments such as legs, antennae, gills, wings, or headless bodies; round worms (Nematoda); microcrustacea (copepods, ostracods, branchiopods); eggs; or winged adult aquatic insects (except Coleoptera). Search inside empty snail and bivalve shells and caddisfly cases for the presence of smaller target organisms. Also, search inside aquatic plant stems and leaves for small invertebrates such as diptera larvae and pupae that mine such tissues. Insects thought to be terrestrial should be verified as such by a taxonomist at sorting time or placed in the labeled bottle for later verification but should not be counted. For segmented worms (Oligochaeta) remove and count only whole bodies and fragments that include a rounded end that could be a head or tail end. Count Oligochaeta end fragments as 1/2 counts (two ends equals one count). Count a whole worm as one count. If unsure as to whether any specimen should be counted, place the organism in the labeled bottle without counting it (the final identity and count will be made by a taxonomist).

5. Record the date each sample was sorted in the appropriate space on the Benthic Macroinvertebrate Sample Sort sheet. Keep a record of the amount of time spent sorting each sample and record it in the appropriate space on the Benthic Macroinvertebrate Sample Sort sheet. Record the total number of grids chosen in the appropriate space on the Benthic Macroinvertebrate Sample Sort Sheet.

6. Prior to discarding the remaining un-sorted sample, briefly look through the sample and record the presence of any large or obviously abundant organisms in the appropriate space on the Benthic Macroinvertebrate Sample Sort Sheet.

7. Place the completed Benthic Macroinvertebrate Sample Sort sheet in the project-specific binder with the Benthic Macroinvertebrate Sample Log-in form.

8. Record the date sorted, initials of the person sorting, and the number of hours spent sorting the sample on the laboratory log sheet on the clipboard in the lab.

Sample cleanup:

1. Clean the gridded pan using dish soap or all-purpose cleaner and a rag if needed and then backwash the screen.

2. Thoroughly wash and rinse all sorting equipment used including pipe-fittings, scoops, bowls, etc.

3. Thoroughly wipe the laboratory table using all purpose cleaner and paper towels.

PADEP *Appendix B Methodology* sample rinsing and set-up, sorting, and cleanup

Rinsing and set-up:

1. Obtain a sample from the appropriate box on the designated shelf.

2. Record the sample label information, date of sample rinse and sorting, and the initials of the person rinsing and sorting the sample on the laboratory log sheet for the designated project. (Keep track of the number of hours spent sorting the sample as this will be recorded on the log sheet as well.)

3. Pour and spread the sample evenly onto a U.S. standard soil sieve #35 (500µm) held above a waste bucket in the sink. Use large forceps if necessary to empty the sample container and spread the sample over the sieve. Drain the alcohol solution into the bucket and gently rinse the sample with water, draining the rinse water into the bucket until the water runs clear. Rinse the sample bottle through the sieve into the bucket to collect any residue remaining in the sample jar. Place used sample jars and lids into the "needs washed" bottle bin in the lab.

4. Remove the rinsed sample from the sieve to the gridded sorting tray, back rinse the sieve to collect all sample residues into the tray and add enough water to the tray to cover the sample.

5. If the sample contains a low amount (one jar) of inorganic substrate such as sand or gravel, proceed in the following manner. Transfer the sample material to the gridded pan and spread the contents evenly across the pan. If the amount of leaf litter or other detritus material exceeds that which fills the gridded pan, then divide the sample among two or more gridded pans.

6. If the sample contains a large amount (two or more jars) of inorganic substrate such as sand or gravel, carefully combine all sample bottles together into one gridded pan.

7. Backwash the sieve to prevent cross-contamination of samples.

8. After the sediment from the sample has settled to the bottom of the bucket, dump the water from the top of the bucket into the drain, reserving the sediment at the bottom of the bucket. Add water to the bucket and dump the bucket and sediment behind the building in the gravel area designated for samples.

9. Transport rinsed samples to the benthic macroinvertebrate laboratory to begin the sub-sampling procedure.

Quantitative samples are sub-sampled to a randomized fixed count of 200 +/-20% benthic macroinvertebrate specimens per sample using standard EPA EMAP laboratory sorting

methods or sorted whole if the target number of 200 is not reached. All sub-sampling is done using the 24-grid white porcelain sorting trays.

Sorting:

1. Remove large objects (sticks, stones, empty clam shells) and carefully inspect them under the 3X magnifying ring light for organisms. Return organisms if found to the sample in the gridded pan and place the large objects into a residue bucket. Gently spread the sample material over the bottom of the gridded pan as evenly as possible. Move the sample material into the corners of the gridded screen using forceps. Gently vibrate or shake the pan to help spread the sample.

2. Use a random number generator (numbered bottle caps) to select four grids (sub-sample) from the gridded tray. The goal is to randomly select at least four of the grids from the 24 grids on the gridded screen in an effort to ensure that the sub-sample material is representative of the overall sample. Record the randomly chosen numbers chronologically on the Benthic Macroinvertebrate Sample Sort sheet (Attachment 4) in the "Random Number Grid ID" column. Mark the "Grid Order" number in the coordinating box at the bottom of the page.

3. Place the plastic dividing frame (pipe fitting) over the sample at the approximate locations of the grids selected for processing based on the numbers marked on the gridded tray.

4. Remove or extract the sample material within the pipe fitting (step 3) from each of the four grids using a white plastic teaspoon, mesh scoop, and/or forceps. Place the extracted sample material in a white bowl or white porcelain pan and add water to cover the sample material. Rinse the extraction tools and the inside surface of the pipe fitting over the photo tray and inspect these tools and the grid for any remaining organisms using the 3X magnifying ring light.

Use the following rules when dealing with organisms that lie on the line between two grids:

- An organism belongs to the grid containing its head.
- If it is not possible to determine the location of the head (i.e., for worms), the organism is considered to be in the grid containing most of its body
- If the head of an organism lies on the line between two grids, all organisms on the top border of a grid and those on the right border of a grid belong in that grid, and are picked with that grid.

5. Set the gridded tray aside until the macroinvertebrates have been sorted and counted from the previously extracted grid material.

6. If the number of organisms within the first four grids appears to be lower than the sample target count (200 +/-20%), proceed in the following manner:
   - If, after sorting and counting all the organisms from the first four grids, the organism count is greater than 140 then: put aside the organisms for that count and randomly choose a new grid following step 2.
   - If four complete grids have yielded fewer than 30 organisms, then group the next four randomly chosen grids and sort and count the target organisms. If the organism count from the first eight grids is lower than 60, then extract the entire remaining sample from the gridded pan and sort and count the benthic macroinvertebrates from it. Write in the "General Comments" section of the Benthic Macroinvertebrate Sample Sort sheet that the remaining grids were combined for sorting. If the organism count from the first six grids is higher than 120, then resume random selection of individual grids until the target count (200 +/-20%) is reached. If it is uncertain whether a sample should be processed whole, it is at the discretion of the laboratory supervisor to determine whether a sample can be processed whole or by individual grids.

7. If the number of organisms in any four grids appears to exceed the target count (200 +20%), spread the sorted organisms in a second gridded pan and randomly choose individual grids, picking the organisms from each grid until the expected target count (200 +20%) is reached. Once a grid is chosen, the entire grid must be picked. Document in the "General Comments" section of the Benthic Macroinvertebrate Sample Sort sheet that this procedure was used.

8. Obtain a 60ml bottle from the bottle storage area and label it with the appropriate sample label from the project clipboard in the laboratory. Add approximately 45ml of 75% Isopropyl Alcohol.

9. Slowly search the entire pan in a systematic pattern to locate all identifiable benthic macroinvertebrates. First search the base of the pan and then search focusing on the surface of the water, looking for surface floating organisms. Remove organisms and place in the appropriately labeled 60ml bottle keeping a tally of the number of organisms placed in the bottle. Record the total number of organisms removed from the sample in the appropriate space on the Benthic Macroinvertebrate Sample Sort Sheet.

10. Do not remove or count: empty snail or bivalve shells; empty caddisfly cases; fragments such as legs, antennae, gills, wings, or headless bodies; round worms (Nematoda); microcrustacea (copepods, ostracods, branchiopods); eggs; or winged adult aquatic insects (except Coleoptera). Search inside empty snail and bivalve shells and caddisfly cases for the presence of smaller target organisms. Also, search inside aquatic plant stems and leaves for small invertebrates such as diptera larvae and pupae that mine such tissues. Insects thought to be terrestrial should be verified as such by a taxonomist at sorting

time or placed in the labeled bottle for later verification but should not be counted. For segmented worms (Oligochaeta) remove and count only whole bodies and fragments that include a rounded end that could be a head or tail end. Count Oligochaeta end fragments as 1/2 counts (two ends equals one count). Count a whole worm as one count. If unsure as to whether any specimen should be counted, place the organism in the labeled bottle without counting it (the final identity and count will be made by a taxonomist).

11. Record the date each sample was sorted in the appropriate space on the Benthic Macroinvertebrate Sample Sort sheet. Keep a record of the amount of time spent sorting each sample and record it in the appropriate space on the Benthic Macroinvertebrate Sample Sort sheet. Record the total number of grids chosen in the appropriate space on the Benthic Macroinvertebrate Sample Sort Sheet.

12. Prior to discarding the remaining un-sorted sample, briefly look through the sample and record the presence of any large or obviously abundant organisms in the appropriate space on the Benthic Macroinvertebrate Sample Sort Sheet.

13. Place the completed Benthic Macroinvertebrate Sample Sort sheet in the project-specific binder with the Benthic Macroinvertebrate Sample Log-in form.

14. Record the date sorted, initials of the person sorting, and the number of hours spent sorting the sample on the laboratory log sheet on the clipboard in the lab.

Sample cleanup:

1. Clean the gridded pan using dish soap or all-purpose cleaner and a rag if needed and then backwash the screen.

2. Thoroughly wash and rinse all sorting equipment used including pipe-fittings, scoops, bowls, etc.

3. Thoroughly wipe the laboratory table using all purpose cleaner and paper towels.

APPENDIX F

Materials List
for Taxonomic Identification
of Samples

Materials List for Taxonomic Identification of Samples

Taxonomic Literature (see Attachment 7)
Storage shelves and boxes for sorted and identified samples
5-gallon plastic storage/dispense container for isopropyl alcohol
Isopropyl alcohol 75% by volume
Dropping pipettes
Fine point jewelers forceps (#5)
Fine point dissecting probes (probe handles with #000, #1, #3 and #7 insect pins)
Plastic 60ml Nalgene bottles and lids
Plastic wash bottles (500ml)
Plastic 500ml bottles with squirt tops
Plastic petri dishes with lid
Glass vials (1 dram) with cone-insert caps
Stereomicroscopes (15X – 90X)
Fiber optic microscope lights
Lens paper
Preprinted sample-vial labels
Calculators
Clear packing tape
Pencils
Paper scissors
Permanent felt-tip markers
Highlighters
Post-it notepads
3-ring binders (2 inch, 3 inch)
Binder dividers
Materials Safety Data Sheets (MSDS) for all chemicals used in the lab
Paper towels
Dishtowels
Hand soap
Dish soap

APPENDIX G

Standard Taxonomic Level-of-Effort for Benthic Macroinvertebrate Identification

Standard Taxonomic Effort List
Wallace & Pancher Inc.

Phylum Mollusca		
Class Gastropoda	snails, limpets	family
Class Bivalvia	clams, mussels	family
Phylum Annelida		
Subclass Oligochaeta	aquatic earthworms	subclass
Subclass Hirudinea	leeches	subclass
Phylum Arthropoda		
Order Amphipoda	scuds, sideswimmers	family
Order Isopoda	slaters, aquatic sowbugs	family
Order Decapoda	crayfish	family
Class Entognatha		
Order Collembola	aquatic springtails	order
Class Insecta		
Order Ephemeroptera	mayflies	genus
Order Trichoptera	caddisflies	genus
Order Odonata	damselfies, dragonflies	genus
Order Plecoptera	stoneflies	genus
Order Megaloptera	fishflies, alderflies, dobsonflies	genus
Order Coleoptera (larvae)	aquatic beetles	genus
Family Elmidae (adult)		genus
Order Diptera	aquatic flies	genus
Except: Chironomidae	midge flies	family

APPENDIX H

Taxonomic Literature
for Benthic Macroinvertebrate Identification

Taxonomic Literature Use by WPI Laboratory Personnel for Benthic Macroinvertebrate Identification

Edmunds, G. F., Jr., Jensen, S.L., and L. Berner. 1976. Mayflies of North and Central America. University of Minnesota Press, Minneapolis.

Epler, J. H. 1996. Identification Manual for the Water Beetles of Florida.

Gelhaus, Jon K. 2002. Manual for the Identification of Aquatic Crane Fly Larvae of Southeastern United States. Prepared for Carolina Area Benthological Workshop.

Johannsen, O.A. and Lillian C. Thomsen. 1970. Aquatic Diptera. Entomological Reprint Specialists, Los Angeles, California.

Merrit, R.W. and K.W. Cummins. 2008. An Introduction to the Aquatic Insects of North America, Second Edition. Kendall/Hunt Publishing Co., Dubuque, Iowa.

Peckarsky, B.L., Frassinet, P.R., Penton, M.A. and D.A Conklin, Jr. 1990. Freshwater Macroinvertebrates of Northeastern North America. Cornell University Press. Ithaca, New York.

Stewart, K.W. and B.P. Stark. 1993. Nymphs of North American Stonefly Genera (Plecoptera). University of North Texas, Denton, Texas.

Thorp, James H. and Alan P. Covich. 1991. Ecology and Classification of North American Freshwater Invertebrates. Academic Press. San Diego, California.

Voshell, J.R., Jr. 2005. A Guide to Common Freshwater Invertebrates of North America. The McDonald and Woodward Publishing Company, Blacksburg, Virginia.

Wiggins, Glenn B. 1977. Larvae of North American Caddisfly Genera (Trichoptera). University of Toronto Press, Toronto.

APPENDIX I

Benthic Macroinvertebrate Sample Identification Form

Wallace & Pancher, Inc. Laboratory
Benthic Macroinvertebrate Sample Identification Form

Project: _____ Sample ID: _____ Collection Date: _____

Season: _____ Round # (If Applicable):_____

ID'd By: _____ ID Date: _____

Time Expenditure: _____ Total # of Organisms: _____

Quality Control:

Sample Used for Quality Control? _____ Yes _____ No

Quality Control Officer Name: _____ Date: _____

Total # of Families in Sample:_____ Total # Correct to Family:_____ Percent Correct:_____

Total # of Genus in Sample:_____ Total # Correct to Genus:_____ Percent Correct:_____

Sample Passes (>90%) _____ Sample Fails (<90%) _____

APPENDIX J

Benthic Macroinvertebrate Identification
Quarterly Quality Control Sheet
for
Appendix B and RBP Methodologies

Wallace & Pancher, Inc. Laboratory
Macroinvertebrate Identification (Appendix B & RBP)
Quarterly Quality Control Sheet

Scientist Name: _____ Year: _____ Quarter: 1 2 3 4

Date of Review with Scientist: _____

Bottle #1 Sample Checked: _____ Project/ Mine: _____

Sample Date: _____

Total Genera in Sample: _____ Total Correct: _____

Total correct to family level: _____ Percent correct: _____

Problem Families and Genus:

_____

_____

_____

Bottle #2 Sample Checked: _____ Project/ Mine: _____

Sample Date: _____

Total Genera in Sample: _____ Total Correct: _____

Total correct to family level: _____ Percent correct: _____

Problem Families and Genus:

_____

_____

_____

Combined percentage correct to family: _____

Combined percentage correct to genus: _____

Scientist Signature: _____

Quality Control Officer: _____

Annual Review Scoring: 97%-100% - 5 90.1%-96.9% - 4 range 87%-90% - 3 range
83%-86.9% - 2 range 78%-82.9% - 1 range <78% - 0

APPENDIX K

Benthic Macroinvertebrate Identification Quality Control Sheet

for *Appendix A Methodology*

Wallace & Pancher, Inc. Laboratory
Macroinvertebrate Identification (Appendix A)
Quality Control Sheet

Scientist Name: _____ Date: _____

Sample Bottle Number:_____

Identify the organisms in the sample to family level.

Plecoptera:	Diptera:	Molluska:
1.	1.	1.
2.	2.	2.
3.	3.	3.
4.	4.	

Ephemeroptera:	Coleoptera:	Megaloptera:
1.	1.	1.
2.	2.	
3.	3.	
4.	4.	

Trichoptera:	Odonata:	Crustacea:
1.	1.	1.
2.	2.	2.
3.	3.	3.
4.		

Quality Control:

Quality Control Officer: _____

Total # of Families in Sample:_____ Total # Correct to Family:_____ Percent Correct:_____

> 90% sample passes _____ < 90% sample fails _____

APPENDIX L

Laboratory Supply Order Form

WPI
WALLACE & PANCHER, INC.
ENGINEERING & ENVIRONMENTAL

Laboratory Supply Order Form

Request Date: _____ **Need by (date): _____**

Requestor's name: _____

#1 WPI catalog number: _____ Quantity needed: _____

#2 WPI catalog number: _____ Quantity needed: _____

#3 WPI catalog number: _____ Quantity needed: _____

#4 WPI catalog number: _____ Quantity needed: _____

#5 WPI catalog number: _____ Quantity needed: _____

#6 WPI catalog number: _____ Quantity needed: _____

#7 WPI catalog number: _____ Quantity needed: _____

#8 WPI catalog number: _____ Quantity needed: _____

If item not in WPI catalog, please provide company name/address, website/email, item number, and approximate cost below, or specific item description:

_____

_____

_____

_____

_____

_____

Requestors Signature: _____

JBB 3/21/07
Revised 5/14/07

Laboratory Supply Order Form

For items not included in the WPI Catalog:

Item# _____ (from Page 1)

Ordering Information (Circle): Phone Order Web Order

Supplier: _____

Contact: _____ Phone: _____

Website/email: _____

Item Number: _____ Quantity: _____

Price per Unit: _____ Total Cost:_____

Confirmation Number: _____

Date Ordered: _____ Date Received: _____

New WPI Catalog Item? Yes No If Yes: WPI Catalog Number: _____

Item# _____ (from Page 1)

Ordering Information (Circle): Phone Order Web Order

Supplier: _____

Contact: _____ Phone: _____

Website/email: _____

Item Number: _____ Quantity: _____

Price per Unit: _____ Total Cost:_____

Confirmation Number: _____

Date Ordered: _____ Date Received: _____

New WPI Catalog Item? Yes No If Yes: WPI Catalog Number: _____

Reviewed By: _____ Date: _____

Approved By: _____ Date: _____

APPENDIX M

Laboratory Purchase Order Form

PURCHASE ORDER

1085 S. Hermitage Road
Hermitage, PA 16148
Office: 724-981-0155
Fax: 724-981-0156

P.O. NO. 08-L004
DATE
Project #:
Account #:

VENDOR

SHIP/BILL TO Wallace & Pancher, Inc.

1085 S. Hermitage Road
Hermitage, PA 16148
Office: 724-981-0155
Fax: 724-981-0156

SHIPPING METHOD	SHIPPING TERMS	PAYMENT TERMS	DELIVERY DATE

QTY	ITEM #	DESCRIPTION	JOB	UNIT PRICE	LINE TOTAL

SUBTOTAL	
SHIPPING	
SALES TAX	6.00%
TOTAL	

Requested by *Date*

Authorized by *Date*

APPENDIX N

Laboratory Supply Tracking Form

Laboratory Supply Tracking Form

(Laboratory management use only)

	Supplier	Item #	Unit Cost	Total Cost	Order Date	Ordered By	Order Type	Rec'd Date	Invoice To/Date	Date Paid
#1										
#2										
#3										
#4										
#5										
#6										
#7										
#8										

Appendix O

Benthic Macroinvertebrate Sample Identification Request Form

Wallace & Pancher, Inc. Laboratory
Benthic Macroinvertebrate Sample Identification Request Form

Requestor:		
Project Name:	**Project #:**	**Methodology:**
Mine/District:	**Season:**	**Round (If Applicable):**
Requested Completion Date:		
Submitted to (Supervisor's Name):		**Date Submitted:**

Sites Needed: **If ALL SITES NEEDED, check here:**_____

If not all sites are needed, list below:

#	Sample ID	#	Sample ID	#	Sample ID
1		15		29	
2		16		30	
3		17		31	
4		18		32	
5		19		33	
6		20		34	
7		21		35	
8		22		36	
9		23		37	
10		24		38	
11		25		39	
12		26		40	
13		27		41	
14		28		42	

Supervisor Use Only:

Supervisor's Signature:
Priority:
Specifications:

Lab Use Only:

Request Received in Lab By: _____ Date: _____

Data Reviewed By: (two scientists) #1 _____ #2_____

Completion Date: _____ Placed in Lab Folder: _____

Verification Email Sent By (name): _____ Date: _____

Requestors Signature: _____ Date: _____

When your data is compiled, an email will be sent to you verifying completion of the samples and notifying you of the location of your data within the lab folder. After you have removed your data from the folder, please sign the bottom of this form and place in the lab administrator's box.

Appendix P

Laboratory Safety Checklist

Wallace & Pancher, Inc.
Laboratory Inspection Checklist

Work Site Location
1085 South Hermitage Road
Hermitage, PA 16148

	Yes	No
1. Housekeeping		
Are emergency exits defined?		
Are exit lights functional?		
Are exits well-marked?		
Are exits clear of debris?		
Are walkways clear?		
Do employees know emergency procedures?		
Location of exits		
Location of first aid kits		
Communication procedures in emergency		
Names of safety trained personnel		
Are fire extinguishers checked monthly?		
Are slop buckets emptied nightly?		
Are rugs taped down?		
Are cabinet and desk drawers kept closed?		
2. Hazard Communication		
Training in proper disposal of lab chemicals?		
Training in proper storage of lab chemicals?		
Training in proper ventilation of chemicals?		
Is MSDS present for each chemical used in lab?		
Are alcohol containers properly labeled?		
Are other chemical containers properly labeled?		

Appendix Q

Safety Report Form

Safety Report

Date/Time of Incident	Date/Time of Report

Detailed Description of Safety Concern (attach additional sheets if necessary):

Reported by: *(optional)* _____

Was the person or people involved made aware of the safety concern? YES NO Date: _____

Action Taken by Supervisor (attach additional sheets if necessary):

Supervisor Signature: _____ Date: _____

Action Taken by Safety Committee (attach additional sheets if necessary):

Chairman Signature: _____ Date: _____

Appendix R

WPI Benthic Macroinvertebrate Laboratory Equipment List

Wallace & Pancher, Inc. Laboratory
General Operating Equipment Inventory

Equipment Type	Quantity
Olympus SZ2-ST Stereo Microscopes	2
SM-2B 7x-45x Binocular Stereo Zoom Microscope	1
Schott Fiberoptic Cold Lamps	2
Dual goose neck fiberoptic illuminator	2
Walter QF Series Stereo Microscope	3
1.3 MP (MD600) Microscope Color Digital Camera	1
Olympus WHsZ 20x Eyepieces	2 pair
Heavy duty magnifier lamp	3

Appendix S

Incident Report Form

Employee Accident / Injury Incident Report

WALLACE & PANCHER, INC.
ENGINEERING & ENVIRONMENTAL

_____

Last Name First Name MI

_____

Street Address (Home)

_____

City State Zip Code

_____

Work assignment at time of incident

_____ _____ _____

Incident Date Incident Location Time of Incident

_____ _____

Job Title Hire Date

Specify Injury: _____

Did injury require treatment: Yes No Specify Treatment: _____

Detailed description of Incident (use additional sheets as necessary): _____

_____

_____

_____

_____

_____

Employee signature: _____

Name of witness(es): _____

_____

_____

Employee Accident / Injury Incident Report
Supervisory Action

Date of notification by employee: _____

If incident involved medical treatment:

Medical treatment: Yes No

Treatment site: _____

Did incident involve third party with injuries? Yes No

Describe: _____

_____

_____

_____

If injury involved motor vehicle:

Did incident require drug screening? Yes No Circle: DOT or Non-DOT

If yes, was testing completed? Yes No If yes, where: _____

If no, explain: _____

Location of results: _____

Supervisor's action: _____

_____

_____

_____

_____

_____

_____

Date: _____ Supervisor's signature: _____

WPI
WALLACE & PANCHER, INC.
ENGINEERING & ENVIRONMENTAL

Employee / Equipment Incident Report

_____
Last Name First Name MI

_____
Street Address (Home)

_____
City State Zip Code

_____
Work assignment at time of incident

_____ _____ _____
Incident Date Incident Location Time of Incident

Was there an injury: Yes No Specify Injury: _____

Did injury require treatment: Yes No Specify Treatment: _____

Was equipment involved: Yes No Specify Equipment: _____

Was equipment damaged: Yes No Specify Damage: _____

Detailed description of Incident (use additional sheets as necessary): _____

_____

_____

_____

_____

_____

Employee signature: _____

Name of witness(es): _____

_____

_____

Employee/Equipment Incident Report
Supervisory Action

Date of notification by employee: _____

If incident involved medical treatment:

Medical treatment: Yes No

Treatment site: _____

If incident involved damaged equipment:

Equipment replacement: Yes No

Replacement history / cost: _____

_____

_____

Equipment repaired: Yes No

Repair information: _____

_____

_____

_____

Repair / Replacement cost: _____

Supervisor's action: _____

_____

_____

_____

_____

_____

_____

Date: _____ Supervisor's signature: _____

Appendix T

Benthic Macroinvertebrate Sample Sorting Form

Wallace & Pancher, Inc. Laboratory
Benthic Macroinvertebrate Sample Sort Sheet

Project: _____ Season: _____

Sample ID:_____ Collection Date: _____

Sorter: _____ Date: _____

Number of Grids Picked: _____ Time Expenditure: _____ No. of Organisms: _____

Indicate the presence of large or obviously abundant organisms:

_____

General Comments: _____

Insect Tally:

Quality Control:

Sample used for Quality Control? _____ Yes _____ No

Quality Control Officer: _____

# organisms originally sorted	# organisms recovered by QC officer	Total # of organisms recovered	% Sorting Efficiency

> 90% sample passes _____

< 90% sample fails, action taken _____