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of DREXEL UNIVERSITY

QUALITY ASSURANCE PROJECT PLAN

Delaware River Watershed Initiative

Version I

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Quality Assurance Project Plan (QAPP)
Delaware River Watershed Initiative

QAPP APPROVALS

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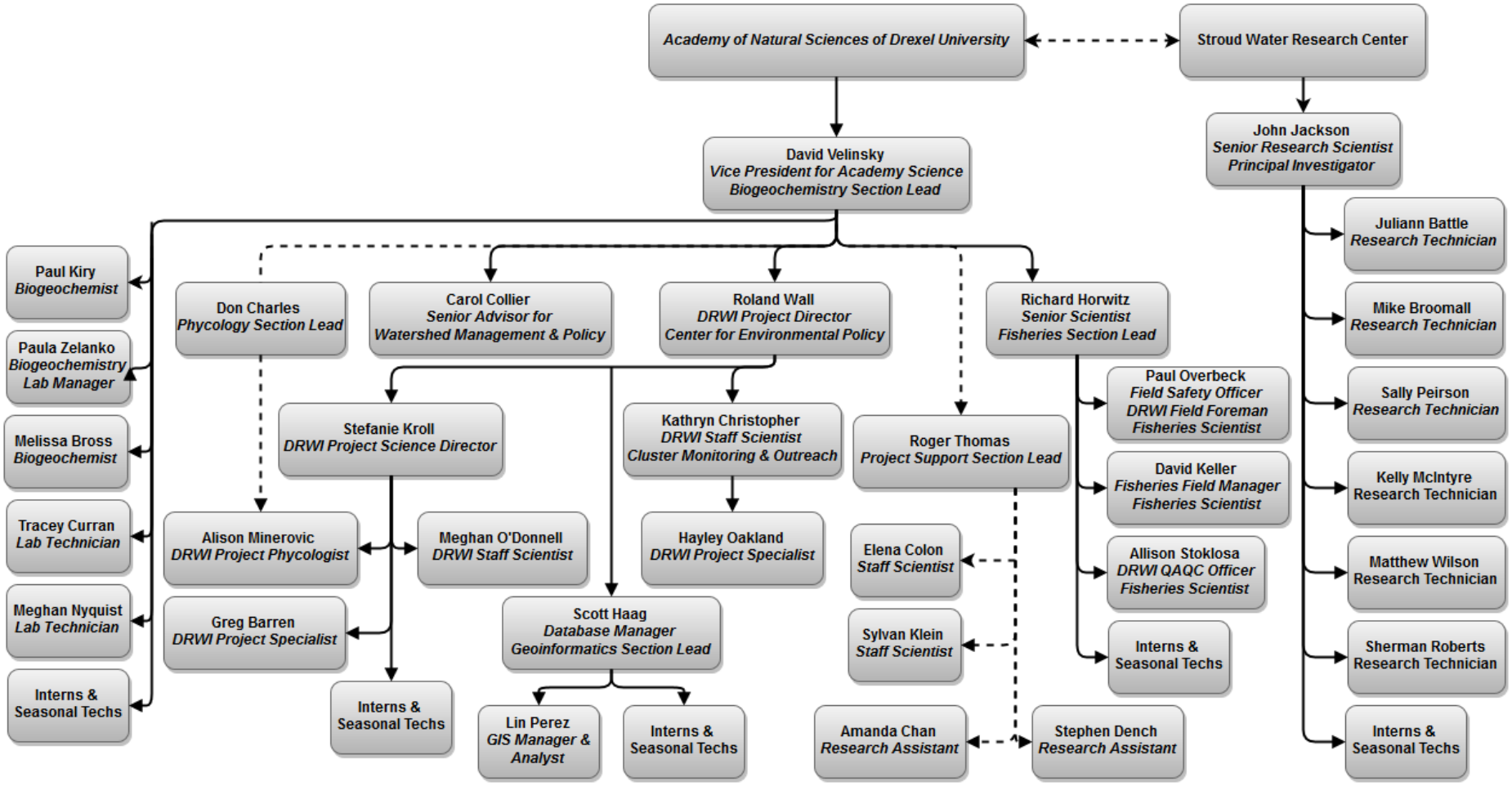
DRWI Personnel Organization Chart and Lines of Reporting



Acronyms

ANSDU	Academy of Natural Sciences of Drexel University
BMP	Best Management Practices
Chl-a	Chlorophyll-a
Clusters	Groups of spatially proximate sub-watersheds
DIC	Differential Interference Contrast
DIW	Deionized Water
DRWI	Delaware River Watershed Initiative
EPA	Environmental Protection Agency
FNUT	Filtered Nutrient water samples
FSP	Field Safety Plan
GLP	Good Laboratory Practice
IACUC	Institutional Animal Care and Use Committee
NADED	North American Diatom Ecological Database
NAWQA	National Water Quality Assessment
NFWF	National Fish and Wildlife Foundation
NHD	National Hydraulic Dataset
NLCD	National Land Cover Dataset
ORDMS	Object-related database management system
OSI	Open Space Institute
PCER	Patrick Center for Environmental Research
PHab	Physical habitat
QAPP	Quality Assurance Project Plan
QAQC	Quality Assurance, Quality Control
RWB	Reachwide benthos
SRID	Spatial Reference System Identifier
SWAMP	Surface Water Ambient Monitoring Protocol
SWRC	Stroud Water Research Center
UNUT	Unfiltered Nutrient water samples
USGS	United States Geological Society
UTM	Universal Trans Mercator
WGS84	World Geodetic System 1984
WPF	William Penn Foundation

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1.1 Purpose

This document is the Quality Assurance Project Plan (QAPP) for the Delaware River Watershed Initiative (DRWI) within the Academy of Natural Sciences of Drexel University (ANSDU) and the Stroud Water Research Center (SWRC). This QAPP provides quality assurance/quality control (QAQC) reasoning and procedures which shall govern project activities at all times. All responsible parties listed here within are expected to comply with the procedural requirements of this QAPP to ensure the generation of accurate, precise, and reliable data.

This QAPP may be modified at any time by the Project Director, Project Science Director, Section Leads, or QAQC Officer as changes within the project occur. Additionally, this QAPP will be reviewed annually in February and March, prior to the onset of the summer field season. Any significant changes to this QAPP will be documented and justified in subsequent versions. Changes to this document must be approved by the QAQC Officer and Project Director before implementation. No field or laboratory work is to be done prior to approval of significant changes. Significant changes include but are not limited to: changes in field sampling methods and laboratory procedures; additions or changes to reasoning behind collection methodology; and the creation and addition of DRWI projects. Minor deviations from this QAPP in relation to unexpected circumstances involving field work shall not require prior authorization but shall be properly documented and shall be made at the discretion of the field crew leader. Minor deviations may include: use of different yet equivalent field equipment if a problem occurs with primary equipment; slight sample timing deviations due to unavoidable site restrictions such as weather events, landowner conflicts, or equipment malfunctions; and any deviations from this QAPP deemed necessary at the time of sample to ensure the overall safety of field crews.

2.0 Overall Project Description

In 2012, the William Penn Foundation (WPF) made a strategic decision to focus its environmental investments on projects that benefit and protect water quality and ecological health in the Delaware River Basin.

This investment resulted in the Delaware River Watershed Initiative (DRWI). The DRWI is a large-scale collaborative program that provides funding and research for conservation projects with the goal of improving water quality. The restoration and protection actions of the DRWI are focused on addressing agricultural and urban stormwater runoff quantity and/or quality, aquifer recharge (in the Kirkwood-Cohansey aquifer, mainly), and reducing fragmentation of forested areas. This work builds on ongoing conservation work, but shifts the focus from other restoration objectives (e.g. habitat for specific species) to water quality.

The projects and strategies envisioned for the DRWI rely on multiple dimensions of scientific information to identify needs, plan actions, evaluate and revise policies, priorities, and outcomes, and articulate funding and resource requirements. Because scientific knowledge is central to successful investment in these goals, WPF has placed a high priority on acquiring and applying the biological metrics and other data that characterize the conditions, stressors, threats, and opportunities in priority sub-watersheds.

In February 2012, ANSDU began working with WPF in partnership with SWRC, the Open Space Institute (OSI) and the National Fish and Wildlife Foundation (NFWF) to provide planning and technical support for the development and implementation of the DRWI. In the first phase of the project, ANSDU's work has focused on identifying groups of sub-watersheds that are candidates for restoration or conservation based on ecological qualities. Merging that with an organizational and feasibility

analysis by the team resulted in high priority watershed "clusters" (i.e. groupings of spatially proximate sub-watersheds) containing the principal candidate sites for WPF investment. Additionally, an extensive review and analysis of techniques and capacities for monitoring ecological characteristics of the watersheds and analysis of the methods for evaluating the effectiveness of projects relative to baseline watershed conditions was executed. Included in this process has been the development of recommended indicator sets which aim to characterize ecological conditions and changes at both the sub-watershed and basin-wide scales.

Since the start of the DRWI, ANSDU has been, and continues to be, responsible for tasks that have included:

1. Site evaluation and site selection for project investment.
2. Evaluating baseline ecological conditions in targeted areas.
3. Data gathering, interpretation and curation on ecological characteristics and water quality.
4. Direct monitoring and ecological evaluation of sub-watersheds and clusters.
5. Support and oversight of project level monitoring by grantees.
6. Translation and communication of scientific and environmental elements of the DRWI.
7. Developing and facilitating work on key research questions related to watersheds.
8. Performing concrete research projects that derive from watershed function and monitoring.
9. Supporting WPF in managing the DRWI, including participation in the Coordinating Committee.

WPF is funding ANSDU to collect, analyze, and report indicators that will reflect ecological conditions relative to the project sites, general conditions of the targeted watershed clusters, and overall conditions of the Delaware Basin. ANSDU is contracting SWRC to conduct the assessment of macroinvertebrates for the DRWI including collection, analysis, and reports. Data from both ANSDU and SWRC will be shared with WPF, the cluster coordinating organizations, and the individual grantees. Although funding for the sub-watershed level projects will focus on restoration and conservation goals at specific sites, WPF is committed to having an impact on the overall ecological health of the Delaware Basin. Therefore, monitoring and evaluation will be used to relate the funded projects to the conditions of the clusters and of the basin as a whole. There is also recognition that for specific projects, the likelihood of dramatic changes in larger watershed conditions is unlikely. For that reason, more intense monitoring will take place in the proximity of projects, while general monitoring will be used at the cluster and basin scale to set the context of the projects and determine major trends. For conservation projects, expected outcomes of the grants will focus on the commitments of the grantees (i.e. "miles of riparian zone restored") rather than firm ecological expectations.

The DRWI at ANSDU has initiated several primary monitoring projects as well as additional research projects that shall be described individually within this document outlining specific project goals, background information, sampling procedures, and QAQC procedures.

2.1 Data Usage

Data collected within this study is to be publicly disclosed (with the exception of sensitive data relating to landowners, personnel, or individuals associated with the DRWI) in accordance with the goals and mission of WPF. Results may also be presented in cases wherein data sharing is legally required (e.g. as part of reporting for scientific collecting permits), as part of a presentation to the scientific or public communities, to permission-granting landowners, or as part of a manuscript or report in a scientific journal.

2.2 Data Storage and Management

All internal data for the DRWI is stored on the ANSDU database server (located at ans-drwi.ansp.org) using PostgreSQL, an open source object-relational database management system (ORDMS) software package. An ORDMS allows internal and external groups or organizations to insert and update databases, extract data, and analyze relationships within and among different datasets. PostgreSQL as an ORDMS demands standards-compliancy, extensibility (programmatically stored procedures, allowing performance of extended custom procedures), and ensured reliability as well as data-integrity. PostGIS extends PostgreSQL with full-bodied spatial database management functionality, turning it into a spatial analysis tool that can store geometry and compare relationships between geometries.

Data collected in the field by DRWI scientists may be queried, analyzed, or mapped using PostGIS by assigning geometry data types (e.g. point, line string, polygon etc.) from GPS acquired site latitudes and longitudes, and a spatial reference system identifier (SRID). All spatial data uses a standard projected World Geodetic System 1984 (WGS 84) reference ellipsoid to model the Earth in a 2-dimensional Cartesian coordinate system Universal Trans Mercator (UTM). UTM is not one single projection system, but rather a system divided into 60 zones. ANSDU delineates spatial data to UTM zone 18 north with SRID 32618, comprising the geographic locations of all DRWI data within the Northeastern United States. Internal sources of spatial data include all information collected by DRWI scientists as well as the watershed/stream modelling software package Stream Hiker.

Ensuring accuracy and reliability of both data entered and subsequent derived calculations is of high importance. QAQC is to be carried out by persons not involved in entering said data or the derived calculations, confirming no erroneous or inaccurate information exists as a result of entry. Data are queried using the ORDMS, and statistical/spatial analyses are implemented to verify the dataset. All SQL code and data is stored on the William Penn Share drive and the ANSDU DRWI server.

Scientists and staff connecting to the database, whether to upload or extract data, should have one to two meetings with the database administrator or a delegated member of the BioGeoInformatics group to review access instructions and database protocol. This provides the ability to remotely access information from either the database itself or through the use of another program (e.g. Microsoft Access, Excel, etc.) as well as the ability to understand the structure of information within the database and how to query datasets. Access to different schemas and tables within the database will be granted by the senior database administrator on a case by case basis to the appropriate scientists or staff member in order to ensure the integrity of ANSDU information. To access the DRWI database on the ANSDU server, staff members will need to install the latest version of PostgreSQL (version 9.3.9 or newer) onto their computer and use the administration and development platform pgAdmin III (included with the PostgreSQL software package).

Geospatial referencing and interpretations done by any BioGeoInformatics group member necessitates proficiency in GIS software. Online manuals can be used for instruction purposes such as the Boundless Introduction to PostGIS 2.1.9 (http://workshops.boundlessgeo.com/postgis-intro/), the PostgreSQL 9.3.9 Documentation and User's Manual (http://www.postgresql.org/files/documentation/pdf/9.3/postgresql-9.3-A4.pdf) and the Quantum GIS 2.8 Documentation and User Guide (http://docs.qgis.org/2.8/en/docs/user_manual/). Reference materials are also located within the BioGeoInformatics group office for further familiarization with pertinent software, including 'Getting to Know ArcGIS Desktop' by Tim Ormsby (version 10.2.1) and 'PostGIS in Action' by Regina O. Obe and Leo S. Hsu. Specific inquiries not found through these resources can be made to either the database administrator or the geospatial analyst.

2.3 Field and Laboratory Safety

All personnel and volunteers must receive proper training and certification as required by ANSDU. All individuals associated with the DRWI must understand and adhere to the associated safety plans for the project, for ANSDU/SWRC, and for each section. All field staff are required to attend the American Red Cross (or similar) First Aid/CPR/AED training. If field staff are handling vertebrates they are additionally required to be trained in animal welfare policy mandated by the Drexel Institutional Animal Care and Use Committee (IACUC). Under this policy the staff must complete an annual Occupational Health physical and complete on-line CITI training in Animal Welfare prior to handling vertebrates. Refer to the DRWI field safety plan (FSP) in Appendix III for specific details and information regarding safety guidelines and restrictions.

3.0 Integrative Site Study

3.1 Background

In 2012, eight sub-watershed clusters were prioritized according to landscape variables (e.g. land use, conservation easements and land trust areas) as well as organizational capacity for potential grantees of WPF. In 2013, "integrative" sites were chosen to be representative of land use and stream conditions within sub-watershed clusters and were integrative of stressors or conservation areas in the drainage basin of these sites. These integrative site stream reaches are to be monitored over 3 to 10 years. They were revisited in 2015 to examine inter-annual variability of these communities. Baseline sampling from 2013 provides initial data sets on the current status of the ecological integrity of these streams for comparison with future samples.

3.2 Integrative Site Selection

Sub-watershed clusters were prioritized according to landscape variables (land use, conservation easements and land trust areas) as well as organizational capacity for potential grantees of WPF. Then, sample sites were chosen within these clusters. The number of sites per sub-watershed varies, depending on the diversity of landscape conditions within the cluster. Once the sub-watershed clusters were defined, sites were selected to characterize "typical" conditions within the clusters. Integrative sites were chosen using the following criteria:

1. The sites encompass significant parts of individual subdrainages, thereby integrating land use and land cover.
2. The sites are located in a sub-watershed with stressors typical of the area and with agricultural or urban land in a percentage that is similar to the overall percentage in the sub-watershed.
3. The sub-watersheds chosen contribute significantly to the amount of water resources and streams are typically 3rd order or larger, with very small tributaries avoided.
4. For most sites in agricultural and protection areas, very large streams were avoided so that point sources of urban input were not captured.
5. As many major tributaries in each cluster as possible are included.
6. In large clusters with many streams, such as the Poconos cluster, a variety of streams were selected, although it was not possible to cover as many major tributaries as in smaller clusters.
7. Larger streams were chosen to reflect general conditions of the watershed.
8. Few known, significant point source inputs were identified upstream using GIS information and expert knowledge, for example:
    a. Site must be far enough downstream of a town to avoid signaling other point source inputs (e.g. storm-induced sewage overflow).
    b. If point sources were observed during site scouting, a site would be relocated.

c. In two cases, sites were located downstream of a point source considered typical for the region (i.e. Musconetcong River and West Branch Brandywine Creek).

9. Sites are on public land, when possible, to ensure access in the future. When necessary, sites are placed on private land and landowners are contacted to request permission.
10. The site has relatively easy access for field crews, for reasons of efficiency and safety.

3.3 Timing of Sampling Events at Integrative Sites

Macroinvertebrate sampling is to be completed by SWRC from March to June in accordance with guidelines set forth by SWRC and approved by ANSDU. Fish surveys are to be conducted between April and October by ANSDU's Fisheries Section as outlined within this document. Algae is to be sampled between June and September by ANSDU's DRWI team and is to be conducted at a site at least ten days after fish sampling has occurred. Water chemistry parameters are to be sampled quarterly by ANSDU's Biogeochemistry Section in accordance with guidelines found herein. All field collections are contingent upon climatic events and stream conditions which may impact timing of said sampling events. All sampling decisions are to be made by the associated section leader in accordance with sampling criteria set forth in this document and/or as determined by field crew leader upon field examination of sampling locations.

3.4 Monitoring of Water Chemistry at Integrative Sites

3.4.1 Biogeochemistry Introduction

Water chemistry is used to assess water quality and validity of biotic indicators (e.g., algae, macroinvertebrates) derived during this study. Specifically, water chemistry provides a baseline of water quality concentrations across all clusters and watersheds and helps to determine sources of nutrients to and within various watersheds and clusters. Water chemistry is also useful to compare water quality parameters with appropriate state and federal water quality guidelines. Routine water chemistry monitoring aids in the assessment of the impact of land protection or restoration practices before and after land protection/restoration which aids in investigating biogeochemical processes that impact water chemistry across different land characteristics.

3.4.2 Water Chemistry Sampling Design

Water chemistry and discharge sampling and collection are to be completed on a quarterly basis every year starting in 2013 by ANSDU personnel and led by the Biogeochemistry Section. A "snapshot" sampling protocol for field work is desired, meaning collecting samples in the shortest time frame in each quarter as possible. A two person crew is sufficient to complete a site, and if possible, having two crews of two is ideal to ensure sites are done in the snapshot time frame. Sampling and collections should be performed during the stream's base flow. If a rain event occurs, personnel must check nearby USGS gauge heights (http://waterdata.usgs.gov) until the water has fallen back to base height before sampling and collection resumes. The duration and intensity of the storm in the area of the stream as well as in areas upstream from the site location should be taken into consideration. Ultimately, it will be the decision of the Biogeochemistry Section or field crew lead to determine when sampling should resume. Additional concerns in winter months may need to be addressed and it may not always be feasible to sample due to ice and/or snow coverage. In the event that winter hazards prevent sampling, sampling at the affected site will wait until thawing occurs or may be cancelled for the season. Water turbidity should also be taken into consideration as increased turbidity can cause errors in the measuring equipment. Sampling should be done when the water is clear, however in the event that this is not possible, notes on the turbidity of the water must be recorded on the field data sheet. A full list of the monitored chemical parameters and detection limits can be found in Table 3.1.

Table 3.1 DRWI monitored chemical parameters, methods, and detection limits

Parameters	Sample Matrix	Method Reference	Description	Detection Limit
Dissolved Oxygen (mg/L)	Water	360.1*	YSI Sensor	
pH	Water	150.1*	YSI Sensor	
Specific Conductance	Water	120.1*	YSI Sensor	
Temperature	Water	170.1*	YSI Sensor	
Dissolved Ammonia+ammonium (as N)	Water	350.1 Rev. 2.0 (1993)	ANS-CAS	0.005 mg/L
Dissolved Nitrate+Nitrite-N***	Water	353.2, Rev. 2.0 (1993) **	ANS-CAS	< 10 µg N or P/L
Total Nitrogen	Water	SM 22nd Ed.4500-N C Persulfate Method	ANS-CAS	0.05 mg/L
TKN	Water	By calculation (TN – (Nitrate+Nitrite))	ANS-CAS	< 100 µg N/L
Soluble Reactive P (orthophosphate)	Water	365.1, Rev. 2.0 (1993) **	ANS-CAS	< 10 µg N or P/L
Total Phosphorous	Water	365.1, Rev. 2.0 (1993) **	ANS-CAS	< 5 µg P/L
Total Suspended Solids	Water	SM20(1998); ANSP SOP	ANS-CAS	< 1.0 mg/L
Total Alkalinity	Water	SM20-2320 B	ANS-CAS	< 1 mg/L
Total Hardness	Water	SM20(1998); ANSP SOP	ANS-CAS	2.00 mg/L
Total Chloride****	Water	SM20(1998)	ANS-CAS	0.61 mg/L, 0.2 mg/L****
Sulfate	Water	EPA Method 300	ANS-CAS	0.1 mg/L
Bromide	Water	EPA Method 300	ANS-CAS	0.4 mg/L
Na, Mg, Ca, K	Water	EPA Method 200.7 ICP	SERC	0.2, 0.02, 0.05, 0.4 mg/L respectively
Ba, Sr	Water	EPA Method 200.7 ICP	SERC	0.02, 0.008 mg/L respectively
Al, Fe, Mn	Water	EPA Method 200.7 ICP	SERC	0.008, 0.003, 0.001 mg/L respectively

* - As documented in EPA Methods for Chemical Analysis of Water and Wastes or ANSDU SOP
**- As documented in EPA Methods for the Determination of Inorganic Substances in Environmental Samples
*** Dissolved nitrate (by difference)
**** Ion chromatography may also be used, EPA Method 300

Additionally, underwater temperature loggers must be deployed at each site and checked for data periodically. Temperature tracking can be used to analyze stream health and biological communities present. The temperature loggers should be checked every four to six months to make sure the device is functioning correctly; although, the logger can record over 42,000 12-bit temperature measurements and may be checked at less regular intervals if necessary.

3.4.3 Water Chemistry Sampling Methods

The following measurements are taken as single, one-time readings of surface water chemistries at each sampling location in a swift moving portion of the stream before any disturbance to the stream by sampling crews has occurred that day. All measurements are recorded on pre-printed data sheets (Appendix IV), as well as date, time, site name, name(s) of collectors, weather 24 hours before, current weather, and any notes that seem beneficial.

The following instructions are adapted from the YSI Professional Plus User Manual.

Dissolved Oxygen

Measured with a YSI 6000DM or equivalent sensor in situ. After turning the meter on, 5-15 minutes must go by before reading should occur. The probe should be placed in a swift moving portion of the stream and given a quick shake to release any air bubbles. The temperature readings should be allowed to stabilize before DO is read. If not able to place the probe in swift waters, stir the probe in the stream to overcome the stirring dependence of the dissolved oxygen sensor. The meter requires a velocity of at least 3 inches per second for 2.0 PE membranes, 6 inches per second for 1.25 PE membranes, and 12 inches per second for Teflon® membranes. Once the values plateau and stabilize, the measurement may be recorded. The dissolved oxygen reading will drop over time if stirring is ceased. If placing the DO sensor into fast flowing waters it is best to place it perpendicular to the flow and NOT facing into the flow .The stated accuracy of the meter per manual of operations is +/- 0.2mg/L.

pH

Measured with a YSI Multi Probe or equivalent sensor in situ. pH readings are typically quick and accurate, however, it may take the sensor a little longer to stabilize if it becomes coated or fouled. To improve the response time of the sensor, follow the cleaning steps in the maintenance section of the user manual. The stated accuracy of the meter per manual of operations is +/- 0.1 pH units.

Conductivity

Measured with a YSI Multi Probe or equivalent sensor in situ. The conductivity sensor will provide quick readings as long as the entire sensor is submerged and no air bubbles are trapped in the sensor area. The probe should be immersed into the stream so that the sensors are completely submerged and then shaken to release any air bubbles. Occasional cleaning of the sensor may be necessary to maintain accuracy and increase the responsiveness. The range of the meter is 0–1000µS/cm with a resolution of 4 digits.

Temperature

Measured with a YSI Multi Probe or equivalent sensor in situ 1m below water surface or just below the water surface in riffles. Calibration is factory set, and requires no re-calibration (accuracy ±0.2° C).

Water column sampling

At each site a sample of water is to be taken for analysis at both the quarterly chemistry sampling and during algae sampling events. All collection equipment (the sample container and any additional sampling equipment – e.g. pitcher – if used) is rinsed three times with site water prior to collection. Between sites, sampling equipment (e.g. pitcher) is disassembled and rinsed with deionized water (DIW), then stored in a clean Zip-Loc bag. At the next site, the sampling equipment is then rinsed three times with site water prior to use. If only the sample container (e.g. cubitainer, bottle) – and no additional sampling equipment – is to be used, it is necessary only to rinse the sampling container three times with site water prior to collection.

Samples should be taken at moving water upstream of bridges, and upstream of any disturbance in the water (i.e. people walking in the water). Samples should be collected at the downstream portion of the study reach before anyone has entered the stream. If any portion of stream reach is disturbed, samples must be taken from the top of the reach. A clean pair of latex or nitrile gloves can be put on at the beginning of each sample collection if the water is of questionable quality. Sample containers are labeled directly on the container with permanent marker or with permanent marker on label tape, which has been wrapped all the way around to avoid peeling. Information on the label should include: DRWI, site ID, date and time of collection.

Samples are taken using a dip method in the middle of the water column. The sample container is submerged until approximately 80% full. It is best to submerge the container at a few locations across the stream and not entirely at one point to catch any variations in water quality throughout. If the stream is too shallow due to low flow conditions, a modified grab sample can be taken using a pre-cleaned Pyrex glass pitcher. In addition, if conditions are encountered where the above method of sample collection is considered to be dangerous (e.g., during high flow events), a modified technique is used, in which samples will be composited from subsamples taken at representative depths and locations along the stream transect.

Two types of blanks and one duplicate sample are collected during the duration of the sampling season.

Equipment Blank

If, in addition to the sample container, an additional bottle/pitcher is used to collect the sample, the equipment blank will be collected on the same day at a rate of 1 blank per 10 samples. After the bottle/pitcher and sample container have been cleaned and rinsed with DIW, the inside of the bottle/pitcher is rinsed with DIW into the sample container enough times to eventually fill it. The sample container is placed on ice. The label should include: “Equip. blank,” DRWI, site ID, date and time of collection.

Field Blank

Collect 1 every 10 samples collected at the beginning of the first field day (any subsampling equipment [pitcher/bottle] had been cleaned the night before). Sample container is rinsed three times with DIW, and then the sample container is filled with DIW. The sample container is placed on ice. The label should include: “Field blank,” “DRWI,” site ID, date and time of collection.

Duplicate

A second, typical stream water sample; collected at the same time as sample at a rate of 1 duplicate per 10 samples. The label should include: “Dup,” “DRWI,” site ID, date and time of collection.

Water Chemistry Sample Preservation

Sample containers containing water column samples must be kept in the dark and on ice at 4 degrees Celsius (± 2 degrees Celsius) after collection until received by laboratory. Nutrient sample bottles (filtered/unfiltered sub-samples) are frozen within 24 hours of water column sample collection, either in a laboratory freezer or in the field using dry ice, and transported to the laboratory for analysis. Dry ice should be stored in a cooler, kept in the packaging from the supplier, with the samples kept up against the ice as much as possible to ensure they freeze. If traveling overnight for multiple nights in the summer, a 25 pound block is acceptable. For an overnight field trip in the other three seasons, a 15 pound block is acceptable. Amounts of dry ice are weather dependent; these values can change depending on what you feel is sufficient to ensure samples stay frozen.

Current Speed

Where feasible, current speeds will be measured with a pre-calibrated current meter (e.g., FlowTracker or River Surveyor). Too shallow waters can affect the ability for either meter to function properly. For the FlowTracker, very shallow water is when the probe cannot be completely submerged underwater. For the River Surveyor, the company states anything under 30cm is too shallow to get accurate readings. Please see the user manuals for the FlowTracker and the River Surveyor for more detailed instructions.

Where meaningful current speed measures cannot be obtained using a current meter, surface current speed will be measured by timing floating objects over a fixed distance. Portions of wooden tongue depressors or similar low-profile floating objects will be timed over a distance of one or more meters, and converted to centimeters per second.. Timing is done with a digital stopwatch accurate to 0.1 second.

Depth

Depth is to be measured using a stainless steel ruler. Measurements are taken to estimate the undisturbed water surface (typically the downstream side of the rule), i.e. the increased depth on the upstream side of the rule will not be included. Accuracy will be to 1cm.

Discharge

Methods adapted from Hauer, F.R. and G.A. Lamberti. 2007. Methods in Stream Ecology. 2nd ed. Chapter 3, Discharge measurements and streamflow analysis. Oxford (UK): Elsevier Inc.

"Stream discharge is affected by conditions within the channel and the channel geometry…In general, the highest stream velocities occur at or near the thalweg [i.e. the deepest part of the channel] and are a function of resistance to flow, usually as a result of streambed material (i.e. bed roughness). Discharge is determined by multiplying the mean velocity by the cross-sectional area of the flow. The cross-sectional area can be measured directly by stretching a measuring tape across the stream and taking several measurements of depth with a meter stick. Several measurements of mean velocity must be taken across the stream, because flow is unevenly distributed across the stream channel" (Hauer and Lamberti 2007). Areas where flow is very irregular or undercut banks, exposed boulders, dense aquatic vegetation, or upstream eddies occur should be avoided. At some sites, the location of transects may be constrained by access; for example, personnel may have access only to a narrow area under or adjacent to bridges.

"The midsection method is a standard technique used by most hydrologists (and recommended by the USGS) for calculating the discharge of most streams and rivers. To measure discharge (*Q*), stretch a measuring tape across the stream and then divide the transect into *n* convenient increments, or cells. In fact, the observation point locates the center of the cell to be examined with cell boundaries halfway to the next observation point (See equation 3.1 and Figure 3.1). As a general rule, cell widths should not exceed 3m. A stream of 30m width, then, should have at least ten observation points (or verticals). It is not necessary to make uniform width cells. If there are any hydraulic irregularities (a protruding boulder, a cascade, a pool, etc.) across the transect, a new cell or observation point should be designated where more uniform conditions resume. If the flow is uniform, the mean velocity is measured at the observation point at a height above the stream bed equal to 0.4 times the depth at that location. At an observation point where the depth exceeds 0.6m, the mean velocity should be calculated as an average between velocities measured at 0.2 and 0.8 times the depth at the observation point"(Hauer and Lamberti 2007). Cells should be selected so that they have approximately equal amounts of discharge, e.g. locations will be closer together in deeper or higher velocity sections of the cross-section. Discharge will be calculated by treating each location as representative of discharge through a polygon which contains the sample point. Data should be checked to confirm that none of the sections of the discharge computation exceed 10% of the total discharge. If the flow in a cell is greater than 10% of the total discharge, the cell should be split in two elements and re-measured. This method of discharge calculations may be done by hand with the Marsh-McBirney, or with use of computer software with the FlowTracker or RiverSurveyor, as described below. Please see the attached field sheet for discharge calculations in Appendix V.

(3.1)

$$q_x = v_x \left[\frac{(b_{(x+1)} - b_{(x-1)})}{2} \right] d_x$$

Where: q_x = discharge through partial section x; v_x = mean velocity at observation point x; $b_{(x-1)}$ = distance from the datum to the preceding observation point $(x-1)$; $b_{(x+1)}$ = distance from the datum to the next observation point $(x+1)$; d_x = depth of the water at observation point x. The total discharge (Q) is the sum of the partial section discharges (q_n).

If no acceptable transect is found at a site, discharge may be estimated by using average current velocities (e.g., by average speed of floating objects) and cross-sectional area.

Discharge may also be continuously monitored using data from nearby USGS gauge stations.

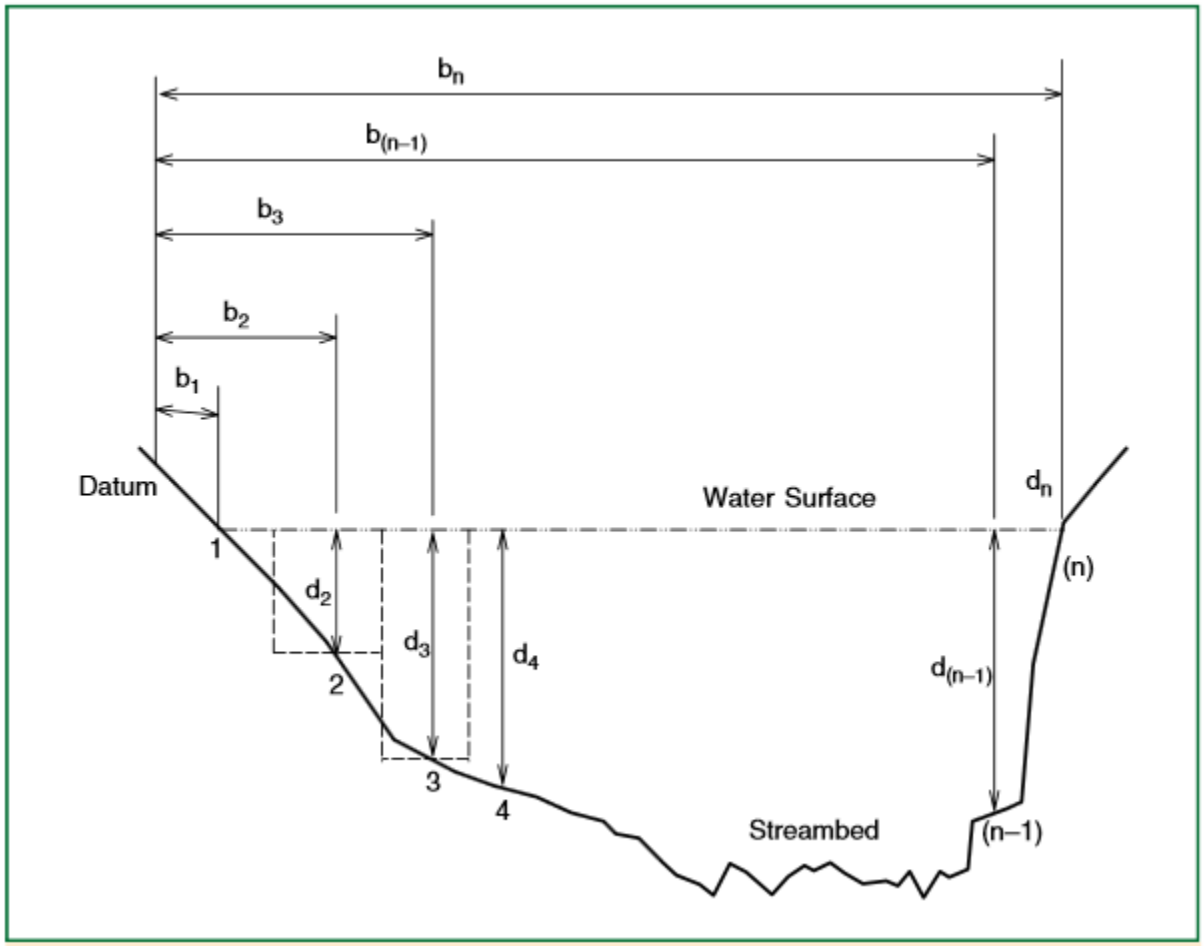

Figure 3.1 Taken from Hauer and Lamberti 2007. Schematic of the midsection method for computing cross-sectional area for discharge computations. Where: 1, 2, 3, ...n are observation points; b_1, b_2, b_3,…b_n are distances (transect intervals) from the datum to the observation points. d1, d2, d3, …d_n are depths of water (surveyed verticals).The dashed lines outline the section (cell) being measured.

FlowTracker

For detailed information on assembling, using, and troubleshooting please see the FlowTracker user's manual and the USGS FlowTracker Quick Sheet in Appendix XI. The following instructions are adapted from the FlowTracker user's manual.

The FlowTracker is suitable for use in measuring surface freshwater discharge in wadeable streams. Pre-measurement diagnostics should be performed both in the office before leaving, as well as the field location to ensure proper system function. Battery power should be checked before leaving for the field. Extra batteries and tools to open the back of the FlowTracker are packed in the FlowTracker carrying case, but it is important to ensure that they are there before leaving for the field. A BeamCheck is performed in the office and requires a connection to an external computer with the FlowTracker software. A BeamCheck should be run about once per week if the FlowTracker is being used for an extended period of time. Refer to the FlowTracker user's manual for detailed information and steps to complete a BeamCheck.

The FlowTracker should be mounted on the wading rod at the field location, ensuring that the X-axis of the probe coordinate system is perpendicular to the tag line, and the red band of receiver arm 1 is facing downstream. Picture examples of probe orientation can be found in the user's manual.

Once a sampling location is chosen in the field, one crew member should walk the proposed transect to ensure that the area is free of large obstacles or debris, such as large boulders, fallen trees, or heavy vegetation, as these will affect the discharge reading. Once an area is deemed appropriate, a tape measure is used to create a straight line transect across the stream. The stream width should be recorded and will be used in determining the number of sites used to measure discharge.

Upon entering the water, the operator turns on the FlowTracker and chooses the Start Data Run option. The operator is then prompted to enter information about the site such as site name and operator initials. Once this information has been entered, the operator is prompted to conduct an automatic QC test, which is an automated version of a BeamCheck. The operator will then follow the on screen instructions to complete the QC test and ensure proper system operation.

After performing the QC test, starting-edge location will be displayed. The operator will then press Set Location to set the starting edge location. This will be the meter measurement on the measuring tape transect that you wish to begin your series of measurements. Note: this value does not have to be located at 1 meter. Once the location information is entered, the operator will press Set Depth to set the starting-edge water depth. This can be measured by the second field crew member and entered in meters. Press LEW/REW to change the starting edge of the water to left or right bank. When complete, press Next Station to continue. Station information for Station 1 will then be displayed on the screen. Twenty to 30 measurements need to be performed within the stream transect to ensure an accurate discharge measurement, so the total width of the stream transect should be divided by 20 to 30 to calculate the measuring interval. This interval is then added to the starting-edge measurement to determine the location of Station 1. Example: if your Starting Edge is 1.5m and the interval is 0.4m, the location for Station 1 should be 1.9m. Once the location is set on the screen, the wading rod should be positioned along the measuring tape at the same location. The probe of the FlowTracker should be positioned so that it is facing upstream. The depth at Station 1 is then measured by the second crew member and entered as depth. The wading rod should then be adjusted to the appropriate corresponding depth (in feet) on the wading rod handle to ensure that the probe is suspended at the center of the water column.

When ready to measure, the operator should make sure the leveling bubble on the wading rod is centered, and that the probe is correctly facing the angle of stream flow. The operator will then press the Measure button. The wading rod should remain as still as possible while the FlowTracker makes measurements, counting down from 30 seconds. During this time, the velocity and signal to noise ratio (SNR) will be displayed on the screen. After 30 seconds, the FlowTracker will display the quality control values and the velocity data. Error codes may appear on the screen, especially if underwater obstacles are present. Refer to the FlowTracker user's manual to determine whether QC measurements are within acceptable range. The angle of probe to stream flow should be within 20 degrees. Measurements may be repeated as many times as necessary if QC parameters such as angle or SNR are not within range. If the measurement is suitable, the operator can press 1 to accept the measurement. The FlowTracker will then move to the next station at the determined interval. Location and depth measurements are repeated until the transect is complete. Once all stations have been completed the operator can press the End Section button to begin the steps to end the transect. Any error(s) that were present during the run will appear and will say at which location(s) the error(s) occurred. If the FlowTracker states that there was a discharge greater than 10%, the field crew must go back to the location(s) to take further measurements. For instance, if there was a discharge reading greater than 10% at 4.5m, a reading before and a reading after that location should be taken, at 4.4m and at 4.6m respectively. The "greater than10% discharge" error usually happens if a jump in velocity occurs between points, so if you see velocities change, it is best to take extra readings while still performing the run so as to not have figure out where to go back afterwards. Once completed, you will have to press End Section again. The FlowTracker will then prompt the operator to input the Ending Edge. The

location and depth of the ending edge should then be entered. The operator will then press the Calculate Discharge key to get the discharge data for the site. The various parameters can be scrolled through by using the Enter key. Crew members should then record all necessary information such as total Q, mean and maximum velocity, maximum depth, width and area on the field data sheet. When finished, the operator should press 9 to exit to the main menu, ensuring that all data is saved to the recorder. The FlowTracker may then be turned off by using the On/Off button.

RiverSurveyor

For detailed information on assembling, using, and troubleshooting please see the RiverSurveyor S5/M9 System Manual. The following instructions are adapted from that manual.

The RiverSurveyor is appropriate for use in discharge measurements if all portions of the stream transect are greater than 30cm in depth, including any areas with boulders, woody debris, or vegetation mats. The RiverSurveyor uses software that is installed on the DRWI field laptop. Before heading to the field locations, all equipment should be checked to make certain all pieces needed for assembly are present and in working order and that the field laptop and River Surveyor battery are fully charged.

The RiverSurveyor should be assembled at the field location according to directions found in the system manual. The Bluetooth dongle has a range of 60m if there is a clear line of sight and good weather, so the laptop must be set up strategically to accommodate accurate reception.

Once at the site, one crew member should walk with a ruler across the proposed stream transect to ensure adequate depths. If this is not possible due to hazardous stream conditions, it is appropriate to attempt to use the RiverSurveyor from the upstream side of a bridge.

Once a transect has been designated, one crew member should stay with the field laptop and another should take the RiverSurveyor into the stream with a ruler. The laptop operator should click the Start a Measurement button to start data collection. Note that this does not record any data, instead it allows the data from the system to be viewed to make sure the system is operating correctly. The other crew member should position the vessel at the start edge of the transect. Click the Start Edge button and collect at least 10 edge samples. The Edge window will be displayed showing information for both edges. The vessel should be kept as stationary as possible during this time. The starting distance from the bank to the middle of the RiverSurveyor is measured along with the edge shape and entered into the Edge dialog that pops up. Click the Start Moving button and the Transect window will be displayed. The vessel speed and direction need to be kept constant as it progresses across the river to the end edge. Click the End Edge button when the vessel reaches the edge of the opposite bank. A dialog box will prompt you to describe the End Edge. Note that the screen changes from the Transect Tab to the Edges Tab. This allows detailed viewing of the edge data collection. Be sure to collect at least 10 samples at the ending edge keeping the vessel as stationary as possible. Click the End Transect button upon completion of the end edge. This automatically opens a new data collection window so you can start a new measurement. The system is still running, so if you need to make another measurement, click the Start Edge button (or F5) to begin again as instructed. If data collection is complete, click the Abort button or press F8 to stop. It is recommended to go to the System tab and download the recorded data files upon completion of all the measurements.

The crew member working the laptop should review the data to make sure that the RiverSurveyor has no data gaps (i.e. areas where discharge was not taken because the depth was too shallow or for other reasons). Data gaps can most easily be identified as black spaces on the bottom graph which shows the measured transect with the vertical profile data included. It may be necessary to re-take the discharge measurements several times to obtain accurate and complete data.

In the event of hazardous stream conditions that prevent crew members from walking the RiverSurveyor across the transect, deployment from the upstream edge of a bridge is acceptable. To deploy the RiverSurveyor from a bridge several ropes should be used with great care given to make certain they are firmly attached to the vessel and are secured to the operator.

Temperature Logger Deployment

Adapted from the TidbiT® v2 Temp (UTBI-001) Manual and the HOBO® Waterproof Shuttle (U-DTW-1) Manual. For detailed directions and troubleshooting see these manuals.

Temperature data is collected using TidbiT® v2 Temperature loggers from Onset. The units are waterproof up to 305m and have enough memory to record over 42,000 12-bit temperature measurements. These units should be installed on a large piece of substrate (i.e. large boulder, bedrock, or foundation) or a heavily embedded boulder.

The temperature loggers are attached to brackets placed on the appropriate substrate. Brackets must be installed when the water temperature is higher than 12.7 ºC and when the stream is at base flow. The brackets need to be installed on a substrate that is neither too shallow so that the temperature logger is exposed to the air at any time, nor too deep so that retrieval is impossible. The loggers should never be installed in lentic areas but in areas that have a moderate and representative flow for the stream.

A two-part boat epoxy is used to attach the brackets to the substrate and must cure for at least 5hours at 12.7 ºC or higher temperatures before the temperature loggers can be affixed. After the bracket has been installed, the GPS location should be recorded and photos of the area upstream and downstream should be taken along with a drawing of significant landmarks in the area for future relocation purposes.

Once the bracket has cured, the temperature logger is deployed and affixed to the bracket. The temperature logger unit should be deployed with a delayed start time as outlined in the manual using the DRWI field laptop and the HOBO Waterproof Shuttle (U-DTW-1). The mounting bail on the logger accepts 1/8 inch (4mm) diameter nylon cord or other strong cable. If wire is used to secure the logger, make sure the wire loop is snug to the bail. Any slack in the loop may cause excessive wear. The battery in the TidbiT v2 temperature logger is a non-replaceable 3-Volt lithium battery and should last on average 5 years.

Temperature Logger Data Retrieval

Adapted from the TidbiT® v2 Temp (UTBI-001) Manual and the HOBO® Waterproof Shuttle (U-DTW-1) Manual. For detailed directions and troubleshooting see these manuals.

Data should ideally be retrieved from the temperature loggers once a quarter at the same time as the quarterly water chemistry sampling, however the memory on the loggers allows this period to be extended if needed.

Before traveling to the site location it is imperative that all parts to the HOBO waterproof shuttle are present and that the DRWI field laptop has full battery. Once the temperature logger has been located at the site, connect to and read out the temperature data in accordance with the TidbiT® v2 Temp (UTBI-001) Manual and the HOBO® Waterproof Shuttle (U-DTW-1) Manual. Make certain that the data has downloaded and saved to the DRWI field laptop and that the temperature logger is functional for the next set of collections before you leave the site.

Steps to readout temperature logger data

1. The logger should be inserted with its mounting bail on the side of the shuttle without the notch. The correct mount will become tighter when pressed in versus looser which means it is on incorrectly.
2. The cable should then be connected to the shuttle and computer.
3. The "Start" button and "Devices and Printers" should then be clicked.
4. The lever on the side of the shuttle should be depressed and the Transfer light should blink yellow then a green light should appear.
5. The Shuttle icon should appear in the Devices and Printers screen after a few moments and should say "TidbiT" underneath of it. If the icon only says "Shuttle" and not "TidbiT" that indicates that the logger is not being read by the shuttle correctly. Steps 1-4 should be repeated until the shuttle is correctly attached to the temperature logger and readout is successful.
6. The HOBOware Pro software should then be opened on the field laptop. If connected correctly, "Tidbit" will be displayed on the bottom left corner of the screen. If incorrect, it will only display "Shuttle".
7. The second icon from the left can then be selected to perform the "Readout".
8. A screen will then pop up asking if you want to stop reading the logger. "Don't Stop" should be selected.
9. A blue progress bar shows the progression of the data download.
10. Another screen will then pop up asking to save the data. Data can be stored in the folder titled "HOBO Data 2015" as the site ID and the date.
11. Another screen will pop up to plot the data, and the "Plot" option should be clicked. A plot will then pop up.
12. Once these steps have been completed the readout is complete and the program can be exited.

3.4.4 Water Chemistry Laboratory Methods

Samples are composited in the field and then packed in ice filled coolers, which are then sent (or hand delivered) to the ANSDU laboratory where filtering and processing will be carried out. In some cases, filtration and initial sample processing will be accomplished in the field laboratory (i.e. hotel). In these cases, samples can be preserved on ice (nutrient samples frozen) and delivered to the main laboratory within 5 working days.

Nutrient sub-sampling

From the water column sample container, a subsample is set aside for nutrient analysis. These samples are analyzed for dissolved and total nutrients. This requires two 125-mL bottles per site – one unfiltered, one filtered. Nutrient samples must be filtered and frozen within 24 hours of collection of water column sampling time. Samples can be filtered in the field using 125-ml, pre-cleaned syringe filtering apparatus and 25 mm x 0.45-um syringe filters or equivalent. All material is cleaned prior to use. Bottles should be labeled before filling. Bottles are wrapped with label tape and identified using permanent marker: "DRWI", site ID, date, and Filtered ("FNUT") or Unfiltered ("UNUT"). Make sure to leave space for biogeochemistry laboratory staff to write login IDs on tape for internal organization. Sample container with water column sample should be shaken before any subsample is extracted from it.

Water is extracted from the sample container using the syringe. The fiber glass filter is attached to the syringe and a small amount of the filtered water is pushed into the FNUT bottle, shaken to rinse, then discarded. Then the bottle is filled, leaving a half inch of space for expansion when frozen, with the filtered water using the syringe. Unfiltered: A small amount of the water column sample is used to rinse out the inside of the UNUT bottle, which is then filled, leaving a half inch of space for expansion when

frozen, with water from the cubitainer/bottle. Nutrient sample bottles are stored in a freezer (or frozen in the field using dry ice) until they can be tested.

3.4.5 Chemistry Quality Control and Assurance

All duplicates must be within 30 RPD. All standards and QCs analyzed within a run must be within 10% of the correct value. Blank QA/QC is determined by the discretion of the analyst, in relation to previous blanks, normal blanks, and circumstances during the run. See Table 3.3 for information on each analyte with the standard used to make calibrations, the range of the calibration, the R2 limit of the calibration, the number of points in the calibration, the amount and placement of QCs, standards, sample duplicates, blanks, and spikes within a run. All standards and QCs are purchased from VWR. QC criteria for water chemistry samples are presented in Table 3.3. These tables include information regarding reporting and detection limits, methods, and frequency of laboratory QC samples. In the field, QC samples will be collected at a frequency of 10% of the total number of sites. These samples will include duplicates and equipment blanks. Detection limits and reporting limits provided will meet the project objectives.

3.4.6 Chemistry Equipment Calibrations

All YSI calibrations, as well as, name, date/time, and unit/probe serial numbers will be written on pre-printed DRWI calibration sheets and kept in a binder for QA/QC purposes.

YSI

Adapted from the YSI Professional Plus User Manual.

The YSI Multimeter has a built in GLP or 'Good Laboratory Practice' file which saves detailed information about calibrations automatically. It also includes diagnostic information about the sensors. A single GLP file is utilized to store all calibration records and is capable of storing 500 records. Once the GLP file is full, the instrument will begin to overwrite the oldest record with each new calibration record. This data can be periodically downloaded or can be used to reference when the last calibration occurred and if it was done correctly. It is acceptable to calibrate the YSI Multimeter within 12 hours before sampling; however, it is best to calibrate each field day morning to ensure the meter is working properly. Post-sampling checks on the meter should be undertaken after each day of use if not using the meter the following day, or in the morning before calibration. If points fall within their acceptable values then calibration is not necessary that morning.

All probe/cable assemblies have a built-in temperature sensor and temperature calibration is not required nor available.

Dissolved Oxygen

The dissolved oxygen % (DO) sensor should have a one point calibration in water-saturated air at each site location to account for barometric pressure differences. The supplied sensor storage container (a grey sleeve for a single port cable or a screw on plastic cup for the dual-port and Quatro cables) can be used for DO calibration purposes. Moisten the sponge in the storage sleeve or plastic cup with a small amount of clean water. The sponge should be clean since bacterial growth may consume oxygen and interfere with the calibration. If using the cup and you no longer have the sponge, place a small amount of clean water (1/8 inch) in the plastic storage cup instead. Make sure there are no water droplets on the DO membrane or temperature sensor. Then install the storage sleeve or cup over the sensors. The storage sleeve ensures venting to the atmosphere. If using the cup, screw it on the cable and then disengage one or two threads to ensure atmospheric venting. Make sure the DO and temperature sensors are not immersed in water. Turn the instrument on and wait approximately 5 to 15 minutes for the storage container to become completely saturated and to allow the sensors to stabilize. Highlight DO %

and press enter to confirm. The instrument will use the internal barometer during calibration and will display this value in brackets at the top of the display. Highlight Barometer and press enter to adjust it if needed. If the barometer reading is incorrect, it is recommended that you calibrate the barometer. Note - the barometer should be reading "true" barometric pressure (see Barometer section for more information on "true" barometric pressure). If the value is acceptable, there is no need to change it or perform a barometer calibration. Wait for the temperature and DO% values under "Actual Readings" to stabilize, then highlight Accept Calibration and press enter to calibrate.

Conductivity

The conductivity sensor should be calibrated with 1413 µS/cm or solutions of similar specific conductance and checked on 718µS/cm or 100 µS/cm. The solution must cover the holes of the conductivity sensor that are closest to the cable. Ensure the entire conductivity sensor is submerged in the solution or the instrument will read approximately of half the expected value! Choose the units in SPC-us/cm and press enter. Highlight Calibration value and press enter to input the value of the calibration standard. Then, once the temperature and conductivity readings stabilize, highlight Accept Calibration and press enter.

pH

The pH sensor should have a three point calibration preformed with pH 4, 7, and 10 standards. Calibration can be accomplished in any buffer order. pH 7 buffer should be used regardless of how many calibration points you use but it does not have to be used first. Highlight ISE (pH) and press enter. The message line will show the instrument is "Ready for point 1". The pH calibration allows up to six calibration points. Place the sensor in a traceable pH buffer solution. The instrument should automatically recognize the buffer value and display it at the top of the calibration screen. If the calibration value is incorrect, the auto buffer recognition setting in the Sensor Setup menu may be incorrect. If necessary, highlight the Calibration Value and press enter to input the correct buffer value. Once the pH and temperature readings stabilize, highlight Accept Calibration and press enter to accept the first calibration point. The message line will then display "Ready for point 2." To continue with the 2nd point, place the sensor in the second buffer solution. The instrument should automatically recognize the second buffer value and display it at the top of the screen. If necessary, highlight the Calibration Value and press enter to input the correct buffer value. Once the pH and temperature readings stabilize, highlight Accept Calibration and press enter to confirm the second calibration point. The message line will then display 'Ready for point 3" and you can continue with the 3rd calibration point. After the 3rd calibration you must press Cal to finalize the calibration and to allow the instrument to update the pH offset and slope. The instrument will not take these cal values into account until Cal has been pressed. Make sure to rinse the probes with DIW after each pH buffer.

The YSI and its sensors will be maintained according to the owner's manual and manufacturer specifications.

Marsh-McBirney Flo-Mate

Adapted from the Marsh-McBirney Flo-Mate Model 2000 Portable Flowmeter Instruction Manual.

The Marsh-McBirney Flo-Mate should be calibrated at the beginning of each field day. A 5 gallon bucket or equally sized container should be used that is at least 10in deep and 10in in diameter. The Flo-Mate should be hung over a secured rod or stick over the container and the meter should be placed so that it is at least 3in from the sides, bottom, or top of the water. To make sure the water is not moving, wait 10 or 15 minutes after you have positioned the sensor before taking any zero readings. Zero stability is ± 0.05ft/sec. To initiate the zero start sequence, press the STO and RCL keys at the same time. You will see

the number 3 on the display. Decrement to zero with the down arrow key. The number 32 will be displayed. The unit will decrement itself to zero and turn off. The unit is now zeroed.

3.4.7 Chemistry Data Entry and Management

An analyst from the Biogeochemistry Section keeps track of QC values, duplicate RPDs, and blank concentration throughout all runs. All raw data is then entered into Excel and every data point and equation is checked, by a different person or by the same person on a different day. Data is then entered into its primary summary file, and then every data point is checked, by a different person or by the same person on a different day. All Excel files are kept in the Geochem_Share folder on the Academy's server. Hard copies are kept in folders and filed away.

3.5 Monitoring and Assessment of Algae at Integrative Sites

3.5.1 Phycology Introduction

Algal assemblages are a useful tool to assess and monitor stream health. Unlike fish or macroinvertebrates, algae can colonize virtually any stream substratum. This serves a unique and particularly useful purpose in the variable stream types throughout the Delaware River Watershed. Algal taxa have high dispersal and growth rates, and relatively short generation times, which allow rapid responses to changes in their environment and complement other biomonitoring efforts. Algal taxonomic information is widely accepted as a powerful water quality assessment tool, especially when combined with other bioindicators, such as fish or benthic macroinvertebrates. Algae tend to be particularly sensitive to ambient water chemistry parameters such as pH, conductivity, and temperature, as well as nutrient enrichment, heavy metal contamination, siltation, or other sources of pollution. Chlorophyll-a can provide insight into identifying potential eutrophication or explain low benthic algal biomass.

3.5.2 Phycology Training Requirements

All field staff must be trained in the field and laboratory under the supervision of an experienced field crew leader. Training is comprised of two parts: an introductory office/laboratory setting in which general protocols are described and training in the use and or calibration of specialized equipment and tools is carried out, and a day of field training at a local site. All paid staff are required to meet with the field safety officer and read, agree, and adhere to the FSP. All permanent and seasonal staff must possess a valid driver's license and complete a Motor Vehicle Request form in order to drive ANSDU vehicles.

3.5.3 Algae Sampling Design

Modified from the Surface Water Ambient Monitoring Protocol Bioassessment Procedures 2010: Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California (Fetscher et al. 2009).

Sampling occurs during peak periods of algal growth, during the summer between the end of June and the end of September.

A site is considered appropriate for sampling when a field crew has safe and legal access to an entire 100m reach. Exceptions include newfall/blocked access (i.e. log jams, active construction, road/culvert crossing reach) that may require a fragmented or shortened reach <100m in length. If possible, no tributaries feeding into the channel are within the monitoring reach. Other features to avoid within a sampling reach include man-made structures like bridges, roads, culverts, or artificial stream bottoms.

Table 3.3 Biogeochemistry QA/QC procedures per analyte

Analyte	Standard	Calib Range	R2 Limit	Point Calib	QC	Start and End of Run	After every 10 samples	Procedure	Instrument
TP	AS-PO4P9-2Y	0 - 0.5mg/L	>0.9995	8	QCI-064	QC, Blank, Std(s)	Duplicate, Std(s), Blank, Spike	Discrete Flow Spectrometry	Smart Chem
SRP	AS-PO4P9-2Y	0.01 - 0.5mg/L	>0.9995	8	USGS N125*, N126*	QC, Blank, Std(s)	Duplicate, Std(s), Blank, Spike	Discrete Flow Spectrometry	Smart Chem
NO3+NO2	AS-NO3N9-2Y, AS-NO2N9-2Y	0.05 - 0.5mg/L	>0.9995	8	QCI-084	QC, Blank, Std(s)	Duplicate, Std(s), Blank, Spike	Discrete Flow Spectrometry	Smart Chem
TKN	AS-NO3N9-2Y, AS-NO2N9-2Y	0 - 0.5mg/L	>0.9995	8	QCI-064	QC, Blank, Std(s)	Duplicate, Std(s), Blank, Spike	Continuous Flow Spectrometry	Alpchem
NH3	AS-NH3N9-2Y	0.005 - 0.5mg/L	>0.9995	8	QCI-084	QC, Blank, Std(s)	Duplicate, Std(s), Blank, Spike	Continuous Flow Spectrometry	Alpchem
TSS	NA	NA	NA	NA	QCI-084	QC, Blank*	Duplicate, Blank	Filtration	In house filtering set up
Total Alk	NA	NA	NA	NA	QCI-136	QC, Blank	Duplicate, Blank	Titration	Bottle top Burrette
Total Hardness	NA	NA	NA	NA	QCI-084	QC, Blank	Duplicate, Blank	Titration	Bottle top Burrette
Total Cl	NA	NA	NA	NA	QCI-136	QC, Blank	Duplicate, Blank	Titration	Bottle top Burrette
Cl	AS-CL9-2Y	1 - 200mg/L	>0.9999	9	QCI-136	QC, Blank, Std(s)	Duplicate, Std(s), Blank, Spike	Ion Chromatography	Dionex
Br	AS-BR9-2Y	0.2 - 5mg/L	>0.9996	7	QCI-136	QC, Blank, Std(s)	Duplicate, Std(s), Blank, Spike	Ion Chromatography	Dionex
SO4	AS-SO49-2Y	1 - 200mg/L	>0.9999	9	QCI-136	QC, Blank, Std(s)	Duplicate, Std(s), Blank, Spike	Ion Chromatography	Dionex
Ba, Sr, Na, Mg, K, Ca, Al, Fe, Mn	SCP Sciences	0.003-200ppm	>0.9996	11	NIST: 1643e, NIST: 1640a, MRC-CNRC: SLRS5	QC, Blank, Std(s)	Duplicate, Std(s), Blank, Spike	Inductively Coupled Plasma Optical Emission Spectrometry	Perkin Elmer Optima 8300

*- As documented in this QAPP

High velocity stream flow following a significant storm event can scour algae from stream substrate. Sampling will occur at least 10 days after a severe storm event. A flood event is arbitrarily defined as a sharp increase in flow that had at least one mean daily flow peak greater than 3 times the average flow over the preceding 7 day stable flow period (Biggs et al 1989). Flow and discharge are estimated using nearest real-time US Geological Survey stream gauges and compare to long-term median values (USGS Current Water Data for Pennsylvania, http://waterdata.usgs.gov/pa/nwis/rt).

Algae sampling follows the reachwide benthos (RWB) method for collecting algae, as outlined in the SWAMP protocol (Fetscher et al 2009). Eleven sub-samples, one sample per transect, are composited into a single sample representing the entire sampling reach. The RWB method is advantageous for monitoring streams in all clusters, regardless of size or dominant substrate type, and allows for a more complete sample of the algal community existing in both epilithic and depositional habitats.

In order to preserve sample integrity, samples are kept out of the sun, protected from heat and desiccation with a black plastic cover, to prevent chlorophyll-a (chl-a) degradation, limit cell division and decay of soft-bodied algae. Sub-sample tubes are covered in aluminum foil, and all samples are put on ice until crews return to the Academy.

To prevent contamination/transfer of algae from other sites, all equipment including dish tubs, scum-getters, and scrub brushes are thoroughly rinsed with stream water before and after each sampling event.

3.5.4 Algae and Chlorophyll-a Sampling Methods

Modified from the Surface Water Ambient Monitoring Protocol Bioassessment Procedures 2010: Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California (Fetscher et al. 2009).

A 100-m reach is established and sampled to collect a representative composite of the algal communities present at each integrative site. The monitoring reach is divided into 11 equidistant transects running perpendicular to stream flow, each 10 m apart. Transects are designated “A” through “K,” with the “A” (0m) transect at the downstream end of the reach (Fetscher et al. 2009). After the reach has been established, GPS coordinates are collected at the bottom (0m) and top (100m) ends of the reach using a Garmin eTrex handheld GPS unit and recorded on the first sheet of the DRWI algae monitoring field sheet packet (see Appendix VI).

Ambient water chemistry measurements (pH, dissolved oxygen (DO) specific conductance, pressure, and temperature) are recorded using a YSI ProPlus multimetric probe just downstream of the monitoring reach before the reach has been entered or disturbed in accordance with section 3.4.3 Water Chemistry Sampling Methods. If the reach has been walked through, the samples must be taken upstream of the disturbed area. A 4-liter cubitainer of stream water is collected and stored on ice until it can be returned to the ANSDU chemistry lab. Analytes related to algal growth and community composition are evaluated using this water sample, including: NO_3, NO_2, NH_3, TN, SRP, TP, and major ions. (Refer to section 3.4.3 and 3.4.4 for specifics and additional directions).

At seven points along each transect, stream depth is measured, substrate size and flow type are classified, and substrate embeddedness is estimated. These points are defined as the left bank, 1m from left bank, left-center, center, right-center, 1m from right bank, and right bank. For descriptions of size classes and flow type classifications, see "Algal size and substrate" field sheet in Appendix VI.

Using the RWB method, the sub-sampling position alternates between the left-center, center, and right-center positions. Algae are sampled prior to physical habitat (PHab) transect and reachwide assessments so as to not disturb benthic substrate prior to sampling.

Quantitative diatom, soft-bodied algae, and chlorophyll-a sample collection

1. Algae subsample collection begins at transect "A," at the left-center point, and proceeds to transect "K," alternating the subsample collection location as described above. As samples are collected, a tally is taken of the number of samples that correspond to each of the classes of sampling device, based on the surface area they sample: a 28274.33 mm^3 Petri dish lid for depositional samples (particle size up to 16mm in diameter), or a 615.75 mm^2 60 mL syringe ("Scum-getter" area; Scum-getter apparatus described below).

   Tallies are recorded on the Algae size and substrate field sheet in the DRWI algae monitoring field packet (Appendix VI). This information is used to calculate cell density, algal biovolume and biomass.

2. For substrates larger than or equal to GC size class (see Appendix VI for size class information) a scum-getter is used to isolate a 615.75mm^2 area, and the underlying substrate is thoroughly scrubbed using the brush end. A 2mL pipette and bulb or 125mL wash bottle filled with stream water is used to remove scrubbed algal material, using a minimal amount of water, and collected in a light-colored composite dish tub*. The number of scum-getter areas collected per piece of substrate depends on substrate size and thickness of algal cover, but areas scrubbed should be non-overlapping and proportional to substrate size and number of substrate surfaces not embedded in streambed sediment. At least one scum-getter area is collected per piece of substrate. If the substrate is between 16 and 28mm in diameter, an additional piece of substrate is collected.

   *For sampling seasons 2013 and 2014, the "top rock" periphyton collection method was used, in which the entire surface of the particle was scrubbed and scrubbed rock surfaces were covered in heavy duty aluminum foil, surface area of each foil piece was measured, and total biomass was estimated. In 2015, the "top rock" collection method was replaced with scum-getters for more consistent and accurate surface area estimates.

   For depositional substrates collected using the Petri dish, sediment or other types of substrate (e.g. macrophytes, dead leaves) should be massaged thoroughly between the fingers to dislodge any clinging algae.

3. The sediment is then rinsed thoroughly but sparingly using stream water to create a suspension of dislodged algal material. The final volume of the composite sample is measured using a 500mL graduated cylinder, pre-rinsed with stream water. Take care to

ensure that the final composite volume does not contain sediment greater than or equal to size class SA (0.6-2 mm in diameter).

The composite volume is recorded next to the "diatom" composite volume on the first page of the DRWI algae monitoring field packet.

4. From the liquid composite sample, 40mL is aliquoted into a pre-labeled 50mL diatom sample tube wrapped tightly in aluminum foil. The new composite volume is recorded next to the "soft algae" composite volume on the first page of the DRWI algae monitoring field packet.

5. 45mL is then aliquoted into a pre-labeled 50mL soft algae sample tube wrapped tightly in aluminum foil. This new composite volume is recorded next to the chl-a composite volume on the first page of the DRWI algae monitoring field packet.

6. 50mL is then aliquoted into the pre-weighed, pre-labeled 50mL chl-a sample tube wrapped tightly in aluminum foil.

7. A Whirl-Pak is then filled with the remaining composite sample, either with the full remaining amount or to the 100mL fill line on a 4-ozWhirl-Pak, whichever is less. The remaining composite sample is discarded. All sampling and collection tools are thoroughly rinsed and scrubbed before leaving the site.

Assessment of algal cover percentage

A rapid algal assessment, (see page 2 of the DRWI algae monitoring field packet), is completed after sampling the entire reach. The rapid algal assessment estimates are agreed upon by the entire field crew before leaving the site in an attempt to negate some of the subjectivity.

Qualitative soft-bodied algae sample collection

During sample collection, any crew member may collect qualitative soft-bodied macroalgal samples within the reach that would otherwise not be collected as part of the quantitative composite algal sample. Qualitative macroalgae are visible to the naked eye and are collected to aid in quantitative soft algal identification and provide a more accurate representation of soft algal diversity in a sample.

Qualitative samples should be collected from all obviously different types of filamentous and mat-forming algal growths, including colonies growing on the surface of sediments. Samples are collected from as many distinctly different locations within the reach as possible. Certain types of green algae may be misidentified as moss or macrophytes; if the field technician is unsure, it is best to collect the sample anyway.

Each qualitative algal grab sample is collected in individual Whirl Paks and all grab samples from a site are stored in a single large Ziploc bag, along with the quantitative subsamples. Because these samples are not preserved, they can decompose rapidly. Field crews must keep these samples out of direct sunlight.

Sample handling and custody

Upon returning from the field, a chain-of-custody form Q-00-11-R2 (ANSP PCER 2004) is filled out for all water samples and chl-a samples. Along with samples, this form is relinquished to the ANSDU Biogeochemistry Section.

A second chain-of-custody is filled out for all qualitative and quantitative algae samples. This chain-of-custody is relinquished to the ANSDU Phycology Section (Frank Acker or Alison Minerovic).

Water cubitainers are stored in the ANSDU Biogeochemistry refrigerator. In instances of overnight travel, filtered and unfiltered nutrient samples (FNUT and UNUT, respectively) are prepared the same day as sampling following the Biogeochemistry protocol in section 3.4.4.

Chl-a samples are to be centrifuged at ≥ 10,000 RPM for ≥ 5 minutes. Water is decanted using a vacuum pump without disturbing the pellet. Samples + tubes are weighed and weight is recorded. Samples are then be re-wrapped in foil and stored in the Biogeochemistry freezer.

Diatom quantitative samples are preserved with 10 mL of 10% buffered formalin. Preservatives are added under a fume hood, following laboratory safety protocols. Soft algae quantitative samples are preserved with 5 mL of 50% glutaraldehyde. Preservatives are added under a fume hood, following laboratory safety protocols.

All algae samples from a site (fixed quantitative and unpreserved qualitative grab samples) are stored in a single large Ziploc bag in the walk-in cooler on the 3rd floor of the Academy. (Overnight trips store samples in a cooler on dry ice until return to the Academy).

Reachwide physical habitat assessments

Transect habitat assessments are modified from the US Environmental Protection Agency's Wadeable Streams Assessment Field Operations Manual (USEPA 2004), High/Low Gradient Chapter 9-1, USEPA 2004 (high/low gradient), and two riparian forest indices, by Munné et al. 1998 (QBR index), and González del Tánago & García de Jalón 2011(RQI).

See Appendix VI for complete field sheet. Habitat assessments are completed in 10m intervals – along each sampling transect and 5m upstream and downstream of each transect. Each bank is assessed separately for canopy cover and structure and human bank alterations. Bankfull and wetted widths are measured at each transect. Canopy cover is estimated using a spherical densiometer at the left bank, center of channel, and right bank at each transect. At each of these three locations, canopy cover estimates are taken facing upstream, towards the left bank, downstream, and towards the right bank. Estimates are recorded and averaged on the field sheets.

At each transect, fish cover and other variables, including ranked percentages of aquatic vegetation and algae, boulders, woody debris and others are also measured. Visual assessments of riparian cover and human influence are included in these measurements.
At transects A, F, and K (0, 50, and 100m transects), photos are taken facing upstream and downstream, respectively, and camera and corresponding photo numbers are recorded on the field sheet. Left and right bank height and angle are measured using a Bosch handheld laser level at transects A, F and K in accordance with the Standard Operating Procedures for Measuring Banks with Bosch Meter. Bank angles >60 degrees are recorded as ">60 degrees."

Three reachwide habitat assessments from are also completed (See Appendix VI). The "high/low" gradient assessment varies at each site; either the "high" or "low" gradient assessment will be completed, depending on valley and streambed substrate type.

3.5.5 Laboratory Phycology Sample Preparation Methods

Samples are logged into the Phycology Section's North American Diatom Ecological Database (NADED) following protocol P-13-47 (ANSP PCER 2012a). Sample preparation and analysis progress is tracked following protocol P-13-58 (ANSP PCER Diatom Prep Lab Manual version 2012b).

Diatom samples are cleaned of organic material and prepared on cleaned cover slips following protocol P-13-42 (ANSP PCER 2009). Dried cover slips are fixed to microscope slides using Naphrax diatom mountant following protocol P-13-49 (ANSP PCER 2012c). Slides are labeled following instructions in the Diatom Prep Lab Manual (ANSP PCER 2012d). Following completion of all slide counts for the year, slides are archived and stored following protocol P-13-56 (ANSP PCER 2015 Currently under revision). Benthic algal chl-a preparation, extraction, and analysis are documented in protocol P-16-117 (ANSP PCER 2002).

3.5.6 Phycology Analytical Methods

Diatoms

Slides are analyzed under 1000x magnification using oil immersion and differential interference contrast (DIC) microscopy. Six hundred valves (300 cells) are counted along one or more transects and identified to the lowest possible taxonomic level. Count data are recorded in the Phycology Section's Tabulator program (ANSP PCER 1999a).

Soft algae

Quantitative soft algae samples are analyzed following the National Water Quality Assessment (NAWQA) soft algae RTH DTH protocol P-13-63 (ANSP PCER 1999b).

Qualitative soft algae samples are observed under 100x or 400x magnification and identified to the lowest possible taxonomic level. Taxa lists are compiled and added to the quantitative soft algae sample data. Macroalgal subsamples are saved in 25mL glass vials, preserved with 1-3mL 50% glutaraldehyde. A portion of each subsample is left unpreserved, but spread and dried onto herbarium sheets.

3.5.7 Phycology Quality Control and Assurance

To ensure taxonomic consistency, diatom and soft algal taxa are photographed using a Nikon microscope camera and NIS-Elements Advanced Research 3.0 imaging software. Image processing and uploading procedures follow protocol P-13-74 (ANSP PCER 2008), modified using Nikon camera software features.
10% of slides are to be re-counted by another ANSDU Phycology Section analyst. At least 70% species richness and diversity similarity between analysts is considered acceptable QA.

For a more detailed QA implementation plan, see Patrick Center for Environmental Research QA/QC Implementation Plan Rev. 1 (ANSP PCER 1998)

3.5.8 Phycology Instrument Calibration

The YSI ProPlus multimetric probe is calibrated in accordance with section 3.4.6 Chemistry Equipment Calibration.

Microscopes are cared for and maintained following protocol P-13-57 (ANSP PCER 1999c)

3.5.9 Phycology Data Management

Before leaving each site, field sheet data is re-checked and reviewed by two separate technicians for completeness and accuracy. Upon returning to the Academy, field sheets are scanned and digital files are saved in the WilliamPenn_Share drive. Field sheet data are uploaded to the ANS DRWI database. Site photos are uploaded to the ANSDU DRWI photo database. Diatom and soft algae subsample count data are entered into the ANSDU Phycology Section's Tabulator program (ANSP PCER 1999a). Data uploaded from Tabulator to the Phycology Section's NADED database are subject to automated data checks.

3.6 Monitoring and Assessment of the Fish Assemblage at Integrative Sites

3.6.1 Fisheries Introduction

Fish are commonly used in stream bioassessments because: fish are important and abundant members of stream food webs; fish support a significant human use of streams through recreational and commercial fishing; and fish assemblages are responsive to a variety of stressors, including stream warming, pollution and oxygen depletion, hydrological modification, nutrient enrichment, habitat modification, acidification and contamination. Many of these stressors are related to different types of watershed and riparian land cover, and relationships between land cover and fish assemblages have been well-established. The distribution of individual fish species and their sensitivity to different stressors have been studied and can be used to define indices of biological integrity (IBI) and develop predictive models of species occurrence. These metrics and models can in turn be used to evaluate individual stream reaches.

A variety of approaches have been used to assess stream fish populations, including abundance and biomass of different species, assemblage structure, anomalies and parasites, and concentrations of contaminants in different tissues. In the standard DRWI fish monitoring, sampling is designed to provide estimates of fish abundance, assemblage structure, and external condition (i.e., anomalies and parasites), allowing a variety of assessment procedures. The estimation of abundance also minimizes effects of variable catchability among sampling events related to site or water level differences.

In addition to fish assessment, this program element monitors crayfish and salamanders. Crayfish are important and often abundant constituents of stream communities. The establishment of non-native crayfish is well-documented and can have large effects on community structure. Salamanders are monitored because of their sensitivity to a variety of stressors and their abundance, particularly in small streams.

3.6.2 Fisheries Training Requirements

Newly hired staff are trained in the laboratory by qualified fisheries scientists on identifying preserved fish, crayfish and salamander specimens, using appropriate taxonomic keys and previously identified and curated vouchers. In addition, when staff commences field work they

are trained in identifying and safely handling live specimens under the supervision of fisheries scientists familiar with the regional fauna.

All field staff are trained by the American Red Cross (or similar) in First Aid/ CPR/AED. If field staff are handling vertebrates they are additionally trained in animal welfare policy mandated by the Drexel Institutional Animal Care and Use Committee (IACUC). Under this policy the staff must complete an annual Occupational Health physical and complete online CITI training in Animal Welfare prior to handling vertebrates.

Staff are also taken into the field prior to actual project sampling and given a demonstration of the electrofishing equipment set-up, use and safety. To acknowledge their understanding, staff sign an electrofishing document outlining the collection methods and safety considerations pertaining to electrofishing.

3.6.3 Fish Assemblage Sampling Design

A 100-m reach will be sampled to assess the fish community using multiple pass depletion electrofishing (minimum of 2 passes; reach lengths other than 100m may be used for compatibility with historical data). Each reach will be blocked at the upper and lower end using nets of 0.25-in mesh, unless natural barriers sufficient to prevent escape of fish are present. Unusual conditions such as tributaries entering the sample reach, nearby juncture of sample reaches with larger tributaries, close proximity to dams, and other large in-stream obstructions will be avoided. Sampling will be done during daylight. The sampling crew will be large enough to provide efficient capture of specimens and maintain captured fish in conditions which will minimize mortality.

Samples will be taken under conditions where the ability to see and capture stunned fish is not compromised by transient site conditions. In particular, samples will not be taken in the period immediately after precipitation if turbidity and water levels are deemed high enough to significantly impede sampling. Operationally, these levels will be determined by visual assessment of bottom visibility (sampling could be conducted if bottom substrates are visible in most riffle and run areas and in most pools (excepting deepest parts of some pools, which may be obscured even in normal conditions) and by assessment of depths (sampling would not be conducted if significant parts of pools have depth greater than 0.9m). The decision to postpone sampling until a later date will be at the discretion of the field leader based on the level of rain and condition of the site.

3.6.4 Fish Assemblage Sampling Methods

The main method of sampling will be by backpack electrofishing (refer to Fisheries SOP #P-14-02) and will be done using Smith-Root Model LR-20 (or similar) backpack electrofishing units. Alternatively, tow barge electrofishing may be done where sites permit the use of a canoe. For tow barge electrofishing, a 2500 watt generator and Smith-Root 2.5 GPP controller is towed in a canoe or similar boat while field staff carry electrode poles and nets used for sampling. Attempts will be made to capture all fishes, lampreys, salamanders, and crayfishes. Notes on frogs observed or captured will be taken.

Fish collecting equipment

A minimum of one dip net (0.25- in mesh) should be used to collect fish. This will usually be carried by the backpack operator, but may be carried by others. Additional dip nets (0.25-in mesh) are commonly used by other staff when sampling with the electrofisher operator.

Polarized sunglasses will be worn by all crew members to reduce sun glare and increase capture rates (sunglasses will not be worn when they decrease visibility, e.g., in dense shade).

Staff will also carry collection buckets filled with ambient water to place the affected fish into when captured. In addition, large tubs (20 gallons) with ambient water are set throughout the reach in order to place effected specimens into for recovery. Aeration in these tubs is maintained (aerators or changes of water) throughout the collection period and during specimen processing to prevent suffocation.

All staff sampling will wear non-conductive waders (no breathable waders permitted) and Type 0 or 00 Electrical Linesman's gloves of non-conductive material. All staff wading in the water in the vicinity of electrofishing should wear non-breathable waders. Sampling will be stopped if people or animals are in the waterbody near the electrofishing.

Backpack Checkout

The output current is adjusted prior to sampling immediately downstream of the sampling area. The current is adjusted according to the conductivity of the waterbody and the effectiveness of the electrofisher to affect fish. To minimize fish mortality, the minimum amperage needed to immobilize fish will be used. The power for the control unit should be placed in the on position. The voltage should be noted in the field notes. The electrodes should be placed in the water without touching. Before further checkout or operation, the operator should check that no persons are vulnerable to the current, i.e., that the only persons in the water in the vicinity of unit are protected by insulating material (waders, gloves, etc.) from being shocked. The unit must be vertical or nearly vertical for further checkout and actual operation. The dead-man's switch on the wand electrode should be closed. The current should be read while the switch is closed. While sampling may be conducted under some situations at lower currents, the low current should be recorded in the field notes. If the ammeter registers a current spike and then indicates no voltage, the unit may be shutting off automatically because of too high current. The voltage control on the control unit may be adjusted to increase or decrease the output voltage in order to increase or decrease the output current. Typically, 100-300 volts will be used to output sufficient amperage of pulsed DC (50Hz, 6ms) to immobilize fish. To minimize fish mortality, the minimum amperage needed to immobilize fish will be used. Higher voltages will be used in streams with conductivities less than 100 µs/cm. The voltage and current at the start of shocking should be recorded, and both should be checked periodically during shocking to monitor loss of generator power.

Backpack Operation

Operation may be done by one or more persons. The main operator carries the backpack, one electrode and usually a dip net, and the second electrode is trailed behind the operator. The operator turns current on and off with the dead-man's switch on the anode pole, and catches fish with the dip net. Additional persons may be used, e.g., to net fish with a second net and handle captured fish.

Enumeration and measurement of the catch and data transcription may be done at intervals during sampling or at the end of sampling. The decision should be based in part on minimization of mortality of fish to be released and maintenance of proper condition for preservation.

While being held prior to data transcription, fish should be held either in ambient water, ice or preserved in appropriate preservative solution (10% buffered formalin or 70-95% alcohol following SOP # P-14-04). A worker will monitor biota for signs of stress while they are being held prior to processing. These procedures may include holding specimens in flow-through containers in the stream, holding in aerated, closed buckets, frequent water changes, and/or maintaining samples at low densities. Specimens to be released should be handled in such a way to minimize stress whenever practical. This includes minimization of time out of water, minimization of handling, manual movement of water through the gills (e.g., by moving the fish through the water prior to release) and frequent renewing of the holding water prior to release.

Larger individuals that do not fit into buckets and/or show signs of stress will be processed immediately. Remaining fish will be processed after these; fish will be enumerated, identified, measured, and condition (disease, anomalies, etc.) or markings (e.g., hatchery) of individuals will be noted. The majority of fish (all, if feasible) will be identified to species on site. Staff making identifications will be skilled in identification of the regional fish fauna.

All fish over 25mm total length will be counted. All fish specimens will be measured, except that groups of similar-sized individuals may be sub-sampled when the number of individuals is extremely high.

Length information will be taken as follows:

1. Up to 100 arbitrarily-selected specimens of each species less than 20 cm in length will be measured (total length).
2. All fish larger than 20 cm total length should be measured; these fish should not be included within the 100-fish count.
3. Where a subsample of all fish is measured, efforts will be made to avoid size-selection in measurement, including:
    a. Fish will be netted in groups with a net sufficient to capture a range of sizes; i.e., picking out fish singly (by hand or small dip net) will be avoided as it would likely involve unintended size bias.
    b. When the 100 fish count is reached for a species, individuals will be counted but no length measurements will be taken.
4. Separate procedures should be followed if a size distribution is encountered which precludes arbitrary selection. For example, if a sample contains a few large fish and a large number of small fish, it would be very difficult to pick fish with an unbiased probability of measuring the larger fish. Options include:
    a. Measure all fish.
    b. Measure larger fish and note that these are non-arbitrarily picked (e.g., as in the case of collection of only a few larger fish).
    c. Measure smaller fish and note that these are non-arbitrarily picked (e.g., as in the case of collection of only a few small fish).

Salamanders and other amphibians will be collected by hand dip nets of soft mesh material. Amphibians will only be directly handled by OSHA and CITI trained staff. Care must be taken

to avoid removal of the protective mucus layer covering the skin of amphibians. It is extremely important for samplers to ensure that they have not applied insect repellents, perfumes, lotions, or other potentially toxic substances that might be absorbed through highly permeable amphibian skin. Disposable non-latex, non-powdered gloves that have been rinsed in ambient water will be worn by trained staff when measuring and identifying amphibians. Wearing disposable gloves when handling amphibians will potentially protect the animals' skin (and investigators skin) from abrasion, chemicals and the spread of infection. Animals should be handled gently and transported in suitable sized individual containers that protect them from trauma and desiccation. Small amphibians can be temporarily restrained in plastic bags or plastic compartmental boxes containing a small amount of water, filled with air and sealed.

Medium and large frogs and toads may be grasped cranial to the hind limbs with the hind limbs fully extended. This helps prevent them from kicking. Larger animals may require a second grip around the forelegs. Larval forms and caudate species with gills should never be restrained around the neck because the gills will be damaged. Similarly, tails should not be used for restraint because they may easily detach.

Sampling may be stopped at the discretion of the crew leader by reason of conditions which would impair crew safety, strongly reduce collecting efficiency, impair comparability of samples, or lead to environmental damage. These include, but are not limited to:

1. Equipment malfunction.
2. Weather or flow conditions which create safety risks.
3. Weather which would impair sampling (rain, fog, etc.).
4. Onset of night (for day sampling).
5. Changes in sampling conditions of water (e.g., tide, conductivity, temperature).
6. Observations of high mortality among non- target groups, encounter with organisms sensitive to the sampling procedure (spawning aggregations, etc.).

The following information about each sample should be recorded on the field data sheets which can be found in Appendix VII:

1. Collection number (specific to each separate sample).
2. Serial number of preserved sample (specific to each separate package or group of packages; depending on the type of sample and project plan, specimens from more than one collection may be preserved under one serial number (e.g., individuals of one species collected in different samples from the same station).
3. Sampling locality.
4. Date and time.
5. Crew.
6. Operating characteristics (voltage and current).
7. Modifications from standard setup, if any.
8. Indices of collecting effort (i.e., sampling time, area, number of crew).
9. General notes on habitats sampled.
10. General notes on collecting efficiency (e.g., visibility, efficacy of stunning of fish)
11. Occurrence and reasons for aberrant termination of sampling, if any.
12. Data on organisms captured or collected
13. Notes on target group (i.e., whether all specimens seen were captured and noted, or whether only some subset of specimens was captured).

Habitat Measurements

Habitat and water chemistry measurements are to be taken during the same time as the fish sample unless unforeseen circumstances (e.g. storm fronts, time constraints, equipment malfunction, etc.) prevent their completion. If habitat measurements are not taken on the same day they should be taken as closely to the date of sample as possible and under similar conditions.

Water chemistry measurements are to be taken using a YSI Professional Plus Multimeter (or similar) instrument upstream of any stream activity or disturbances caused by the field crew. The YSI meter should be placed in a flowing portion of the stream, preferably riffle, and measurements should be taken in accordance with section 3.4.3 Water Chemistry Sampling Methods. Measurements (conductivity, specific conductance, pH, water temperature, dissolved oxygen (%), dissolved oxygen (mg/L), and time of day should be recorded on the field data sheet.

Latitude and longitude in decimal degrees should be taken at the bottom of the reach and recorded, along with GPS accuracy, on the field data sheet. If the GPS measurement is taken at a different point on the stream, that should be noted. Additional notes about reach location (e.g. distance from a landmark such as a tree, road, or bridge) should be recorded to aid in future reach relocation.

Habitat measurements will be taken for bankfull and wetted width and maximum depth at 10 points within the 100 m reach. These measurements are taken at transects that are perpendicular to the stream at ten meter intervals starting at the 5m mark. Widths are measured with a 50m open reel tape. Depth is measured with a 1.22m meter stick. Bankfull width is defined as the width at which the stream would overflow its banks in the event of flooding. The point on the bank at which this occurs is typically marked by the first occurrence of terrestrial vegetation.

Stream gradient will be measured at each site using a Pacific Laser Systems HLE 1000 Rotary Laser Level (or similar) following procedures outlined in the user manual and using the Gradient Field Sheet found in Appendix VIII. One measure of stream gradient will be sufficient for the life of the project unless there is reason to suggest a substantial change.

Photos will be taken in both the upstream and downstream directions at 0m, 25m, 50m, 75m, and 100m. Photo numbers should be written clearly on a field data sheet.

3.6.5 Fisheries Sample Handling and Custody

Some fishes, salamanders and crayfish will be preserved (10% formalin, 95% ethanol, or dry ice, depending on eventual sample use) for laboratory identification or as voucher specimens. Collected fish will also be preserved if sample processing cannot be completed by the end of the day or if sampling needs to be terminated due to other causes. A chain of custody will be prepared for all samples brought to the laboratory following SOP # Q-00-11r1.

3.6.6 Fisheries Analytical Methods

Specimens brought back to the laboratory will be identified (SOP # P-14-03) and measured (Fisheries SOP #P-14-06) by a staff scientist with the aid of taxonomic keys and use of identified specimens from the ANSDU Ichthyology Collection. Numbered and labelled laboratory bench

sheets will be maintained and data recorded on these sheets will be entered into the Fisheries Access Database and checked.

3.6.7 Fisheries Quality Control and Assurance

In addition to proper training of the field crews, data is rigorously checked at several points. Firstly, field sheets are reviewed by the crew leader for completeness and accuracy the same day as or at least within several days of the sample. The next check occurs when data is entered into the database by the individual(s) doing the entry that check for any missed values or any values that do not seem accurate or plausible. After the data is entered, 100% of the data entered is checked for transcription errors against the data sheets. Additionally, data is checked through a series of queries involved in the data analysis that highlight any missed outliers and inaccuracies.

3.6.8 Fisheries Instrument Calibration

The YSI meter should be calibrated in accordance with section 3.4.6 Chemistry Equipment Calibrations.

The Marsh-McBirney Flo Mate should be calibrated each day before use following instructions in the user manual and in accordance with section 3.4.6 Chemistry Equipment Calibrations.

3.6.9 Fisheries Data Management

Upon arrival at ANS, all field sheets are scanned and a digital copy is archived on the Academy's server in G:\Fisheries_Share\Data Archive. Photographs are downloaded from the camera and archived in G:\Fisheries_Image_Library\Projects\Watershed Protection. The "WP_site_photo.xlsx" file is updated with descriptions of each photograph is located in the same location. Hard copy data sheets should be grouped by site and date and given a data check tag. The tag should include when the datasheets were scanned and archived and by whom, entered and by whom, when the data was checked and by whom, and when, if at all, the data was corrected and by whom. After entry, datasheets are to be stored in alphabetical order by year in the appropriate filing cabinet for the foreseeable future.

Data is to be entered into the fisheries Access database (FshCrntv03.mdb).

Data is then to be uploaded into the Delaware River Watershed Initiative PostGRES database.

3.7 Monitoring and Assessment of Macroinvertebrates by Stroud Water Research Center at Integrative Sites

3.7.1 Macroinvertebrates Introduction

Aquatic macroinvertebrates are commonly used in water quality monitoring because: (i) as a group, they are sensitive to environmental change and stress; (ii) their limited mobility and relatively long life spans (approximately a year) makes the presence or conspicuous absence of macroinvertebrate species at a site a meaningful record of environmental quality during the recent past, including short-term infrequent events that might be missed by periodic chemical sampling; (iii) they are an important link in the food web, functioning as primary consumers (herbivores and detritivores) of plant and microbial matter that are then available to secondary consumers such as fish; and (iv) their abundance lends itself to statistical analysis, which can play an integral role in water quality assessment programs. Macroinvertebrates in this study will

be collected in early spring because they can be used as a measure of both habitat and water quality conditions of a given stream or river for the previous nine month period (reflecting actual exposure period of collected specimens).

3.7.2 SWRC Training Requirements

The methods described herein are focused on the efforts of highly trained professionals, most of whom have bachelors or masters degrees with extensive experience in both the field and laboratory collection and identification of macroinvertebrates. All new staff will meet with and be trained by experienced staff members at SWRC.

3.7.3 SWRC Macroinvertebrates Sampling Design

The macroinvertebrate sampling methods described here involve quantitative composite sampling in riffle habitats using a Surber sampler. The compositing technique involves combining 4 random Surber samples in the field to form one composite sample. The composite sample is then split into four quarters and ¼ becomes the preserved sample that is taken back to the laboratory. In rare instances where riffle habitat is limited the composite sample may be made of two Surber samples. The compositing technique has several advantages over standard (non-compositing) macroinvertebrate sampling. Compositing increases the accuracy of the desired description by increasing the number of samples collected (i.e., the area sampled) relative to the number of samples processed; for example, if four samples are combined per composite sample, then four times more samples are collected than processed. At the same time, compositing homogenizes spatial variation when these samples are combined, which generally reduces variance among samples in statistical analyses.

The reach of stream/river to be studied is initially defined as the length of stream/river that includes representative habitat diversity (e.g., riffles and/or pools) as well as sufficient area for the collection of samples. The length of the study reach is typically 100 m but may be shorter (e.g., 20-50m) if there are access issues or longer (e.g., 100-200 m) if riffle habitat is limited. If the sites are to be sampled repeatedly and samples from microhabitats (e.g., riffles, pools) are not to be combined. In addition, a rough map is drawn of the site that delimits the microhabitats (e.g. riffles, pools) based on their positions along the length of stream can be drawn.

Random sampling locations are chosen based on their longitudinal position (e.g., along the length of the study reach) and their position relative to the stream bank. Macroinvertebrate sampling is commonly focused on riffles because riffles generally support a macroinvertebrate assemblage with a high density of individuals representing a wide variety of species that includes many of the pollution-sensitive taxa (if not removed by environmental stressors). For example in a small stream, a riffle sampling location might be designated as 17-25, which would represent a sampling location in a riffle 17 m upstream from the starting point and 25% across the stream (from the right bank). Each sample is, a priori, assigned to a specific composite sample. Random sampling locations must pertain to an appropriate sampling location in that microhabitat (e.g., riffle). Because each composite sample consists of 2-4 samples, the number of sampling locations far exceeds the number of composite samples collected. For example, if one was to collect four composite samples, each consisting of four samples from riffles, then a minimum of 16 sampling locations in riffles would be chosen *a priori* plus 4-8 alternative locations in case some of the original sampling locations are not satisfactory (e.g., wrong habitat or impossible to sample). The choice of number of samples composited, number of composite samples collected, and microhabitat(s) sampled depends on the project protocol and field conditions.

3.7.4 SWRC Macroinvertebrates Field Sampling Methods

Operation of Surber Sampler

Sampling should start at the downstream end of the sampling area and proceed in an upstream direction. The operator should identify the location of each sampling area based on the longitudinal and lateral position. The precise location of the sample within a given sub-sampling area is a subjective decision made by the operator. The center of the randomly chosen area is preferred but the presence of boulders or large woody debris may require movement off-center (laterally or longitudinally). If it is impossible to obtain a good sample from this location, an alternative sampling site should be used for this composite sample.

The operator sets the bottom of the metal frame of the net into the substrate until there is a tight seal across the bottom to prevent animals from migrating under the sampler. The operator then lays the square bottom frame out on the stream bed. This defines the sample area. If rocks are only partially in the frame than only the section that is in the sample area is scrubbed. Large rocks that cannot be moved are scrubbed in place. The operator picks up the larger substrate particles (> 6 cm in longest dimension) one by one, scrubbing each one with a soft bristled brush under the water (in front of the net) to remove most organisms (n.b. the water current moving through the sampler carries these dislodged organisms into the sample net). After all particles have been scrubbed and removed, the enclosed benthic area is rapidly stirred and agitated for at least 20 seconds to swirl any residual organisms up into the water column and subsequently into the sample net. The sampler is then removed from the bottom and stream water is splashed onto the outside of the net in order to wash clinging animals into the bottom of the net. The net in inverted and the contents are washed into a plastic bucket filled with stream water designated for that composite sample. Remember, sets of four random samples have been designated *a priori* for inclusion in specific composite samples.

After all of the composite samples have been collected, they must be subsampled before they are preserved. Each composite sample (contents of four samples) is washed into a large sample splitter. The mixture of macroinvertebrates, detritus, and sediments is homogenized and resuspended by stirring, agitating, and pushing water into the subsampler. The material is then allowed to resettle across the bottom of the subsampler by slowly drawing it out of the barrel. If the material does not appear evenly distributed, repeat the resuspension and settling process. Separate the net-covered bottom from the rest of the subsampler, and push the plastic separator into the sample material, dividing the material into four equal slices. Use the spatula and scissors to separate one slice of material and transfer that material into a labeled sample jar filled with 95% ethanol. One slice represents 1/4 of the composite sample, which means it represents 1/4th of the stream bottom area combined in the composite sample. If 4 Surber samples were combined in the composite sample, then the 1/4 slice represents the area of a single Surber sample.

Each field sample must be properly labeled, showing the project name, project number, study site, sampling device, sample date and time, sample number, and sample type (e.g., ¼ of a composite of 4 Surber samples), and preservative (e.g. 95% ethanol). This same information is repeated on subsample jars produced in the laboratory, with the addition of subsample size.

3.7.5 SWRC Macroinvertebrates Laboratory Sampling Methods

Pour the sample from the sample jar into a 250-µm mesh sieve; any detritus or macroinvertebrates remaining in the sample jar are rinsed with water into the sieve. Rinse the

sample thoroughly with water to remove fine particles. The target is at least 200 macroinvertebrates per subsample.

The following outlines the procedures for splitting samples into four (or factors of four, e.g., 1/4, 1/16, 1/64, or 1/256) subsamples:

1. Using the three clamps in Section A (Figure 3.2) of the subsampler, Section B (with two black lines drawn on the screen, dividing it into 4 slices) is affixed with the screen end up, being careful to align arrows on each piece.

2. A water bath is created so that the sample can be suspended in the water and the A/B Section is placed into the container.

3. Decanted benthos sample is poured into Section A/B. Using a spatula, the sample is stirred with a back and forth motion across the diameter of Section A/B. Do not create a vortex. While the material is suspended, Section A/B should be carefully lifted out of the water while rotating, so that sample is distributed evenly on the screen on Section B. Again, avoid creating a vortex, as this will cause material to settle toward the center of the screen.

4. Blot excess water from the underside of the screen using a sponge or towel and carefully undo the fasteners holding Sections A and B together. Remove Section A.

5. Using spatula and scissors (to cut leaves if present), the four slices are separated. Depending on the desired subsample size, one or more slices (e.g., 1/4th or 1/2 of the sample) should be transferred to a separate vial or jar with the appropriate label information (see above). The unused material goes back into the original sample jar. If smaller subsamples are desired, repeat the above procedure until the desired size is reached. For example, if the target subsample size is 1/64th of a sample, the first 1/4th subsample can then be split into four slices, each representing 1/16th, and then one of these 1/16th slices would be split into four slices, each representing 1/64th of the original subsample. Likewise, subsample slices can be combined to create other subsample sizes (e.g., 1/32, 3/16, 1/8th or 1/2). All samples are then preserved in 95% ethanol.

Macroinvertebrate Sorting procedures

Macroinvertebrates are separated from detritus by taking a small portion (approximately 1/4 teaspoon) from the sample or subsample and placing it in a glass petri dish or sorting tray partially filled with water. This material is then carefully examined with the aid of a dissecting microscope (12 X magnification), which is the most accurate way of examining the sample material. All other methods that rely on less magnification (e.g., in a white enamel pan without the aid of magnification, or with the aid of a desktop magnifying glass (1.75 X magnification)) result in more specimens being missed (greater sorting errors). All macroinvertebrates are removed from the detrital material collected in the sample and placed in a vial containing 95% ethanol.

A permanent label containing the appropriate information (project name, project number, study site, sampling device, sample number, sample date, name of individual who sorted and identified sample, preservative used) is placed in the vial containing the macroinvertebrates.

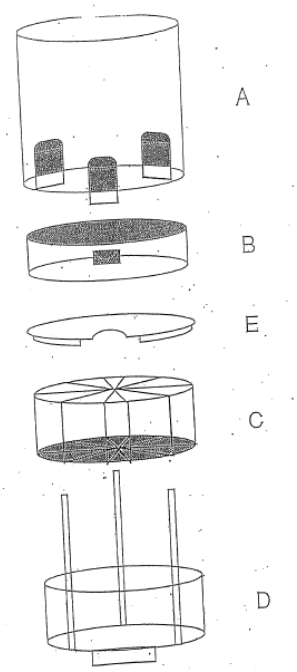

Figure 3.2 SWRC macroinvertebrate subsampler

Macroinvertebrates Identification

All macroinvertebrates are identified to the lowest taxonomic level possible. For aquatic insects, this is generally genus or species. Chironomids are subsampled before identification, and the number examined represents the percentage of chironomids in a sample of 200 individuals. For example, if a sample contained 300 macroinvertebrates and 100 of them were chironomids, then 67 chironomids are identified to genus/species and these identifications are applied proportionally to the remaining 33 of the original 100 chironomids. Other macroinvertebrates (e.g. crustacea, mites, flatworms, oligochaetes, and nematodes) are commonly left at higher taxonomic levels (e.g. order, family). Specimens that are damaged or extremely small are identified to the lowest taxonomic level possible. Identified macroinvertebrates are placed in vials containing 95% ethanol and a permanent label.

Notes to be recorded in the laboratory notebook include: project name, project number, study site, sampling device, sample number, sample date, name of individual who sorted and identified sample.

3.7.6 SWRC Sample Handling and Custody

The most important step in handling collected samples is making sure that the necessary information is on the label, and the label is affixed to the proper sample jar or subsample jar. It is essential that this is checked as the samples are collected. If whole or portions of samples are to be transferred to another lab, a Chain of Custody form can be used to make an inventory list documenting the transfer of samples.

3.7.7 SWRC Quality Control and Assurance

Errors for macroinvertebrate data are measured in three ways: sorting errors, identification/count errors, and identification accuracy. Sorting error (or efficiency) is measured by resorting through

the processed detrital material looking for macroinvertebrates that were not found in the first sort. All samples processed by "new" employees are rechecked by superiors until the new employee has three sorts in a row that have <10% error. After that, random checks are done to ensure continued accuracy in sorting.

Error in macroinvertebrates identifications are minimized by having difficult specimens examined by multiple taxonomists, and by checking all identifications of less experienced taxonomists. Selected voucher specimens are incorporated into the permanent macroinvertebrate collection at SWRC as part of the record of those difficult identifications. Error in macroinvertebrates identifications and counts is quantified by reexamining the specimens identified in at least 5% of the samples in each year. Errors may be due to incorrect identifications or counts or placing an individual in the wrong vial. Macroinvertebrate specimens (sorted and unsorted material) are archived by SWRC for at least 10 years after the collection date.

All identification data are initially recorded on paper worksheets, and later transferred to digital excel files. The excel file is then brought into SAS to be managed and analyzed. The first step in managing the data is to check each line of data against the original desktop data sheets.

3.7.8 SWRC Data Management

SWRC has a four-person Information Services group to oversee hardware, software and network functions and to manage and analyze data. Data management and analysis is implemented primarily with SAS and more recently Python, using MS Excel and MS Access for data entry along with several multivariate analysis software packages to complement data analysis in SAS. Data are backed up nightly or weekly (depending on data type) via an HP StorageWorks 1/8 autoloader with eight data cartridge slots and an HP Proliant DL100G2 Storage Network Access Storage (NAS). Backup tapes are regularly stored off site.

3.8 Rapid Salamander Monitoring and Habitat Assessment at Integrative Sites

3.8.1 Salamander Introduction

Salamanders are recognized as being abundant, important members of stream communities and sensitive to habitat loss and stream impairment. However most states do not have monitoring programs/protocols for assessing stream salamanders and their habitats. Existing state protocols use minimal salamander-specific habitat assessment. As a result, determinations from salamander monitoring may confound water quality impairment with habitat quality. To address this need, ANSDU is developing protocols to assess salamanders and their habitats. Our protocols have shown relationships of salamander metrics with watershed impairment. However, our protocols need additional work to insure detectability of uncommon and rare species, to determine the appropriate spatial scale for sampling, and to improve habitat assessment. ANSDU is continuing to refine its salamander and habitat assessment protocols by sampling a range of stream sizes, sampling longer reaches, and using an internally developed rapid habitat assessment protocol.

3.8.2 Salamander Training Requirements

See 3.6.2 Fisheries Training Requirements

3.8.3 Salamander Experimental Design

Four 20m reaches are sampled at a subset of sites designated for biological monitoring to determine the ideal sample length for determining species richness at the site. Habitat variables are assessed for each reach to characterize the habitat quality and to account for these factors when interpreting salamander richness and catch-per-unit effort.

3.8.4 Salamander Sampling Methods

Four 20m reaches will be sampled for 20 minutes by two staff. Available cover (i.e., cobble) will be turned by hand both in the stream and within 1m of the wetted edge. Aquarium dip nets are used to aid salamander capture. All salamanders captured are identified to species (excepting that some larval salamanders may not be able to be identified to species level and will be identified to the lowest level possible).

Typically, each reach is to be separated by 5m and is contained in a 100m long stretch of stream. The location of the reach will be noted to allow for relocation.

Salamanders and other amphibians will be handled in accordance with section 3.6.4. Fish Assemblage Sampling Methods. All salamanders encountered are enumerated, identified to the lowest taxonomic level possible and measured for total length and snout-vent length if able to be captured.

Habitat is assessed after sampling and on the same day. Variables denoted with an asterisk (*) will be scored using the following scale: 0 to 4 where: 0= absent, 1= sparse (<10%), 2= moderate (10-40%), 3= heavy (40-75%) and 4= very heavy (<75%). The following parameters will be measured at 2, 6, 10, 14, and 18m of each reach:

1. Site dimensions: Wetted width, Bankfull width, Max depth, Bar width, Undercut distance on the LB, Undercut distance on the RB
2. In-stream parameters: Dominant substrate, Mesohabitat (Pool, Riffle, or Run), Aquatic macrophytes* (moss will be noted when present), big woody debris (>0.3 m in diameter)*, small woody debris (<0.3 m in diameter)*, Average embeddedness of particles*
3. Parameters out-of-stream (wetted edge to 1 m) and including bars: Average embeddedness of particles*, % of area comprised of small boulders*, % of particles moist or wet underneath*, and % of new fall*.

3.8.5 Salamander Sample Handling and Custody

See 3.6.5 Fisheries Sample Handling and Custody

3.8.6 Salamander Analytical Methods

In accordance with 3.6.6 Fisheries Analytical Methods excepting that identified salamander specimens from the ANSDU collections may be used.

3.8.7 Salamander Quality Control and Assurance

See 3.6.7 Fisheries Quality Control and Assurance

3.8.8 Salamander Data Management

See 3.6.9 Fisheries Data Management

4.0 Project Site Study

4.1 Background

In 2014, the first rounds of funding were awarded by NFWF and OSI to DRWI partners for on-the-ground projects. ANSDU communicated with these partners as well as the grant receiving organizations to place monitoring sites downstream of project site locations. This process took place in 2014 before funding was awarded, based on applications for funding. Project sites were sampled in 2014 to capture conditions before project implementation. A subset of these, as well as newly funded project sites, will be sampled in 2016 to target specific geographies or Best Management Practices (BMPs) of interest, to be determined and defined by February 2016.

In 2014, the DRWI at ANSDU sampled as many project sites as possible. In 2016, the DRWI at ANSDU will work to refine the number of sample sites to answer specific research and Initiative questions to better support the goals of collecting data which shows measurable results of project implementation.

4.2 Project Site Study Design and Site Selection

ANSDU is working closely with all project participants as grants are awarded to cluster groups for restoration and conservation projects to identify where priority sites should be for project monitoring.

In conservation clusters, only downstream sites are monitored. For any restoration projects, the BACI experimental design (Before-After, Control-Impact (Stewart-Oaten, 1986)) is implemented. Although this approach requires a significant amount of resources, in practice twice as many monitoring sites compared to downstream only, it provides information on stream communities upstream of the project (i.e. control sites) as well as downstream (i.e. impact sites) to gauge the relative response in the downstream reach associated directly with project implementation. The use of an upstream control site for comparison is important for considering any inter-annual variability on in-stream communities and in avoiding attributing ecosystem responses to the project actions when differences in biota over time or space may actually be due to weather events, climate, or other factors. Sampling in 2014 provides “before, control-impact” sampling (within the BACI design, Stewart-Oaten et al., 1986, Bence et al., 1996) for restoration sites. The “after, control-impact” sampling will be performed after project implementation (2016-2017) and likely in subsequent years.

Control sites are geographically as close to the impact sites as possible and have similar land use percentages and geology. Incorporating additional control sites in other locations in the cluster will strengthen the analysis of project effects by increasing sample size and may provide insurance against future changes that can affect the upstream control site. Additional controls may be temporal (multiple baseline samples at the same site) or may be sites on other streams with similar characteristics. Additional control sites are addressed in more detail below.

Upstream controls may not be feasible in some situations, e.g., where there are significant changes in topography, habitat, etc., upstream of the project site; in these cases negative control

sites on other streams are used. Upstream control sites are also not appropriate where restoration is expected to change upstream conditions (e.g. dam removal could change upstream fish assemblages). Sites with these concerns will be evaluated for specific restoration practices as they are encountered.

After land parcels are identified for project implementation, the plausibility of sampling downstream of the projects is evaluated. Current cluster monitoring plans do not cover all land acquisitions, leaving a very large number of sites without monitoring. While the DRWI at ANSDU hopes to take on as many of these sites as possible, realistic limitations placed on sampling efforts by seasonality, staffing, and monetary restrictions force a number of sites to be dropped from our monitoring efforts. Options for addressing this problem include:

1. Choosing certain land acquisition sites to monitor only once, with future sampling events recommended after the grant period
2. Choosing a subset of ecological indicators to sample at certain land acquisition sites to monitor as many sites as possible with a reduced subset of indicators
3. Focusing monitoring efforts on sites that provide a range of land areas and characteristics for studying the effects of land conservation on water quality and omitting sites with less representative characteristics
4. Where acquisitions are clustered in a single sub-watershed, single stations may be relevant to a number of individual acquisitions

Additional Control Sites

Additional control sites will be compared with cluster and project sites and have the potential to add to the statistical rigor of future analyses of the results of projects. Using upstream control sites in BACI design will offer valuable information on local conditions, but by adding additional control sites located in other parts of the watershed, we add information as well as assurance that there will be data at another, unchanged control site if something should change at the primary control site. This increases the likelihood of demonstrating change compared with areas not addressed by projects through this program.

Additional control sites are being identified as project locations are communicated with ANSDU. The Stream Hiker software developed and adapted by researchers at ANSDU will be tailored to identify sites based on whether they are located in restoration or conservation project areas. For each cluster, appropriate land use percentages for control sites will be determined and will become the last criterion for control site selection in Stream Hiker. The number of control sites will be determined by the number and type of restoration and protection activities; for example, a smaller number of sites may serve as controls for a number of restoration or protection sites. Control sites should be located within the Delaware Basin, but in exceptional cases may be located in nearby drainages.

Each set of project-control sites will have similar geology, drainage areas and other characteristics not related to land use. For active restoration projects (agriculture and storm water), both negative and positive controls on other streams will be designated. Negative control sites have no restoration being implemented (i.e. “no treatment” in statistical terms; similar to upstream sites for the BACI design). Positive control sites have effective restoration being applied (i.e. “treatment applied;” ideally, these would be areas where a set of known, state-of-the-art techniques had been successfully implemented). It is possible that are no suitable control sites for the types of restoration activities planned, in which case sites in watersheds without

these specific stressors will be considered as representing the benchmark for assessing the magnitude of restoration. For example, positive controls for storm water sites could be sites in areas where storm water had been controlled during initial development. As an example for protection areas, negative controls will be sites on other streams without development. To test for regional changes in watershed conditions, a single site or group of sites may serve as controls for a number of protection activities.

No positive controls (i.e. other known conserved areas) will be designated for land protection areas because of the uncertainty of land development potential at candidate control sites, therefore the consequences of not protecting areas will be assessed using the extensive literature on effects of development and by modeling.

To add to the amount of data available on water quality before and after land protection, sampling may be done during multiple years to provide more baseline information on natural variability.

4.3 Timing of Sampling Events at Project Sites

Macroinvertebrate sampling is to be completed by SWRC from March to June in accordance with guidelines set forth by SWRC and approved by ANSDU. Fish surveys are to be conducted between April and October by ANSDU's Fisheries Section as outlined within this document. Algae is to be sampled between June and September by ANSDU's DRWI team and is to be conducted at a site at least ten days after fish sampling has occurred. Water chemistry parameters are to be sampled at the same time as algae by ANSDU's DRWI team in accordance with guidelines found herein. All field collections are contingent upon climatic events and stream conditions which may impact timing of said sampling events. All sampling decisions are to be made by the associated section leader in accordance with sampling criteria set forth in this document and/or as determined by field crew leader upon field examination of sampling locations.

4.4 Monitoring of Water Chemistry at Project Sites

In accordance with sections 3.4.1 to 3.4.7, excepting that there is to be no quarterly sampling at project sites, instead water chemistry parameters (i.e. cubitainers and YSI stream side parameters) are to be collected at the time of algae sampling or at another time by the Biogeochemistry section. Discharge is not to be taken and temperature loggers are not to be installed at project sites.

4.5 Monitoring and Assessment of Algae and Diatoms at Project Sites

In accordance with sections 3.5.1 to 3.5.9 for sampling designs, training requirements, methodology, QAQC procedures, instrument calibrations, and data management.

4.6 Monitoring and Assessment of the Fish Assemblage at Project Sites

In accordance with sections 3.6.1 to 3.6.9, sampling for fish will be conducted in wadeable streams for the purpose of evaluating the success of individual projects upstream of integrative sites. Individual projects addressing specific stressors unique to the clusters under study may be evaluated for their effect on water quality as determined by changes in fish assemblages.

4.7 Monitoring and Assessment of Macroinvertebrates by SWRC at Project Sites

In accordance with sections 3.7.1 to 3.7.8 for sampling designs, training requirements, methodology, QAQC procedures, and data management.

5.0 Adventive Site Study

5.1 Background

In discussions with DRWI partners, the question arose of how to prioritize land preservation in headwaters, which can comprise up to 80% of a watershed. Therefore, this study considers the effect small tributaries (first or second order) can have on larger streams (third to fourth order or higher). If adventive tributaries are found to improve water quality of large streams, it can help inform land preservation efforts in areas further downstream in the drainage than the headwaters where first order streams are flowing into other first order or second order streams. This would mean that adventive streams have the capacity to improve water quality in areas downstream where some impairment has occurred due to human activities in larger drainages.

5.2 Adventive Site Study Design and Site Selection

Preliminary sites for the adventive study were selected by combining outputs created using the ANSDU internal watershed/stream modelling software package Stream Hiker and the National Hydraulic Datasets (NHD) Plus. Stream hiker was used to sum upstream watershed areas, Land Use and Land Cover National Land Cover Dataset 2011 (NLCD) United States Geological Society (USGS), geology (from Jerry Mead), and Digital Monitoring Report data from the EPA.

Stream segments were spatially joined where small and large streams met. The size of large streams was limited to less than a 200 km^2 watershed. Small streams were defined as having watersheds <20% of the large stream's watershed area (limiting small stream watershed size to 40 km^2). Small streams contained at least 85% forested or wetland land cover (NLCD) for the upstream watershed and were presumed to have good water quality. Larger streams were 20-60% forested or wetland land cover and were presumed to have lesser water quality relative to the associated small stream. Streams that had greater than 5% limestone bedrock in either the small or large stream watershed were eliminated. Larger streams with major upstream point sources and small streams with minor or major upstream point sources were also excluded. 15 sites were selected of the 121 sites that met the above criteria. Due to landowner permission and field safety, 13 of these sites were sampled upstream, downstream and within the tributaries.

5.3 Timing of Sampling Events at Adventive Sites

Macroinvertebrate sampling is to be completed by SWRC from March to June in accordance with guidelines set forth by SWRC and approved by ANSDU. Fish surveys are to be conducted between April and October by ANSDU's Fisheries Section as outlined within this document. Algae is to be sampled between June and September by ANSDU's DRWI team and is to be conducted at a site at least ten days after fish sampling has occurred. Water chemistry parameters are to be sampled at the same time as algae by ANSDU's DRWI team in accordance with guidelines found herein. All field collections are contingent upon climatic events and stream conditions which may impact timing of said sampling events. All sampling decisions are to be made by the associated section leader in accordance with sampling criteria set forth in this

document and/or as determined by field crew leader upon field examination of sampling locations.

5.4 Monitoring of Water Chemistry at Adventive Sites

In accordance with sections 3.4.1 to 3.4.7, excepting that there is to be no quarterly sampling at project sites, instead water chemistry parameters (i.e. cubitainers and YSI stream side parameters) are to be collected at the time of algae sampling or at another time by the Biogeochemistry section. Discharge and temperature loggers are not to be installed or taken at project sites.

5.5 Monitoring and Assessment of Algae and Diatoms at Adventive Sites

In accordance with sections 3.5.1 to 3.5.9 for sampling designs, training requirements, methodology, QAQC procedures, instrument calibrations, and data management.

5.6 Monitoring and Assessment of the Fish Assemblage at Adventive Sites

In accordance with sections 3.6.1 to 3.6.9, sampling will be conducted in wadeable streams for the purpose of evaluating the contribution of adventive streams to downstream water quality excepting that single-pass electrofishing may be used for some cases. The type of electrofishing (single or multi-pass) will be determined on a project by project basis by the Fisheries Section Lead or Fisheries Project Coordinator.

5.7 Monitoring and Assessment of Macroinvertebrates by SWRC at Adventive Sites

In accordance with sections 3.7.1 to 3.7.8 for sampling designs, training requirements, methodology, QAQC procedures, and data management.

6.0 References

All references can be provided in electronic format upon request and may also be found in the ANSDU WilliamPenn_Share drive on the internal ANSDU server. (\\drexel.edu\fs\ans_science_cas\science_cas\WilliamPenn_Share\QAPP)

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