QUALITY ASSURANCE PROJECT PLAN

STAFF BASELINE WATER QUALITY MONITORING NEAR THE POTENTIAL PENOKEE IRON ORE MINE – CONTINUOUS TEMPERATURE, MACROINVERTEBRATE, and CONDUCTIVITY

BAD RIVER WATERSHED ASSOCIATION
P.O. Box 875
Ashland, WI 54806

REVISION: 0
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A1. APPROVALS

Donalea Dinsmore Digitally signed by Donalea Dinsmore
DN: cn=Donalea Dinsmore, o=Wisconsin DNR, ou=Quality Assurance, Office of the Great Lakes, email=donalea.dinsmore@wisconsin.gov, c=US
Date: 2012.04.04 10:33:43 -05'00'

Donalea Dinsmore:_____
Great Lakes Funding and Quality Assurance Coordinator
Wisconsin Department of Natural Resources

Kurt Schmude:_____
Lake Superior Research Institute Project Manager
University of Wisconsin-Superior

Matt Hudson Digitally signed by Matt Hudson
DN: cn=Matt Hudson, o=Bad River Watershed Association, ou, email=matt@badriverwatershed.org, c=US
Date: 2012.03.05 16:24:16 -06'00'

Matt Hudson:_____
Project Manager
Bad River Watershed Association

QUALITY ASSURANCE PROJECT PLAN REVIEW TEAM

The Bad River Watershed Association's (BRWA) *Staff Baseline Water Quality Monitoring Near the Potential Penokee Iron Ore Mine – Continuous Temperature, Macroinvertebrates, and Conductivity* Quality Assurance Project Plan was developed in response to the need to establish a credible and consistent set of criteria to ensure the quality and usability of data collected by BRWA staff. The QAPP format required by the United States Environmental Protection Agency (EPA) for all EPA-funded projects (BRWA's project is not EPA-funded) was used as the template for this document (EPA 2011).

The BRWA assembled a team of technical experts to review and comment on the sample collection techniques, quality objectives, and overall management of this program. The following individuals provided review of this QAPP:

Donalea Dinsmore, Wisconsin Department of Natural Resources
Tracey Ledder, BRWA Technical Advisor; Wisconsin Department of Natural Resources
Mike Miller, Wisconsin Department of Natural Resources
Cindy Koperski, Wisconsin Department of Natural Resources
Kris Stepenuck, Wisconsin Department of Natural Resources and University of Wisconsin-Extension
Naomi Tillison, Bad River Natural Resources Department
Kurt Schmude, University of Wisconsin-Superior, Lake Superior Research Institute
Joan Elias, National Park Service
Randy Lehr, Northland College

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GROUP A. PROJECT MANAGEMENT

A3. DISTRIBUTION LIST

1. Water Resources Specialist, Bad River Natural Resources Department
2. Wisconsin Department of Natural Resources Bureau of Watershed Management
3. Wisconsin Department of Natural Resources Lake Superior Basin Team Leader
4. University of Wisconsin-Superior Lake Superior Research Institute Project Manager
5. BRWA Project Managers
6. BRWA Executive Director

A4. PROJECT TASK/ORGANIZATION

Below is a list of individuals and organizations that will participate in this project along with their specific roles and responsibilities.

Watershed Action Director – BRWA Project Manager

a) Write QAPP
b) Contact analytical labs as needed
c) Implement the QAPP
d) Field data collection and management
e) Contact person for analytical laboratories
f) Data entry, validation
g) Data analysis and interpretation
h) Assist with project outreach and communication
i) Project reporting

Citizen Involvement Coordinator – BRWA Assistant Project Manager

a) Project outreach and communication
b) Assist with QAPP implementation
c) Assist with field data collection and management
d) Assist with data analysis and interpretation
e) Assist with project reporting

Executive Director

a) General administration and oversight
b) Track budget
c) Assist with field work and reporting as needed

Seasonal Staff

a) Assist with field data collection, entry, and management

QAPP Review Team

a) Assist with writing QAPP
b) Assist with data verification and validation

A5. PROBLEM DEFINITION/BACKGROUND

A5.1 Watershed Description and Project Need

The Bad River Watershed drains over 1,000 square miles along Wisconsin's shore of Lake Superior (Figure 1). The Chequamegon-Nicolet National Forest is found at the headwaters. The streams and rivers of the watershed are important spawning grounds for sturgeon, lake-run trout, salmon and walleye as well as many other fish species. The unique wetland known as the Kakagon Slough/Bad River Slough is located at the mouth of the watershed on Lake Superior. This freshwater estuary is the largest and possibly most pristine remaining on Lake Superior. The lower one-third of the Watershed lies within the Bad River Band of Lake Superior Tribes of Chippewa Indians Reservation. The Kakagon Slough provides abundant habitat for wild rice, which is highly important to the Tribe's culture. It is the only

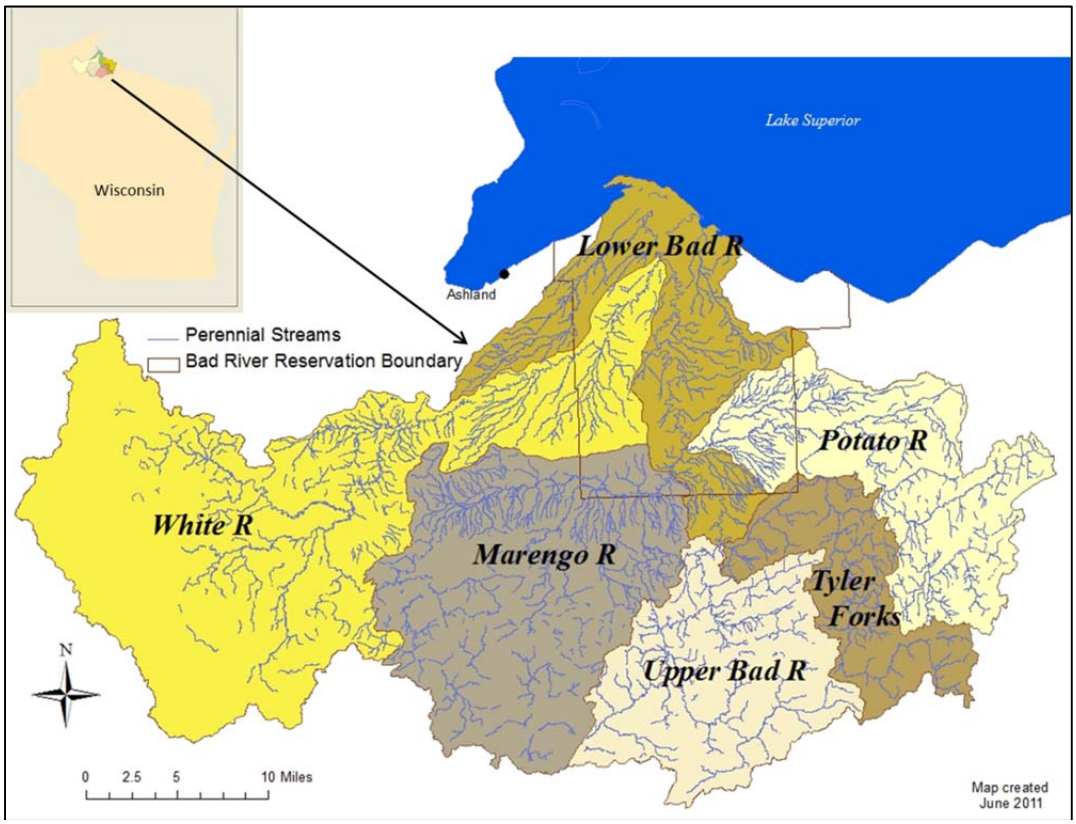

Figure 1. Map of the Bad River Watershed, its location in Wisconsin, the six HUC 5-level subwatersheds, within it, and the approximate reservation boundary of the Bad River Band of Lake Superior Tribes of Chippewa Indians.

remaining extensive coastal wild rice wetland in the Great Lakes Basin, and it provides exceptional habitat for a variety of wildlife.

The southern and southeastern headwaters area of the watershed contain the Gogebic iron range, one of the most significant known and undeveloped taconite iron ore deposits in the United States (Marsden 1978). The range is also referred to as the Penokee Range and will be referred to as such throughout this document. Mining companies own over 22,000 acres of forest land and the underlying mineral rights in a band 22 miles long across the Marengo, Upper Bad, Tyler Forks, and Potato River subwatersheds

(Figure 2). The economic potential and possibility of mining this deposit has been discussed for decades. Marsden (1978) estimated that the Penokee range contains 3.7 billion metric tons of taconite iron ore reserves. Recently, a developer, the Cline Group, has purchased an option to lease mineral rights in the area and have established a subsidiary called Gogebic Taconite to proceed with developing a mine.

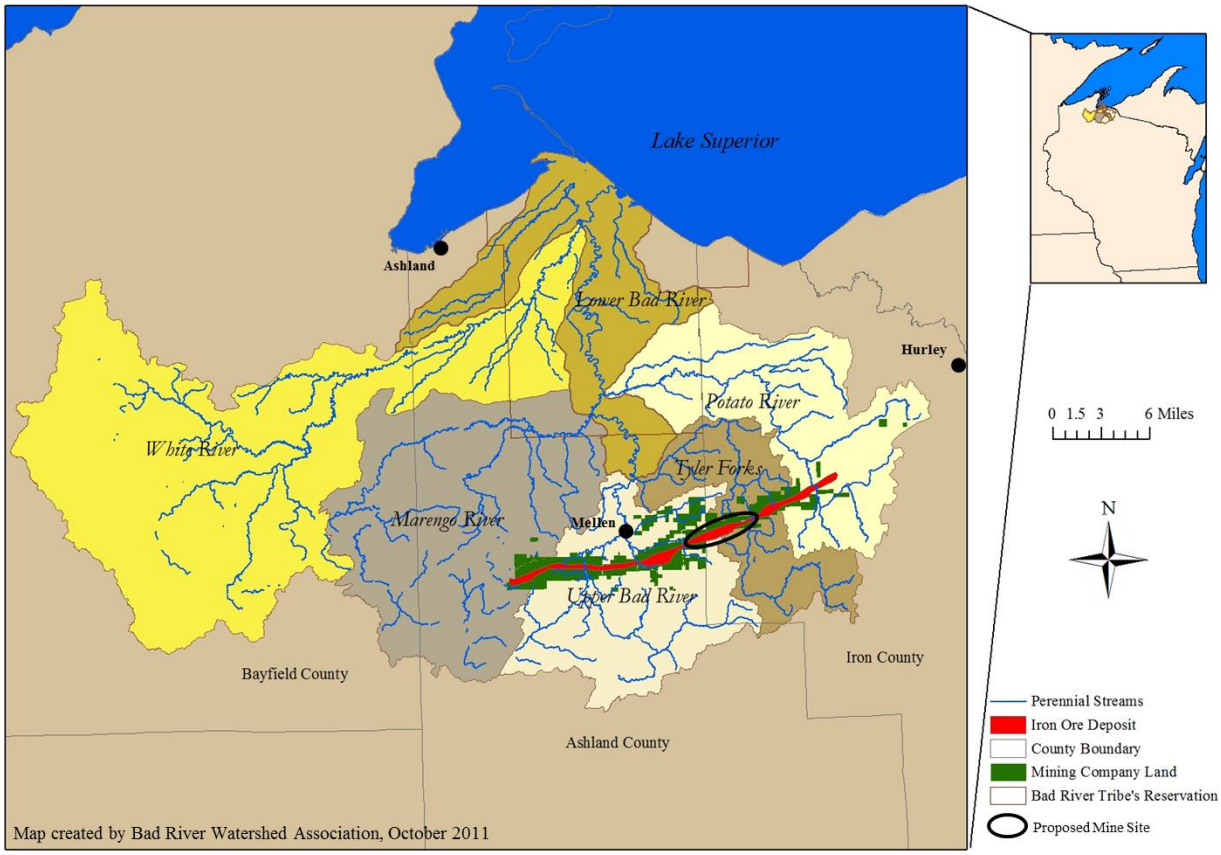

Figure 2. Map of the Bad River Watershed, location of the Penokee/Gogebic iron ore deposit, and the area currently being proposed for mining the iron ore.

Taconite is a low-grade iron ore (MNDNR 2011). To be mined economically, significant amounts of material must be moved and processed. Because of this, taconite is mined via large, open-pits. The footprint of existing taconite mines can be as large as the Hull-Rust-Mahoning taconite mine near Hibbing, MN, with a pit approximately 3½ miles long, 1½ miles wide and 535 feet deep and an even larger tailings basin (MNDNR 2011). Gogebic Taconite has indicated the first phase of its mine would include a pit approximately 4½ miles long and 1½ miles wide (GTAC 2011). This does not include area that would be disturbed for waste rock and tailings storage and for building ore processing facilities.

In addition to the large footprint of open-pit taconite mines, significant amounts of water are needed for processing the taconite ore, and significant amounts of groundwater will likely need to be removed from the open pit to allow for mining activities.

If an open-pit taconite mine is developed in the Penokee Range, the scale of changes to the landscape and water resources in the area would be enormous. The area surrounding the potential mine site currently provides habitat for wide ranging mammals, such as timber wolves and pine martens, and for

breeding populations of migrant songbirds, such as the black-throated blue warbler (TNC 2011). The Penokee Range is identified as an important area of high conservation significance due to its unique geology; many rare plants, animals and forest communities; and high quality recreational opportunities (Pohlman *et al*. 2006). Wisconsin's Strategy for Wildlife Species of Greatest Conservation Need (also known as the Wisconsin Wildlife Action Plan, [WDNR 2005]) identified the Penokee Range as an Important Bird Area and a Conservation Opportunity Area of Continental Significance to maintain a large continuous climate change resistant forest.

Some of Wisconsin's highest quality streams, designated by statute as Outstanding and Exceptional Resource Waters, originate near or flow through the potential mine site. Examples include: Ballou, Devils, and Javorsky Creeks, and the Tyler Forks River. These streams are also classified as Class I or II trout streams and they are not allowed to be degraded from baseline conditions.

Unfortunately, little data exist to characterize baseline conditions in these and other streams, lakes, or groundwater in the area that would be affected by an open-pit taconite mine. Baseline data that documents current conditions in streams, lakes, groundwater, air, and on the landscape needs to be collected ahead of any mine development in order to understand how a mine development would affect these resources. It is needed for activities such as permit evaluations, deciding how to manage mine operations and waste to minimize environmental degradation, or in making decisions about where mining is not a compatible landuse with existing natural resources.

This project will focus on collecting baseline continuous temperature, macroinvertebrate, and conductivity data from wadeable streams. The selected streams are designated as Outstanding and Exceptional Resource Waters or trout streams by the State of Wisconsin and most likely to be either directly impacted or non-impacted (reference sites) by the proposed location for a taconite iron ore mine in the Penokee Range.

A5.2 Project Development

The Bad River Watershed Association (BRWA), a 501(c)3 non-profit organization, retains professional-level and well-qualified staff to carry out natural resources work, including surface water monitoring.

Recognizing the need to collect baseline data ahead of a possible mine development, BRWA contracted with the Town of Morse government (a portion of the possible mine site is located within the Town of Morse) to collect continuous temperature and macroinvertebrate monitoring data from streams near the potential site of the open-pit iron ore mine during 2011. With additional support from the Wisconsin Department of Natural Resources (WDNR), Iron County Land and Water Conservation Department (LWCD), and the Great Lakes Indian Fish and Wildlife Commission (GLIFWC), BRWA staff were able to expand the geographic scope of the original data collection effort beyond the Town of Morse and add conductivity monitoring.

From the beginning, BRWA has sought to have the data collected through this project to be of a quality that will be recognized and utilized by WDNR, BRNRD, and other natural resource management agencies responsible for evaluating mine permit applications. BRWA staff contacted and worked with numerous WDNR, BRNRD, and GLIFWC technical staff (some of whom were on the review team for this QAPP) to identify methods that would be most appropriate for collecting baseline data ahead of a mine development that would also be comparable or identical to methods used by these agencies to conduct

baseline monitoring for temperature, macroinvertebrates, and conductivity. The following is background information on the methods selected for use in this project.

Continuous Temperature Monitoring
Temperature has an important influence on pH, density, specific conductance, the rate of chemical reactions, and solubility of constituents in water. Also, the biological activity and species composition of a waterbody is largely determined by water temperature. Temperature data could be used in several ways to evaluate potential impacts from an open-pit, taconite iron ore mine including:

- Document baseline conditions ahead of a mine development.
- Determine or re-evaluate a stream's temperature category - cold, cool, or warm water.
- Determine trends in stream temperature over time.
- Aid in identifying groundwater-influenced streams.
- Aid in development of a groundwater model to evaluate potential mine impacts.
- Aid in documenting and determining the effects of thermal discharges on aquatic biota (taconite mines use large amounts of water and could change whether a stream receives groundwater or surface water and thus the biological community that the stream can support).

Collecting continuous water temperature data using individual thermistors allows for accurate and detailed information to be collected from streams of all sizes to be used for purposes such as those stated above.

The BRWA has developed a standard operating procedure (SOP) for collecting continuous temperature monitoring data from streams and rivers based on methods used by the WDNR (Appendix A) and using the same thermistor models as WDNR. As of the completion of this document, WDNR was in the process of revising its continuous temperature monitoring protocol. Portions of the 2004 version of the *Guidelines and Standard Procedures for Continuous Temperature Monitoring* and a draft version of the updated *Temperature-Continuous* procedure were utilized in developing the BRWA SOP (WDNR 2004, 2011). Portions of the WDNR protocols are excerpted throughout this QAPP.

Changes in the WDNR protocol for continuous temperature monitoring will not affect data interpretation for this project. The new draft WDNR protocol provides fewer details (particularly on thermistor models and accuracy checks) than the previous version, but the advised logging interval for collecting baseline data and thermistor deployment location considerations remain the same. The deployment period in the new WDNR protocol is about a month longer than the old protocol and BRWA's protocol reflects the new WDNR protocol.

As it was developing its continuous temperature monitoring SOP, BRWA felt there were some additional quality control items that should be added given the possibility that the data collected using these methods could be used as part of a mine permitting process or evaluation of potential mining impacts in the Penokee Range. This primarily included providing more detail on clarifying what was meant by a certified reference thermometer and conducting accuracy checks of thermistors both before and after field deployment.

Macroinvertebrates
Data derived from aquatic macroinvertebrate samples provide valuable information on the biological and physical condition of streams. Most aquatic macroinvertebrates, such as immature insects, live for

one or more years in streams, integrating the effects of environmental stressors over time. Since the majority of aquatic invertebrates has limited mobility (relative to fish), they can be good indicators of local conditions as well as upstream land and water resource factors.

Examples of aquatic macroinvertebrates include insects in their larval form, insects in their adult form, crayfish, snails, and worms. The advantages of using this type of biological assessment as part of collecting baseline data ahead of an open-pit, taconite iron ore mine development include:

- Fluctuating environmental conditions can be monitored in the long-term.
- Biological communities can be used as indicators of general ecological integrity.
- Macroinvertebrates are thought to be good indicators of multiple environmental stressors over time.
- Macroinvertebrates are usually abundant in streams and sampling will have no detrimental effect on the community.
- Individuals are easily identified and established tolerance values are available.
- Due to the relatively short life cycle of the organisms within a community, impacts are easily measured and ecological changes can be seen.
- Bio-assessment identifies problems within an area. Chemical testing and/or a physical assessment can then be done to determine the exact problem or possibly identify a source.

Sampling techniques vary depending on how quantitative the resulting data need to be to answer the desired question/s. When the objective is to collect baseline data, a single-habitat (typically sampling only riffles) or a multi-habitat (sample all available habitats in proportion to their occurrence within a stream reach) approach are often used. These sampling techniques produce semi-quantitative data and are less expensive and time intensive than techniques needed to produce quantitative data typically used to detect population changes in response to a stream impact.

BRWA Project Managers used a proportional, multi-habitat sampling approach to collect composite macroinvertebrate samples from wadeable streams for this project. The protocol was developed with significant input from WDNR (Mike Miller), LSRI (Kurt Schmude), and other members of the QAPP review team. The BRWA SOP (Appendix B) is primarily based on the United States Environmental Protection Agency's *Rapid Bio-assessment Protocols for Use in Streams and Rivers* (Barbour et al. 1999), the Minnesota Pollution Control Agency's *Invertebrate Sampling Procedures* (MPCA 2011), and the Wisconsin Department of Natural Resources *Guidelines for Collecting Macroinvertebrate Samples from Wadeable Streams* (WDNR 2000).

Various metrics and indices are used to interpret macroinvertebrate taxonomy data. In general, as the level of environmental degradation increases within a stream there is a corresponding decrease in environmentally sensitive macroinvertebrate species and an increase in a few environmentally tolerant species (WDNR 2000). The multi-habitat collection procedure used by BRWA will allow a broad set of metrics to be calculated with the taxonomy data (see examples on page 13). These metrics will initially be calculated by WDNR's Surface Water Integrated Monitoring System (SWIMS) database. Because care must be taken to account for strengths, weaknesses, and data assumptions used in order to correctly apply and interpret metric data, BRWA will rely on the expertise of Dr. Kurt Schmude and WDNR biologists to interpret metrics derived from the taxonomy data.

Conductivity

Conductivity is a measure of the capacity of water (or other media) to conduct an electrical current (EPA 2010). Conductivity is strongly influenced by water temperature and measurements are often corrected to 25 degrees Celsius, which is called specific conductance (APHA *et al*. 1998). Conductivity in streams and rivers is affected primarily by the geology of the area through which the water flows, but can also be affected by point and nonpoint discharges (such as industrial, agricultural, residential septic systems, etc.). Conductivity is useful as a general measure of stream water quality and can provide important baseline information about a stream site ahead of a potential open-pit, taconite iron ore mine development. Conductivity can be a quick indicator of whether a point or nonpoint effluent discharge may be impacting stream health.

BRWA Project Managers used a handheld conductivity meter received from GLIFWC to collect preliminary baseline specific conductance (will be referred to as "conductivity" throughout this document) data approximately monthly from the same site locations as the continuous temperature and macroinvertebrate monitoring. The BRWA has developed a SOP for conductivity based on the manufacturer's instruction manual for the instrument and through consultation with the QAPP review team (Appendix C).

A5.3 Coordination With Other Monitoring Efforts

In addition to BRWA's surface water monitoring plans, a United States Environmental Protection Agency-funded (EPA) project (led by GLIFWC and the United States Geological Survey [USGS]) to collect baseline stream data from Lake Superior Basin watersheds with the potential for mining occurred in 2011. The project was part of the EPA's Year of Intensive Monitoring effort in the Lake Superior Basin in 2011. The Bad River Natural Resources Department (BRNRD), WDNR, and Northland College also planned to collect data in the vicinity of the potential mine site during 2011. As a result, BRWA staff worked to bring these partners together to discuss and coordinate sample sites and share resources where possible.

During spring 2011, BRWA, GLIFWC, and Northland College staff conducted site visits to determine the most suitable locations for baseline monitoring efforts. Final site selections and monitoring plans were shared to avoid overlap where necessary (for instance, both GLIFWC and BRWA planned to collect continuous temperature monitoring data) and maximize data comparability where possible (for instance, WDNR fisheries staff tried to collect fisheries data in the same locations as BRWA's monitoring). BRWA staff coordinated this effort.

Overall, this QAPP was developed in order to document the systematic planning that has occurred and provide a basis for collecting and evaluating data for BRWA's project. The QAPP details methods and procedures used by BRWA staff to collect baseline continuous temperature monitoring data using thermistors, baseline biological assessment data using macroinvertebrates, and baseline conductivity data using a handheld meter. In addition, it provides rationale and context for site selection and it provides context for BRWA's project as part of the other monitoring efforts that occurred near the vicinity of the potential Penokee range open-pit iron ore mine.

A6. PROJECT TASK/DESCRIPTION

A6.1 Project Objective and Site Selection

The objective of this study is to collect baseline continuous temperature, macroinvertebrate and conductivity monitoring data from reference streams and streams likely to be impacted should a taconite iron ore mine be developed in the Penokee Range between Mellen and Hurley, WI. The data will be useful in understanding stream conditions and characteristics prior to any development and operation of a mine at this location, may be useful in informing permitting decisions for a mine development, and provide a reference from which to evaluate any future impacts if a mine development occurs.

Sample sites were selected based on several factors including:

- Proximity to the potential mine site and likelihood of being impacted by mining activities.
- Preference was given to streams designated by the State of Wisconsin as Outstanding and Exceptional Resources Waters and/or trout streams.
- Locations upstream of the potential mine or on streams similar in size and character to potentially impacted streams, but less likely to be directly impacted by mining activities (reference sites).
- Five sites needed to be located within the Town of Morse to satisfy BRWA's contract with the Town.
- Location of partner monitoring efforts.
- Ease of access.

Nine sites for continuous temperature monitoring and 11 sites for macroinvertebrate monitoring were selected by BRWA during 2011. Conductivity monitoring was conducted at all sites (Table 1, Figure 3). Streams that flowed through the potential mine site were sampled above and below the expected impact area (i.e. sites on Tyler Forks River and Ballou Creek). Streams were sampled above major confluences (i.e. sites on Devils and Ballou Creek).

The sites on Javorsky Creek, Bull Gus Creek, and the Tyler Forks River at Hwy. 77 and Stricker Rd. were not monitored for continuous temperature by BRWA because the parameter was covered by partner efforts. Additional partner financial support allowed BRWA to collect macroinvertebrate monitoring data from Javorsky Creek and the Tyler Forks River at Hwy. 77 and Stricker Rd. The sites on Bull Gus Creek and Rouse Creek were not sampled for macroinvertebrates because they had been sampled in recent years by WDNR.

On sites where streams flowed through private property, landowner permission was sought either verbally or in written form to install the continuous temperature monitoring devices because they involved driving rebar into the stream bottom. On sites where streams flowed through public property, local government officials or land managers were notified of BRWA's sample plans.

Table 1. Sites monitored by the Bad River Watershed Association during 2011 near the site of a potential open-pit iron ore mine between Mellen and Hurley, WI. Also listed are stream designation*, parameters measured at each site, and whether the site was considered a reference or impact site with respect to the potential mine.

Stream	Site Location	Longitude (DD)	Latitude (DD)	Stream Designation*	Stream Order	Temp Site	Macro Site	Conduct Site	Reference Site	Impact Site
Tyler Forks River	Caroline Lake Rd.	-90.502850	46.277116	ORW, Class II Trout	3	X	X	X	X	
Tyler Forks River	Hwy. 77	-90.494603	46.347492	ORW, Class II Trout	4		X	X		X
Tyler Forks River	Stricker Rd.	-90.590000	46.394720	ORW, Class II Trout	4		X	X		X
Potato River	Upson Park	-90.412086	46.370889	ORW, Class II Trout	4	X	X	X	X	
Erickson Creek	Casey Sag Rd.	-90.465233	46.372039	Class II Trout	2	X	X	X	X	
Rouse Creek	Casey Sag Rd.	-90.465767	46.360833	Class III Trout	2	X		X	X	
Javorsky Creek	Hwy. 77	-90.518417	46.344836	ERW, Class I Trout	1		X	X		X
Bull Gus Creek	FR 703	-90.500208	46.303732	Class III Trout	2			X		X
Opergard Creek	Near Revai Rd.	-90.586017	46.341617	Class II Trout	2	X	X	X		X
Devils Creek	Upstream Ballou confluence	-90.579234	46.318659	ERW, Class I Trout	2	X	X	X		X
Ballou Creek	Red House Rd.	-90.575977	46.305917	ERW, Class I Trout	3	X	X	X	X	
Ballou Creek	Upstream Devils confluence	-90.579573	46.318298	None	3	X	X	X		X
City Creek	Lake Dr.	-90.644975	46.308677	Class II Trout	3	X	X	X	X	

*Stream designation: The State of Wisconsin designates its highest quality waters as Outstanding or Exceptional Resource Waters (ORW/ERW). Trout streams are classified as Class I (highest quality, natural trout reproduction), Class II (some natural trout reproduction), or Class III (no natural reproduction, trout populations maintained by stocking).

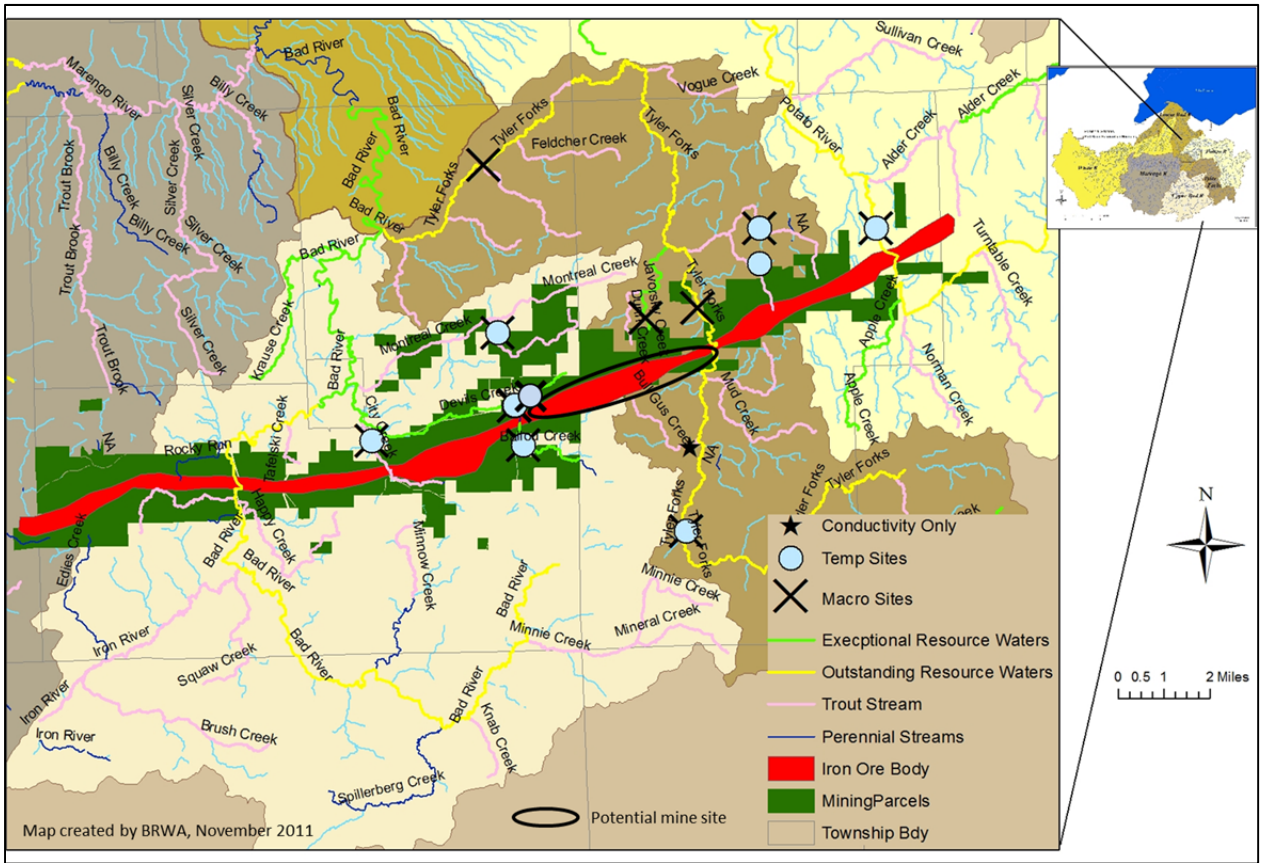

Figure 3. Sites monitored for continuous temperature, macroinvertebrates, and conductivity by the Bad River Watershed Association during 2011. Subwatersheds of the Bad River watershed are shaded, the location of a potential open-pit iron ore mine, property held by mining interests, and the iron ore body are displayed.

A6.2 Data Collection and Evaluation

Continuous Temperature Monitoring
BRWA used TidbiT v2 thermistor models from the Onset Computer Corporation to collect continuous temperature data for this project. This thermistor model is also used by WDNR. BRWA received several TidbiT v2 thermistors from WDNR staff for use in this project.

BRWA collected baseline continuous temperature data using a 1-hour logging interval. WDNR has determined that a 1-hour logging interval is adequate for collecting baseline data to assess thermal extremes and to determine if a stream should be classified as cold, cool, or warm. The deployment period for collecting these data should be (at minimum) between May and September (a minimum of four months; WDNR 2011). During 2011, thermistors were deployed in late May and retrieved in November. The thermistors were checked and data downloaded on a monthly basis.

Resulting data will be compared to criteria used by WDNR (i.e. maximum daily mean temperature, Lyons *et al*. 1996) to determine stream temperature classification (i.e. cold, cool, warm).

Macroinvertebrates
Benthic macroinvertebrates were collected systematically from all available instream habitats by jabbing or kicking the substrate with a D-frame dip net. A total of 20 jabs (or kicks) were taken from all major habitat types in the reach. For example, if the habitat in the sampling reach was 50% snags, then 50% or 10 jabs were taken in that habitat. An organism-based subsample of 300 organisms was sorted in the laboratory and identified to the lowest practical taxon, generally genus or species.

The samples were collected by BRWA Project Managers during September and October of 2011, preserved in 80% ethyl alcohol, and sent to Dr. Kurt Schmude, Aquatic Entomologist with the University of Wisconsin-Superior Lake Superior Research Institute (LSRI), to be identified to species-level where possible. Several indices will be automatically calculated by entering the data into WDNR's SWIMS database (including the Hilsenhoff Biotic Index (HBI), HBI Max 10, Hilsenhoff Family Biotic Index (FBI), Species Richness, Genera Richness, Percent EPT (Ephemeroptera, Plecoptera, Trichoptera) individuals, Percent EPT Genera, Percent *Chironomidae* individuals, Benthic Index of Biotic Integrity (IBI), percent functional feeding groups (scrapers, filterers, shredders, gatherers), Shannon's Diversity Index), based on the species-level data. Other indices may be calculated as appropriate. BRWA will rely on the expertise of Dr. Kurt Schmude and WDNR biologists to interpret metrics derived from the taxonomy data.

Macroinvertebrate samples will be archived at the Lake Superior Research Institute and will be available for future analysis.

Conductivity
Conductivity data were collected from the same site locations as the continuous temperature and macroinvertebrate monitoring during monthly site visits to download continuous temperature monitoring data, during fall 2011 macroinvertebrate sampling, and side-by-side data were collected with GLIFWC partner instruments in September 2011.

Conductivity data will be compared to other available conductivity data and continuous temperature data to give a preliminary characterization of stream quality and variability of this parameter both within and across these sites. Because conductivity can be a highly variable parameter depending on factors

such as runoff, a more detailed picture of baseline conductivity at these sites can be achieved by installing continuous conductivity data loggers, such as those installed at four project sites (Tyler Forks River at Stricker Rd. and Hwy. 77, Javorsky Creek at Hwy. 77, and Bull Gus Creek at FR703) through partner efforts during 2011.

All data that meets quality parameters outlined in this document will be entered into WDNR's Surface Water Integrated Monitoring System (SWIMS) database and shared with other resource managers and the public.

The project schedule for implementation is provided in Table 2.

Table 2. Project schedule for BRWA staff water quality monitoring.

Parameter	Data Collection	Validation & Reporting
Continuous Temperature Monitoring	Minimum of four months – deployed in May and collected after September 2011.	Late fall 2011/early winter 2012
Macroinvertebrates	Fall 2011 (between September 15 & end of November)	Late fall 2011/early winter 2012
Conductivity	In conjunction with monthly continuous temperature and fall 2011 macroinvertebrate monitoring events.	Late fall 2011/early winter 2012

A7. QUALITY OBJECTIVES AND CRITERIA

Continuous Temperature Monitoring
An accuracy check of any thermistors to be used in the field must occur both pre- and post-deployment to document instrument accuracy within the manufacturer's specifications and performance at representative temperatures (see section B7). An exception to this can occur if the thermistors being used are brand new, have not been deployed in the field, and have a National Institute of Standards and Technology (NIST) certificate of accuracy available. All of the TidbiT v2 thermistors used for this project meet these three criteria.

A field check of stream temperature at all monitoring sites will occur upon deployment, during regular site visits, and during instrument retrieval at the end of the study period using a field thermometer checked using the same protocol as the thermistors. Air temperature data and instream temperature data for each site will be compared for evidence that the instream thermistor was exposed to the air due to stream stage falling below the installed depth of the thermistor. Further data quality objectives and corrective actions for continuous temperature monitoring are described in Table 3.

Table 3. Quality objectives, criteria, and corrective actions for continuous temperature monitoring.

Data Quality Indicator	**Measurement**	**Data Quality Objective**	**Corrective Action**
Alternative Measurement Sensitivity (AMS)*	Conduct lab test pre- & post-field deployment in ice bath and at room temperature. Collect 10 measurements at a 1-minute	Optimum target AMS of ≤ ± 0.2°C	Evaluate sample data for usability.

Data Quality Indicator	Measurement	Data Quality Objective	Corrective Action
	interval. Multiply the standard deviation of the 10 measurements by 3.250 to obtain 99% confidence intervals.		
Bias	Thermistors should be shaded from sunlight to prevent measurement bias. Place each thermistor in a well-mixed area of a stream where it will ideally remain submerged and free of sedimentation during the period of deployment. Field check of stream temperature and thermistors on a monthly basis.	Mean difference between field thermometer and thermistor temperature readings within the certified accuracy of the field thermometer (minimum 3 field thermometer measurements).	1. Evaluate sample data for usability. 2. Flag and remove data points from use in analysis that suggest thermistor was exposed to air, buried in sediment, etc.
Accuracy	Conduct lab test pre- & post-field deployment with NIST-traceable reference thermometer (certification current) in ice bath and at room temperature. Not necessary if thermistor is new, never field deployed, and has NIST certificate available.	≤ ± 0.2 °C between 0 and 50 °C (manufacturer's stated accuracy for TidbiT v2).	Evaluate sample data for usability (see discussion in section A7).
Representativeness	Thermistors placed in field prior to June 1 and retrieved after September 15 (a minimum of 4 months deployment). Program thermistor to collect hourly measurements.	Thermistor captures annual maximum daily mean temperature (MDMT).	1. If fewer than 4 months of data are collected, evaluate temperature profile for likelihood of having captured MDMT. Compare to thermistor data from nearby sites (same year) or to nearby air temperature records to aid in determination. 2. Flag data and note whether sufficient evidence exists that MDMT was captured. 3. If sufficient evidence does not exist, flag data and attempt to re-deploy thermistor in a

Data Quality Indicator	Measurement	Data Quality Objective	Corrective Action
			subsequent year.
Comparability	Using same type of thermistors and protocol as WDNR staff.	Evaluate thermistor check documentation to ensure each instrument meets quality criteria.	Evaluate sample data for usability.
Completeness	[Total number of thermistors deployed that are retrieved and capture MDMT / total number of thermistors deployed] * 100	>90% of deployed thermistors should be retrieved and capture MDMT.	1. Adjust sample location and collect data in a subsequent year. 2. Adjust thermistor deployment method depending on site conditions.
Sensitivity	See "Alternative Measurement Sensitivity"		
Precision	See "Alternative Measurement Sensitivity"		

* Alternative Measurement Sensitivity (AMS) and AMS+: AMS and AMS+ are alternative ways to control and document measurement sensitivity at the QC level when low level detection limits (like Method Detection Limits) are not needed. AMS can be used to estimate the interval of uncertainty around each single data point, recognizing that no single data point is perfect. The difference between AMS and AMS+ is that AMS is based on at least 7 replicate measures of one sample (like would be done in a lab setting), while AMS+ is based on measures of at least 7 measures of different samples (like would be done in a field setting; Irwin, 2008).

Macroinvertebrates

Precision/Accuracy/Bias

The multi-habitat method of collecting macroinvertebrates does not support quantitative precision, accuracy, or bias calculations. Instead, two qualitative methods will be used to assess these parameters.

1. BRWA will ensure accuracy by contracting with a qualified invertebrate taxonomy expert. During fall of 2011, BRWA will work with Dr. Kurt Schmude, Aquatic Entomologist with the University of Wisconsin-Superior Lake Superior Research Institute (LSRI) Taxonomy Laboratory. Samples will be collected in the field by BRWA Project Managers, but all processing and identification of the samples will be conducted by Dr. Schmude and the LSRI Taxonomy Laboratory. Protocols used by LSRI are in Appendix D. BRWA Project Managers will receive training in proportional, multi-habitat sampling techniques from Dr. Schmude (occurred on 9/19/2011).
2. Precision/variability with the field collection method will be assessed by collecting at least one duplicate macroinvertebrate sample with Dr. Schmude. Duplicates are collected from the same proportional habitats (but not the same exact locations) within the designated stream reach. BRWA's macroinvertebrate SOP further details the protocol for collecting duplicate samples (Appendix B). A target value for the relative percent difference (RPD) between the duplicate samples will be less than 40%. All calculated index values (e.g. HBI, HFBI, etc.) received for each duplicate sample from Dr. Schmude will be used to assess RPD. RPD is calculated as follows:

   RPD = [(X1 – X2) / (mean of X1 and X2)] x 100

where X1 and X2 are each of the duplicate values.

Actual results will be evaluated to determine if the 40% RPD is appropriate or if additional variability or methods need to be considered to evaluate precision.

Completeness

It is anticipated that a complete and QA/QC validated macroinvertebrate sample will be collected from all sites selected as part of this project.

Representativeness

As indicated, all available habitats within a designated stream reach will be sampled proportionally and documented to assure that a macroinvertebrate community sample representative of the habitats available at the site is collected. Resulting data will be used to summarize the biological conditions of the sites that were sampled. The sampling protocols used by BRWA Project Managers are based on standard methods for collecting representative baseline macroinvertebrate samples from wadeable sections of streams and rivers (Barbour *et al*. 1999, MPCA 2011, WDNR 2000).

Comparability

Comparability of data collected by BRWA Project Managers will be assured in several ways.

1. The methods used by BRWA Project Managers are considered standard for collecting proportional, multi-habitat, baseline macroinvertebrate samples and are primarily based on those recommended by the US EPA and MPCA (Barbour *et al*. 1999, MPCA 2011).
2. By collecting proportional, multi-habitat samples and establishing a reference sample reach at each site, data can be compared both spatially and temporally.
3. BRWA Project Managers have been previously trained in using similar protocols and will be trained on using the multi-habitat protocol by Dr. Schmude (occurred 9/19/2011).
4. BRWA will work with Dr. Schmude, who also does contract work for WDNR and BRNRD, to help ensure comparability of taxonomic work.

Conductivity

Table 4. Quality objectives, criteria, and corrective actions for conductivity monitoring.

Data Quality Indicator	**Measurement**	**Data Quality Objective**	**Corrective Action**
Bias/Accuracy	1. Calibrate meter using the HI7031 (1413 µS/cm) calibration solution recommended for the meter prior to each field day. Calibration check with HI70033C (84 µS/cm) calibration solution and deionized water. 2. Measure two calibration solutions (1413 & 84 µS/cm) and deionized water with meter at end of field day. 3. Deionized water blank measured during initial calibration, once every 10 field measurements, and at the end of	1. Successful calibration required to use instrument. 2. Calibration check should be within 10% of certified value. Deionized water check should be less than 10 µS/cm. 3. Blanks should be less than 10 µS/cm.	1. Initial Calibration a. Re-calibrate instrument and re-do calibration checks. b. Perform instrument maintenance. c. Return to manufacturer for maintenance. 2. End-of-day Check a. Re-measure calibration

Data Quality Indicator	Measurement	Data Quality Objective	Corrective Action
	the field day. If fewer than 10 field measurements are collected, the blank measurements during initial calibration and end of field day are sufficient.		solutions and blank. b. Evaluate sample data for usability. 3. Blanks a. Clean electrode, exchange deionized water, and re-analyze. b. Evaluate sample data for usability.
Representativeness	Samples represent preliminary baseline data to give range and variability of parameter at each site approx. once per month during 2011 field season and provide preliminary comparison of parameter across sites (including partner efforts in 2011).	Identify sites for more detailed conductivity baseline assessment in future.	Evaluate instrument for usability in future monitoring efforts.
Comparability	Side-by-side measurements will be taken with partner instruments at least once during 2011 field season.	BRWA meter readings within 10% of partner instruments.	Evaluate instrument for usability in future monitoring efforts.
Completeness	[Total number of samples analyzed found to meet or exceed quality control criteria / total number of samples analyzed] * 100	>95% samples should pass quality control criteria	1. Evaluate sample data for usability. 2. Perform instrument maintenance. 3. Make program adjustments as necessary.
Alternative Measurement Sensitivity Plus (AMS+)*	Collect a minimum of 7 field conductivity measurements one right after the other (at one field site) twice annually.	Optimum target AMS/AMS+ of ± 5 µS/cm or ± 3% (whichever is greater)	Evaluate sample data for usability.
Precision	Duplicate field measurement taken once every 10 field measurements or once per field day if fewer than 10 measurements collected.	RPD between duplicate measurements 10% or less.	1. Rinse electrode with deionized water and repeat duplicate measurement. 2. Re-calibrate instrument.

Data Quality Indicator	Measurement	Data Quality Objective	Corrective Action
			3. Re-evaluate precision criteria.

* Alternative Measurement Sensitivity Plus (AMS+): See Table 3 for discussion.

A8. SPECIAL TRAINING/CERTIFICATION

BRWA Project Managers will work with Dr. Kurt Schmude to collect side-by-side macroinvertebrate samples during fall 2011 and as a check of correct implementation of the multi-habitat sampling protocol. No additional training or certifications for collecting continuous temperature and conductivity data are necessary by those conducting this study.

Typical qualifications for BRWA full-time salaried or hourly staff conducting water quality monitoring are at least a Bachelor's Degree in natural resources management or related field. BRWA also hires limited term staff that may conduct water quality monitoring. These staff members typically have some course work or training towards a degree in natural resources management or related field.

A9. DOCUMENTS AND RECORDS

The final original QAPP will be retained by BRWA at its main office in hard copy and electronic portable document format (PDF). Copies of the QAPP will be made available to personnel at the WDNR, BRNRD, and LSRI as listed in section A3.

All hard copy field data sheets, instrument calibration data sheets, and chain of custody forms will be archived at the main BRWA office and will be scanned into PDF format for electronic storage. All data will be entered into an electronic database (e.g., Microsoft Access, Excel) kept by BRWA, with the exception of data provided by Dr. Schmude, which will be entered and sent to BRWA as a spreadsheet. Macroinvertebrate and continuous temperature data will be submitted to WDNR for inclusion in the State's SWIMS database. Conductivity data may be submitted for inclusion in SWIMS. Macroinvertebrate samples will be archived by LSRI according to their protocols for sample storage. A BRWA Project Manager will proof all data entered by BRWA staff against the hard copy of the final data sheets. Copies of the digital database will be stored in two locations not on the same computer.

GROUP B: DATA GENERATION AND ACQUISITION

B1. SAMPLING PROCESS DESIGN (Experimental Design)

Location of sampling sites and rationale for site selection are described in detail in section A6.1. The objective of this study is to collect baseline continuous temperature, macroinvertebrate and conductivity monitoring data from reference streams and streams likely to be impacted should a taconite iron ore mine be developed in the Penokee Range between Mellen and Hurley, WI. Continuous temperature data are intended to capture baseline conditions at each site over the course of the

warmest months of the year. Data can be used in determining stream thermal and natural community classification and assessing stream condition for management purposes. Macroinvertebrate data are intended be a representative baseline sample of the macroinvertebrate community at each site and will be useful for assessing stream condition and natural community characteristics for management purposes. The data also could be useful in informing permitting decisions for a mine development, and provide a reference from which to evaluate any future impacts if a mine development occurs.

The conductivity data are not collected frequently enough to provide a thourough baseline assessment of this parameter, but the data will provide a preliminary assessment of the range and variability of the parameter at each site, across sites, and compared to results from other partner efforts. They will provide an indication of areas where future, more detailed monitoring of this parameter could be conducted.

Instrumentation and methodologies were selected based on consultation with individuals on the QAPP Review Team (see pg. 2) and discussions with other partner efforts described in section A5.3.

B2. SAMPLING METHODS

Continuous Temperature Monitoring
BRWA will collect continuous temperature monitoring data with the use of individual thermistors available from the Onset Computer Corporation (HOBO TidbiT v2 [part # UTBI-001]). BRWA uses software and thermistor data management tools available from the manufacturer for programming and managing the thermistors and downloading data from them.

BRWA uses LaMotte non-mercury thermometers (Code: 1066, accuracy +/- 1.0°C) for doing air and water temperature readings during site checks of the thermistors. Field sampling methods are described in detail in BRWA's Continuous Temperature Monitoring SOP (Appendix A).

Macroinvertebrates
Macroinvertebrate samples are collected from the stream or river bottom using a 500 micron mesh size D-frame dip net. Staff will collect samples from pre-determined stream sites in fall 2011.

Composite, proportional, multi-habitat samples will be collected at each site. A total of 20 jabs (or kicks) are taken from all major habitat types within a 100-meter reach. For example, if the habitat in the sampling reach is 50% snags, then 50% or 10 jabs will be taken in that habitat. Samples will be preserved whole in 80% ethyl alcohol and transferred to Dr. Kurt Schmude at LSRI for processing and analysis. An organism-based subsample of 300 organisms is sorted in the laboratory and identified to the lowest practical taxon, generally genus or species. Further details on macroinvertebrate sampling methods are in BRWA's SOP in Appendix B.

Conductivity
BRWA uses a Hanna Instruments Model HI 98129 pH/EC/TDS handheld meter to collect conductivity data in the field. The meter has automatic temperature compensation to 25°C. Samples are taken by submerging the electrodes of the meter directly into the stream to a depth of approximately 40% of the total stream depth above the bottom of the stream (or 60% of the total depth below the water surface). Further method details are described in BRWA's Conductivity SOP (Appendix C).

B3. SAMPLING HANDLING AND CUSTODY

B3.1. Field Handling Procedures

Continuous temperature monitoring
Field deployment, maintenance, and data collection procedures are detailed in BRWA's Continuous Temperature Monitoring SOP (Appendix A).

Figure 4. Example thermistor deployment housing used by BRWA.

The deployment period for collecting baseline continuous temperature monitoring data is typically between May through September, for a minimum of four months (WDNR 2011). Each thermistor will be placed in a well-mixed area of a stream where it will ideally remain submerged and free of sedimentation during the period of deployment. The thermistors also should be shaded from sunlight to prevent bias in temperature readings. WDNR's 2004 protocol outlines several methods that can be used to deploy the thermistors (WDNR 2004). An example deployment method used by BRWA is shown in Figure 4.

Adjustments to the thermistor placement in the stream may need to be made if it becomes exposed to the air or buried in sediment during deployment. Thermistors will be checked periodically throughout the deployment period to ensure they are working properly (evidenced by a red blinking light), are not exposed to air or buried in sediment, and to download data.

Macroinvertebrates
Field handling procedures are detailed in BRWA's SOP (Appendix B). In short, samples are collected and preserved in the field by BRWA Project Managers. Samples are preserved in clean, plastic containers (such as a 5-quart plastic container or ice cream pail). A label containing information about the sample will be affixed to the outside of the container (Table 5). An identical label will be placed inside the container and a chain of custody form filled out for each sample (Appendix E).

Table 5. Example macroinvertebrate field sample label.

Bad River Watershed Association Macroinvertebrate Sample	
Sample ID Number: 20110925-887	Coordinates: Lat. 46.388 Long. 90.601 Datum: WGS84
Waterbody Name: Tyler Forks River	Subwatershed: Tyler Forks
Collector/s Name: Jane Doe, John Doe	Road Crossing: Will Rd.
Date: 09/25/2011	Replicate Number: 1 of 1
Preservative: 80% ethyl alcohol	Split-Sample Designation: None

Conductivity
All conductivity measurements are taken in the field using a Hanna Instruments Model HI 98129 handheld meter. There is no sample collection or transport. Sampling procedures are discussed in section B2, B4, and in Appendix C.

B3.2 Laboratory Handling Procedures

Continuous temperature monitoring
There is no laboratory handling procedure associated with continuous temperature monitoring.

Macroinvertebrates
Sample processing and analysis at the LSRI will be completed according to their established protocols (Appendix D). Samples will be received at LSRI with proper labels and chain of custody forms. Upon completion of analysis, samples will be archived according to procedures used by LSRI.

Conductivity
There is no laboratory handling procedure associated with the conductivity measurements.

B4. ANALYTICAL METHODS

Continuous temperature monitoring
There are no analytical methods associated with continuous temperature monitoring.

Macroinvertebrates
Sample processing and analysis will be conducted at the UW-Superior LSRI according to their established protocols (Appendix D).

Conductivity
There are no analytical methods associated with the conductivity measurements.

B5. QUALITY CONTROL

Continuous temperature monitoring
An accuracy check of any thermistors to be used in the field must occur both pre- and post-deployment to document instrument accuracy within the manufacturer's specifications and performance at representative temperatures (see section B7). An exception to this can occur if the thermistors being used are brand new, have not been deployed in the field, and have a National Institute of Standards and Technology (NIST) certificate of accuracy available.

A field check of stream temperature at all monitoring sites will occur upon deployment, during regular site visits, and during instrument retrieval at the end of the study period using a field thermometer checked using the same protocol as the thermistors. Air temperature data and instream temperature data for each site will be compared for evidence that the instream thermistor was exposed to the air due to stream stage falling below the installed depth of the thermistor. Further data quality objectives and corrective actions for continuous temperature monitoring are described in section A7.

Macroinvertebrates
A minimum of one field duplicate macroinvertebrate sample will be collected with Dr. Schmude. Duplicates are collected from the same proportional habitats (but not the same exact locations) within the designated stream reach. A target value for the relative percent difference (RPD) between the duplicate samples will be less than 40%. All calculated index values (e.g. HBI, HFBI, etc.) received for each duplicate sample from Dr. Schmude will be used to assess RPD. Duplicates are also described in section A7. Quality control parameters used by LSRI are described in Appendix D.

Conductivity

A deionized water field blank will be measured during initial calibration, once every 10 field measurements, and at the end of the field day. If fewer than 10 field measurements are collected, the blank measurements during initial calibration and end of field day are sufficient. Blanks should be less than 10 µS/cm.

Duplicate field measurements will be taken once every 10 field measurements or once per field day if fewer than 10 measurements collected. RPD between duplicate measurements should be 10% or less. In addition, side-by-side measurements will be taken with GLIFWC partner calibrated conductivity instruments at least once during 2011. RPD between the side-by-side measurements should be within 10%.

Alternative Measurement Sensitivity Plus (AMS+) will be measured to give an idea of measurement precision and sensitivity (See Table 3 for more details). AMS+ will involve collecting a minimum of 7 field conductivity measurements one right after the other (at one field site) twice annually. Optimum target AMS+ will be ± 5 µS/cm or ± 3% (whichever is greater).

The BRWA Program Manager is responsible for ensuring that quality control measures associated with this project are implemented and evaluating QC results compared to data quality objectives. Independent review of quality control data compared to data quality objectives will also be conducted by a member of the QAPP Review Team (see pg. 2 and section D1).

An annual quality control report will be generated by the BRWA Project Manager. The report will summarize quality control checks, instrument calibrations, any response actions taken based on quality control checks, deviations from the QAPP, and independent data review done by a member of the QAPP Review Team. This report will typically be completed in late fall or winter when all temperature, macroinvertebrate, and conductivity data for a typical field season have been received and validation completed.

B6. INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

Continuous temperature monitoring
See the discussion of thermistor accuracy checks in sections A7, B5, and in Appendix A.

The BRWA maintains a Microsoft Excel spreadsheet that lists all thermistors in use, model numbers, serial numbers, date received at the BRWA office, and results of all accuracy checks for each thermistor. All thermistors are stored at the BRWA office when not deployed in the field. Prior to field deployment, thermistors are checked for damage, battery life, that all data have been downloaded, any needed firmware upgrades, and that the units are functioning properly.

The TidbiT v2 thermistors do not have replaceable batteries. When battery life is indicated at less than 20%, the thermistors should not be deployed in the field and should be retired. Damaged units will either be retired or sent to the manufacturer for maintenance.

Macroinvertebrates
Equipment used for field macroinvertebrate collections will be inspected prior to each sampling event to ensure it is in working order. Inspect nets for rips and organisms remaining from previous sampling; sample containers for cracks and cleanliness; preservative solutions that they are not expired and that

there is adequate volume for the expected samples that will be collected. All damaged equipment will be replaced.

Conductivity
Prior to field use, the Hanna Instruments meter should be inspected for damage to the electrode and the meter housing. If there is visible damage, the meter should not be used and the manufacturer should be contacted for repair options.

Battery life should be checked by turning the meter on by holding the "MODE" button for 2-3 seconds. The remaining battery life is displayed immediately. A low battery symbol warns the user when the batteries need to be replaced. In addition, the instrument is equipped with a Battery Error Prevention System that avoids erroneous readings caused by low voltage by turning the meter off.

Replace batteries according to manufacturer instructions in the meter Operational Guide. The instrument should be re-calibrated (see section B7) prior to field use after the batteries have been changed.

B7. INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

Continuous temperature monitoring
BRWA has adopted procedures for conducting thermistor accuracy checks based on those used by the Washington State Department of Ecology for continuous temperature monitoring of fresh water rivers and streams conducted in a Total Maximum Daily Load (TMDL) project for stream temperature (WSDE 2009). The procedure is described briefly here and in detail in BRWA's existing SOP for continuous temperature monitoring (Appendix A).

A reference thermometer with a current NIST certification must be used for the check. The reference thermometer should have accuracy greater than or equal to the manufacturer-stated accuracy of the thermistors being checked (certified Accuracy for TidbiT v2 is less than or equal to ± 0.2 degrees Celsius (°C) between 0 and 50 °C). This procedure may be conducted in a laboratory or similar environment and is accomplished by submerging the reference thermometer, thermistors, and any field thermometers in an ice bath (near 0 degrees Celsius) and an ambient temperature bath (near 20 degrees Celsius). At the completion of the monitoring, the raw data will be assigned a measurement accuracy (mean of ice bath and ambient bath accuracy) for both the pre- and post-study calibration results (Table 3). The desired accuracy for the continuous temperature monitoring data collected during this project is less than or equal to ± 0.2 °C.

If the average (of the ice bath and ambient bath) temperature difference for a thermistor or a field thermometer, compared against the NIST-certified thermometer, is less than or equal to ± 0.2 °C the data from the instrument can be used without further qualification. If the average temperature difference for a thermistor is greater than the stated accuracy, then a second check should be performed to determine whether the same results are achieved.

If the second result is still greater than the manufacturer accuracy, and if this is the pre-study check, then the thermistor or field thermometer should not be used. If the second result is greater than the manufacturer accuracy, and if this is the post-study check, then the stated accuracy for the data should

be the mean difference of the pre- and post-study calibration values from the NIST reference thermometer.

If the mean difference of the pre- and post-study calibration values is greater than ± 0.2 °C and less than or equal to ± 1 °C, the raw temperature data from the thermistor should be adjusted by the mean difference of the pre- and post-calibration check results to correct for the instrument bias. If the thermistor is off by greater than ± 1 °C, or the field thermometer by greater than ± 2 °C, the data should be flagged and a decision should be made whether or not to include the data set from the thermistor or field thermometer in any analyses and reports.

Macroinvertebrates
There are no instrument or equipment calibrations conducted with the macroinvertebrate monitoring.

Conductivity
Calibration instructions are in Appendix C. The Hanna Instruments Model HI 98129 pH/EC/TDS handheld field meter must be calibrated prior to each field day and recorded on the Conductivity Meter Calibration Record sheet (Appendix C). A calibration check will be conducted at the end of each field day by measuring the two calibration solutions (1413 & 84 µS/cm) and deionized water (see Table 4).

B8. INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

All supplies and consumables required for continuous temperature, macroinvertebrate, and conductivity monitoring will be received at the BRWA office and inspected to ensure they are the appropriate supplies needed and that no damage has occurred during shipping. Any damaged supplies and consumables should be returned immediately to the manufacturer for replacement.

A list of monitoring supplies and consumables, including dates of purchase and projected replacement, is maintained by the BRWA Program Managers and stored at the BRWA office.

B9. NON-DIRECT MEASUREMENTS

Results from this project will be compared to available metrics used by WDNR and BRNRD to evaluate and interpret these kinds of data. Possible metrics that will be calculated include: 1) continuous temperature monitoring - maximum daily mean temperature (Lyons *et al*. 1996); 2) macroinvertebrates – the Hilsenhoff Biotic Index (HBI), HBI Max 10, Hilsenhoff Family Biotic Index (FBI), Species Richness, Genera Richness, Percent EPT (Ephemeroptera, Plecoptera, Trichoptera) individuals, Percent EPT Genera, Percent *Chironomidae* individuals, Benthic Index of Biotic Integrity (IBI), percent functional feeding groups (scrapers, filterers, shredders, gatherers), Shannon's Diversity Index; 3) conductivity data may be compared to other partner data to give an indication of variability of this parameter at sites monitored in 2011.

B10. DATA MANAGEMENT

Field data sheets for continuous temperature (field checks of thermistors), macroinvertebrate, and conductivity monitoring will be kept in separate binders and stored at the BRWA office. Field data will be

collected by BRWA Project Managers and entered into an electronic spreadsheet (e.g. Microsoft Excel) immediately following each field day. Once field data have been entered, the staff person who entered the data will place their initials in the appropriate column on the field data sheet to indicate the data have been entered.

Following each field season (usually late fall or winter), field data sheets will be scanned into electronic PDF or similar format for electronic storage. Hard copies will be stored in a file cabinet in the BRWA office. All database entries from the field season will be checked against the field sheets by a BRWA Project Manager. All BRWA files are backed up on an external hard drive, typically on a weekly basis. The external hard drive is stored at the home of a BRWA staff person.

Following data review and validation (described in section D2), data are ready for analysis and sharing with intended users (i.e. WDNR, BRNRD) and other partners, etc.

GROUP C: ASSESSMENT AND OVERSIGHT

C1. ASSESSMENTS AND RESPONSE ACTIONS

The responsibility for maintenance of quality for a project lies with every BRWA staff member and contracted personnel associated with this project. All project personnel shall aid in identifying perceived problems that may affect quality and report such problems to their supervisor or to the BRWA Project Manager.

Any laboratory or field problems will be reported to the BRWA Project Manager and will be discussed with laboratory personnel, WDNR, or other technical support staff as needed to determine the appropriate response action.

Deviations from the QAPP will immediately be reported to the BRWA Project Manager for review and approval. Deviations from the QAPP will be documented on a deviation form by the BRWA Project Manager (Appendix F).

Data collected as part of this project may be compared to or assessed alongside data collected by other partners to help give a more complete picture of baseline conditions in streams near the potential mine site. The data can be used to assess stream condition and natural communities, identify gaps or future monitoring needs, and lay the foundation for collaborative efforts to provide a thourough and comprehensive assessment of baseline stream conditions prior to any potential mining development.

C2. REPORTS TO MANAGEMENT

An annual quality control report will be generated by the BRWA Project Manager. The report will summarize quality control checks, instrument calibrations, any response actions taken based on quality control checks, and deviations from the QAPP. This report will typically be completed in late fall or winter when all temperature, macroinvertebrate, and conductivity data for a typical field season have been received and validation completed.

Once data have been validated for use by following and documenting their quality via this QAPP, they will be ready to be shared as needed. It is the intent, at minimum, that the continuous temperature and macroinvertebrate monitoring data be shared with WDNR and BRNRD for use in their statewide (SWIMS) or tribal water quality databases and reporting and decision-making related to those databases. Reporting to agencies such as WDNR and BRNRD will occur per the requirements for the agency. For instance, WDNR's protocol for continuous temperature monitoring describes specific data formatting and submission requirements. The BRWA Project Manager will contact appropriate agency staff to ensure data submission requirements are met as needed.

GROUP D: DATA VALIDATION AND USABILITY

D1. DATA REVIEW, VERIFICATION AND VALIDATION

Continuous temperature, macroinvertebrate, and conductivity monitoring data will be verified and validated by the BRWA Project Manager and independently reviewed by a member of the QAPP Review Team (see pge 2). This will be done by evaluating quality control results compared to the data quality objectives described in this QAPP (section A7). Decisions to reject or qualify data are made by the Project Manager in consultation with the independent reviewer. Other technical experts will be contacted as needed if questions arise about data interpretation and usability. Decisions made during data review, verification, and validation will be discussed in the annual quality control report (see section C2).

D2. VERIFICATION AND VALIDATION METHODS

Upon completion of the post-deployment accuracy check, all continuous temperature monitoring thermistor data will be evaluated for usability with the criteria discussed in section A7. Field temperature measurements conducted during site checks will be compared to thermistor data for evidence that the thermistor became exposed to air or impacted by sediment during any point of its deployment. Erroneous and suspect data points will be flagged in the continuous temperature data record so they are not used in analyses. In order to ensure each thermistor was equilibrated with ambient temperature following deployment, the first data point in the deployment period will be removed from the data record used in analyses.

Chain of custody sheets for macroinvertebrate sample handling will be evaluated for any problems that may have occurred during sample transfer. Duplicate samples will be evaluated for meeting RPD criteria in section A7. If duplicate samples exceed the RPD criteria, the BRWA Project Manager will discuss and decide on data usability in consultation with Dr. Schmude.

The Conductivity Calibration Record, blanks, and calibration check records will be evaluated for meeting criteria in section A7. Results not meeting criteria will be flagged for further review.

All database entries from the field season will be checked against the field sheets by a BRWA Project Manager. Errors in data entry will be corrected. Outliers and inconsistencies will be flagged for further

review, or discarded. Problems with data quality will be discussed in the interim and final reports to data users.

D3. RECONCILIATION WITH USER REQUIREMENTS

Data quality objectives will be reviewed on an annual basis to ensure that objectives are met. Any data quality problems will be reported to the BRWA Program Manager for assessment and corrective actions. In addition, data quality issues will be recorded as a separate item in the database and made available to all data users. Specific response to and reconciliation of problems that occur in data quality are outlined in section C1.

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APPENDICES

Appendix A: Standard Operating Procedure: Bad River Watershed Association Staff Continuous Temperature Monitoring

Appendix B: Standard Operating Procedure: Bad River Watershed Association Staff Macroinvertebrate Monitoring

Appendix C: Standard Operating Procedure: Bad River Watershed Association Staff Field Conductivity Analysis

Appendix D: Lake Superior Research Institute Taxonomy Laboratory Standard Operating Procedures

Appendix E: Bad River Watershed Association Macroinvertebrate Sample Chain of Custody Form

Appendix F: Bad River Watershed Association Deviation Form

Appendix A:

Standard Operating Procedure: Bad River Watershed Association Staff Continuous Temperature Monitoring

Standard Operating Procedure
Bad River Watershed Association Staff Continuous Temperature Monitoring

Continuous Temperature Monitoring

A. Scope and Applicability
Temperature has an important influence on pH, density, specific conductance, the rate of chemical reactions, and solubility of constituents in water. Also, the biological activity and species composition of a waterbody is largely determined by water temperature. Collecting continuous water temperature data using individual thermistors allows for accurate and detailed information to be collected from streams of all sizes to be used for many watershed management purposes.

The Bad River Watershed Association (BRWA) has adopted protocols used by the Wisconsin Department of Natural Resources (WDNR) for most aspects of its staff continuous temperature monitoring program. As of the completion of this document, WDNR was in the process of revising its continuous temperature monitoring protocol. Portions of the 2004 version of the *Guidelines and Standard Procedures for Continuous Temperature Monitoring* and a draft version of the updated *Temperature-Continuous* procedure were utilized in developing this Standard Operating Procedure (SOP; WDNR 2004, 2011). The SOP provides additional details related to thermistor operation, deployment, data management, and quality assurance/quality control to provide more detail specific to BRWA's equipment and monitoring needs related to data collected by staff.

B. Summary of Method
BRWA uses thermistors from the Onset Computer Corporation, such as the TidbiT v2 and Pendant models. BRWA will focus on collecting baseline continuous temperature data. For collection of baseline data, WDNR has determined that a 1-hour logging interval is adequate and the deployment period should be a minimum of four months, typically between May and September (WDNR 2011).

An accuracy check of any thermistors to be used in the field must occur both pre- and post-deployment to document instrument accuracy within the manufacturer's specifications and performance at representative temperatures. Each thermistor will be placed in a well-mixed area of a stream where it will ideally remain submerged and free of sedimentation during the period of deployment. The thermistors also should be shaded from sunlight to prevent bias in temperature readings. Adjustments to the thermistor placement in the stream may need to be made if it becomes exposed to the air or buried in sediment during deployment. Thermistors will be checked periodically throughout the deployment period to ensure they are working properly (evidenced by a red blinking light), are not exposed to air or buried in sediment, and to download data.

C. Definitions
Continuous temperature monitoring – Monitoring conducted with the use of an electronic temperature logger or thermistor that is placed in the stream to gather temperature measurements at a defined temporal interval.

D. Health and Safety
Protect feet with waders, boots or sneakers while working in streams. Wash hands after working and before eating.

If you feel that working in the stream will put you at personal risk – DO NOT SAMPLE.

E. Cautions

Ensure that thermistors are securely deployed and well-hidden in the field using a method described in this or the WDNR's SOP (2004) to avoid losing them during high flow events or to curious stream users. Make sure the deployment location is well-documented with GPS coordinates, photos, and/or landmarks in the area so the thermistors can be easily re-located by staff, but not others.

F. Interferences

Thermistors may become exposed to air or buried in sediment or other debris during deployment. Frequent checks of the thermistors and air and water temperature will help minimize these potential interferences and allow staff to account for them during data validation.

G. Personnel Qualifications

All BRWA staff should undergo training in the use of this method prior to sampling. Typical qualifications for BRWA full-time salaried or hourly staff conducting water quality monitoring are at least a Bachelor's Degree in natural resources management or related field. BRWA also hires limited term staff that may conduct water quality monitoring. These staff members typically have some course work or training towards a degree in natural resources management or related field.

H. Field Equipment

1. Thermistors
    a) Onset TidbiT v2 (part # UTBI-001)
    b) Onset Pendant (part # UA-002-64)
2. Field Kit
    a) Waterproof data shuttle with lanyard
    b) All data shuttle couplers
    c) USB cable for data shuttle
    d) Extra AA batteries for data shuttle
    e) Extra thermistors
    f) Alcohol-filled thermometer that has been checked for accuracy with NIST-traceable thermometer.
    g) Laptop with HOBOWare Pro software loaded
    h) Field forms on Rite-in-the-Rain paper
    i) Lab-grade silicone seal lubricant for Pendant
    j) Extra 3-volt CR-2032 lithium battery
    k) Digital Camera (with batteries and memory card)
    l) Metal Clipboard
    m) 3 pencils
    n) 3 pens
    o) 3 black Sharpie markers
    p) Waterproof wrist watch or other suitable field clock
    q) Rebar
    r) 2 and 3 inch PVC pipe painted a dark color for camouflage
    s) Saw for cutting PVC
    t) Wire rope and extra rope clips
    u) Wire cutters
    v) Metal hose clamps
    w) Flat-head screwdriver

x) Socket wrench with 8mm and 9/32" sockets
y) Toothbrush (for cleaning TidbiT sensors if needed)
z) Duct tape
aa) Sledge hammer or similar device for pounding rebar
bb) GPS
cc) Pictures and/or locations of sites

I. Accuracy Check of Instruments

The continuous temperature monitoring thermistors cannot be calibrated, but they can be checked for accuracy. The purpose of the accuracy check of thermistors is to see if they are producing temperature readings within the manufacturer's specifications. The WDNR protocol provides general guidelines on how to conduct an accuracy check (WDNR 2004). The following additional procedures provide more specific guidance and continuity for quality assurance and control. The procedures are based on those used by the Washington State Department of Ecology for continuous temperature monitoring of fresh water rivers and streams conducted in a Total Maximum Daily Load (TMDL) project for stream temperature (WSDE 2009).

Instruments must be checked both pre- and post-deployment. Any field thermometers that will be used during field checks of thermistors (see section J4) should also be checked with this procedure. A reference thermometer with a current National Institute of Standards and Technology (NIST) certification must be used for the check. The reference thermometer must have accuracy greater than or equal to the manufacturer-stated accuracy of the thermistors being checked. At the completion of the monitoring, the raw data will be assigned a measurement accuracy value based on the pre- and post-study calibration results. A calibration log should be kept that includes instrument serial numbers and measured accuracy based on the following methods.

If the average temperature difference for a thermistor, compared against the NIST certified thermometer, is equal to or less than the manufacturer stated accuracy of the instrument (i.e., certified Accuracy for TidbiT v2 is +/- 0.2 degrees Celsius (°C) between 0 and 50 °C, and for the Pendant is approximately +/- 0.5 °C between 0 and 40 °C – refer to manufacturer's specifications for certified accuracy of other temperature sensors) the instrument can be used without further qualification. If the average temperature difference for a thermistor is greater than the stated accuracy, then a second check should be performed to ensure there wasn't a problem with the calibration method.

If the second result is still greater than the manufacturer accuracy, and if this is the pre-study check, then the thermistor should not be used. If the second result is greater than the manufacturer accuracy, and if this is the post-study check, then the stated accuracy for the data should be the mean difference of the pre- and post-study calibration values from the NIST thermometer. If the thermistor is off by a degree or more, a decision should be made whether or not to utilize the data set from the thermistor in any post-data collection analyses.

Use the following accuracy check procedure:

a) Prepare two containers for water baths (preferably distilled water) at least four hours before the thermistors are actually calibrated and conduct this test in a room where the air temperature can be held constant for the duration of the test. One bath should be at ambient temperature (typically around 20 degrees Celsius) and the other should contain crushed ice and water for a cool bath. The cool bath should be in a cooler.

b) Program the thermistors using a delayed launch so they all begin at the same time and use a 1 minute sample interval to measure temperatures. Keep thermistors at room temperature until the baths are ready.
c) Soak the thermistors in the ambient bath first for 20 minutes before beginning comparison temperature measurements using the NIST reference thermometer.
d) Stir the bath constantly to maintain homogeneous water temperature. After the soak time has lapsed, record the bath temperature using the NIST reference thermometer every 1 minute beginning at the time the thermistors record their temperature measurements. Record the time and temperature of each NIST thermometer measurement. Keep stirring. An example data logger accuracy check form can be found in Appendix 1.
e) Record 10 readings in the ambient bath.
f) Transfer all thermistors to the cool water bath and soak for 20 minutes. Keep the bath stirred to ensure a well-mixed (non-stratified) water bath.
g) After the soak time has lapsed repeat steps *d* through *e*.
h) Upon completion of the test, download the data from each of the thermistors and calculate the difference between the certified reference thermometer and each thermistor or field thermometer for each of the 10 observations in the ambient and cool water baths.
i) Calculate the mean difference between the 10 comparison readings for each thermistor or field thermometer in the ambient and cool water baths to check accuracy.

J. Sample Collection

1) Deployment period: A 1 hour interval between temperature readings is adequate for acquiring baseline data (WDNR 2011). This interval will allow determination of whether a stream should be considered cold, cool, or warm as well as the temperature extremes the waterbody experiences. If the objective is to collect data for a purpose other than baseline information, the logging interval should be selected based on the needed objectives for the project. Minimum deployments for collecting baseline data are four months (typically from May through September); with a deployment start no later than June 1 and retrieve no earlier than August 31.

2) Before going out in the field:
a) Make sure an accuracy check has been completed on the thermistors being used (Section I).
b) Make sure battery life in thermistors is adequate for field deployment.
c) Place batteries in waterproof data shuttle.
d) Launch waterproof data shuttle by connecting to a computer with HOBOWare® Pro software loaded. Connect with USB cable.
    a. Make sure all firmware is updated.
    b. Make sure computer clock and data shuttle clock show the same time.
e) Launch any thermistors that will be deployed by connecting each to the waterproof data shuttle with the appropriate coupler. Choose launch time close to anticipated field deployment time.
f) Obtain appropriate field forms for the installation of continuous monitoring stations (Appendix 2). Keep field forms in a binder.

3) Site selection and thermistor deployment methods:
a) For baseline monitoring, choose a site where other data (i.e. macroinvertebrates, water chemistry) are collected by BRWA or other partner agencies or groups. If a site has not been established via other data collection, choose a site that meets the desired objectives of the specific project.
b) Obtain landowner permission if thermistor will be deployed on private property.

c) Each thermistor will be placed in a well-mixed area of a stream where it will ideally remain submerged and free of sedimentation during the period of deployment. The latter is an important consideration when monitoring in low-gradient streams, or alluvial high and moderate gradient streams with aggrading or shifting substrates (e.g. sand). A riffle or run is preferred to a pool. Seek shade so as to minimize any radiant heat from the sun.

d) BRWA uses a deployment design that has a piece of 2-inch diameter PVC pipe attached to a piece of rebar that is driven into the bed of the stream. The PVC is secured to the rebar with a metal hose clamp above and below it. This allows the PVC to move with the flow of the current, but cannot move vertically on the rebar. The thermistor is attached inside of the PVC with a removable wire and wire clip (Figure 1).

e) In highly unstable or "flashy" streams, extra care should be taken to secure the thermistor in place. One option would be to run a piece of wire through the loop on the thermistor and attach it to a sturdy object (such as a tree) above bankfull height on the streambank.

f) Other deployment options are described in the WDNR protocol (WDNR 2004).

Figure 1. Example thermistor deployment housing used by BRWA.

4) <u>Field checks and data collection</u>:

a) Locate thermistor.
b) Attach appropriate coupler to waterproof data shuttle.
c) Remove wire clip from top of PVC pipe and pull out sensor far enough to insert into coupler.
d) Clean sensor nodes with toothbrush if dirty or have an organic residue on them.
e) Insert sensor into coupler and push in lever on coupler far enough to engage the yellow light on the waterproof shuttle. The green light should come on if data transfer initializes properly.
f) Once transfer is complete, place thermistor back into PVC and replace wire clip. The thermistor should be hanging firmly in PVC, but not loose so it will bang into PVC or rebar.
g) PVC openings should be parallel with stream flow.
h) Preferably, download data on laptop before going to next site to check for any abnormalities or need to change thermistor, etc.
i) Collect both air and water temperature data using a field thermometer that has been checked for accuracy with the thermistors in the pre-and post-deployment checks (Section I). Fill out the field data form in Appendix 2.

K. Handling and Preservation
No entry.

L. Sample Preparation and Analysis
No entry.

M. Troubleshooting
Thermistors may be exposed to the air or missing completely during a site visit. If thermistors are exposed, attempt to find a new deployment location within the stream channel as close to the original location and reinstall the deployment apparatus to the stream bottom. Take a new GPS reading of the location and thoroughly document the move on the field data sheet.

Attempt to locate missing thermistors. A metal detector can be helpful in locating the deployment apparatus. If the thermistor/s cannot be found, a decision will need to be made on whether to deploy another thermistor at the site.

If a thermistor is not working, either replace the battery (if the thermistor has a replaceable battery) or consider installing a new thermistor at the same location. Any changes made must be thoroughly documented on the field data sheet.

If data cannot be downloaded from a thermistor that is not working, contact the manufacturer for options.

N. Data

Post-field data management:

a) If data were downloaded onto the laptop in the field, transfer file to appropriate data folder on the BRWA network. If data were not downloaded in the field, connect waterproof shuttle to computer with USB cable and download data to appropriate folder on the BRWA network.
b) Save as an Excel file and in the HOBOware® format.
c) Remove batteries from waterproof shuttle once downloading has been completed (to conserve power).
d) Enter data collected on the field data form (Appendix 2) into an electronic spreadsheet (e.g. Microsoft Excel) immediately following each field day.
e) A second person should check 100% of the hand-entered data prior to validation.

O. Hardware and Software

BRWA uses the most recent version of HOBOware® Pro software and Onset's Underwater Data Shuttle (part no. U-DTW-1) for programming and managing the thermistors and downloading data from them. Software updates for HOBOware® Pro and firmware updates for the thermistors should be completed as indicated within the program.

P. Data and Records Management

Field data sheets for continuous temperature monitoring (Appendix 2) will be kept in a binder and stored in the BRWA office. Field data collected by BRWA staff are entered into an electronic spreadsheet (e.g. Microsoft Excel) immediately following each field day. Once field data have been entered, the staff person who entered the data will place their initials in the appropriate column on the field data sheet to indicate the data have been entered. All data entered will be checked by a second person (100% check of hand-entered data).

Following each field season (usually late fall or winter), field data sheets are scanned into electronic PDF or similar format for electronic storage. Hard copies are stored in a file cabinet in the BRWA office. All BRWA files are backed up on an external hard drive on a weekly basis. The external hard drive is stored at the home of a BRWA staff person.

Original electronic data downloaded from the thermistors are stored in a separate folder on the BRWA network in both the HOBOware® Pro format and Microsoft Excel. A separate folder on the BRWA network is used to store data that has been quality control-checked and will be used in any analyses. The data used for analysis is entered into an Excel spreadsheet format that allows import and analysis within an electronic database, such as Microsoft Access.

Q. Quality Assurance and Quality Control

Quality Assurance and Quality Control objectives are described in detail in BRWA's *Quality Assurance Project Plan – Staff Water Quality Monitoring – Continuous Temperature, Macroinvertebrate, and*

Conductivity (BRWA 2011). In short, the pre- and post-deployment checks will be used to evaluate the accuracy of the thermistors and the field thermometers. Upon completion of the post-deployment accuracy check, all continuous temperature monitoring thermistor data will be evaluated for usability with the criteria discussed in section B5 of the QAPP. Field temperature measurements conducted during site checks will be compared to thermistor data for evidence that the thermistor became exposed to air or impacted by sediment during any point of its deployment. Erroneous data points, including the first data point in the deployment period of each thermistor, will be removed from the continuous temperature monitoring data record that will be used in all post-quality control data analysis.

References

Bad River Watershed Association 2011. Quality Assurance Project Plan – Staff Water Quality Monitoring – Continuous Temperature, Macroinvertebrate, and Conductivity. Rev.0. 80 pp.

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Wisconsin Department of Natural Resources (WDNR) 2011. Temperature - Continuous - Field Procedures Manual. Draft Revised 7/13/2011. 3 pp.

APPENDIX 1- Temp SOP

Bad River Watershed Association Temperature Data Logger Accuracy Check Form

Bad River Watershed Association Temperature Data Logger Accuracy Check Form

		Temperature (deg C)				
Date	Time	NIST Ref. Therm.	Field therm. BRWA T1	Field therm. BRWA T2	Field therm. BRWA T3	Notes

APPENDIX 2 – Temp SOP

Bad River Watershed Association Field Sheet for Continuous Temperature Thermistors

BRWA Field Sheet for Continuous Temperature Thermistors

Site:					Thermistor Type:		
BRWA Site ID:					Thermistor #:		
Activity	Who	Date mm/dd/yy	Time	Air Temp. (C)	Water Temp. (C)	Stream Flow (Low, Average or High)	Notes

Activity examples = deploy, download, visual check, etc
Notes examples = logger buried in debris/sediment, logger in good condition, etc.

Form based on Appendix C in: *Guidelines and Standard Procedures for Continuous Temperature Monitoring Wisconsin DNR, May 2004 (Version 1).*

Appendix B:

Standard Operating Procedure: Bad River Watershed Association Staff Macroinvertebrate Monitoring

Standard Operating Procedure
Bad River Watershed Association Staff Macroinvertebrate Monitoring

Macroinvertebrate Monitoring

Scope and Applicability
Benthic macroinvertebrates differ in their tolerances to pollution and other watershed stressors in streams and lakes. Macroinvertebrate monitoring involves collecting a representative sample of macroinvertebrates from a waterbody and then using the resulting laboratory taxonomic data to assess the environmental conditions of the waterbody. Macroinvertebrates are organisms lacking a backbone that are large enough to be seen with the naked eye. Examples of aquatic macroinvertebrates include insects in their larval form, insects in their adult form, crayfish, snails, and worms. The advantages of using this type of biological assessment include;

1) Fluctuating environmental conditions can be monitored in the long-term.
2) Biological communities can be used as indicators of general ecological integrity.
3) Macroinvertebrates are thought to be good indicators of multiple environmental stressors over time.
4) Macroinvertebrates are usually abundant in streams and sampling will have no detrimental effect on the community.
5) Individuals are easily identified and established tolerance values are available.
6) Due to the relatively short life cycle of the organisms within a community, impacts are easily measured and ecological changes can be seen.
7) Bio-assessment identifies problems within an area. Follow-up chemical testing and/or a physical assessment can be done to determine the exact problem or possibly identify a source.

Summary of Method
Background information and the procedures for field collection and lab analysis used in this standard operating procedure (SOP) are primarily based on the United States Environmental Protection Agency's *Rapid Bio-assessment Protocols for Use in Streams and Rivers* (Barbour *et al*. 1999), the Minnesota Pollution Control Agency's Invertebrate Sampling Procedures (MPCA 2011), and the Wisconsin Department of Natural Resources *Guidelines for Collecting Macroinvertebrate Samples from Wadeable Streams* (WDNR 2000).

Staff will collect samples from pre-determined stream sites in fall and/or in spring. WDNR prefers fall sample collection for data to be used as part of water quality assessments, so fall collections will be prioritized if resources are limited. The rationale for prioritizing fall sampling is that fall low flows tend to be the most stressful period for invertebrates and fall samples likely represent the poorest invert assemblages and most accurate assessments of local habitat and water quality impacts (Miller, personal communication).

Composite, proportional, multi-habitat, samples will be collected at each site. Benthic macroinvertebrates are collected systematically from all available instream habitats by kicking the substrate or jabbing with a 500 micron mesh size D-frame dip net. A total of 20 jabs (or kicks) are taken from all major habitat types in the reach. For example, if the habitat in the

sampling reach is 50% snags, then 50% or 10 jabs should be taken in that habitat. An organism-based subsample of 300 organisms is sorted in the laboratory and identified to the lowest practical taxon, generally genus or species. Data sheets on stream habitat and macroinvertebrate identifications will be kept. Macroinvertebrates are used to indicate water quality based on known tolerance/intolerance of different types to polluted waters. Metrics, such as those calculated by WDNR's Surface Water Integrated Monitoring (SWIMS) database (the Hilsenhoff Biotic Index (HBI), HBI Max 10, Hilsenhoff Family Biotic Index (FBI), Species Richness, Genera Richness, Percent EPT (Ephemeroptera, Plecoptera, Trichoptera) individuals, Percent EPT Genera, Percent *Chironomidae* individuals, Benthic Index of Biotic Integrity (IBI), percent functional feeding groups (scrapers, filterers, shredders, gatherers), Shannon's Diversity Index), will be calculated with the macroinvertebrate data to give an indication of water quality at the sampled sites.

Definitions
Macroinvertebrate – Invertebrates are all animals that do not have internal skeletons or backbones. Aquatic biologists use the term "macroinvertebrate" to indicate those invertebrates that can be seen without magnification. Examples include insects, worms, mollusks, crayfish, and freshwater shrimp.

Health and Safety
A solution of 80% ethyl alcohol is used as a preserving agent and should be kept out of reach of children.

Avoid contact between the alcohol solution and skin, eyes, nose and mouth. In the event of an accident or suspected poisoning, immediately call the Poison Control Center at 1-800-222-12222.

Clean up any spills immediately.

Protect feet with waders, boots or sneakers while working in streams. Wash hands after sampling and before eating.

If you feel that sampling in the stream will put you at personal risk – DO NOT SAMPLE.

Cautions
Avoid prolonged exposure of equipment and solutions to direct sunlight.

Interferences
Heavy spring melt floods or extreme storm events can disturb stream communities and mask habitat types due to turbid water. Thirty days should be allowed to pass after an extreme flood event to allow macroinvertebrate populations to re-colonize. Silt in depositional areas and autumn leaves in the stream can plug the sampling net and cause potential interferences.

Personnel Qualifications
Typical BRWA staff qualifications for conducting macroinvertebrate and other water quality monitoring are at least a Bachelor's Degree in natural resources management or related field. Staff must be trained in multihabitat sampling and use of field datasheets by another trained staff member or professional familiar with multihabitat sampling.

Field Equipment

Waders
D-frame dip net (500 micron)
Sample containers (e.g. 5-quart ice cream pails with lids)
Sample labels
2-3 liters ethyl alcohol (80%) per sample
Forceps or tweezers
Datasheets
Chain of custody forms
Previous notes about sample site locations (if available)
Magnifying glass
100 meter tape measure
Clipboard
Float (orange or other neutrally buoyant object that is biodegradable)
Stopwatch
Pencil
Clear packing tape & duct tape
Flagging tape
Plastic tub for carrying samples and equipment

Instrument or Method Calibration

No instrument calibration needed

Sample Collection

1) Timing

Spring: April – May
Fall: September 15 – November
Ideally, thirty days should be allowed to pass after an extreme flood event to allow macroinvertebrate populations to re-colonize.

2) Site Selection and Establishment

Sample sites are usually selected at road crossings because of ease of access. The site should be established upstream of the road crossing, unless the upstream section of stream does not appear to be representative of the available habitats at the site. This will be up to the professional judgment of staff. The start of the station should be outside any zone of influence the road crossing may have on the physical habitat of the stream (e.g. ponding, scour pools, rip-rap, etc.). In addition, there should be no major tributaries discharging to the stream in the study area.

Establishing the site is best done in pairs. The site reach will be 100 meters (m) in length. Using the 100-meter tape, measure a distance of 10 m upstream from the culvert or bridge (or downstream if site must be below road crossing). If this point is outside the area of influence of the culvert or bridge, begin measuring the 100 m site from this point. If additional distance is needed to be outside the area of bridge or culvert influence, measure this additional distance. Mark the distance from road crossing on the data sheet (Appendix 1). This information will be helpful for future samplers to be able to return to the same general reach.

Complete measurement of the 100 m site. Measure the distance within the stream channel or on the stream bank. Care should be taken to avoid disturbing substrates that will be sampled. Place a piece of flagging tape on a tree branch or other marker on the stream bank to identify the end of the reach.

3) Selecting Habitat Proportions

The multihabitat method entails collecting a composite sample from up to five different habitat types. These habitat types include (as described in the MPCA protocol):

Hard bottom (riffle/run/pool/cobble/boulder)
This category is intended to cover all hard, rocky substrates, not just riffles. Runs and wadable pools often have suitable "hard" substrates, and should not be excluded from sampling. The surfaces of large boulders and areas of flat, exposed bedrock are generally quite unproductive, avoid including these habitats in the sampling area if possible. This is a general rule, if a particular stream has productive exposed bedrock, or boulder surfaces, those habitats should be considered for sampling.

Aquatic Macrophytes (submerged/emergent vegetation)
Any vegetation found at or below the water surface should be considered in this category. Emergent vegetation is included because all emergent plants have stems that extend below the water surface, serving as suitable substrate for macroinvertebrates. Do not sample the emergent portion of any plant.

Undercut Banks (undercut banks/overhanging vegetation)
This category is meant to cover in-bank or near-bank habitats, shaded areas away from the main channel that typically are buffered from high water velocities.

Snags (snags/rootwads)
Snags include any piece of large woody debris found in the stream channel. Logs, tree trunks, entire trees, tree branches, large pieces of bark, and dense accumulations of twigs should all be considered snags. Rootwads are masses of roots extending from the stream bank.

Leaf Packs
Leaf packs are dense accumulations of leaves typically present in the early spring and late fall. They are found in deposition zones, generally near stream banks, around logjams, or in current breaks behind large boulders.

Before sampling can begin, staff must determine which habitats are present in the reach. This is done by walking the length of the reach, determining which productive habitats dominate the stream reach, and documenting the habitat proportions on the field data sheet (Appendix A). Ideally the stream should be viewed from the top of the stream bank, but this is generally the exception rather than the rule. For this reason, great care must be taken to walk carefully through the stream so as to disturb as little substrate as possible.

It is difficult to estimate total stream coverage of certain habitats due to their linear or three dimensional natures. Undercut banks and overhanging vegetation appear linear, snags are

three dimensional, as are vegetation mats, and emergent vegetation. For these reasons best professional judgment must be used to determine what level of effort is adequate to equal one "sample effort" for any given substrate.

4) Collecting the Organisms

Sampling begins at the downstream end of the reach and proceeds upstream. A total of 20 jabs or kicks will be taken over the length of the reach; a single jab consists of forcefully thrusting the net into a productive habitat for a linear distance of 0.5 m, accompanied by sweeping the area with the net to capture all dislodged macroinvertebrates. A kick is a stationary sampling accomplished by positioning the net and disturbing the substrate for a distance of 0.5 m upstream of the net.

Different types of habitat are to be sampled in approximate proportion to their representation of surface area of the total macroinvertebrate habitat in the reach. For example, if snags comprise 50% of the habitat in a reach and riffles comprise 20%, then 10 jabs should be taken in snag material and 4 jabs should be taken in riffle areas. The remainder of the jabs (6) would be taken in any remaining habitat type. Habitat types contributing less than 5% of the stable habitat in the stream reach should not be sampled. In this case, allocate the remaining jabs proportionately among the predominant substrates. The number of jabs taken in each habitat type should be recorded on the field data sheet (Appendix 1).

The jabs or kicks collected from the multiple habitats will be composited to obtain a single homogeneous sample. Every 3 jabs or so (more often or less depending on the amount of material trapped in the net), wash the collected material by running clean stream water through the net two or three times. Thoroughly wash large sticks, stones, leaves and other sizeable organic debris into the net to remove any organisms and discard these materials. Deposit the remaining sample material into the sample container. If clogging does occur that may hinder obtaining an appropriate sample, discard the material in the net and redo that portion of the sample in the same habitat type but in a different location. Do not spend time inspecting small debris in the field.

5) Preserving the Sample

Transfer the sample from the net to the sample container(s) and preserve in enough 80% ethyl alcohol to cover the sample. Make sure all organisms are transferred. Forceps may be needed to remove organisms from the dip net or bucket. Place a label indicating the sample identification code, coordinates, waterbody name, subwatershed, collector name, road crossing, date, replicate number, split-sample designation (if more than one container is needed for a sample), and type of preservative into the sample container. The outside of the container should include the same information. Place a lid on the container and secure with duct or packing tape. Chain-of-custody forms must be filled out and include the same information as the sample container labels. Forms and labels are available in BRWA's *Quality Assurance Project Plan – Staff Water Quality Monitoring – Continuous Temperature, Macroinvertebrates, and Conductivity* (BRWA 2011).

After sampling has been completed at a given site, all nets, buckets, etc. that have come in contact with the sample should be rinsed and cleaned thoroughly of debris. The equipment should be examined again prior to use at the next sampling site.

Attempt to collect duplicate samples at the rate of 10% (i.e., 1 duplicate for every 10 samples) of the sites as a qualitative check on in-stream variability of the macroinvertebrate community and repeatability of the sampling technique between individuals or collection teams. Collect the sample in the same habitat types but in different locations.

6) Habitat Assessment

The Field Data Sheet (Appendix A) includes additional habitat assessment information from the selection of proportional habitats for sampling. This information is critical for the analysis of macroinvertebrate data. Fill out the sheet on-site according to the following (after macroinvertebrates have been collected);

a) Composition of river bed: estimate the percentage of the stream bed in the different substrate categories.
b) Percent embedded: estimate (as a percentage) how surrounded or buried by fine silt and sand the larger rocks and particles are.
c) Flow: percentage of the stream channel bottom presently filled with water.
d) Overhead canopy: stand as near to the center of the stream as possible and hold your hands straight out to the side. Now move your hands upward until your outstretched fingertips are pointing to the edges of the canopy. The percent of the 180 degrees that your hands moved from the straight out to straight up is the percentage canopy cover.
e) Water odor: circle the best approximation.
f) Algal growth: use the index to rate the amount of algae growth on a scale of 0 to 2. Look at rocks and vegetation as well as slow water areas.
g) Bank vegetation: estimate the percentage and type of bank vegetation that is present at the sampling site.
h) Depth: select a spot typical of the riffle area, measure depths at 1 step intervals from bank to bank. If the water becomes too deep, please do not try to measure all the way across.
i) Current velocity: measure the time it takes a neutrally buoyant float (e.g. an orange) to travel 10 feet, twice in each of two fast sections and two slow sections of the sample reach (stopwatch, tape and orange method).

Handling and Preservation

Samples will be kept in 80% ethyl alcohol. Handle specimens as gently as possible as some parts, especially external gills, are fragile and easily broken off. Be sure jars, data sheets, and chain of custody are properly labeled.

Samples will be shipped or transferred to a qualified taxonomic expert (contractor) for picking, subsampling and identification to the lowest taxonomic level possible (usually species). Chain of custody forms will accompany the samples to the contractor.

If samples are to be stored for more than two months prior to laboratory analysis, the initial 80% ethyl alcohol in the sample must be replaced with fresh 80% ethyl alcohol to improve long term preservation of the organisms.

Sample Preparation and Analysis

Sample picking, subsampling and identification will be completed according to the contractor's established protocols. Upon completion of analysis, samples will preferably be archived in glass vials in an appropriate solution (e.g. 80% ethanol and 5% glycerin) and stored through agreement with the contractor or at the BRWA main office.

Trouble Shooting
No Entry.

Data
Macroinvertebrate data will be entered into an electronic spreadsheet (e.g. Microsoft Excel) and stored in an electronic database (e.g. Microsoft Access). Metrics, such as those calculated by WDNR's Surface Water Integrated Monitoring (SWIMS) database (the Hilsenhoff Biotic Index (HBI), HBI Max 10, Hilsenhoff Family Biotic Index (FBI), Species Richness, Genera Richness, Percent EPT (Ephemeroptera, Plecoptera, Trichoptera) individuals, Percent EPT Genera, Percent *Chironomidae* individuals, Benthic Index of Biotic Integrity (IBI), percent functional feeding groups (scrapers, filterers, shredders, gatherers), Shannon's Diversity Index), will be calculated with the macroinvertebrate data to give an indication of water quality at the sampled sites.

Hardware and Software
No Entry.

Data and Records Management
Field data will be entered into an electronic spreadsheet (e.g. Microsoft Excel) immediately following each field day. Once field data have been entered, the initials of the staff person who entered the data are placed in the appropriate column on the field data sheet to indicate the data have been entered. All hand-entered data will be checked by a second person.

Laboratory data will be received from the contractor according to terms set in the contract. An electronic spreadsheet (Microsoft Excel) of all archived macroinvertebrate samples will be kept by BRWA staff.

Following each field season (usually late fall or winter), field data sheets will be scanned into electronic PDF or similar format for electronic storage. Hard copies will be stored in a file cabinet in the BRWA office. All of the database entries from the field season will be checked against the field sheets by a BRWA staff person. All BRWA files are backed up on an external hard drive on a weekly basis. The external hard drive is stored at the home of a BRWA staff person.

Following data review, verification, and validation, data are ready for analysis and sharing with intended users (e.g. Wisconsin Department of Natural Resources, Bad River Natural Resources Department) and other partners.

Q. Quality Assurance and Quality Control
Quality Assurance and Quality Control objectives are described in detail in BRWA's *Quality Assurance Project Plan – Staff Water Quality Monitoring – Continuous Temperature, Macroinvertebrates, and Conductivity.* In short, all BRWA staff conducting macroinvertebrate work will be trained initially on sample collection and sample identification by other trained staff, BRWA's Technical Advisor, WDNR Biologists or other qualified person familiar with BRWA's macroinvertebrate protocols. Attempt to collect duplicate samples at 10% (i.e., 1 duplicate for

every 10 samples) of the sites as a qualitative check on in-stream variability of the macroinvertebrate community and repeatability of the sampling technique between individuals or collection teams. Laboratory protocols for picking, subsampling, and identification will be provided by the contractor.

References

Bad River Watershed Association 2011. Quality Assurance Project Plan – Staff Water Quality Monitoring – Continuous Temperature, Macroinvertebrate, and Conductivity. Rev.0. 80 pp.

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Wisconsin Department of Natural Resources. 2002. Guidelines for Evaluating Habitat of Wadeable Streams. Madison, WI.

APPENDIX 1 – Macro SOP

Bad River Watershed Association Staff Macroinvertebrate Monitoring Field Data Sheet

Bad River Watershed Association Staff Macroinvertebrate Monitoring **Page 1**
Field Data Sheet

Sample ID #_____ **Waterbody**_____ **Subwatershed** _____
Date_____ **Samplers**_____

Weather:
Today Clear Partly Cloudy Overcast Light Rain Steady Rain Snow
Last 48 Hours Clear Partly Cloudy Overcast Light Rain Steady Rain Snow

Selecting Habitat Proportions

Establish 100 meter Stream Reach:

Distance from culvert or bridge to start of reach (m)
_____

Instream Habitat Proportions:

Habitat	Percentage	Number of Jabs (total must be 20)
Hard bottom (riffle/cobble/boulder)		
Aquatic Macrophytes (submerged/emergent vegetation)		
Undercut Banks (undercut banks/overhanging vegetation)		
Snags (snags/rootwads)		
Leaf Packs		

Habitat Assessment

River Bottom Composition:
%Bedrock_____ % Boulder_____ %Cobble_____ %Gravel_____
% Sand_____ % Silt_____ % Organic_____

%Embedded _____ (Estimate % of large rocks or particles covered with silt)

Flow: _____% (Estimate % of stream bottom currently filled with water)

Overhead Canopy_____% **Algal Growth:** None Little Lots

Water Odor:
None Fish Organic Sewage Oil Other_____

Habitat Assessment Continued…

Left Bank Description: facing upstream
Shrubs____% Grass/forbs_____% Conifer_____% Deciduous_____% Clear_____%
Erosion_____% (of 100)

Right Bank Description: facing upstream
Shrubs____% Grass/forbs_____% Conifer_____% Deciduous_____% Clear_____%
Erosion_____% (of 100)

Estimation of Flow

Depth: Select a spot typical of the sampling site and measure in one step intervals

1._____	6._____	11._____	16._____	21._____
2._____	7._____	12._____	17._____	22._____
3._____	8._____	13._____	18._____	23._____
4._____	9._____	14._____	19._____	24._____
5._____	10.____	15._____	20._____	25._____

Current Velocity: Velocity = 10 feet/number of seconds **Wetted Width:** _____
Fast 1: _____ seconds velocity: _____ feet/second
Fast 2: _____ seconds velocity: _____ feet/second
Slow 1: _____ seconds velocity: _____ feet/second
Slow 2: _____ seconds velocity: _____ feet/second

Average site velocity Total _____/4 = _____ feet/second

Site Sketch (Next page…)

Site Sketch

Sketch major features and mark areas from which samples were taken in the stream reach.

Appendix C

Standard Operating Procedure: Bad River Watershed Association Staff Field Conductivity Analysis

Standard Operating Procedure
Bad River Watershed Association Staff Field Conductivity Analysis

Conductivity Analysis

A. *Scope and Applicability*
Conductivity is a measure of the capacity of water (or other media) to conduct an electrical current (EPA 2010). Conductivity is strongly influenced by water temperature and measurements are often corrected to 25 degrees Celsius (°C), which is called specific conductance (APHA *et al*. 1998). Conductivity in streams and rivers is affected primarily by the geology of the area through which the water flows, but can also be affected by point and nonpoint discharges (such as industrial, agricultural, residential septic systems, etc.). Conductivity is useful as a general measure of stream water quality and can provide important baseline information about a stream site, as well be a quick indicator of whether a point or nonpoint effluent discharge may be impacting stream health.

B. *Summary of Method*
BRWA uses handheld field meters from the Hanna Instruments Company (such as Model HI 98129) to collect measurements of conductivity directly from streams and rivers. The meter has built-in temperature compensation (to 25°C) and provides conductivity results as specific conductance (will be referred to as "conductivity" throughout this document. Samples are collected at approximately 40% of the total stream depth above the bottom of the stream (or 60% of the total depth below the water surface).

C. *Definitions*
Specific Conductance = conductivity measurements that have been temperature-corrected to 25 degrees Celsius.
mL = milliliter
µS/cm = micro Siemens per centimeter

D. *Health and Safety*
Conductivity standardizing solutions (1413 µS/cm, 84 µS/cm)

These solutions contain water (>98%) and potassium chloride (<1%). Potassium chloride: CAS# 7447-40-7. The solutions are not considered hazardous, but users should avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation. Can be safely disposed of as ordinary refuse.

Protect feet with waders, boots or sneakers while working in streams. Wash hands after sampling and before eating.

If you feel that sampling in the stream will put you at personal risk – DO NOT SAMPLE.

E. *Cautions*
Before taking any measurements, make sure the meter is calibrated (See section I, below for more details). Rinse the electrodes thoroughly with deionized water and/or sample water. Rinse the electrodes thoroughly after calibration and between samples. Wait until the stability symbol on the LCD display disappears before recording conductivity readings from the meter.

F. Interferences

Temperature variations and corrections represent the largest source of potential error.

Oil, grease, algae, or dirt can interfere by coating the electrodes, causing a sluggish response and incorrect readings.

G. Personnel Qualifications

All BRWA staff should undergo training in the use of this method prior to sampling. Typical qualifications for BRWA full-time salaried or hourly staff conducting water quality monitoring are at least a Bachelor's Degree in natural resources management or related field. BRWA also hires limited term staff that may conduct water quality monitoring. These staff members typically have some course work or training towards a degree in natural resources management or related field.

H. Apparatus and Materials

Hanna Instruments Company Model HI 98129 meter
Deionized water in a wash bottle
Conductivity calibration solution (1413 µS/cm) in 20 mL sachets
Conductivity calibration check solution (84 µS/cm) in 20 mL sachets
Plastic container (1-quart) for collecting samples from deep streams
Conductivity Meter Calibration Record form
BRWA Field Sheet for Conductivity Measurements

I. Instrument or Method Calibration

Successful calibration is required to use the conductivity meter. The conductivity meter displays "CAL" on the lower left corner of the LCD screen when it is calibrated. The meter should be calibrated prior to each field day and checked for calibration at the end of each field day. The meter is calibrated at one point for conductivity and checked with a lower conductivity standard. Use the HI7031 (1413 µS/cm) calibration solution recommended for the meter to calibrate and the HI70033C (84 µS/cm) calibration solution for the calibration check. The calibration solutions are provided in 20mL disposable pouches and are certified traceable to NIST Standard Reference Material referenced at 25°C. Make sure the calibration solution is not expired before using.

Remove the protective cap over the meter electrodes and rinse with deionized water. From the measurement mode of the instrument, press and hold the "MODE" button until "CAL" is displayed on the lower LCD.

Release the button and immerse the electrodes in the HI7031 (1413 µS/cm) calibration solution. Once the calibration has been automatically performed, the LCD will display "OK" for one second and the meter will return to normal measurement mode. Write down the calibration solution temperature on the Conductivity Meter Calibration Record form (Appendix 1).

The "CAL" symbol on the LCD means that the meter is calibrated. Rinse the electrodes with deionized water.

Shake off excess deionized rinse water and immerse the electrodes in the HI70033C (84 µS/cm) calibration solution. Record the conductivity and temperature readings on the Conductivity Meter Calibration Record form (Appendix 1). Rinse the electrodes with deionized water and shake off

excess. The conductivity calibration solutions can be discarded down the drain. Complete the remainder of the Conductivity Meter Calibration Record form.

The calibration check should be within 10% of the certified value. If the check is outside this range, re-calibrate the instrument and re-do calibration checks. If calibration continues to fail, perform maintenance on the meter or return to the manufacturer for maintenance.

The calibration checks at the end of each field day should also be within 10% of the certified values for the high and low standards. If one or both are outside of this range, the solutions should be re-measured. If they continue to be greater than 10%, a decision should be made on how to use the data collected during that field day. Additional details on quality objectives and criteria are included in BRWA's *Quality Assurance Project Plan – Staff Water Quality Monitoring – Continuous Temperature, Macroinvertebrate, and Conductivity* (BRWA 2011).

In addition to calibration for conductivity, the meter's internal temperature sensor (thermistor) should be verified against a National Institute of Standards and Technology (NIST) traceable thermometer annually and noted on the Conductivity Meter Calibration Record form (Appendix 1). If the temperatures do not agree within ± 4°C, the unit must be repaired or replaced.

J. Sample Collection

Field measurements are taken using a handheld field meter from the Hanna Instruments Company (such as Model HI 98129). Measurements are taken at approximately 40% of the total stream depth above the bottom of the stream (or 60% of the total depth below the water surface). No laboratory samples are collected.

K. Handling and Preservation

All conductivity measurements are taken in the field with a handheld meter. There is no sample collection, transport, or preservation needed.

L. Sample Preparation and Analysis

Field measurements are taken by turning on the meter and selecting "EC" mode ("EC" stands for "electrical conductance") with the "SET/HOLD" button.

Remove the protective cap from the meter and submerge the electrodes in the main current, or thalweg, of the stream. Hold the electrodes upstream or to the side of where you are standing and wait until any disturbance to the bottom sediments have cleared before taking measurements. The electrodes should be submerged to approximately 40% of the total stream depth above the bottom of the stream (or 60% of the total depth below the water surface).

Do not submerge the meter into the water below the digital screen. If the stream is too deep to submerge the electrodes to 40% of the total stream depth, use the 1-quart plastic container to collect a water sample from 40% of the total stream depth. Rinse the container two times before collecting the sample. Measure the conductivity immediately by placing the electrodes of the meter into the sample container, while swirling the container as the meter stabilizes.

All measurements should be taken when the stability symbol on the top left of the LCD screen disappears.

The conductivity value automatically compensated for temperature is shown on the primary LCD, while the secondary LCD shows the temperature of the sample. Write the conductivity and temperature readings on the field data sheet (Appendix 2). Rinse the electrodes with deionized water, shake off the excess water, and replace the protective cap.

M. Trouble Shooting

If problems occur during sampling or analysis, report them on the data sheet. Contact the BRWA Program Director for problem correction.

N. Data

Fill out the BRWA Field Sheet for Conductivity Measurements with site observations and analytical results (Appendix 2).

O. Hardware and Software

There is no hardware or software associated with operating the conductivity meter.

P. Data and Records Management

It is the responsibility of the BRWA Program Director to enter field data into a computer (i.e. Microsoft Excel or Access) database and generate any data summaries. All data entered will be checked by a second person (100% check of hand-entered data). Paper records (data sheets) will be scanned into electronic format (i.e. PDF) and kept in a binder in the BRWA main office.

Q. Quality Assurance and Quality Control

Training in the use and maintenance of the Hanna Instruments handheld meter is mandatory. The Hanna Instruments meter must be calibrated before each field day and checked for calibration at the end of each field day. Calibration is completed with the 1413 µS/cm solution (HI7031) from Hanna Instruments and checked with the 84 µS/cm solution (HI70033C) both before and after each field day. A blank solution of deionized water should be measured once every ten field samples. Additional details on quality objectives and criteria are included in BRWA's *Quality Assurance Project Plan – Staff Water Quality Monitoring – Continuous Temperature, Macroinvertebrate, and Conductivity* (BRWA 2011).

References:

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APPENDIX 1 – Conductivity SOP

Bad River Watershed Association Conductivity Meter Calibration Record

Bad River Watershed Association

Conductivity Meter Calibration Record

Meter Type:				Model #:		
Who	Date mm/dd/yy	Time	Calibration Solution	Calibration Solution Temp (C)	Calibration Successful? (Y or N)	Notes

APPENDIX 2 – Conductivity SOP

Bad River Watershed Association Field Sheet for Conductivity Measurements

BRWA Field Sheet for Conductivity Measurements

Date:	Sampler(s):				Meter:	
Site	Time	Air Temp. (C)	Water Temp. (C)	Sample Type	Conductivity (µS/cm)	Notes

Sample Type = sample, blank, duplicate, AMS test, etc

Appendix D

Lake Superior Research Institute Taxonomy Laboratory Standard Operating Procedures

Procedure No: FS/25
Issue Date: September 09, 2011
Number of Pages: 09

STANDARD OPERATING PROCEDURE

PICKING BENTHIC INVERTEBRATES FROM QUALITATIVE SAMPLES USING CATON TRAYS

SOP Written by Kurt Schmude

Signature: Kurt Schmude Digitally signed by Kurt Schmude DN: cn=Kurt Schmude, o=University of Wisconsin-Superior, ou=Lake Superior Research Institute, email=kschmude@uwsuper.edu, c=US Date: 2011.09.09 14:57:09 -05'00'

Title: Senior Scientist

Date: 09-Sep-2011

Reviewed and Approved by Lana Fanberg

Signature: Lana Fanberg Digitally signed by Lana Fanberg DN: cn=Lana Fanberg, o=UWS, ou=LSRI, email=lfanberg@uwsuper, c=US Date: 2011.09.09 15:44:03 -05'00'

Title: Associate Research Specialist

Date: 09-Sep-2011

Cleared For Issue by Kelsey Prihoda

Signature: Kelsey R. Prihoda Digitally signed by Kelsey R. Prihoda DN: cn=Kelsey R. Prihoda, o=Lake Superior Research Institute, ou=Quality Assurance/Quality Control, email=kprihoda@uwsuper.edu, c=US Date: 2011.09.12 08:07:16 -05'00'

Title: Quality Assurance/Quality Control Manager

Date: 12-Sep-2011

DISTRIBUTION LIST:

LSRI Taxonomy Laboratory staff and students, Quality Assurance staff, LSRI Director, and any individual responsible for picking invertebrates from processed field samples that occur in Caton trays.

RECORD OF REVISIONS:

No.	Date	Type	No.	Date	Type
1			7		
2			8		
3			9		
4			10		
5			11		
6			12		

PICKING BENTHIC INVERTEBRATES FROM QUALITATIVE SAMPLES USING CATON TRAYS

INTRODUCTION

This standard operating procedure (SOP) describes the method for extraction (picking) of benthic invertebrates from processed, qualitative samples that are spread onto a Caton tray. This method closely follows the methods established by the United States Environmental Protection Agency for wadeable streams assessment (US EPA 2004). Benthic invertebrate samples are collected in the field and are transported to the Taxonomy Laboratory at the Lake Superior Research Institute (LSRI) for processing. Samples are rinsed through a sieve to remove the preservative and debris, and organisms retained by the sieve are transferred onto a Caton tray. The sample is evenly distributed within the tray, grids are randomly chosen, the debris from the grid is removed, and the organisms are removed from the debris using forceps while viewing through a dissecting microscope. The animals are separated into taxonomic groups (depending on the project requirements) and placed into vials containing ethyl alcohol.

REFERENCES

United States Environmental Protection Agency. 2004. Wadeable Streams Assessment: Benthic Laboratory Methods. EPA841-B-04-007. US EPA, Office of Water and Office of Research and Development, Washington, DC.

EQUIPMENT LIST

- 3.7-mL Scintillation Vials
- 70%-80% Denatured Ethyl Alcohol Solution (sample preservative)
- Caton Tray and Accessories (metal square, metal spatula, forceps)
- Chemical Splash Goggles or Safety Glasses
- Data Sheets (see Appendix 1 for example) or Laboratory Notebook
- Dishpans
- Dissecting Microscope
- Fine Forceps
- Flexible Fiber Optic Lights
- Jar or Plastic Container (for saving sample preservative)
- Lab Coat
- Light Box
- Permanent Marking Pen and Label Tape
- Petri Dish, Round and Gridded
- Plastic Spoon
- Processed Macroinvertebrate Samples
- Safety Gloves (**Note:** Special gloves may be required when working with high hazard materials. Consult the UW-S Environmental Health and Safety Director prior to starting.)

- U.S. Standard Sieve, Number 120 (for catching fine silt)
- U.S. Standard Sieve, Size is Project-Dependent
- Wash Bottle (Tap Water)

Reagents

Sample Preservative (70% to 80% Denatured Ethyl Alcohol Solution): To prepare a 1-L solution of sample preservative (reduce or increase volumes proportionally if less or more is prepared), add 30 mL glycerol and 700 mL (for 70%) or 800 mL (for 80%) of denatured ethanol to a 1000-mL volumetric flask (or similar container). Dilute to volume with water and mix well. Store the prepared preservative in an appropriately labeled container within a flammable storage cabinet. The denatured ethanol is a denatured 3A ethanol that includes a mixture of 90.48% ethanol and 4.52% methanol, with 5.00% isopropyl alcohol added.

SAMPLE HANDLING REQUIREMENTS

1. Preserve samples using sample preservative (70-80% denatured ethyl alcohol solution).

2. Samples may be stored indefinitely in a flammable storage cabinet once preserved.

PROCEDURE

1. A lab coat must be worn at all times; chemical splash goggles (or safety glasses) and gloves must be worn during procedures 4-11 and 25-26.

2. Workers are advised to see their supervisor *immediately* if there are any questions throughout the entire SOP.

3. Follow proper waste disposal practices during the picking procedure.

4. Take a labeled sample jar from the Flammable Storage Cabinet where it is stored to a fume hood. The sample jar should have been properly labeled by the individual(s) who collected the sample.

5. Decant excess preservative through a U.S. Standard Sieve (size deemed appropriate for a given project) into a separate container (e.g., jar or plastic container) to be saved.

6. The saved preservative should be in a sealed or covered container and properly labeled with the name of the preservative, concentration, date saved, initials, and any hazard communication information (e.g., “Danger – Flammable”).

7. Fill the sample jar with tap water and pour the contents onto a clean U.S. Standard Sieve (size is project-dependent). A dishpan must be underneath the sample at all times to prevent debris and organisms from being lost. Rinse the jar and lid thoroughly and gently with a water wash bottle and pour contents onto the sieve; no debris or organisms should be left in the jar or stuck to the lid.

8. Remove larger debris, such as sticks and rocks, from the sieve. Check the debris thoroughly for attached organisms. Rinse any attached organisms into the sieve, and dispose of the large debris in the trash if no attached organisms remain.

9. Partially fill a clean dishpan or plastic tray with tap water and gently rinse and sieve (i.e., gently circulate the contents of the sieve in water) the sample to eliminate the preservative and fine debris. The sample should be rinsed and sieved for a few seconds, or up to 10-15 minutes, depending on the amount of debris. Sieving can stop when the fine debris that passes through the sieve becomes negligible/water is clear. Pour the fine debris left in the dishpan/tray onto a U.S. Standard No. 120 Sieve to be collected and properly disposed in the trash. The rinse water may go down the drain.

10. Place all of the debris from the sample onto the Caton tray. Add enough tap water to the tray to allow the debris to spread out and/or float. Thoroughly and carefully stir the mixture with a spoon or forceps to evenly spread out the debris and organisms across all grids. Slowly lift the metal portion of the tray out of the water. Examine the sample for uneven distribution. If the sample is unevenly distributed, reimmerse the sample and spread out the sample again, repeating the process. When the sample has been determined to be evenly spread, discard the water remaining in the wooden tray, and place the metal tray back into the wooden tray. Take the tray to the workstation.

11. Thoroughly clean the hooded work station and equipment so they are ready for the next sample.

12. Label an appropriate number of 3.7-mL scintillation vials with the sample identification code and the words “Danger-Flammable” (a hazard communication label may also be used), and fill with 70-80% denatured ethyl alcohol solution.

13. At the work station, randomly choose three numbered grids using a table of random numbers. Three grids are processed from the sample to ensure that the subsampled debris is representative of the overall sample. Record the numbers of the three grids that were randomly chosen on a project-specific datasheet (see Appendix 1) or laboratory notebook. Remove all of the debris from the three grids using the metal square and spatula, and place each subsample into separate, gridded Petri dishes. Add just enough water to each dish to evenly spread out the debris and organisms.

14. Examine the contents by eye to determine if the number of organisms in the **first** subsample will exceed the target number for the project (e.g. 125, or 300, or 500 organisms). If the number of organisms will clearly **not** exceed the target number, then the subsample can be processed in its entirety. If the number of organisms will clearly exceed the target number, then the subsample needs to be quartered. One-quarter of the subsample needs to be randomly chosen. Remove the contents of the one-quarter and place it into another gridded Petri dish.

15. View the debris and organisms under a minimum of 6x and a maximum of 12x magnification using a dissecting microscope on a light box and with flexible fiber optic

lights. The macroinvertebrates to be removed from the sample, as well as, the quantification of these organisms is project dependent. Therefore, remove all appropriate macroinvertebrates and count them if required. Start at one end of the Petri dish and slowly move from grid to grid, searching through the debris, inside empty snail and bivalve shells, and plant stems and leaves for small invertebrates. Place the organisms into the labeled vials containing 70-80% denatured ethyl alcohol solution. Fragments of organisms that allow for identification should be removed (e.g., head + first thoracic segment). Do not remove: insect exuviae, empty snail or bivalve (Mollusca) shells, eggs or egg masses, unless required by the project.

16. If the number of specimens in the one-quarter of the first subsample exceeds the target number, then the sample is finished and no other subsample needs to be processed. If the target number is not reached, then the same one-quarter from the **second** subsample needs to be processed in the same fashion.

17. If the cumulative number of specimens after processing the one-quarter of the second subsample exceeds the target number, then the sample is finished and no other subsample needs to be processed. If the target number is not reached, then the same one-quarter from the **third** subsample needs to be processed in the same fashion.

18. If the cumulative number of specimens after processing the one-quarter of the third subsample exceeds the target number, then the sample is finished and no other subsample needs to be processed. If the target number is not reached, then the next one-quarter from the first subsample needs to be processed in the same fashion. Subsequently, corresponding one-quarters need to be processed until the target number is reached, or all four quarters from each of the first three grids are processed.

19. If the target number is not reached after all three Caton tray grids are processed, then another grid is randomly chosen, and the entire grid is processed. Additional grids are randomly chosen and processed in their entirety until the target number is reached.

20. Place the debris in the Petri dishes into a jar labeled exactly as the sample jar and include the phrase “Sorted Debris” on the label.

21. If the worker cannot complete the sample before the end of the day, or must leave for more than one hour, enough water must be added to the Caton tray to keep the sample moist. The Caton tray must be covered, and it must be refrigerated with the "Sorted Debris" jar and the labeled vial(s). If a longer delay is expected and the sample will not be picked for several days, then the sample must **NOT** be started in the first place.

22. Place the finished, labeled, 3.7-mL scintillation vials in a Flammable Storage Cabinet.

23. Enter the following information (as applicable) on the project-specific laboratory bench sheet (see Appendix 1) or laboratory notebook:
    a. Waterbody name
    b. Site identification number

c. Serial identification number
d. Collection date
e. Collector's name
f. Collection method
g. Sorter's name
h. Sort date
i. Each grid number that was processed
j. Number of specimens in each grid
k. Cumulative number of specimens

24. Place the data sheet (Appendix 1) in the appropriate, project-specific binder.

25. Take the "Sorted Debris" container to a fume hood. Decant excess water through the appropriate U.S. Standard sieve (size is project dependent) and into the drain; return any debris on the sieve into the labeled "Sorted Debris" jar. Pour the preservative that was saved in an earlier procedure into the jar, cover it, and place it into the appropriate Flammable Storage Cabinet. These jars may be returned to the granting agency or contractor for disposal if so stipulated in the contract. Otherwise, this material will be disposed as agreed upon with the UW-S Environmental Health & Safety Director.

26. Take any "Unsorted Debris" remaining in the Caton tray to a fume hood. Place all of the remaining debris and organisms into an appropriately labeled "Unsorted Debris" jar for preservation. These jars may be returned to the granting agency or contractor for storage or disposal if so stipulated in the contract. Otherwise, this material will be disposed as agreed upon with the UW-S Environmental Health & Safety Director.

27. Thoroughly clean the work station and equipment so they are ready for the next sample.

Quality Assurance and Quality Control

Note: Quality assurance/quality control of benthic invertebrate picking is project-specific; the following procedures are best-practices that should be implemented whenever possible.

1. Benthic invertebrate picking should only be conducted by personnel who have read and understood this SOP, who have been properly trained, and who have demonstrated competency in following this procedure (initial competency is achieved when personnel have passed a QC check of five consecutive samples). All procedures outlined in this SOP should be followed exactly; any deviations from this SOP should be approved (prior to sample picking) by a supervisor or project principal investigator.

2. Record data on pre-printed datasheets and/or in project-specific laboratory notebooks, following the documentation procedures outlined in the LSRI Quality Management Plan. Data storage time is project-specific, but typically does not exceed five years from the date the project is completed (i.e., final report is signed) or terminated.

3. A quality control (QC) check must be performed by qualified personnel who are

experienced in sorting and picking benthic invertebrate samples. All QC checks must be performed immediately following picking of the sample.

4. A QC check should be conducted on 10% (1 out of 10, randomly selected) of an individual's picked samples for each project.

5. The individual performing the QC check must go through the "Sorted Debris" container for the randomly chosen sample and count the number of benthic invertebrates found in the debris.

6. Calculate the percent picking efficiency for each sample using the following calculation:

$$\textbf{\textit{Percent Picking Efficiency}} = [A/(A+B)] \times 100\%$$

Where: A = the number of organisms found by the primary picker
B = the number of organisms missed by the primary picker and found during the QC check

7. Ensure that a >90% picking efficiency is achieved. If an individual fails to achieve a >90% picking efficiency on a QC check, then QC checks should be performed on that individual's next five consecutive samples until a >90% efficiency is achieved.

8. If an individual fails to meet the >90% picking efficiency on all five consecutive samples, corrective actions should be taken, such as re-training the individual.

9. Allow a reduced accuracy (i.e., lower percent picking efficiency) in the following two situations (and based on the project objectives):

    a. When a sample contains a low density of benthic invertebrates; low numbers of organisms can produce artificially high percentages of error. For example, if three organisms were found during the first pick of a sample, and two additional specimens were found during the QC check, then 40% of the organisms were missed during the first pick. However, only two specimens were missed overall.
    b. When the percent picking efficiency does not have any effect on the interpretation of the data samples do not need to be repicked.

APPENDIX 1

EXAMPLE DATASHEET FOR QUALITATIVE BENTHIC INVERTEBRATE PICKING

BENTHIC MACROINVERTEBRATE LABORATORY BENCH SHEET (FRONT)

Project Name : _____

Serial ID#: _____ Waterbody Name: _____

Sorter (initially spread sample) _____ Site ID#: _____

Sort Date _____ Collection Date: _____

GRID ORDER	SORTER'S INITIALS	RANDOM # GRID ID	NUMBER OF SPECIMENS/GRID	CUMULATIVE # OF SPECIMENS/GRID
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				

Check off grids as selected

	1	2	3	4	5	6
1						
2						
3						
4						
5						

Collector: _____

Collection Type: _____

BENTHIC MACROINVERTEBRATE LABORATORY BENCH SHEET (BACK)

SUBSAMPLING/SORTING INFORMATION

Sorter _____

Date _____

Additional Sorters:

1 _____

2 _____

3 _____

4 _____

Number of grids picked: _____ whole _____ quarters

Time expenditure _____ No. of organisms _____

Indicate the presence of large or obviously abundant organisms

_____

_____

QC: ☐ **YES** QC Checker _____

# organisms orginally sorted	# organisms recovered by QC checker	# organisms originally sorted	% sorting efficiency

≥ 90% sample passes _____

≤ 90% sample fails, action taken: _____

_____

TAXONOMY

ID _____

_____

_____

DATE _____

Explain TCR ratings of 3-5:

Other Comments (e.g. condition of specimens):

_____

_____

QC: ☐ **YES** QC Checker _____

Organisms recognition ☐ pass ☐ fail

Verification complete ☐ YES ☐ NO

General Comments (use this space to add additional comments):

Procedure No: FS/13
Issue Date: August 20, 1996
Number of Pages: 4

STANDARD OPERATING PROCEDURE
IDENTIFICATION OF BENTHIC INVERTEBRATES

SOP Written by Kurt Schmude

Signature: Kurt L. Schmude Digitally signed by Kurt L. Schmude DN: cn=Kurt L. Schmude, o=University of Wisconsin Superior, ou=Lake Superior Research Institute, email=lrschmude@uwsuper.edu, c=US Date: 2010.05.21 14:05:02 -05'00'

Title: Senior Scientist

Date: May 21, 2010

Reviewed, Approved, and Cleared For Issue by Kelsey Prihoda

Signature: Kelsey R. Prihoda Digitally signed by Kelsey R. Prihoda DN: cn=Kelsey R. Prihoda, c=US, o=Lake Superior Research Institute, ou=Quality Assurance/Quality Control, email=kprihoda@uwsuper.edu Reason: I am approving this document Date: 2010.05.21 14:17:04 -05'00'

Title: Quality Assurance/Quality Control Manager

Date: May 21, 2010

DISTRIBUTION LIST:

LSRI Taxonomy Laboratory staff, quality assurance staff, LSRI director, and any individual responsible for identification of benthic invertebrates.

RECORD OF REVISIONS:

No.	Date	Type	No.	Date	Type
1	04/08/2003	Added more references.	7		
2	05/21/2010	Formatted SOP. Added text to "Introduction". Added reference for SOP and updated taxonomic references. Added "Syracuse Watch Glass" to "Equipment List". Added ¶1, ¶3, ¶5-¶7, ¶11, and ¶12. Added "Quality Assurance and Quality Control" section.	8		
3			9		
4			10		
5			11		
6			12		

IDENTIFICATION OF BENTHIC INVERTEBRATES

INTRODUCTION

This standard operating procedure (SOP) describes the method used for identification and enumeration of benthic invertebrates from samples received by the Lake Superior Research Institute's (LSRI) Taxonomy Laboratory. Samples are collected and then transported to LSRI for processing, subsampling, and/or picking (extraction). The following LSRI SOPs may be used to prepare samples for identification: LSRI/SOP/FS/16 – *Processing Hester Dendy Samples*, LSRI/SOP/FS/12 – *Subsampling Benthic Invertebrate Samples in the Laboratory*, and/or LSRI/SOP/FS/14 – *Picking Benthic Invertebrates from Samples*.
Benthic invertebrates are identified to the lowest taxonomic level possible based on current literature, or they are identified to the taxonomic level required by the project. Genus/species identification provides more accurate ecological and environmental information, but family-level identification provides a higher degree of precision among samples and taxonomists, requires less expertise to perform, and accelerates assessment results. Regardless of the taxonomic level of identification, only those taxonomic keys that are peer-reviewed and available publically (i.e., published) should be used (Barbour et al., 1999).

REFERENCES

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

REFERENCES: TAXONOMIC LITERATURE

Note: Numerous, additional taxonomic keys/publications, especially for the Chironomidae, are routinely consulted.

Epler, J.H. 2001. Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina. North Carolina Department of Environmental and Natural Resources.

Hilsenhoff, W.L. 1995. Aquatic Insects of Wisconsin. Keys to Wisconsin Genera and Notes on Biology, Distribution, and Species. Publication of the Natural History Council, University of Wisconsin-Madison, No. 3.

Kathman, R.D. and R.O. Brinkhurst. 1998. Guide to the Freshwater Oligochaetes of North America. Privately Published. Aquatic Resources Center, College Grove, TN.

Klemm, D.J. 1985. A Guide to the Freshwater Annelida (Polychaeta, Naidid, Tubificid, Oligochaeta, and Hirudinea) of North America. Kendall/Hunt Publishing Co., Dubuque, Iowa.

Merritt, R.W., K.W. Cummins, and M.B. Berg (Eds.). 2008. An Introduction to the Aquatic Insects of North America. 4th Edition. Kendall/Hunt Publishing Co., Dubuque, Iowa.

Pennak, R.W. 1989. Freshwater Invertebrates of the United States. Protozoa to Mollusca. 3rd Edition. John Wiley & Sons, Inc., New York. xvi + 628 pp.

Smith, D.G. 2001. Pennak's Freshwater Invertebrates of the United States. Porifera to Crustacea. 4th Edition. John Wiley & Sons, New York. x + 638 pp.

Thorp, J.H. and A.P. Covich. 2001. Ecology and Classification of North American Freshwater Invertebrates. 2nd Edition. Academic Press, Inc., New York. xvi + 1056 pp.

Wetzel, M.J., S.V. Fend, K.A. Coates, R.D. Kathman, and S.R. Gelder. 2006. Taxonomy, Systematics, and Ecology of the Aquatic Oligochaeta and Branchiobdellidae (Annelida, Clitellata) of North America, with Emphasis of the Fauna Occurring in Florida. A Workbook. 10 September 2006. vi + 269 pp + color plates.

Wiederholm, T. (Ed.). 1983. Chironomidae of the Holarctic region. Keys and Diagnoses. Three volume series. Entomologica Scandinavica Supplements.

EQUIPMENT LIST

- Data Sheets
- Dissecting and Compound Microscopes
- Empty Scintillation Vials
- Fine-Tipped Forceps
- Permanent Marking Pen and Label Tape
- Syracuse Watch Glass (see Figure 1)
- Tally Counter
- Taxonomic References
- Vials Containing Samples

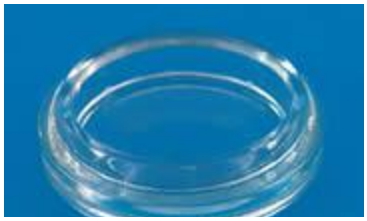

Figure 1. Syracuse watch glass for taxonomic identification of benthic invertebrates. Accessed from: http://www.emsdiasum.com/microscopy/products/grids/images2/71570.jpg, March 2010.

PROCEDURE

1. This procedure must only be conducted by taxonomists who have the appropriate training and experience in the identification of freshwater benthic invertebrates. Identifications are made by the senior taxonomist, trained biologist(s), or trained biology student(s). All identifications made by students and a proportion made by the biologists are verified by the senior taxonomist for accuracy.

2. Remove the appropriate benthic invertebrate samples (preserved in 70-80% denatured ethyl alcohol in labeled vials) from the Flammable Storage Cabinet and bring to a laboratory work station.

3. Record the sample label information on the project-specific data sheet.

4. Benthic invertebrates are identified and enumerated separately by taxonomic group while viewing through a compound microscope (e.g., Oligochaeta or larvae of Chironomidae), or dissecting microscope (e.g., all other invertebrates) using fine-tipped forceps. Only one

sample should be opened and processed at a single work station at a time; this will avoid mixing specimens among samples.

5. Pour the specimens from the vial into a Syracuse watch glass. Rinse the vial into the watch glass using 70-80% ethanol in a wash bottle. Add enough ethanol to the watch glass to cover the specimens.

6. Examine the vial label, vial, and its lid under a compound microscope for attached specimens.

7. Examine the sample under the compound or dissecting microscope and use taxonomic keys and other supportive taxonomic literature to identify the specimens.

8. Taxonomic identification level depends on the specimen. Benthic invertebrates are identified to the following taxonomic levels (unless otherwise specified by project requirements):

    8.1. Oligochaeta are identified to lowest taxonomic level possible, usually species. All other specimens are identified as pieces (without heads), immature tubificids (without chaetae), immature tubificids without hair chaetae, or immature tubificids with hair chaetae.

    8.2. Larvae and pupae of Chironomidae are identified to subfamily or tribe (very immature or damaged specimens), genus, species group, or species.

    8.3. Other macroinvertebrates are identified to the following taxonomic levels: insects to genus or species; Mollusca to family, genus, or species; Crustacea to genus or species; Hirudinea to genus or species; Nematoda to phylum; and Cnidaria to genus.

9. Place each taxon into separate 3.7-mL scintillation vials, or place all specimens from one sample into a single vial, depending on the objectives of the study. Vials are filled (one-half to three-quarters full) with 70%-80% ethyl alcohol for preservation.

10. Enumerate specimens as they are identified by manually marking on a data sheet or by using a counter.

11. Immediately record the following information on a project-specific datasheet: family, genus, or species; counts of larvae, pupae, and adults as appropriate for the taxonomic group; and any comments. Store the completed datasheets in a project-specific, three-ring binder.

12. Create a label for each vial that includes: sample identification code, collection date, taxon, number of individuals, initials of individual responsible for identification, date of identification, and any hazard communication information (e.g., “Danger – Flammable”).

13. Sample vials are stored together in a Flammable Storage Cabinet.

Quality Assurance/Quality Control

1. Identification and enumeration of invertebrates will be the responsibility of LSRI's Senior Invertebrate Taxonomist, Dr. Kurt Schmude.

2. All identifications made by students and a proportion made by the biologists are verified by the senior taxonomist for accuracy

3. All identifications will be based on current taxonomic literature.

4. Confirmation by outside expert taxonomists will be obtained if deemed necessary.

5. All invertebrates will be housed and maintained at LSRI upon completion of the project, or returned to the granting agency if required.

Appendix E

Bad River Watershed Association Macroinvertebrate Sample Chain of Custody Form

CHAIN-OF-CUSTODY/DATA FORM – MACROINVERTEBRATE MONITORING

BAD RIVER WATERSHED ASSOCIATION

SECTION A: SAMPLE COLLECTION

Sample ID Number:	Coordinates: Lat. Long.
Waterbody Name:	Road Crossing:
Date:	Replicate Number (i.e. 1 of 2):
Study Name:	Split-Sample Designation (i.e. 1 of 2):
Sample Container:	Preservative:

SECTION B: SAMPLE STORAGE AND CUSTODY

1. Sample Collector Name:_____ Date:_____ Time:_____ Preservative: _____
2. Custody given to : _____ Date:_____ Time:_____ Preservative: _____
3. Custody given to : _____ Date:_____ Time:_____ Preservative: _____
4. Custody given to : _____ Date:_____ Time:_____ Preservative: _____

Comments:_____

_____

_____

APPENDIX F

Bad River Watershed Association Deviation Form

DEVIATION FORM - BAD RIVER WATERSHED ASSOCIATION

Project Title:_____ **Date/Time:_____**

Explanation of Deviation:

Corrective Procedure:

Signature:_____Date:_____

_____

Route to BRWA Project Director for Evaluation.

Impact on this Study:

Signature:_____Date:_____