Appendix A.1

Wisconsin Department of Natural Resources

Wadable Smallmouth Bass Warmwater Stream Assessments

WADABLE SMALLMOUTH BASS WARMWATER STREAM ASSESSMENTS

Guidance at-a-glance

SAMPLING

Equipment. One or more standard WDNR DC stream ("tow-barge") shockers with three anodes An AC shocker can be used instead when conductivity is low (< 100 µS). Shocking should proceed in an upstream direction over the entire width of the stream channel during daylight. One shocker is recommended for streams less 60 ft wide, two shockers for streams 60-89 ft wide, and three shockers for streams 90 ft or wider. In the "in-between" waters, a hybrid sampling design may be used in which deep areas are sampled with a miniboom and shallow areas with a stream shocker. Catch rates are then calculated separately for each gear.

Time: Shocking will be most efficient and representative during summer months at baseflow - between May 24 and early September 15 in the southern two-thirds of Wisconsin and between June 7 and August 31 in the northern third.

Sample Length: Fish community composition (IBI), species relative abundance, and SMB (gamefish) are estimated over the first 35x the mean wetted width with a 100 meter minimum and 400 meter maximum. The SMB (gamefish) assessment is then extended to the full 800-meter (0.5-mile) site.

Data and Metrics: Length frequency (lengths of all gamefish), counts, and effort (IBI, Catch per effort (CPE), RSD). Weights and collection of age structures for gamefish are optional but recommended.

STREAM SELECTION

1) Stratified random sampling within the various smallmouth bass stream classes
2) Biologist chosen, non-random trend and or target streams (1 trend/1 target stream per biologist). *Trend streams* are surveyed annually. *Targeted streams* are pulled out of order of the random list and surveyed for a specific management purpose (Chapter 30 recommendations, spills/kills, habitat alterations, pending threats, ID new water, etc.). For trend streams, biologists may choose exact locations, including emphasizing prime smallmouth bass habitat. You must, however, document very clearly where you started and stopped and how far you sampled. Nonrandom sampling is for a biologist's own management needs so trend waters should be of high value to each biologist.

The warmwater stream class will be sampled at a higher percentage than the coolwater or nursery classes. We are recommending a 3:1:1 sample ratio (warmwater, nursery, coolwater) when working off the random list.

SMB Wadable Streams Master List

1) The SMB Wadable Streams Master List includes known smallmouth bass waters and potential smb waters (predicted from an ongoing GIS-based computer modeling study that estimates the fishery potential of Wisconsin streams and rivers by drainage area, estimated flow, estimated maximum temperature, and gradient). This information was used to classify streams into the appropriate class (wadable warmwater, wadable coolwater, and wadable nursery – color coded orange, blue, and green in the list, respectively).
2) Classified streams have been sorted by Fish Team and separated further by biologist within Fish Team.
3) The number of suggested sample sites for each Fish Team for each year of the biennium (FY08, FY09) is in parentheses after the Fish Team title (Central Wisconsin (17); Headwaters (10); Lower Chippewa (12), etc.).

The list should accurately reflect (as much as possible) the true smallmouth bass resource. If streams are listed that are definitely entire trout streams, they should be removed. If a stream, beyond a shadow of a doubt, doesn't have smallmouth it should be dropped. If you are not sure, it should remain on the list.

Work Plan Example for Upper Chippewa River

FY08 – The UC Fish Team was given a proposed workload of 17 warmwater wadable sites to sample.

No trend sites were selected (TRND column). Keep in mind, a trend stream will only have one site, regardless of the number of sites actually listed (e.g., the length of the Brunet River in Sawyer County calls for 3 sample sites. As a trend stream, the Brunet River would have only one sample site).

The team identified two target streams per biologist that would be sampled in FY08 – identified by X in TRGT column (remember target streams are those streams that were identified to be sampled sooner rather than later. After sampling, they will be put back into the sampling rotation):

Pratt 2 sites on the Couderay River; 2 sites on the West Fork Chippewa River;
Roth 2 sites on the Bad River; 3 sites on the Manitowish River; and
Scheirer 6 sites on the North Fork Jump River, and 4 sites on the South Fork Flambeau River.

The workload above identifies 19 sites and the UC Team can work plan accordingly.

FY09 – Again the UC Fish Team was given a proposed workload of 17 warmwater wadable sites to sample.

Because the target streams were sampled in the first year of the biennium and there are no trend sites (a site that is sampled annually), the UC Fish Team will now use the "Random" column to define the sampling schedule in FY09.

We are recommending that the warmwater class receive higher priority (more potential as a fishery). Ideally, we'd recommend classes be sampled at a 3 warmwater to 1 coolwater to 1 nursery ratio on the random list.

The random list would be followed sequentially by smbclass.
So, on the random list in the SWWARM class, Frank Pratt could do number 1 – 2 sites on the East Fork Chippewa River; number 2 – 1 site on the Thornapple River and in the SWNURSERY class number 1 – 2 sites on the Thornapple River. Jeff Roth could do, in the SWWARM class number 1 – 2 sites on the West Fork Montreal River, number 2 – 2 sites on Tyler Forks in Iron County, and in the SWNURSERY class number 1 – site on the Montreal River. Jeff Scheirer could do, in the SWWARM class number 1 – 3 sites on Main Creek, number 2 – 1 site on Little Jump River, and in the SWNURSERY class number 1 – 4 sites on Deer Tail Creek.

The FY09 workload, as outlined above, would identify 18 sites to work plan.

Because each biologist has a classified list of randomly numbered streams, workload can be somewhat flexible as long as the random number is followed in sequence (1, 2, 3, etc.). One biologist might have more of lake workload or coldwater workload in a given year and desires less warmwater wadable work. The list, in its current form, would allow that biologist to do less and another biologist to pick up the slack (i.e., would move further down the random list) to satisfy the Fish Team workload. Some biologists might have a higher proportion of coolwater streams and, consequently, might put more emphasis on them. Bottom line: stream classes can be sampled in various proportions – just continue to use random number list.

Other things to consider

1) More streams sampled = reduced rotation length
2) Number of sites per stream varies, so number of streams sampled each year will vary.
3) A complete census of nursery streams not necessary.
4) If stream is not accessible, or can't be sampled for some reason, move down the random number list to the next available stream.

SITE SELECTION

Fish teams will receive a list of randomly chosen streams within each SMB stream class (warmwater, coolwater, and nursery) with stream segment length and number of sites to be sampled (sample at least 10% of the stream segment in 0.5-mile length sites). A segment must have reasonable field vehicle access (bridge, farm crossing, etc) for it to be sampled. Similarly, if there is access but the segment is unwadable (too deep, bottom too soft, too many logs, etc.) then the next access point is sampled. If access exists and the segment is wadable, the water is sampled, regardless of the quality of the habitat. If there are multiple access points, a coin may be flipped to decide the access point. The sample should start (or end) at least 10 X the channel width from a bridge or other hydraulic control, such as a low head dam or wier (or even further if the influence of the structure, such as pooled water or an atypical riffle, extends further).

DATA COLLECTION

Fish Community and SMB/Gamefish Evaluation. Four different data sheets will be used in the Fish Community and SMB/Gamefish evaluation: **Site Summary, Catch Summary, Individual Fish Data Sheet**, and **Individual Game Fish Data Sheet**. http://infotrek.er.usgs.gov/doc/wdnr_biology/Fishproto_dataforms_20000711.pdf

All four data sheets apply to the whole site. There is one **Site Summary** sheet per site and one or more of each of the other data sheets, depending on the number and diversity of fish captured. Guidelines for filling out each data sheet and examples of blank and completed sheets can be found in the Guidelines for Assessing Fish Communities of Wadable Streams in Wisconsin http://infotrek.er.usgs.gov/doc/wdnr_biology/Fish_IBI_Protocols_20010324.pdf.

Appendix A.2

Wisconsin Department of Natural Resources

Monitoring Protocol for Tier 1 Coldwater Wadeable Streams

Monitoring Protocol for Tier 1 Coldwater Wadeable Streams

Monitoring Design: All classified trout streams have been divided into segments based on trout classification (1, 2, or 3) and stream order (small: 2-3, large: 4-5). Streams of the same order and trout class were found to be similar in terms of fisheries metrics (CPE and PSD) of interest. Streams and segments to sample in 2007 will be identified on the web surface water viewer. All segments on a stream should be sampled sequentially. Sites are the continuous length of stream that will be sampled within segments.

Sampling Frequency: The number of sites to sample on a segment vary by length of the segment: 0 sites on segments less than 0.5 miles, 1 site on segments 0.5 to 1.5 miles, 2 sites on segments from 1.5 – 3.0 miles, and 1 site per 3 miles (minimum of 3 sites) on segments greater than 3.0 miles. Trout streams of management interest less than 0.5 miles should have 1 site. Sites should be chosen to represent the variety of habitat in the segment.

Site Length: Length of sites should be 35 times mean stream width on segments greater than or equal to 3 meters wide, and 100 meters on streams less than 3 meters wide.

Timing: Monitoring should take place between June 15 and September 15 to allow capture of young-of-year. Sites that will be compared over time, e.g. trend sites, should be sampled as close as possible to the same date each year.

Data Collection: At all sites collect and measure lengths of all gamefish, exotics, and threatened and endangered species (if large numbers of gamefish are encountered at a site, only the first 200 fish of each species need to be measured). Collection of fish weights is optional. At one site per segment collect and count all fish species for calculation of an IBI. Streams may be scheduled for Clean Water Act evaluation and will have exact sites identified and require IBI's. Watershed staff should be consulted prior to sampling Clean Water Act sites so that habitat and fish station locations and lengths are identical and habitat and fish data are collected within the same time period (ideally within 2 weeks of each other). For IBI's, follow the detailed procedures in the Guidelines for Assessing Fish Communities of Wadeable Streams in Wisconsin.

Habitat procedures are being developed for a qualitative analysis following the methods of Simonson et al (1994) and will be posted on the FM database website. Continuous temperature measurements are suggested when assessing streams for potential trout stream classification. Temperature data is to be entered in the SWIMS database (contact Joanna Griffin for data entry questions).

Appendix A.3

Wisconsin Department of Natural Resources

Guidelines for Assessing Fish Communities of Wadable Streams in Wisconsin

State of Wisconsin
Department of Natural Resources

Guidelines for Assessing Fish Communities of Wadable Streams in Wisconsin

Revised March 2001

Bureau of Fisheries Management and Habitat Protection
Monitoring and Data Assessment Section
101 S. Webster St.
Madison, WI 53707.

(Modified from Simonson and Lyons 1993.
Evaluation of the Wisconsin Priority Watershed Program for
Improving Stream Fish Communities

GUIDELINES FOR ASSSESSING FISH COMMUNITIES

GENERAL SAMPLING PROCEDURES

Mean stream width (MSW) is an important characteristic of each fish community and stream habitat assessment station. The MSW is used to define the length of the station, and the spacing of habitat measurements i.e., distances between transects. The MSW is based on the mean of 10 preliminary measurements of stream width from throughout the station (within approximate station boundaries), including all types of macro-habitats such as runs, riffles, and pools (see: Guidelines for Evaluating Habitat of Wadable Streams in Wisconsin). If the water level appears to be substantially **(greater than 0.15 m**) above normal, sampling should not occur (see: **Station Summary**, in Guidelines for Evaluating Habitat of Wadable Streams in Wisconsin, for determination of water levels). Once the MSW for a station has been determined, this value is used for **all** future sampling, including future years when riparian land use or instream habitat improvements may have changed the actual stream width.

Stations for assessing fish communities in wadable streams are each approximately **35** times the MSW in length. This length is based on the distance necessary to sample more than 3 pool-riffle sequences thereby capturing most fish species present in a wadable stream. If a stream has well-developed pool-riffle structure, then each station should start and end at the base of a riffle to help facilitate fish capture, even if this requires that the distance between the second last and last transect is somewhat more or slightly less than 3 times the MSW in length. Ideally stations should not contain permanent tributaries or hydraulic controls (e.g., dams, old bridge abutments), and should be a sufficient distance away from bridges and other man-made structures that alter the natural stream morphology which may influence the fish and macroinvertebrate community found there. The fish community assessment is done in the exact same stream reach in which stream habitat is evaluated.

Fish community composition and species relative abundance are estimated over the entire length of each station using catch per effort (CPE) sampling procedures. A single electrofishing run is made from the downstream to upstream end of the station. No blocking nets are used. This constitutes the one and only sampling pass. All fish greater than 26 mm in total length are collected. At the end of the pass, all trout, esocids, smallmouth bass, largemouth bass, sauger, and walleye total lengths are measured. The weighing of individual or aggregate gamefish is optional (Table 1). The measuring or weighing of carp, creek chubs, catostomids, bullheads and catfishes, centrarchids, and selected percids (non-darters) is also optional (Table 1). If more than 200 fish of any given gamefish species are captured, a random subsample of 100 are measured. For trout, esocids, smallmouth bass, largemouth bass, sauger, and walleye, if recorded, individual weights may be taken from five fish for each 10 mm length interval.

Fish should be handled carefully to minimize mortality. After processing, the fish are returned to the stream. If fish are being processed before the end of the assessment reach (e.g. fish holding tub is full) fish should be released immediately downstream of a riffle to reduce the chance that fish will be recaptured during the same sampling pass. Small numbers of each species may be preserved as voucher specimens or to check identifications.

DATA COLLECTION

Fish Community Evaluation Four different data sheets are used in the Fish Community Evaluation: **Station Summary, Catch Summary, Individual Fish Data Sheet**, and **Individual Game Fish Data Sheet**. All four data sheets apply to the whole station. There is one **Station Summary** sheet per station and one or more of each of the other data sheets, depending on the number and diversity of fish captured. Guidelines for filling out each data sheet and examples of blank and completed sheets are given on the following pages.

STATION SUMMARY DATA SHEET

This sheet summarizes location, sampling characteristics, and gear used for the entire station. Some of the data on this form are derived from maps or from other data sheets. The location information should be identical to that collected during the Stream Habitat Evaluation for Wadable Streams. The parameters on this sheet are as follows:

Location **----------**

Stream Name The name of the stream as shown on the most recent USGS 7.5' topographic map. The name used here should be identical to that used on the other data sheets, and to that used for all other stations on the same stream. Make sure the spelling of the name is accurate and include all parts of the stream name (e.g., "West Branch", "Middle Fork", "River", "Creek", "Brook", "Run", etc.) to avoid confusion. Other commonly used names for the stream can be written here in parentheses.

Waterbody ID Code A unique seven-digit number that identifies each stream; all streams, rivers, and lakes in Wisconsin should have an assigned number. These numbers are available on the DNR intranet, under the listing for "DNR Tabular Database Service" for the WDNR Register of Waterbodies (ROW). As with Stream Name, waterbody ID code should be the same for all stations on a stream.

Site Mile The distance along the stream channel from the mouth of the stream to the downstream end of the station. This distance is a useful shorthand for indicating and identifying the location of the station. Site mile should be measured on the most recent USGS 7.5' topographic map to the nearest 0.1 mile using a map wheel.

Station Number If a stream has two or more stations, the downstream station is number 1, the next upstream is number 2, and so on. If there is only one station, the number is 1.

Date Fill in the date when the fish community data were collected for the station, use the YYYYMMDD format (e.g., 19900607 equals June 7, 1990).

Starting Location A precise narrative description of the point on the stream where the fish sampling began (i.e., the downstream end of the station). The description should include the exact distance and direction from the start to a "permanent" landmark such as a bridge, building, road marker, rock formation, etc. **Avoid using landmarks that might be lost during future years** (e.g., don't use tree or fence lines). Make the description as specific and precise as possible so that someone visiting the station for the first time can easily find the starting point.

Township, Range, Section, 1/4 - 1/4 Section, 1/4 Section Legal description for the Starting Location of the station within the Public Lands System. These can be determined from recent USGS 7.5' topographic maps or a detailed county map. On a topographic map, a "land locator" template is useful for determining the 1/4 - 1/4 and 1/4 Sections, which are indicated by a compass direction (NW, NE, SW, or SE). Note that in Wisconsin, all Townships are "N" (north), but Range can be either "E" or "W" (east or west). Make sure the appropriate letter is included for both Township and Range.

Latitude and Longitude It is important that geographic coordinates of the **start** of the station are recorded, along with the Method Used to determine latitude and longitude (e.g. USGS map, mapping software, global positioning system (GPS) units). Also the geodetic Datum Used upon which the coordinates of the map, mapping software or GPS coordinates are based (e.g. North American Datum 1983 (NAD 83), should also be recorded.

7.5' Quad Map Name The name of the most recent USGS 7.5' topographic map on which the station is found.

Basin Name The name of the Basin in which the station is found, following WDNR designations (e.g., Lower Grant, Middle Trempealeau, Upper Wisconsin, etc.).

Watershed Name The name of the watershed in which the stream is located.

County The name of the county in which the station is located.

Sampling Description ----------

Sampling Type The type of fish sampling done at the station. Check the appropriate category. Generally, during Baseline Monitoring Assessments, single pass catch per effort (CPE) sampling is done. In special cases, other types of sampling, such as "Depletion" or "Mark-Recapture" may also be done at a station.

Station Length The length of the stream assessment station. Measure with a tape measure to the nearest 1 m following the middle of the stream channel.

Number of Passes The total number of times a shocker is passed through the station during fish sampling. Normally, for "CPE" sampling there will be only one upstream pass, and for "Depletion" sampling there will be four or more passes. During depletion sampling, an upstream and then downstream run is usually regarded as "1" pass, but the fish captured are processed separately (see Type of Pass below).

Time The time range during which the electrofishing was conducted. "Start" refers to the time when the first electrofishing pass was started, and "Finish" refers to the time when the last pass was completed. Use the "military" format (i.e., 9:30 AM is 0930 hrs. and 9:30 PM is 2130 hrs.), to the nearest 10 minutes.

Type of Pass A description of the sampling direction through the station during a pass. "Upstream Only" refers to a pass that begins at the downstream end of the station, proceeds upstream, and then ends at the upstream end of the station. This is the type of pass used for "CPE" sampling. "Upstream, then Downstream" refers to a pass that begins at the downstream end of the station, proceeds upstream to the upstream end of the station, and then proceeds back downstream to the downstream end of the station. This is the type of pass used in "Depletion" sampling, and constitutes "1" pass; a minimum of 4 passes are completed for depletion sampling.

Gear Description ----------

Gear A description of the number and type of electrofishing units used in sampling. Specify the number of each type of gear that applies. Sampling will normally involve one or two backpack shockers or one stream shocker.

Number of Anodes per Unit The number of anodes per shocker. Normally there is one for backpacks and two or three (three preferred) for stream shockers.

Blocknets Record the number and mesh size of any blocknets used. For "CPE" sampling, no blocknets are used. For "Depletion" sampling, use a single downstream and a single upstream blocknet.

For Backpacks and Stream Shockers:

Anode Size The length of the long axis of the anode (the diamond-shaped or circular stainless steel tip on the hand-held probe), measured with a tape measure to the nearest 1.0 mm. If multiple anodes are used on a shocker, they must all have the same Anode Size, Shape, and Material Thickness. Anode Size can be changed (by replacing the tip with a larger tip, or covering part of the tip with electrical tape) if necessary to maintain a relatively constant voltage and amperage.

Anode Material Thickness The thickness (diameter) of the metal used to form the tip of the anode. Measure with calipers or a ruler to the nearest 0.1 mm.

Anode Shape The general shape of the metal tip of the anode.

For Mini-Boom Shockers:

Anode Length The length of the exposed metal portion of the cylindrical dropper(s) that come off the boom and dangles into the water. Measure with a tape measure to the nearest 0.01 m.

Anode Diameter The outside diameter of the exposed metal portion of the droppers on the front boom. Measure with calipers or a ruler to the nearest 1.0 mm.

Number of Front Droppers The number of individual droppers on the front boom.

Meter Readings ----------

Type of Electroshocker Current The type of electrical current (AC, DC, or pulsed DC) that the shocker puts into the water (this will often be different from that put out by the generator in the shocker). Check the appropriate category.

Electroshocker Control Box Meter Readings The typical output readings (i.e., not the extreme high or low readings) observed during sampling. Note the units of amperage and voltage for the meters when recording the meter values. Efforts should be made to keep readings fairly constant during shocking within a station, between stations within a stream; and if possible, among samples of the same station over time. Preliminary sampling just downstream of the station may be necessary to determine the output readings associated with the most effective electrofishing. As a rule of thumb, try to keep voltage above 150 V and **average** amperage above 2 A. Voltage can be adjusted by changing the number and surface area of anodes (see above), and amperage can be adjusted by increasing generator output (adjusting generator throttle, using a boost switch if present, or using a generator with a different power rating). For AC or pulsed DC, some control box output ammeters read peak rather average amperage; if this is the case, this should be noted on the sheet. Peak amperage equals four times the average amperage. If output meters are not present or are broken, note this on the sheet. Always try to use shockers with functioning output meters.

If Pulsed DC This refers to two important parameters, "Pulse Rate" and "Duty Cycle", of pulsed DC current. Some shockers allow values for these parameters to be varied, whereas others have a single fixed value for each parameter. If values can be varied, they should be set to the appropriate level at the beginning of sampling and not changed during sampling. This may require preliminary sampling just outside the station to determine the values where shocking is most effective. The same values should be used for all sampling within a station, between stations within a stream, and among samples of the same station over time. Sampling for many species is most effective and least harmful at Pulse Rates of 40-80 per second and at Duty Cycles of 10-20%.

Person(s) Who Collected Fish Data The **full** names of the person(s) who actually identified and measured the fish collected during the fish community evaluation.

COMMENTS / NOTES Any and all information that seems to be relevant to the fish community survey but is not recorded anywhere else on the data sheets. This information should include weather, water, and habitat conditions (e.g., glare, wind, precipitation, water clarity, unusually deep or shallow areas) and gear performance (e.g., problems with generators or meters) that influenced sampling effectiveness. Any evidence of fish kills (i.e., dead fish in the water or on the bank) or angler use of the stream (e.g., hooks and lines caught in bushes; evidence of cleaned fish on the bank; footprints from waders) should also be noted. Don't hesitate to make comments; **if in doubt, write it down**.

CATCH SUMMARY DATA SHEET

This data sheet is for summarizing and recording the numbers and aggregate weights, by species, of fish captured during each sampling pass. There should be a separate Catch Summary data sheet for each upstream and each downstream run. In other words, if an upstream and then downstream run are completed, the runs should be kept separate; fill out a data sheet for the upstream run, and then fill out an additional data sheet for the downstream run. Thus, depending on the number passes and species captured; there may be more than one of these data sheets for each station. The parameters on this sheet are as follows:

Stream Same as for **Station Summary** data sheet.

Waterbody ID Code Same as for **Station Summary** data sheet.

Site Mile Same as for **Station Summary** data sheet.

Station No Same as for **Station Summary** data sheet.

Date Same as for **Station Summary** data sheet.

Pass Number The pass that this data sheet refers to. The first pass through a Station is "1", the second pass is "2", the third pass is "3", and so on. For Baseline CPE sampling, only one pass is used. For depletion sampling, 4 or more passes should be used.

Time The starting and ending time of actual electrofishing for the pass should be recorded here. If the shocking run is interrupted (e.g., to work up fish when the fish holding tub is full, or due to equipment failure, etc.) the time of the interruption should be noted as the End time; the time actual shocking was resumed and finally ended should be recorded in the parentheses. Elapsed shocking time (in minutes) should be recorded after Total.

Pass Direction Record the direction of the pass (either upstream or downstream) used to capture the fish recorded on this data sheet. There should be a separate **Catch Summary** data sheet for each upstream and downstream run. In other words, if an upstream and downstream run are completed, the runs should be kept separate; fill out a data sheet for the upstream run, and then fill out an additional data sheet for the downstream run.

Catch Summary **----------**

This section of the data sheet is used to summarize the identity, total number, total weight, number of fish with deformities, eroded fins, lesions, and tumors (DELT), number of handling mortalities, the number of voucher specimens retained, and the number of marked or recaptured fish for each species captured. For species that are individually measured (Table 1), transcribe these totals from the **Individual Fish** and **Individual Game Fish Data Sheets**.

Species The identity of each species captured during the pass. Only accepted American Fisheries Society common names should be used (see Robins et al. 1991. Common and scientific names of fishes from the United States and Canada. AFS Special Publication No. 20). If any abbreviations are used, they should be used consistently and explained somewhere on this data sheet. If an exact species identity is unknown, identify fish to lowest possible taxon (i.e., to genus or family), and preserve all the specimens of that species for later complete identification.

Number Caught The total number of a species captured during the pass.

Weight (Optional) The total wet weight (g) of all fish of a species captured during the pass. Weigh to the nearest 0.1 g or to the nearest 1 % of total weight, whichever is larger. For example, for a species with an aggregate weight of about 8 g, weigh to the nearest 0.1 g; for a species with an aggregate weight of about 60 g, weigh to the nearest 1 g; for a species with an aggregate weight of about 250 g, weigh to the nearest 3 g; for a species with an aggregate weight of about 1450 g, weigh to the nearest 15 g; and so on. Weigh groups of fish in a net or plastic bag using an appropriately sized Pesola® spring balance (Gross weight), **and don't forget to subtract the weight of the net or bag** (Tare weight) to get the actual weight of the fish (FINAL weight).

Number DELT The total number of fish of a species that have Deformities, Eroded fins or scales, Lesions, or Tumors ("DELT"). Only obvious deformities, eroded fins or scales, lesions, and tumors observed on live fish should be counted. Electrofishing sometimes causes wounds or burns; do not count these as DELT.

Handling Mortalities The total number of fish of a species killed as a result of the sampling. Every effort should be made to reduce the number of fish killed, but some mortality is inevitable. Only fish that are definitely dead should be counted.

Number of Vouchers The total number of fish of a species that were retained as vouchers or to check identification. All fish that cannot be identified to species with certainty should be preserved in 10% formalin. As many as 25 fish per species may be preserved one time at each station to serve as vouchers and checks on identifications.

Number Marked The number of fish of a species marked or tagged and released alive (for mark and recapture population estimates or for movement studies) during the pass.

Number Recaptured The number of fish of a species from the pass which possess a mark or tag from a previous sampling event.

Lab Check (Vouchers) When voucher specimens are preserved, verify the Number of Vouchers retained and record a check in the "Number" column. If the number preserved (after a lab count) does not match the Number Vouchers, then record the correct number under Number Vouchers. Verify the identification of vouchers and record a check in the "ID" column. If the field identification (under Species) was incorrect, based on a lab examination, change Species to the correct identification.

INDIVIDUAL GAME FISH AND INDIVIDUAL FISH DATA SHEETS

The **Individual Game Fish** and **Individual Fish** data sheets are used to record total lengths, weights, and other information for individual fish captured during a pass. Thus, depending on the number of fish captured and the number of passes made, there may be from one to more than four of these data sheets for each station. Only fish for which total lengths are to be measured need to be recorded on these sheets (Table 1). Juvenile and adult trout, esocids, smallmouth bass, largemouth bass, sauger, and walleye should be measured individually and the data recorded on the **Individual Game Fish** data sheet. The recording of the individual or aggregate weights of individual adult or young of the year (YOY) gamefish listed above is optional. The recording of the weights of carp, creek chubs, catostomids, bullheads and catfishes, rock bass, sunfish, crappies, and yellow perch is also optional and can be weighed in aggregate (Table 1). If more that 200 of any species listed above are captured, a random subsample of 100 fish can be measured. All other species not listed above should be counted. The total number, aggregate weight, and number DELT, number of handling mortalities, number of vouchers, number marked, and number recaptured of each species should be transcribed to the **Catch Summary** data sheet. The parameters on this sheet are as follows:

Stream Same as for **Station Summary** data sheet.

Waterbody ID Code Same as for **Station Summary** data sheet.

Site Mile Same as for **Station Summary** data sheet.

Station No Same as for **Station Summary** data sheet.

Date Same as for **Station Summary** data sheet.

Pass Number Same as for **Catch Summary** data sheet.

Species The identity of each species captured and measured during the pass. To make data summary and computer data entry easier, try to record only one species per **Individual Fish** data sheet. Follow guidelines from the **Catch Summary** data sheet for recording species identities.

Total Length The distance from the tip of the snout to the posterior tip of the longest caudal (tail) lobe of each individual fish. The caudal lobes should be pinched together slightly when measuring this distance. Measure, to the nearest 0.001 m, using a meter stick or measuring board.

Weight (Optional) The wet weight of each individual fish. Weigh to the nearest 0.1 g or 1 % of body weight, whichever is larger (see explanation under Aggregate Weight for the **Catch Summary** data sheet) with an appropriately-sized Pesola® spring balance. Weights should only be taken for up to five fish from each 10 mm total length interval, and then only for the following species: trout, esocids, smallmouth and largemouth bass, sauger, and walleye (Table 1) and recorded on the **Individual Game Fish** data sheet.

Scales (Optional) An indication (Yes or No) of whether a fish had scales removed and saved. Scales are removed to aid in aging fish. If age information is being collected, at least 6 scales should be removed from a spot on the right side of the fish, several scale rows above the lateral line at a position just posterior to the tip of the pectoral fin when that fin is laid flat along the body. For each species of fish, always take scales from approximately the same location. Scales should only be taken from up to 10 fish per 10 mm total length class, and then only for trout, esocids, smallmouth and largemouth bass, sauger and walleye (see "Weight" above). The tally sheet can be used to keep track of the number of fish for each size class that have had scales removed. Scales need not be taken from fish less than 80 mm total length. Scales should be saved in scale envelopes (available from most WDNR offices) that have accurate location, date, species identity, length, weight, and capture information recorded on them.

DELT A brief description of the location and types of Deformities, Eroded fins or scales, Lesions, or Tumors ("DELT") observed on a fish. Only obvious DELTs observed on live fish should be recorded. Electrofishing sometimes causes wounds or burns; do not count these as DELTs. If no DELTs were apparent, leave this space blank.

Table 1: Species or groups of species that should be measured individually in total length (mm). Individual weights are optional and should be recorded for five fish from each 10 mm total length interval. Collection of scales is optional and may be taken from five fish for each 10 mm total length interval. All other species not listed should be counted.

INDIVIDUAL SPECIES Total counts only	***INDIVIDUAL SPECIES LENGTHS***
Cyprinids Carp (Cyprinus carpio) Creek chub (Semotilus atromaculatus) **Catostomids** Carpsuckers (Carpiodes spp.) Longnose sucker (Catostomus catostomus) White sucker (Catostomus commersoni) Blue sucker (Cycleptus elongatus) Chubsukers (Erimyzon spp.) N. hog sucker (Hypentelium nigricans) Buffalos (Ictiobus spp.) Spotted sucker (Minytrema melanops) Redhorses (Moxostoma spp.) **Ictalurids** Bullheads (Ameiurus spp.) Channel catfish (Ictalurus punctatus) Flathead catfish (Pylodictis olivaris) **Centrarchids** Rock bass (Ambloplites rupesteris) Sunfish (Lepomis spp.) Crappies(Pomoxis spp.) **Percids** Yellow perch (Perca flavescens)	**Trout** Rainbow trout (Onchorhynchus mykiss) Brown trout (Salmo trutta) Brook trout (Salvelinus fontinalis) **Esocids** Grass pickeral (Esox americanus) Northern pike (Esox lucius) Muskellunge (Esox masquinongy) **Centrarchids** Smallmouth bass (Micropterus dolomieu) Largemouth bass (Micropterus salmoides) **Percids** Sauger (Stizostedion canadense) Walleye (Stizostedion vitreum)

Appendix A.4

Wadable Stream Fish Assessment Data Sheet

Water:_____ WBIC:_____ Date:_____ County:_____ Collectors:_____

Start Time:_____ End Time:_____ Processing Time:_____ Survey Type:_____ Type of Pass (Upstream/Downstream)

Gear Type:_____ Mean Stream Width:_____ Distance:_____ Target Fish:_____

Volts:_____ Amps:_____ Current Type (AC/DC/Pulsed DC) Pulse Rate:_____ Duty Cycle:_____ # Dippers:_____ # Probes ____

H_2O Temp:_____ H_2O Level (low/normal/high) H_2O Clarity (clear/turbid/very turbid) Weather Conditions:_____

Starting Location:_____ Ending Location:_____

Starting Lat:_____ Long:_____ Ending Lat:_____ Long:_____

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									
31									
32									
33									
34									
35									
36									
37									
38									
39									
40									
41									
42									
43									
44									
45									
46									
47									
48									
49									
50									

Appendix A.5

Wisconsin Department of Natural Resources

Baseline Monitoring – Non-Wadable Streams Protocols

Baseline Monitoring – Non-Wadable Streams Protocols

IBI procedures: Lyons et al. (2001) describe data collection methods for calculating the large river IBI, and the process is reiterated here. The protocol requires sampling main-channel-border habitats, which are relatively shallow shoreline areas along the river channel that carry the majority of the river flow. Depending upon the project goals, it may be informative to sample the borders of major side channels if the channel carries a substantial amount (> 15%) of river flow. Standard shocking occurs in daylight and in a downstream direction as close to the shoreline as possible. Fish collections are made between 15 June and 30 September. Sampling should be avoided if the river stage is > 1m above normal, but it can occur during below-normal flows.

Standard equipment is a boat-mounted, pulsed-DC electrofishing unit. Typically a 5m long aluminum boat powered by a 15-25hp outboard motor, with the boat hull serving as the cathode, works well. The anode is a single 4m boom with a "Wisconsin ring" from which 16 cylindrical, 17mm-diameter stainless steel droppers are suspended. In normal operation, about 125mm of each dropper is in contact with the water. A gas-powered generator rated at ~3500 W provides adequate electricity. The control box converts AC to DC and allows standardization of the pulse rate at 60 Hz and a 25% duty cycle. Depending upon water chemistry, sampling can typically be done at ~3000 W output from the control box.

While sampling, a single person uses a 17mm-mesh (stretch) dip net and attempts to capture all of the fish seen. This mesh size consistently retains fusiform species such as cyprinids >75mm total length and longitudinally compressed species like centrarchids >50mm, but smaller individuals are often collected. Sampling techniques are biased are against small (e.g., cyprinids) and nocturnal species (e.g., catfish, walleye), but collect large numbers of suckers and centrarchids, including smallmouth bass. Captured fish are identified to species, counted, and weighed. Game fish should have individual lengths and weights measured, but other species do not need length information and can be weighed in aggregate. Specimens should be released after processing unless a sample is needed to confirm species identifications. The data sheet in Appendix 1 should be used to document fish information.

Each sampling site is to be sampled for 1 mile of contiguous shoreline, a distance at which estimates of species richness were asymptotic and insensitive to variation in sampling effort. For 187 large river sites sampled statewide, the average shocking "on time" was 40 minutes. Most sites had 20 – 30 individuals representing an all-inclusive list of game species. One Fish Technician or Biologist is expected to be sampling at all times with 1 – 2 LTEs.

Game fish procedures: To reiterate, IBI sampling procedures should be followed if the methods for sampling game fish entail daytime electrofishing. Game fish assessments typically target one species and are tailored to meet the management goals for individual rivers. The most anticipated species of management concern include smallmouth bass, walleye, sauger, and catfish. If the primary game species of management concern is the smallmouth bass, then IBI electrofishing runs may yield data efficiently. Species-specific sampling protocols include extended daytime electrofishing, nighttime electrofishing, tailwater electrofishing in the fall, or hoop netting.

Extended daytime electrofishing.- If enough game fish individuals are not caught after the 1-mile IBI run, then it may be useful to extend the shocking run to sample an additional 1 – 4 miles. Collect and process all game, threatened, and endangered species.

Nighttime electrofishing.- Nighttime shocking poses many logistical and safety concerns, particularly in reaches with poor access, numerous obstructions, or fast, turbulent water. However, if nighttime shocking is opted for then site reconnaissance during the day is encouraged strongly. Studies indicate that night shocking yields more total fish species and biomass than day shocking (for references see Lyons et al. 2001). Most game species are found in greater number, and larger individuals are caught during nighttime sampling compared to daytime (Lyons pers. comm.). The catch differences are pronounced for walleye, sauger, catfish, and esocids, but somewhat less notable for centrarchids.

Fall tailwater electrofishing.- Fish migrations to tailwater areas for intense fall feeding or overwintering pose an opportunity to collect walleye, sauger and esocid data efficiently in some river systems. Consider electrofishing during the daytime if the site poses serious logistical and safety problems, but nighttime shocking may provide higher catch rates. The optimal time of year to sample may vary by river and weather conditions but mid- to late-October is probably appropriate. On the Lower Wisconsin River for example, fall tailwater electrofishing at night is much more efficient than summer IBI runs for collecting walleye, sauger, and esocid data. The walleye catch rate jumps from ~15 fish/hour during summer runs to ~300 fish/hour during fall tailwater sampling.

Hoop netting.- Catfish can be targeted by sampling with hoop nets. Sampling during spring migration typically maximizes catfish catch rates. The optimal time for spring sampling varies by river and weather conditions but it generally ranges from mid-March to mid-May. Depending on management interests, a summer sampling option may be preferred to focus on resident fish. Vokoun and Rabeni (2001) provide a standardized hoop net sampling protocol for sampling channel catfish in prairie streams. Pellett et al. (1998) discuss channel catfish movements and sampling procedures they found useful on the Lower Wisconsin River. Protocols for catfish sampling can be explored by Regional staff and the Nonwadeable Stream Subteam on a river-specific basis.

Water chemistry and habitat procedures: Information on water chemistry, instream habitat, and riparian conditions should be collected to supplement fisheries data. Station location, water chemistry, and habitat variables should be recorded on the Nonwadeable data sheet found in Appendix 2. Water chemistry data should be collected prior to sampling, but habitat information can be recorded during the shocking run or while motoring to or from the boat launch.

Site Selection

Some river reaches may have special local importance to the public and Fish Biologists, whereas other reaches may be primarily important in the statewide scheme. However, the river

reaches that meet both the local concerns and statewide interests will have the highest priority for the establishment of a sampling site.

A river reach may be of particular local concern because, for example, it is a popular fishery, experiencing development pressures, or under consideration for management changes in fishing regulations, dam operations, or habitat structure. Describing the effects of changes on river integrity or game fish is valuable at a statewide level as well. A river reach also becomes of particular interest at the broad scale if it represents a type of river or disturbance that is not being assessed elsewhere. Having many sites on one river becomes an over-sampling issue if there are few current or anticipated differences in habitat or disturbances among sites. We consider 64 sites on 22 Wisconsin rivers practical for sampling annually as part of the Baseline effort (Table 1). The Subteam will entertain suggested river additions to the list and river mile adjustments.

Prioritizing exactly which sites to sample requires discussions among the Subteam and Regional staff. Little flexibility exists for establishing sites on rivers that are only marginally large enough for miniboom shocking, or because of difficult access. However, multiple sites on larger river systems need to be prioritized according to river type, public interest, the kind of human disturbances present, the intensity of human disturbances, river ecosystem integrity, management concerns, and likelihood of change in management or disturbance regime. Sites from Lyons et al. (2001) IBI work depict river ecosystem integrity throughout Wisconsin (Fig. 1). More sites cover the Chippewa River system than are currently depicted (Benike, unpublished data). Sites with low IBI scores should be selected for monitoring to determine if changes in management can improve the conditions. Reaches actively or predicted to undergo management changes should be selected to quantify the effects of the alterations. Some least-impacted sites should also be monitored to identify natural year to year variation due to weather conditions, for example.

Table 1. Working list of Wisconsin's nonwadeable rivers with estimated nonwadeable riverine miles. Numbers of sites to be sampled per river annually are indicated under Reaches. Rivers in *Italic* font have very difficult access or are otherwise considered a low priority.

River	Upstream boundary of nonwadeable reach	Miles	Reaches
Mississippi River Basin			
Mississippi	IL border to confluence with St. Croix R. (MN border)	115	11
Fox (Illinois River trib)	IL border to town of Waterford (Racine Co.)	20	1
Rock	IL border to town of Horicon (Dodge Co.)	65	2
Bark	Mouth of Scuppernong Cr. (Jefferson Co.)	10	
Crawfish	Mouth of Beaverdam R. (Dodge Co.)	37	
Pecatonica	IL border to town of Calamine (Lafayette Co.)	15	1
Sugar	IL border to town of Brodhead (Green Co.)	15	1
Grant	Mouth of Rattlesnake Cr. (Grant Co.)	19	
Wisconsin	Otter Falls Dam (near town of Eagle River, Vilas Co.)	190	9
Kickapoo	Town of LaFarge (Vernon Co.)	30	1
Baraboo	Town of La Valle (Sauk Co.)	74	2
Lemonweir	CTH "H" bridge (near Camp Douglas, Juneau Co.)	30	
Yellow	Dexterville Dam (Wood Co.)	30	1
Tomahawk	Willow Flowage (Oneida Co.)	15	
Black	Lake Arbutus (Jackson Co.)	76	3
LaCrosse	Neshonoc Lake (LaCrosse Co.)	14	1
Trempealeau	Town of Arcadia (Trempealeau Co.)	19	1
Buffalo	CTH "B" bridge (near town of Gilmanton, Buffalo Co.)	17	
Chippewa	Chippewa Flowage (Sawyer Co.)	88	6

Red Cedar	Town of Rice Lake (Barron Co.)	45	2
Eau Claire	Lake Eau Claire (Eau Clarie Co.)	20	
Flambeau (inc N. Fk)	Flambeau Flowage (Iron Co.)	60	2
S. Fk Flambeau	Blockhouse Lake outlet (near State Hwy 182, Price Co.)	23	
St. Croix	Burnett - Douglas Co. border	144	5
Namekagon	CTH "K" (just below Trego Lake, Washburn Co.)	30	
Lake Superior Basin			
St. Louis	MN border, just below Fond du Lac dam (Douglas Co.)	6	1
Bad	Mouth Marengo R. (Bad River Indian Res., Ashland Co.)	34	
White	White River Flowage (Ashland Co.)	11	
Lake Michigan Basin			
Menominee	Confluence of Michigamme and Brule R. (Florence Co.)	65	5
Peshtigo	Sandstone Flowage (near town of Crivitz, Marinette Co.)	32	2
Oconto	Town of Oconto Falls (Oconto Co.)	10	1
Fox	State Hwy 33 bridge (in town of Portage, Columbia Co.)	95	3
Wolf	Town of Keshena Falls (Menominee Co.)	80	3
Little Wolf	State Hwy 22/110 bridge (Waupaca Co.)	11	
Embarrass	Mouth of Pigeon R. (Waupaca Co.)	15	
Milwaukee	Near Lincoln Park (Milwaukee Co.)	6	
Menomonee	Near I-94 (Milwaukee Co.)	1	

Cost estimate

A typical IBI run takes about 3 hours considering it usually takes 40 minutes for shocking, <1 hour for fish processing, and <1 hour for miscellaneous work at the boat launch and boating to and from the sampling station. Data entry may take 1 – 2 hours largely depending upon the number of individual game fish weights and lengths that need entering. Travel distances from the Regional offices to the rivers are usually within 60 miles one-way. Considering these time estimates, travel expenses, and general maintenance, the per site cost to collect an IBI sample is less than $200 (Table 2). A second LTE is useful for sampling rivers with high numbers of suckers present so one person can process fish while shocking. The second LTE may require about 5 hours per site, but the site cost will likely remain under $250. Game fish sampling runs will need to be estimated according to the specific sampling scheme proposed, but they too are likely to be less than $200 per event.

Table 2. Estimated cost per index of biotic integrity (IBI) run on nonwadeable streams.

LTE expenses at typical $10/hour wage		
Travel time to and from the boat launch	2 hours	$20
IBI sampling	3 hours	$30
Data entry	2 hours	$20
General maintenance	1 hour	$10
	LTE subtotal	$80
Travel		
Mileage at ~$0.32/mile	120 miles round trip	$40
Meals for 2 person crew	$9 lunch	$20
	Travel subtotal	$60
Supplies and minor maintenance		
Boat and generator gas		$ 2
Weighing nets, waders, scales		< $40
	S&M subtotal	$40
	Grand total per site	**< $180**

Field methods orientation

Data consistency can be problematic considering personnel turnover and annual LTE hires. Large river sampling techniques take practice and efficiency can vary depending upon experience. Furthermore, some fish species are difficult to identify even for experienced personnel. As such, field staff should plan on devoting at least one day for refreshing their miniboom shocking and fish identification techniques on nonwadeable rivers. An experienced person from the Subteam will have a training day with the Regional staff on one of their area streams (e.g,. Brian Weigel, Heath Benike, or John Lyons).

References

Lyons, J., R.R. Piette, and K.W. Niermeyer. 2001. Development, validation, and application of a fish-based index of biotic integrity for Wisconsin's large warmwater rivers. Transactions of the American Fisheries Society 130:1077-1094.

Pellett, T.D., G.J. VanDyck, and J.V. Adams. 1998. Seasonal migration and homing of channel catfish in the Lower Wisconsin River, Wisconsin. North American Journal of Fisheries Management 18:85-95.

Vokoun, J.C., and C.F. Rabeni. 2001. A standardized sampling protocol for channel catfish in prairie streams. North American Journal of Fisheries Management 21:188-197.

Legend for Figure 1 (attached in IBIrating.jpg file). Nonwadeable streams shown within WDNR Basin boundaries with the index of biotic integrity (IBI) rating depicted. Site points are adapted from Lyons et al. (2001), J. Lyons (unpublished data), and R. Piette (unpublished data).

Appendix A.6

Wisconsin Department of Natural Resources

Nonwadeable Baseline Monitoring Electrofishing Data Sheets

Appendix 1. Site location, water chemistry, and habitat data sheet.

WDNR-Nonwadeable Baseline Monitoring – Electrofishing Data Sheet – NW2

River: _______________ Station: __________ County: _______________ Page ___ of ___

Date: ________________ Runtype: IBI GET Gear: mini big Bank: R L

Shocking Start Time: __________ Shocking End Time: _______ Jars Prsvd: __

	No.		Gross	Tare	Net		
Species	Caught	Length	Wt	Wt	Wt	Delt	Presvd.

Appendix 2. Site location, water chemistry, and habitat data sheet.

WDNR-Nonwadeable Baseline Monitoring – Electrofishing/Habitat Data Sheet –NW1

River: _____ WBIC: _____ Station: _____ County: _____

Date: _____ Runtype: __IBI __GET Bank sampled (as looking upstream): R L

Collector(s): _____

River Mile (0.1): Start _____ End _____

GPS Location:
Start _____° _____._____ End _____° _____._____ Shocking
_____° _____._____ _____° _____._____ Distance (0.01 mi): _____

Time (24hr): Start _____ End _____ Shocking Start Time: _____ Shocking End Time: _____

Water Level: __ Normal __ Above ____ (0.1m) __ Below ____ (0.1m)

Air temp (C): ____ Water temp (C): _____ Generator Output @ 60 pulse 25 Duty cycle

Conductivity (umhos): _____ Turbidity (NTU): _____ Amps: _____ Volts: _____

Habitat Description **(Qualitative categories: N = none, R = rare, O = occasional, C = common)**

Mean width estimate (m): _____
Max Depth estimate (0.1m): _____
Mean Depth estimate (0.1m): _____

Pools:	N	R	O	C
Runs:	N	R	O	C
Riffles:	N	R	O	C
Sand bars:	N	R	O	C
Gravel shoals:	N	R	O	C
Sand flats:	N	R	O	C

Other: _____

Substrate:

Clay:	N	R	O	C
Detritus:	N	R	O	C
Silt:	N	R	O	C
Sand:	N	R	O	C
Gravel:	N	R	O	C
Cobble:	N	R	O	C
Boulder:	N	R	O	C
Other:	N	R	O	C

_____

Gradient: Low Moderate

Channel Features (m)
Natural: _____
Channelized: _____

Artificial Bank Features

Rip Rap:	N	R	O	C	_____ (m)
Seawall:	N	R	O	C	_____ (m)
Piers/docks:	N	R	O	C	_____ (# counted)

Overall Cover: N R O C

Boulders:	N	R	O	C	
LWD:	N	R	O	C	_____ (# counted)
OH Veg.:	N	R	O	C	
Undercut:	N	R	O	C	
Aq. Plants:	N	R	O	C	
Em. Plants:	N	R	O	C	

Riparian Land Use / Vegetation:

Wetland:	N	R	O	C
Grass:	N	R	O	C
Shrubs:	N	R	O	C
Forest:	N	R	O	C
Pasture:	N	R	O	C
Row Crop:	N	R	O	C
Resident:	N	R	O	C
Urban:	N	R	O	C

other: _____

Bank Erosion: N R O C

Natural Cutbank: _____ (m)

Disturbed Cutbank: _____ (m)

Appendix A.7

Wisconsin Department of Natural Resources

Fisheries Assessments – Lakes 2007-09 Sampling Procedures

Fisheries Assessments - Lakes
2007-09 Sampling Procedures

Lakes Assessment Team
Tim Simonson, FH/4
Andy Fayram, FH/4
Joe Hennessy, FH/4
Ted Treska, FH/4

REVISED 3/3/2008

All lake assessments conducted by Fish Teams will follow these guidelines. Some surveys will be more comprehensive than others, depending on objectives for a particular lake.

All lakes should be designated as one of the following:

1) "**High Profile**" lakes (lakes sampled most intensively on a relatively short rotation of about 4 years; generally lakes > 1,000 acres). "Treaty" sampling plans are included in the "High Profile" rotation. If a lake is sampled in your area the fish team is required to provide assistance. Creel Surveys will be conducted on up to 5 "High Profile" lakes outside the ceded territory (according to the standard protocols followed in the ceded territory) each year. Aging should be completed for species of importance.

2) "**Public Access**" lakes (lakes sampled less intensively on a somewhat longer rotation of about 8 years – generally lakes between 100 and 1,000 acres); aging should be completed on about 25% of these lakes;

3) "**Minor Fishery**" lakes (lakes of relatively low importance that are sampled on a much longer rotation of about 10 years). Aging is not needed on these lakes.

Lake assessments are designed to meet the following objectives for each lake sampled:

- Estimate the abundance, size- and age-structure of adult walleyes on "high profile" and treaty lakes;
- Estimate the relative abundance and size structure of other selected gamefish, panfish and non-game fishes; and
- Estimate the relative abundance of YOY and yearling walleye and muskellunge on lakes with natural reproduction or potential for natural reproduction.
- Visually screen for the occurrence of **VHS** in wild fish populations.

The first objective is met by conducting mark/recapture surveys during, and shortly after, peak walleye spawning activity. Gamefish are captured predominately with fyke nets during the marking phase. Recapture sampling is done via electrofishing with a boom shocker shortly after the marking period. The second objective is met by estimating CPE of selected gamefish and panfish during late spring, primarily by electrofishing with a boom shocker. The third objective is determined from CPE estimates conducted via fall electrofishing.

At this time, no emphasis is being placed on sampling non-game fish species except common carp.

VHS – Be familiar with the external signs of VHS (http://dnr.wi.gov/fish/pages/vhs.html). Examine sampled fish for external symptoms of VHS during all fisheries assessments. Any fish exhibiting symptoms should be put on ice and delivered to the Fish Health Lab in Madison within 24 hours, or frozen. Immediately contact the Fish Health Specialist.

Boat, Equipment and Gear Disinfection Protocol

Be sure to follow disinfection protocol (see Manual Code).

Sampling Procedures

The sampling is divided into six distinct periods. Most of these sampling periods are optional and are generally conducted on high profile lakes. Most lakes (typically bass-panfish lakes) will be sampled once in the spring by electrofishing (Spring Electrofishing II; see below). Many lakes with walleye will be sampled twice in spring by electrofishing (spring electrofishing I and spring electrofishing II; see below). These procedures were adapted from the Treaty Fisheries Assessment Team's (TFAT) protocols and closely follow them whenever possible. If you are conducting sampling for the TFAT, follow their specific protocol.

1. Spring Netting I (Walleye Netting; optional): The primary objective for this sampling period is to capture, measure, and mark adult walleye for use in estimating their abundance. The secondary objective is to measure and, if needed, mark other gamefish (northern pike and muskellunge).

- Gear - Set 4' x 6', ¾" bar mesh fyke nets at ice-out and run them daily through the peak of walleye spawning. Water temperature will be approximately 45^{o} F. Set enough nets to sample most of the available walleye spawning habitat. Remove all nets from the lake prior to the conducting other sampling. Record the data from each set separately.

- Marking - The objective is to mark approximately 10% of the estimated population. When no preliminary estimate is available, one walleye per

acre should be marked. Netting should continue until that objective is met or exceeded. Marking more may improve the accuracy and precision of the resulting population estimate, but it is inefficient to spend more than one week netting if the R/C ratio of your catches is 10% or greater. When marking gamefish with a fin clip, make sure it will be recognizable for the appropriate time interval (i.e., 10 months on lakes where creel surveys are planned). Removing about ½ of a fin will leave a mark that lasts for more than one year. Mark adult gamefish with a primary clip. Mark juvenile gamefish with a secondary fin clip. The secondary mark will be a top caudal (TC) fin clip unless otherwise specified. Release marked gamefish away from nets and inlets or outlets, preferably at a mid-lake location.

- For each net on each day, all walleye, northern pike, and muskellunge will be sexed, measured (total length – nearest 0.1 inch), and, if needed, marked (for population estimates or creel survey). Record data from each net separately.

  Mark **all** sexable gamefish and unknown-sex gamefish ≥ the applicable size cutoff. Mark unknown-sex gamefish < the applicable size cutoff with a secondary fin clip (TC unless other specified).

Size cutoffs for primary versus secondary clips on unknown-sex gamefish.

	Primary	Secondary
Walleye	≥ 15"	7" - 15"
Northern Pike	≥ 12"	< 12"
Muskellunge	≥ 30"	< 30”

- Aging is required for high profile lakes. See Appendix B for details on aging.

2. Spring Electrofishing I (Walleye CPE; required on walleye waters):
The primary objective for this sampling period is to count and measure adult walleye (and, when conducting a population estimate, record marks for adult walleye for use in estimating their abundance).

- Boom shocker electrofishing is conducted at night. Two experienced people should dip fish. Dip nets should have 3/8” mesh bags. Shocking should be conducted at or near the peak of walleye spawning; water temperatures will be 45 to 50 F.

- When conducting a recapture for a walleye population estimate, attempt to shock the entire shoreline, including islands.

- When conducting a walleye CPE, if possible, shock the entire shoreline. If difficult to accomplish, shock at least **50%** of the shoreline. This is done by dividing the shoreline into 2-mile segments and shocking at least 50% of them. The minimum coverage needed is as follows:

Total Lake Shoreline (miles)	Minimum Sampling Required (2-mile segments)
8 miles or less	Entire shoreline
8 to 16 miles	4 segments
> 16 miles	5 segments

- The first 2-mile segment should be selected at random and the remaining 2-mile segments should then be chosen at equally spaced intervals around the lake to achieve uniform coverage.

- Actual distance sampled will be recorded and may be determined by GPS or by shoreline landmarks; mark the sampled segments on a lake map. Also, be sure to record time sampled.

- Within each 2-mile segment, all walleye will be collected, sexed, and measured. If conducting a population estimate, examine for and record marks. Record the data from each 2-mile segment separately.

- If aging walleye, collect aging structures only from gamefish in size ranges needed to complete the collection of 5 samples per ½-inch group for the entire sampling season. Review the tally of previously sampled fish. See Appendix B for details on aging.

3. Spring Netting II (Muskellunge Netting; optional): The primary objective for this sampling period is to capture and measure adult muskellunge, and, if a population estimate is needed, mark adult muskellunge. Muskellunge population estimates should be conducted using fyke nets during the spawning period in two consecutive years, with the first year for marking and the second year for recapture. The secondary objective is to count and measure northern pike captured incidental to muskellunge netting.

- Gear - Set 4’ x 6’, ¾” bar mesh fyke nets prior to and during the peak of muskellunge spawning. Water temperatures will be approximately 50^{o} to 55^{0} F. Set enough nets to sample most of the available muskellunge spawning habitat. Remove all nets from the lake prior to the conducting other sampling. Record the data from each set separately.

- If the objective is to conduct a population estimate, attempt to mark approximately 10% of the estimated population. If no preliminary estimate is available, mark one muskellunge per 10 acres. Netting should continue until that objective is met or exceeded. When using a fin clip, make sure enough of the fin is removed to leave a mark that will be recognizable for at least one year (usually, removing about ½ of a fin).

- Mark adults (≥ 30") with a primary clip. Mark juveniles (< 30") with a secondary fin clip to differentiate fish during the recapture period that recruit (become > 30 in). Release marked fish away from nets and inlets or outlets, preferably at a mid-lake location.

- Measuring and Marking Gamefish - Sex, measure for total length (to the nearest 0.1 inch), weigh, and mark with a primary or secondary fin clip, all fish captured during fyke netting. Record length, sex and fin clip data, including recaptured gamefish, on an appropriate data sheet. Record the data from each net separately.

    - Determine sex by presence of milt or eggs, or by visual inspection of the urogenital pore. Fish of unknown sex usually constitute a small portion of the total muskellunge handled.

    - Weights should be taken on as many muskellunge as possible, and as time permits. Accurate weight determination of adult muskellunge is not easy, and is relatively time consuming per fish handled. If muskellunge are abundant, sampling can be stratified within length groups. Because sampling occurs over a two-year period, some flexibility exists for collecting as many weights as possible. If fish are weighed during both periods, do not re-weigh any fish during the recap period that are marked.

4. Spring Electrofishing II (Centrarchid CPE): The primary objective for this sampling period is to count and measure adult bass and panfish. The secondary objective is to count common carp. The minimum requirement is to count "catchable" adult carp (individuals that **could have been** dip-netted) - you don't have to actually bring them into the boat. A tally-counter mounted on the front rail of the shocker boat would be one way to easily keep track of "catchable" carp.

- Boom shocker electrofishing will be conducted at night. Two experienced people should dip fish. Dip nets should have 3/8" mesh bags. Shocking should be conducted prior to peak bass spawning at water temperatures of 55 to 70 F.

- The entire shoreline should be divided into 2-mile segments. Within each 2-mile segment, all bass will be collected in a 1-½-mile **Gamefish station** and

all bass, panfish and “catchable” carp will be collected in a ½-mile **Panfish station**. The minimum coverage needed is as follows:

Total Lake Shoreline (miles)	Minimum Sampling Required (2-mile segments)
< 6 miles	Entire shoreline/2 panfish stations
6 to 12 miles	3 gamefish stations/3 panfish stations
12.1 to 24 miles	4 gamefish stations/4 panfish stations
> 24 miles	5 gamefish stations/5 panfish stations

- The first 2-mile segment should be chosen randomly and the other 2-mile segments should be equally spaced around the lake to achieve uniform coverage.

- Actual distance sampled (miles) will be recorded and may be determined by GPS or by shoreline landmarks; mark the sampled segments on a lake map. Also, be sure to record time sampled.

- Within each 1-½ mile Gamefish station, all bass will be collected, counted and measured to the nearest 0.1 of an inch or centimeter. The investigator should record the data from each 1-½ mile gamefish station and each ½-mile panfish stations separately.

- Within each ½-mile Panfish station all bass and panfish, are collected. A minimum of 100 individuals of each species will be randomly selected (dipped from tub) and measured. Also record the number of “catchable” carp. Data from each ½ - mile Panfish station (including any gamefish species collected) should be recorded separately and should not be combined with data from the larger 1-1/2 mile Gamefish station. If you choose to pick up and count all species, please indicate “all species” as the target in the database.

5. Summer Netting (Panfish; optional): The primary objective for this sampling period is to capture and measure panfish.

- Set 4’ x 6’, ¾” bar mesh fyke nets at water temperatures between 65 and 80 F. Set enough nets to sample most of the available habitats. Remove all nets from the lake prior to the conducting other sampling. Record the data from each set separately.

- All panfish will be counted and at least 250 of each species will be measured (total length – nearest 0.1 inch), preferably from various nets. Record the data from each net separately.

- See Appendix B for details on aging.

6. Fall Electrofishing (Juvenile Assessments; NR walleye/muskellunge lakes): Fall electrofishing is intended to provide an indication of walleye and/or muskellunge recruitment (young-of-year catch rate). The sampling is conducted at water temperatures from 50 to 60 F. Walleye lakes should be completed first and muskellunge lakes should be completed last (catch rates of muskellunge young-of-year tend to increase as temperatures decline). The sampling should be conducted according to the following protocols:

- Boom shocker electrofishing is conducted at night. Two experienced people should dip fish. Dip nets should have 3/8" mesh bags. Shocking should be conducted at water temperatures from 50 to 60 F.

- If possible, shock the entire shoreline. If difficult to accomplish, shock at least 50% of the shoreline. This is done by dividing the shoreline into 2-mile segments and shocking at least 50% of them. The <u>minimum</u> coverage needed is as follows:

Total Lake Shoreline (miles)	Minimum Sampling Required (2-mile segments)
8 miles or less	Entire shoreline
8 to 16 miles	4 segments
> 16 miles	5 segments

- The first 2-mile segment should be selected at random and the remaining 2-mile segments should then be chosen at equally spaced intervals around the lake to achieve uniform coverage.

- Actual distance sampled (miles) will be recorded and may be determined by GPS or by shoreline landmarks; mark the sampled segments on a lake map. Also, be sure to record time sampled.

- Within each 2-mile segment, all walleye < 10" and muskellunge < 20" will be collected and measured. Record the data from each 2-mile segment separately.

- Scales should be collected from enough fish to determine the size-breaks between 1) young-of-year and yearlings; and 2) yearlings and age 2+ fish. See Appendix B for details on aging.

Summary of Sampling Procedures for Fisheries Assessments in Lakes

Protocol	Gear	Waters	Temp. Range	Species	Data	Metrics
Spring Netting I (Walleye Netting)	4'x6', ¾" bar mesh	High Profile/ Treaty Walleye lakes; optional - northern pike lakes	Ice out to 40-50 F	Measure all walleye, northern pike, and muskellunge; mark walleyes.	Mark/recapture; length frequency; ageing structures	PE (walleye); PSD/RSD (other gamefish); age & growth (walleye)
Spring Electrofishing I (Walleye CPE)	Boom shocker, 3/8" mesh dip net bags	Walleye Lakes	45 – 50 F	Measure all walleye; (walleye recapture on Treaty/High Profile Walleye lakes)	Length frequency; counts; distance and time shocked	CPE; PSD/RSD; age & growth
Spring Netting II (Muskellunge Netting)	4'x6', ¾" bar mesh	Musky Lakes	50 - 55 F	Measure/weigh all muskellunge. Mark muskellunge (optional).	Length frequency	PSD/RSD (combine with Spring Netting I data), PE (optional)
Spring Electrofishing II (Centrarchid CPE)	Boom shocker, 3/8" mesh dip net bags	All waters	55 – 70 F	Measure all largemouth bass and smallmouth bass; count all panfish in index stations and measure 250 of each species; count common carp	Length frequency; counts; distance and time shocked; aging structures	CPE, PSD/RSD, age and growth
Summer Netting (Panfish)	4'x6', ¾" bar mesh	All waters	65 – 80 F	Count all panfish and measure 250 of each species	Length frequency	PSD
Fall Electrofishing (Juvenile Assessments)	Boom shocker, 3/8" mesh dip net bags	Walleye and Musky Lakes; NR or potential NR	50 - 60 F	Count and measure all walleye < 10" and musky < 20"	Length frequency; counts; distance and time shocked; aging structures	Juvenile CPE; age

Appendix A - DNR Field Forms

WDNR Field forms are available on the FH Database Web Site (http://infotrek.er.usgs.gov/wdnr_biology/static/wdnr_home.shtml). Make sure you collect all mandatory variables required in the FH database.

Appendix B – Aging and Aging Structures

Aging: Age and growth should be completed on all High Profile lakes and on assigned Public Access lakes. Standard metrics for comparison are from the von Bertalanffy's growth equation: Ultimate length (L_∞), growth coefficient (K), and growth rate ($w = L_\infty * K$). Weigh all fish that are aged.

It is recommended that any aging include back-calculation of length-at-age to maximize the information obtained from the effort. Use Lee's equation to back-calculate length-at-age:

$$L_n = a + [(L - a)(V_n)] / V_r$$

where: Ln = length in year n
a = constant that approximates fish length at time of scale formation
L = fish length at time of capture
Vn = scale radius distance from focus to nth annulus
Vr = scale radius from focus to scale edge

Walleye: Weigh and take spines (2nd or 3rd complete spine from leading edge of dorsal fin), five per half-inch group per sex, from all walleye $\geq$ 12 inches. Take scales from walleye < 12 inches. Take scales from five walleye per half-inch group per sex (including unknowns). Scales must be taken from below the lateral line and just beyond the tip of the pectoral fin.

Muskellunge and Northern Pike: Weigh and take scales from five fish per half-inch group per sex (including unknowns). Scales must be taken from the nape of the fish's neck at the point where the scales are largest. Cleithra are the preferred aging structure but sacrifice of the fish is required. Therefore, routine aging by cleithra is not recommended.

Largemouth and Smallmouth Bass: Weigh and take scales from five bass per half-inch group (> 4" in length). Scales must be taken from below the lateral line and just beyond the tip of the pectoral fin.

Panfish: Weigh and take scale samples from 5 fish in each half-inch group. Scales must be taken from below the lateral line and just beyond the tip of the pectoral fin.

Appendix C - Fish Contaminants

Each spring a collection schedule for fish contaminants is developed. This schedule should be examined before conducting planned field work to see if fish are needed for contaminant analysis. See the following folder for the collection schedule and for collection procedures or contact Candy Schrank (608) 267-7614 (candy.schrank@wisconsin.gov):

FHCOMMON:>MONITORING\Fish Contaminants

Appendix D - Safety, Training , and Quality Assurance

All staff working on boats are required to pass the Boating Safety exam. Electrofishing training is required for anyone who participates in the sampling activity. See section 18, Fish Management Handbook for electrofishing safety procedures. For a complete list of safety training requirements, contact Dennis Schenborn.

Training of field staff for consistency in data collection and recording is critical to the success of the fisheries assessment program. Training in taxonomy, deployment of field gear, disinfection, and general program implementation will be made available to all seasonal staff each year by the crew leader. Data quality assurance procedures include required fields, automated rejection of values outside the normal range, a data screening process built into the statewide database, and mandatory proofreading of entered data. All sampling procedures employed will, at a minimum, meet the Department's data standards as developed by the Aquatic and Terrestrial Resources Inventory (ATRI) Team.

Appendix A.8

Fish Netting Data Sheets

Lake: _____

Lift Date: _____

Collectors: _____

Survey Type: _____

Gear Type: _____

Number of Nets: _____

Hours Each Net Set: _____

Water Temp: _____

Number of _____ Caught _____	Number of _____ Caught _____
Number of _____ Caught _____	Number of _____ Caught _____
Number of _____ Caught _____	Number of _____ Caught _____
Number of _____ Caught _____	Number of _____ Caught _____

Lake Name:_____ Date:____/_____/_____ Survey Gear:_____

Survey Type:_____ Water Temp:_____ Net Nights:_____

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									
31									
32									
33									
34									
35									
36									
37									
38									
39									
40									
41									
42									
43									
44									
45									
46									
47									
48									
49									
50									

Appendix A.9

Fish Collection Voucher Label

Fish Collection Voucher Label Rev: 12/30/2010

Basin (Code): Sheboygan (060) Watershed (Code): Sheboygan (SH03) County (Code): Sheboygan (60)

Waterbody: Sheboygan River WIBC: 50700 Project: Sheboygan AOC Pathway to Delisting Project

Collector(s): _____

Sample Date: _____/_____/_____

Location: _____

Station ID: _____

Number of Specimens Preserved: _____

Comments: _____
_____
_____
_____
_____

Laboratory Identification
Taxonomist Name (Last, First): _____, _____
Date: _____/_____/_____

Common Name	Number	Specimen Retained or Disposed
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

Appendix A.10

Wisconsin Department of Natural Resources

Fish Management Handbook
Draft Update – Chapter 11, Sections J-P, October 2009

Fish Management Handbook
***DRAFT* Update – Chapter 11, Sections J - P**
October 2009

IV. Data Collection and Analysis Procedures

J. Fisheries Management Database

The fisheries biologists or designated fisheries technicians and their fish team supervisors are ultimately responsible for the long-term maintenance of all records in the Fisheries Management Database within their management area.

Fisheries and habitat data collected from Tier 1 and Tier 2 fisheries management assessment surveys, habitat improvement surveys, stocking and regulation evaluation surveys, tournament permits, stocking permits, fish kill investigations and stocking planning and activities are stored in the Fisheries Management Database http://infotrek.er.usgs.gov/wdnr_bio. Information stored in the Fisheries Management Database is used to generate statewide stocking or fisheries summaries, to evaluate fisheries status and trends, to evaluate the effectiveness of regulation strategies or stocking practices, or in regulatory decision making, permitting and policy decisions.

All DNR employees have read-only access to the Fisheries Management Database. Those who have had training and are required to enter data in the system may also be given read-write access. All other interested parties need to request data from the local biologist, if the request is localized, or from the Database Coordinator.

K. Database Training

Annual training is required to enter data into or retrieve from the Fisheries Management Database. Supervisors are responsible for ensuring that at least one of their permanent staff is proficient and current in their training. Large group training courses will be offered in January of each year at a central location. Small group/individual training sessions will be offered at other locations throughout the year as needed.

L. Data Entry

The integrity of the database and every analysis from it depends on the quality of the data collection and its accurate entry into the system. The Fish Team Supervisor or Supervisor's designee is ultimately responsible for data entry. General instructions for data entry and reporting are available on the web site.

Biologists in the field are responsible for collecting the required metrics and measurements. All mandatory fields must be populated on field sheets to minimize error during data entry. All permanent staff will be notified of updates to data entry forms, reports and requirements and to new modules in the form of a newsletter or e-mail.

If the person entering the data was not involved in the sampling, care must be taken when transferring the field data sheets to ensure that all field forms are correctly interpreted and data are entered correctly into the database. Interpretation of field forms can be

confusing and requires some understanding of how fisheries data are typically collected and summarized.

Information stored in the Fisheries Management Database may be accessed and downloaded by anyone within the Water Division as soon as it is entered into the database. Because downloads are possible even if the data entry is not complete, we established a Data Entry Survey Status Code to indicate the status of data entry for anyone downloading information from the database. Except for the default status of "Data Entry Not Complete", the status codes need to be manually updated in the survey data entry form. The following Date Entry Survey Status Codes exist in the database:

> **Data entry not complete**: All survey records have a status code of "Data entry not complete" as the default to prevent someone from downloading and using records that are incomplete and in the process of being entered.
>
> **Data entry complete**: The person entering data has finished entering all information for that survey.
>
> **Data entry complete and proofed**: Data entry is final and the Fisheries Biologist or Fish Team Supervisor's Designee has reviewed the data entry.

M. Data Proofing

Updating the "Data Entry Status Code" in the Survey Data Entry form from "Complete" to "Proofed" should **only** be done by the Fisheries Biologist or Fish Team Supervisor's Designee who will be responsible for the survey records after review.

This person should review the raw data from an export or directly in the data entry form. In addition, the automated summary reports should be reviewed

Before the Data Entry Status Code is updated to "Data entry complete and proofed", the Fisheries Biologist or Fish Team Supervisor's Designee should ask and answer several questions.

1. Does the database accurately reflect the records on the field data sheets? If not, then there might be a typing error in data entry (e.g., is it really a 60 inch bluegill, or should it be a 6.0 inch bluegill?).
2. Is the summary of information from the database's automated reports an accurate reflection of the field data sheet? If not, then there might be an error in how the field data sheet information was organized and entered into the database.
3. Is the summary of information in the database an accurate reflection of the fish population in the lake or stream? The data entry and summary reports may be correct, but something about the sampling event may be confounding the summary results (e.g., a sudden storm or suspected equipment malfunction). Any suspected problems should be documented in the Survey Description area of the Survey data entry form.

N. Data Entry Error Checking Procedures

Error checking procedures are run nightly for all data. Raw data and summaries are also exported and examined for data structure interpretation and other errors that cannot be caught during the error checking procedure. Data entry and data structure interpretation errors will be flagged and visible in reports and in the data entry form. All corrections will be made by the responsible biologist, the data entry person or with the help from the database team at the Central Office.

O. Data Reporting

The Fisheries Management Database produces standard summary reports and raw data reports. The summary reports and calculations are based on standard protocols. These protocols and metadata associated with the reports are located on the Fisheries Management Database web site.

P. Data Use

Not all fisheries sampling information may be used for all purposes. If fisheries data are readily accessible to Water Division staff and eventually the public, care must be taken to prevent misuse and misinterpretation by the unfamiliar user. Metadata is being developed to describe general database structure, field protocols and sampling techniques, assumptions behind sampling techniques, and decision rules to assess the validity of assumptions for each survey. Data Use Codes are based on assumptions for particular sampling techniques. For example, targeted surveys (e.g., walleye fall young-of-year electrofishing) should not be used if the objective is to determine species presence and absence. Data Use Codes are being developed for all survey techniques in the Fisheries Management Database and will be expanded to include all modules.

Appendix A.11

Wisconsin Department of Natural Resources

A sampling framework for smallmouth bass in Wisconsin's streams and rivers

A sampling framework for smallmouth bass in Wisconsin's streams and rivers

Prepared by John Lyons for the
Smallmouth Bass Rivers Assessment Team

December 2006

INTRODUCTION

In summer 2006, an ad hoc committee of WDNR biologists was convened to develop field sampling and data interpretation guidelines for smallmouth bass fishery management in Wisconsin's stream and rivers. The committee was given three specific tasks:

1) Develop a simple yet accurate stream and river classification based on smallmouth bass population potential for determining the distribution of sampling effort and for the interpretation of sampling results;

2) Recommend appropriate sampling techniques and effort and an associated sampling design for each stream/river class and provide estimates of sampling accuracy and precision; and

3) Propose quantitative criteria based on the sampling techniques for each class to determine if fisheries management objectives were being met.

The committee exchanged information electronically and met face-to-face on two separate occasions. Team members worked effectively together and should be commended for their professionalism and dedication to the task. The fruits of their labor are presented here as *A sampling framework for smallmouth bass in Wisconsin's streams and rivers*.

METHODS

Recommendations were generated from review and interpretation of over 20 years of published research on the distribution, habitat, life history, and fishery management of smallmouth bass in Wisconsin's streams and rivers, coupled with new analyses of four existing smallmouth bass datasets managed by WDNR Fish Research. Two of the datasets covered wadable streams and rivers and two covered non-wadable rivers, and all four contained smallmouth bass catch and size data collected by daytime electrofishing during the summer months. The first, termed the "wadable spatial variation" (WSV) dataset, was from stream shocker surveys of 48 different wadable 0.3-1.2-mile long sites on 40 smallmouth bass streams located throughout the state, each sampled once between 1987 and 1992 (Lyons and Kanehl 1993). The second, termed the "wadable temporal variation" (WTV) dataset, had stream shocker surveys from single wadable 0.75-1.2-mile long sites on five streams in southwestern Wisconsin sampled annually from 1989 through 2005 (Lyons 2006a). The third, termed the "non-wadable spatial variation" (NSV) dataset, had miniboom shocker data from 67 non-wadable one-mile-long sites on 10 rivers located throughout the state and surveyed once each between 1996 and 1999 (Lyons et al. 2001). The fourth, termed the "non-wadable temporal variation" (NTV) dataset, had miniboom data from 10 non-wadable one-mile-long sites on the Lower Wisconsin River sampled annually from 1999 through 2006 (Lyons 2006b) and three non-wadable one-mile sites on the Menominee River and one non-wadable one-mile site on the St Croix River each sampled annually from 1996 through 1998 (Lyons 2004). All of the sites in these four datasets were classified as "least-impacted"; that is they were thought to have relatively low levels of human impacts (including angler harvest), and smallmouth bass populations were believed to be relatively close to their potential (i.e., maximum abundance and size structure given current landscape conditions). The two spatial variation datasets were used to compare abundance and size structure among stream/river classes and to generate expected abundance and size structure values for each class. The two temporal variation datasets were used to

quantify the variation among samples within individual sites and to estimate the number of samples necessary to detect changes in smallmouth bass abundance and size structure.

STREAM AND RIVER CLASSIFICATION

The classification scheme was developed primarily from published research on the classification of Wisconsin's warmwater streams and rivers based on fish communities in general and smallmouth bass in particular. These studies, involving over 1,000 different stream and river reaches, have identified four broad-scale factors, listed here in order of importance, that in large part determine the occurrence, abundance, and size structure (and hence fishery potential) of smallmouth bass in Wisconsin streams and rivers:

1) Stream size (drainage area, stream order, flow, width, depth)

2) Summer water temperature (maximum, average)

3) Gradient (velocity, substrate, and channel morphometry)

4) Location in state (climate and geology)

5) Land cover and land use (especially forest, agriculture, and urbanization)

The first four factors are primarily “natural” and determine the potential (i.e., maximum possible) smallmouth population a particular stream or river can support in the absence of human influences, although clearly human activities have modified stream flow, temperature, gradient, and climate in many areas of the state. The fifth factor, human land use, helps determine actual population status, and often prevents a stream or river from reaching its full fishery potential.

Smallmouth bass in Wisconsin occur over a wide range of stream sizes, including the largest rivers in the state, but normally avoid relatively small streams (Forbes 1985; Lyons et al., 1988, 2000, 2001; Lyons 1991, Lyons and Kanehl 1993; Wang et al., 2003). For purposes of classification, the committee broke streams and rivers containing smallmouth bass into two categories, wadable and non-wadable, reflecting fundamental differences in sampling procedures necessary to characterize the smallmouth bass population in each (Lyons and Kanehl 1993; Lyons et al., 2001; Weigel et al., 2006a). Wadable stream and river reaches typically have drainage areas of less than 500 square miles (sqm) (usually less than 350 sqm), are less than 6th order (at the 1:24,000 scale), and have long-term mean annual flows less than 300 cubic feet per second (cfs), mean widths less than 175 ft (usually less than 120 ft), and pool depths that average less than 4 ft (although maxima may be much greater). Non-wadable rivers are generally larger than these threshold values and have been previously defined for the purposes of the baseline monitoring program (B. Weigel and J. Lyons, WDNR Fish Research, unpublished data). Many river reaches with size values close to the thresholds cannot be easily categorized as strictly wadable or non-wadable; they may have wadable stretches intermingled with non-wadable stretches within the same reach or may be wadable during relatively dry years but non-wadable during relatively wet years.

Smallmouth bass occur in all sizes of non-wadable rivers but are absent from many wadable streams (Lyons 1989, 1991, 1996; Lyons et al. 1988, 2001). Smallmouth bass are usually not found in streams with

drainage areas less than 20 sqm, stream order less than 2, mean annual flows less than 10 cubic feet per second (cfs), mean width less than 15 ft, and mean pool depths depth less than 2 ft. Streams only slightly larger than these thresholds typically do not have a fully developed smallmouth bass population, as they are dominated by small juveniles and lack adults, except perhaps briefly during spring spawning (Forbes 1989; Lyons and Kanehl 1993, 2002). These streams, which we term "nursery streams", usually cannot support a fishery directly, but the young they contain may be critical to the maintenance of fisheries in larger stream reaches or lakes further downstream. In general, nursery streams have drainage areas of 20-50 sqm, range from the largest 2nd to the smallest 4th order, have mean annual flows from 10-30 cfs, mean widths from 15-40 ft, and pools that average 2-3 ft deep. Some streams with substantial larger drainage areas and widths may nonetheless also be classified as nursery streams if the geology and soils of their watershed keep their flow and maximum depth within the nursery range. Such larger streams may have many juvenile smallmouth bass but few adults and thus little direct fishery potential (J. Lyons, WDNR Fish Research, unpublished data).

Wadable streams larger than the nursery thresholds are capable of supporting a fully developed smallmouth bass population and associated fishery (Forbes 1985, 1989; Lyons and Kanehl 1993; Lyons et al., 1996; Mason et al., 1993). These "wadable warmwater" streams typically have drainage areas from 50-500 sqm, are 4th-5th order, have mean annual flows of 30-300 cfs, mean widths of 40-175 ft, and mean pool depths of 3-4 ft.

Water temperature limits the distribution and abundance of smallmouth bass in streams. Essentially, all non-wadable rivers are warm enough for fully developed smallmouth bass populations, but many wadable streams are not (Lyons 1989, 1996; Lyons et al., 2001). Although smallmouth bass can tolerate water temperatures down to freezing during the winter, streams that remain cold during the summer hamper smallmouth bass spawning and greatly reduce growth and survival of larvae and juveniles (Lyons 1997). True coldwater streams, with maximum daily mean water temperatures of 70 F or less, only rarely contain smallmouth bass (Lyons et al., 1996; Lyons 1997). "Coolwater" streams, with maximum daily mean water temperatures of 70-77 F, often have smallmouth bass, but the population is usually not fully developed. Reproduction and recruitment is typically limited and erratic because of temperature constraints on spawning and early growth and survival, and very large adults are usually relatively scarce because of slow growth. (Lyons 1997). Nonetheless a fishable population may be present, often because of migration from other warmer waters, consisting mainly of small to medium-sized adults (Forbes 1985; J. Lyons, WDNR Fish Research, unpublished data).

Gradient influences occurrence and abundance of smallmouth bass in both wadable streams and non-wadable rivers. Non-wadable rivers with a relatively high gradient, more than 3.1 ft mile, usually have a relatively high proportion (>10%) of their substrate as rubble/cobble or boulder and have the potential to support higher densities of smallmouth bass than non-wadable rivers with lower gradients and less rubble/cobble substrate (Lyons 1991, 2004, 2005a, 2005b; Weigel et al., 2006b). The higher-gradient "coarse-substrate" rivers are usually at the smaller end of the non-wadable river size spectrum and often change to lower-gradient "fine-substrate" rivers as they get larger.

Among wadable streams, many waters have a gradient too low to support a smallmouth bass population. Streams with gradients less that 4.2 ft/mile usually lack adequate pool-riffle development and rocky substrate to support smallmouth bass populations (Lyons et al., 1989; Lyons 1989, 1991, 1996).

Smallmouth bass are ubiquitous in Wisconsin, and no part of the state lacks the species (Lyons et al., 2000). However, differences in climate and geology across the state create inherent regional differences in the length and suitability of the growing season and in basic aquatic productivity, suggesting that there might be regional differences in the potential abundance, size structure, and growth rate of smallmouth bass populations (Forbes 1985; Lyons and Kanehl 1993; Lyons et al., 2001). Based on previous studies, the most likely differences would occur between streams in the northern third of the state and those located further south (Lyons 1989, 1996; Lyons and Kanehl 1993; Lyons et al., 2001; Weigel et al., 2006b).

CLASSIFICATION

From the above summary of the literature, a classification of Wisconsin's streams and rivers can be proposed with 14 categories, 10 of which have the potential to support a smallmouth bass population (Table 1). The classification recognizes five smallmouth bass stream/river types: wadable warmwater, wadable coolwater, wadable nursery, non-wadable coarse-substrate, and non-wadable fine-substrate, in each of two regions, north and south. The remaining four categories encompass the many wadable streams in the state that are too cold, too small, or have too low a gradient to have the inherent capability to support a smallmouth bass population. All non-wadable rivers in the state have the potential to support a smallmouth bass population.

The WSV and NSV datasets were used to test whether this proposed classification explained significant amounts of the variation in smallmouth bass abundance and age/size structure among stream sites. Catch-per-mile data were first log transformed for three different nested age/size categories, all fish age 1 or older ("age-1"), all fish 8 inches or larger ("8-inch"), and all fish 14 inches or larger ("14-inch"). The log transformation reduced the influence of a few unusually large values and created a more normal distribution of values to satisfy assumptions required for parametric statistical tests. Two-way analyses of variance (ANOVA) were ran with abundance as the dependent variable and stream type (wadable warmwater, wadable coolwater, wadable nursery for the WSV dataset, non-wadable fine-substrate and non-wadable coarse-substrate for the NSV dataset) and region (north versus south) as main effects and including the stream type-region interaction. Duncan multiple range tests were used to compare values among stream/river classes.

The ANOVAs indicated that there were statistically significant differences ($P < 0.05$) in smallmouth bass catch-per-mile between stream types and regions (Table 2). For wadable streams, the wadable warmwater class had significantly greater numbers of smallmouth bass than either the wadable coolwater or the wadable nursery classes for all three size/age categories (Age-1: $F = 7.98$; P , 0.0001; 8-inch: $F = 6.93$; $P < 0.0001$; 14-inch: $F = 2.73$; $P = 0.0320$). The wadable nursery and coolwater classes did not differ significantly from each other. For age-1 fish, southern streams had a significantly higher CPE than northern streams, but there were no regional differences in CPE for 8-inch and 14-inch fish.

For non-wadable streams, there were significant differences in CPE between coarse- and fine-substrate rivers and the northern and southern regions for all three age/size categories (Age-1: F = 3.65; P = 0.0172; 8-inch: F = 3.59; P = 0.0183; 14-inch: F = 5.04; P = 0.0034). Coarse-substrate rivers tended to have higher CPE than fine-substrate rivers and southern

Table 1 Classification of the inherent smallmouth bass fishery potential of Wisconsin streams and rivers with the physical criteria that define each class. Northern refers to the northern third of the state; southern to the remainder.

Stream class	Drainage area (square miles)	Mean annual flow (cfs)	Mean pool depth (ft)	Maximum daily mean water temp. (F)	Gradient (ft/mile)
Have the potential for a smallmouth bass population to be present					
Northern non-wadable fine substrate	> 500	> 300	> 4	> 77	< 3.1
Northern non-wadable coarse substrate	> 500	> 300	> 4	> 77	> 3.1
Northern wadable warmwater	50-500	30-300	3-4	> 77	> 4.2
Northern wadable coolwater	50-500	30-300	3-4	70-77	> 4.2
Northern wadable nursery	20-50[1]	10-30	2-3	> 77	> 4.2
Southern non-wadable fine substrate	> 500	> 300	> 4	> 77	< 3.1
Southern non-wadable coarse substrate	> 500	> 300	> 4	> 77	> 3.1
Southern wadable warmwater	50-500	30-300	3-4	> 77	> 4.2
Southern wadable coolwater	50-500	30-300	3-4	70-77	> 4.2
Southern wadable nursery	20-50[1]	10-30	2-3	> 77	> 4.2
Do not have the potential for a smallmouth bass population to be present					
Wadable coldwater	< 500	< 300	< 4	< 70	Any
Wadable warmwater headwaters	< 20	< 10	< 2	> 77	Any
Wadable coolwater headwaters	< 20	< 10	< 2	70-77	Any
Wadable low-gradient	20-500	10-300	2-4	> 70	< 4.2

[1]A stream can still be classified as wadable nursery if the drainage area is greater than 20-50 sqm but mean annual flow is 10-30 cfs and mean pool depth is 2-3 ft.

Table 2 Summary statistics for the within-class distribution of smallmouth bass catch per effort (CPE; number per mile of stream length) and relative stock density (RSD; %) among least-impacted sites from the two spatial-variation datasets. Age-1 refers to all fish age 1 or older, 8-inch to all fish 8 inches or larger, and 14-inch to all fish 14 inches or larger. N = number of sites; Min = minimum value, and Max = maximum value. The 50th percentile value is equivalent to the median value. N. = northern region and S. = southern region.

Stream class	Population metric	N	Percentile Min	25th	50th	75th	Max	Mean
Non-wadable rivers (NSV dataset)								
Northern fine substrate	Age-1 CPE	12	0.0	1.5	2.5	5.0	16.0	3.9
	8-inch CPE	12	0.0	0.5	1.5	2.0	5.0	1.6
	14-inch CPE	12	0.0	0.0	0.0	0.5	1.0	0.3
	RSD	9	0	0	0	20	50	13
Northern coarse substrate	Age-1 CPE	13	0.0	2.0	5.0	9.0	27.0	7.7
	8-inch CPE	13	0.0	1.0	3.0	5.0	24.0	4.9
	14-inch CPE	13	0.0	0.0	0.0	1.0	6.0	1.1
	RSD	11	0	0	0	33	50	14
Southern fine substrate	Age-1 CPE	38	1.0	3.0	7.0	9.0	34.0	7.3
	8-inch CPE	38	0.0	2.0	4.0	6.0	20.0	4.3
	14-inch CPE	38	0.0	0.0	1.0	2.0	6.0	1.4
	RSD	35	0	8	30	57	100	38
Southern coarse substrate	Age-1 CPE	4	10.0	10.0	11.0	32.0	52.0	21.0
	8-inch CPE	4	3.0	5.0	7.0	9.0	11.0	7.0
	14-inch CPE	4	1.0	2.0	3.0	3.0	3.0	2.5
	RSD	4	27	30	38	43	43	37
Wadable streams and rivers (WSV dataset)								
N. wadable warmwater	Age-1 CPE	8	6.4	20.9	29.0	86.2	285.0	70.4
	8-inch CPE	8	4.8	7.2	19.3	46.7	173.9	40.7
	14-inch CPE	8	0.0	0.0	0.0	1.6	3.2	0.8
	RSD	8	0	0	0	2	13	2
N. wadable coolwater	Age-1 CPE	5	0.0	4.8	6.4	14.5	19.3	9.0
	8-inch CPE	5	0.0	0.0	1.6	3.2	12.9	3.5
	14-inch CPE	5	0.0	0.0	0.0	0.0	0.0	0.0
	RSD	3	0	0	0	0	0	0
Northern wadable nursery	Age-1 CPE	2	0.0	0.0	0.8	1.6	1.6	0.8
	8-inch CPE	2	0.0	0.0	0.8	1.6	1.6	0.8
	14-inch CPE	2	0.0	0.0	0.0	0.0	0.0	0.0
	RSD	1	0	0	0	0	0	0
S. wadable warmwater	Age-1 CPE	21	9.7	25.8	51.5	109.5	978.9	119.1
	8-inch CPE	21	3.2	8.1	17.7	49.9	392.8	49.0
	14-inch CPE	21	0.0	0.0	1.6	3.2	45.1	6.3
	RSD	21	0	0	3	15	60	11
S. wadable coolwater	Age-1 CPE	7	0.0	1.6	6.4	8.1	12.9	5.8
	8-inch CPE	7	0.0	0.0	1.6	3.2	3.2	1.6
	14-inch CPE	7	0.0	0.0	0.0	0.0	1.6	0.2
	RSD	4	0	0	0	25	50	13
Southern wadable nursery	Age-1 CPE	5	0.0	32.2	45.1	59.6	183.5	64.1
	8-inch CPE	5	0.0	0.0	16.1	32.2	66.0	22.9
	14-inch CPE	5	0.0	0.0	0.0	0.0	0.0	0.0
	RSD	3	0	0	0	0	0	0

rivers tended to have higher CPE than northern rivers (Table 2).

At present, the smallmouth bass classification has not yet been applied to the streams and rivers of the state. However, existing inventories of known smallmouth bass waters (WDNR 1968; Forbes 1985), coupled with results from an ongoing GIS-based computer modeling study to estimate

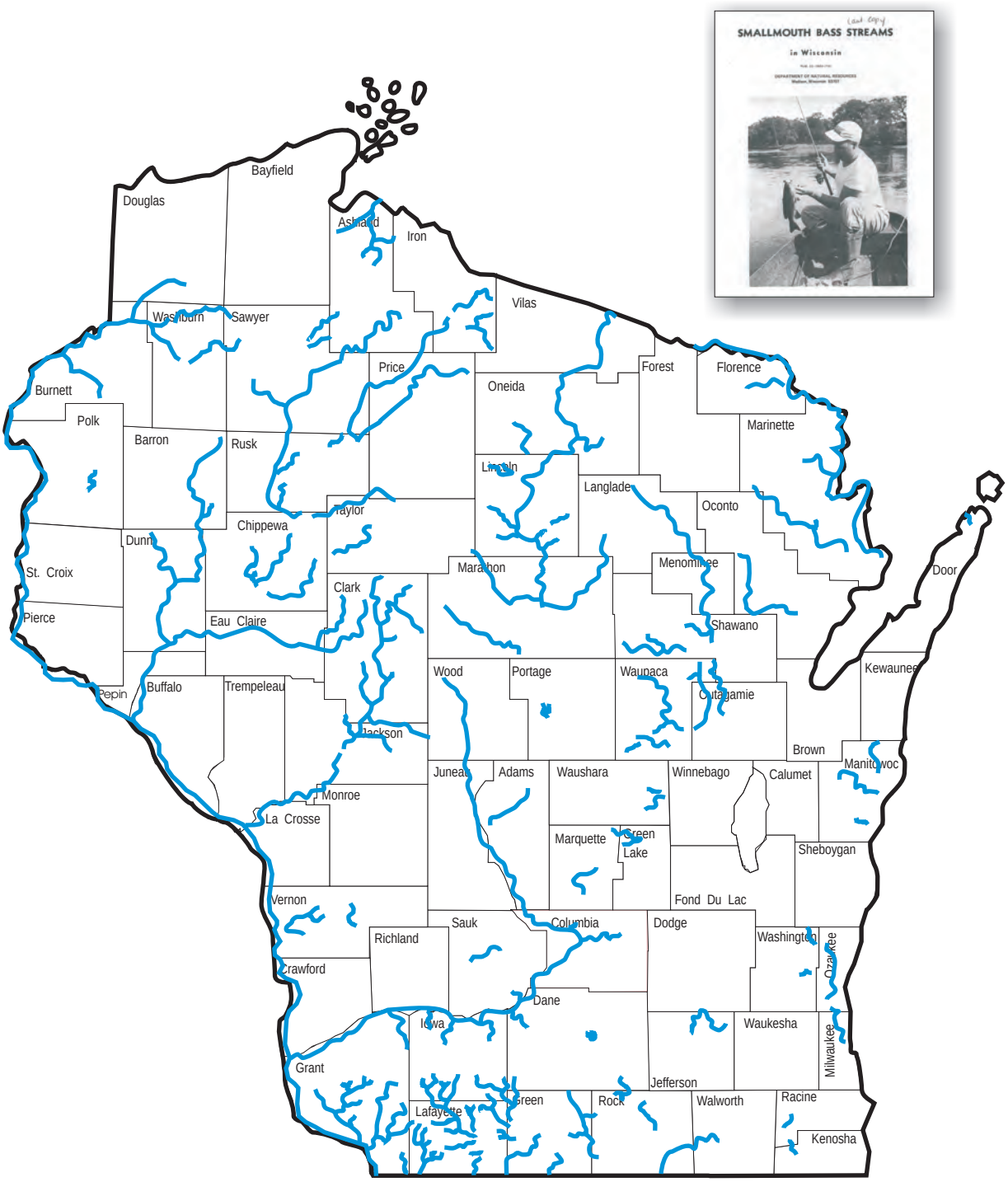


Figure 1. Distribution of smallmouth bass streams and rivers in Wisconsin. (taken from the 1978 publication *Smallmouth Bass Streams of Wisconsin)*.

the fishery potential of Wisconsin streams and rivers that provides drainage area, estimated flow, estimated maximum temperature, and gradient for all stream and river reaches in the state (Lyons and Mitro 2006), make application and mapping of the classification relatively straightforward and rapid.

Human land-use in the watershed and riparian areas of streams and rivers can alter smallmouth bass populations (Forbes 1989; Lyons et al., 1989; Mason et al., 1993; Wang et al., 1997, 2003; Wang and Lyons 2002). Intensive agriculture and urbanization are responsible for the decline of many smallmouth bass populations, particularly in southern Wisconsin. Inappropriate land-uses, along with pollution, direct habitat modifications (e.g., dams, dredging, flow alterations), and angling over-harvest, are the main factors that prevent smallmouth bass populations from achieving the potential of their particular stream or river class.

RECOMMENDED SAMPLING

The committee concluded that two types of data, abundance and size/age structure, are needed for effective management of smallmouth bass in streams and rivers. Abundance can be estimated either through catch-per-effort (CPE) or population estimate parameters. Based on previous studies, CPE data expressed as the catch per length of stream or river sampled, rather than surface area or duration of sampling, are recommended as the standard to assess abundance (Lyons and Kanehl 1993; Lyons 2004). Catch-per-effort data, although often relatively imprecise (see below), accurately track true fish numbers, as demonstrated in direct experiments (Lyons and Kanehl 1993; Simonson and Lyons 1995) and in evaluations of smallmouth bass fishery regulations (Lyons et al., 1996) and smallmouth bass abundance relative to human habitat modifications (Kanehl et al., 1997; Lyons 2004, 2005a, 2005b; Weigel et al., 2006a).

Population estimates provide a more precise measure of abundance, but may not be as accurate or cost-effective as CPE data. An examination of existing population estimates by Lyons and Kanehl (1993) indicated that mark-recapture population estimates were highly biased and inaccurate because of apparent changes in smallmouth bass behavior following marking. Depletion (=removal) population estimates were more accurate but required substantially more time and effort (> 5 times) than CPE data in wadable streams, making them cost prohibitive in most cases. Population estimates were completely impractical in non-wadable rivers because of the huge amounts of labor involved and substantial biases due to fish movements (Lyons 2006b).

Size/age structure is determined through direct measurement of length and weight from sampled fish and through estimation of age from hard structures, such as scales or fin spines, taken from these fish. Length is the easiest of the three types of data to collect, can be used to approximate weight and age, and is essential in most management contexts as it is the basis for many fishery regulations (i.e., length limits). The committee recommended that collection of length data from all captured smallmouth bass be mandatory, but that weight and age data could be collected at the discretion of the biologist. Length data should be summarized as catch per ½ inch length interval in a length frequency histogram and from there summarized as Proportional Stock Density (PSD) and Relative Stock Density (RSD), ratios of the total catch of relatively large fish to the total catch of all medium and large fish (Anderson and Neumann 1996). For

smallmouth bass, PSD has been defined as the ratio of fish 11 inches ("preferred size") or greater to fish greater than 7 inches ("stock size"), whereas RSD has a fish length in the numerator that is specified by the user. The committee recommends using a variant of RSD with a numerator of 14 inches or greater and a denominator of 8 inches or greater because 14 inches is the length for legal angler harvest in nearly all smallmouth bass waters in Wisconsin and 8 inches is the typical size at first maturity (i.e., adulthood) for smallmouth bass in Wisconsin streams and rivers (Forbes 1989; Lyons and Kanehl 1993).

Stream and river smallmouth bass in Wisconsin are captured most efficiently by electrofishing. Recommendations for sampling techniques are based on previously published analyses of electrofishing performance (Lyons 1992; Lyons and Kanehl 1993; Simonson and Lyons 1995; Lyons et al., 2001). In non-wadable rivers, a single standard WDNR pulsed-DC mini-boom shocker with one netter is recommended (Lyons et al., 2001; Lyons 2004, 2006b; Weigel et al., 2006a). Shocking should proceed in a downstream direction along one shoreline during daylight. In coarse-substrate rivers with rapids, an inflatable raft mini-boom shocker can be substituted for the standard aluminum solid-hull version. In wadable streams and rivers, one or more standard WDNR DC stream ("tow-barge") shockers with three anodes will be most effective (Lyons 1992, 2006a; Lyons and Kanehl 1993; Simonson and Lyons 1995). Shocking should proceed in an upstream direction over the entire width of the stream channel during daylight. One shocker is recommended for streams less 60 ft wide, two shockers for streams 60-89 ft wide, and three shockers for streams 90 ft or wider. For both wadable and non-wadable streams and rivers, shocking will be most efficient and representative during summer months at baseflow (Lyons and Kanehl 1993; Lyons 2004).

Sampling effort for stream and river shocking is best expressed as the length of stream channel sampled (Lyons 1992; Lyons and Kanehl 1993; Lyons et al., 2001). Previous studies indicated that a sampling length of 40 times the mean stream width was the minimum appropriate to characterize the fish community of a wadable stream site but that longer lengths would often be necessary to achieve an adequate sample size for smallmouth bass length-frequency analyses (Lyons 1992; Lyons and Kanehl 1993). Based on a consensus among committee members that at minimum of 16-25 fish were needed for a meaningful length analysis, a fixed distance of 0.5 miles (2640 ft) was recommended for wadable streams. At this distance, based on an analysis of the distribution of CPE values within the WSV dataset (Table 2), the probabilities that at least 16 age-1 smallmouth bass will be collected are approximately 67% for southern warmwater, 75% for southern nursery, 0% for southern coolwater, 50% for northern warmwater, 0% for northern nursery, and 0% for northern coolwater streams. These values are for least-impacted streams where smallmouth bass populations have not been depressed by human activities, and in impacted streams, where populations are lower, the probabilities would be reduced. Obviously, increasing the sampling length beyond 0.5 miles would improve the chances that 16 smallmouth bass would be collected, but the committee felt that the benefits of longer lengths were offset by the increased time and labor required and the ultimately the smaller number of sites that could be sampled within any given time period.

For non-wadable rivers, the minimum sampling distance necessary to characterize the fish community is one mile

(Lyons et al. 2001), and this distance has been the minimum standard for WDNR baseline monitoring for the last five years. The committee agreed that this distance should be retained as a minimum for smallmouth bass in rivers, with the option to substantially increase sampling length depending on smallmouth bass CPE and the necessity of a sample of 16 fish for length analyses. In large rivers, particularly the fine-substrate class, smallmouth bass are highly patchy in distribution but generally present at low densities (Lyons 2004, 2005a, 2006b; Weigel et al. 2006a), and assuring a catch of at least 16 age-1 smallmouth bass usually requires a very long station. From an analysis of the NSV dataset (Table 2), with a station length of one mile, the probability of collecting 16 age-1 smallmouth bass would be approximately 45% for southern coarse-substrate, 13% for southern fine-substrate, 8% for northern coarse-substrate, and 8% for northern fine-substrate rivers. To have a 50% chance of catching at least 16 smallmouth bass would require sampling lengths of 1.3 miles for southern coarse-substrate, 2.3 miles for southern fine-substrate, 3.2 miles for northern coarse substrate, and 6.5 miles for northern fine-substrate rivers.

The precision of the CPE and RSD data determines how large a change in a smallmouth bass population must occur before it will be likely to be detected by sampling or, conversely, how many samples must be taken to have a reasonable chance of detecting a given change in the population. Sampling precision is estimated from the variability in CPE and RSD among different sampling sites (spatial variation) and among samples taken at different times at individual sites (temporal variation). Thus, spatial variation in CPE and RSD represents inherent differences in potential and sampling effectiveness among classes and among waters within classes, and temporal variation represents typical year-to-year fluctuations in CPE and RSD owing to natural environmental variation, primarily climate, and variation in sampling effectiveness.

For assessing responses of smallmouth bass populations to human impacts (e.g., riparian land-use changes) or management activities (e.g., changes in fishing regulations), as well as to better understand general trends in smallmouth bass abundance and age/size structure, a variant of the before-after-control-impact design (BACI; Underwood 1994) is recommended. For this approach, multiple independent sites, some of which have the impact or management activity and others of which do not ("control"), are sampled before and after the impact or activity occurs. Comparisons are made between time periods (i.e., before and after activity in question) for each stream site, and the different streams act as "blocks" (in a statistical sense) to provide replication and to account for variation in response among sites. The key comparison is how the impact sites change before and after relative to the control sites. If the change at the impact sites is large relative to any change at the control sites (which may occur due to a natural population fluctuation, for example), then it can be concluded that the impact or management activity had a meaningful effect. In a statistical sense, you test for a significant interaction between the type of stream (impact vs control) and time period (before vs. after).

The statistical power of the BACI design, which is the ability to detect a real change in the parameter of interest, is high relative to other sampling designs, but can be complicated to determine when there are multiple years of before and after data (Osenberg et al., 1994). For the simple case where there is only one sample per site

from before the impact/activity and one from afterwards and the response is expressed as the difference between the before and after period, it is possible to use a standard t-test power analysis formula to determine how many different pairs of sites (i.e., one control and one impact) are needed to have a known probability of detecting a response of a given magnitude. This determination assumes no temporal congruence among sites, which is simplistic given that smallmouth bass populations often fluctuate in synchrony over large geographic areas because of climate induced regional variation in reproductive success (Mason et al., 1993; Lyons et al., 1996; Lyons 2006a). However, positive synchrony in natural population fluctuations among control and impact sites reduces spatial variation and makes population changes easier to detect, so the simplified power analysis may be conservative for many situations.

The t-test statistical power formula is:

$$N = (2s2/ä2) (tá,í + tÂ(1), õ)2$$

where N is the number of pairs of sites needed, s is the standard deviation of the variation among years (i.e., temporal variation) for individual sites, ä is size of the effect of interest, i.e., the magnitude of the difference between before and after values for impact sites and before and after values for the control sites, and tá,í and tÂ(1), í are t-statistic values for specified probabilities, á referring to the probability of type I error, that is, concluding that there has been a change when in fact one has not taken place, set at 5% for this analysis, and Â referring to the probability of type II error, that is, concluding that there has been no change when in fact one has occurred, set at 20% for this analysis, and í is the degrees of freedom for each t statistic (number of pairs of sites minus one).

Temporal variation was estimated from analyses of the WTV and NTV datasets. Mean and standard deviation was calculated for each individual site across all years for each of the three CPE values and for RSD. Where there was more than one site per stream class, the mean of the means and the standard deviations were determined for all sites within each class. Values are given in Table 3.

Using the power formula, estimated temporal variation, and a relative effect size of either 50% (i.e., the impact sites increased by 50% relative to the control sites from the before to the after sample), 100% or 200%, the sample size necessary to detect the effect for each of the stream classes that had any temporal variation data available was determined (Table 4). The smaller the size of the effect, the more pairs of sites were needed to have a reasonable chance of detecting a change. However, the number of pairs needed dropped rapidly as the size of the effect increased. For a 50% change, 22-106 pairs were needed, but for a 100% change, 3-27 were required, and for a 200% change, only 2-10 were necessary. Because the standard deviations of the 14-inch CPE and RSD categories were higher relative to their mean values than the those for the Age-1 and 8-inch CPE categories, detecting a given change in 14-inch CPE and RSD values required substantially more pairs of sites.

QUANTITATIVE ASSESSMENT CRITERIA

Based on analyses of the WSV and NSV datasets, criteria have been proposed for assessing the status of smallmouth bass populations in Wisconsin's streams and rivers (Table 5). Each stream class has its own criteria, and they are derived from data in Table 2. The 25-75% percentiles of the distribution of CPE and RSD values from the

Table 3 Means and standard deviations of CPE (number per mile) and RSD (%) values across years for smallmouth bass at selected stream and river sites.

Site	Years	Mean/Standard deviation			
		Age-1 CPE	8-inch CPE	14-inch CPE	RSD
Southern wadable warmwater streams (WTV dataset)					
Ames Branch	1991-2005	4.0/2.8	2.4/1.4	0.2/0.2	7/6
Galena River	1990-2005	8.3/4.8	4.6/2.1	0.9/0.7	20/11
Little Platte River	1990-2005	8.6/5.0	4.3/2.0	0.6/0.3	17/10
Rattlesnake Creek	1990-2005	6.7/3.2	3.5/1.1	0.4/0.2	13/6
Mean		6.9/4.0	3.7/1.7	0.5/0.4	14/8
Southern wadable nursery streams (WTV dataset)					
Mineral Point Branch	1989-2005	5.5/4.1	2.3/1.2	0.1/0.1	7/7
Southern non-wadable fine-substrate rivers (NTV dataset)					
Wisconsin R. (4.4)	1999-2006	3.3/2.1	1.8/0.9	0.9/0.8	50/41
Wisconsin R. (16.4)	1999-2006	2.2/1.1	1.3/1.3	0.4/0.7	23/32
Wisconsin R. (17.6)	1999-2006	3.4/2.1	2.1/2.1	0.4/0.8	23/43
Wisconsin R. (36.5)	1999-2006	15.4/9.7	8.3/6.2	1.4/1.8	17/21
Wisconsin R. (43.1)	1999-2006	10.0/5.1	7.0/3.1	1.8/1.2	26/21
Wisconsin R. (45.5)	1999-2006	16.3/6.5	9.1/3.4	2.3/1.8	25/20
Wisconsin R. (50.2)	1999-2006	4.0/2.6	2.6/2.5	0.6/0.9	12/16
Wisconsin R. (67.9)	1999-2006	3.8/1.5	2.6/1.3	0.9/0.8	32/35
Wisconsin R. (75.9)	1999-2006	5.5/3.2	3.6/2.2	1.5/1.4	38/26
Wisconsin R. (89.9)	1999-2006	17.3/7.1	8.5/5.0	1.5/1.2	17/9
Mean		8.1/4.1	4.7/2.8	1.2/1.1	26/26
Northern non-wadable fine-substrate rivers (NTV dataset)					
St. Croix River	1996-1998	4.7/0.6	1.3/0.6	0/0	0/0
Northern non-wadable coarse-substrate rivers (NTV dataset)					
Menominee R. (QF2)	1996-1998	15.3/19.7	3.7/2.5	0.7/1.2	11/19
Menominee R. (SF1)	1996-1998	10.7/5.0	8.0/6.6	3.3/3.1	32/29
Menominee R. (SF2)	1996-1998	19.3/8.0	16.7/7.5	2.7/1.5	16/7
Mean		15.1/10.9	9.5/5.5	2.2/1.9	20/18

WSV and NSV datasets, rounded up to the nearest whole number per mile, determine the criteria and are considered acceptable ranges for smallmouth bass populations. For the wadable nursery class, criteria are not provided for the 8-inch CPE, 14-inch CPE, and RSD population metrics. By definition, nursery streams do not have the capability to support a fully developed population of adult (i.e., 8 inches or larger) smallmouth bass, so it is inappropriate to assess such streams based on adult abundance and size structure. Similarly, for the wadable coolwater classes, criteria are not provided for the 14-inch CPE and RSD metrics, as coolwater streams inherently tend to lack larger adults, and for the age-1 CPE metric, as reproduction is often limited by cold water temperatures.

DISCUSSION

The committee has developed an easily understood and applied classification of

Table 4 Number of pairs of sites (impact site plus control site) necessary in a Before-After-Control-Impact (BACI) experimental design with one before sample and one after sample in order to detect a relative change in smallmouth bass population CPE (number/mile) and RSD (%) of either 50% or 100% with a 5% chance of Type I error and a 20% chance of Type II error for different stream classes. A "" indicates that a calculation was not possible because of division by zero.

Stream class	Population metric	Number of pairs to detect change of 50%	100%	200%
Southern wadable warmwater	Age-1 CPE	36	8	3
	8-inch CPE	22	6	3
	14-inch CPE	68	17	4
	RSD	35	9	5
Southern wadable nursery	Age-1 CPE	59	15	5
	8-inch CPE	29	8	3
	14-inch CPE	106	27	8
	RSD	106	27	8
Southern non-wadable fine-substrate	Age-1 CPE	27	8	3
	8-inch CPE	38	10	4
	14-inch CPE	89	22	10
	RSD	106	27	8
Northern non-wadable fine-substrate	Age-1 CPE	4	3	2
	8-inch CPE	23	7	3
	14-inch CPE	--	--	--
	RSD	--	--	--
Northern non-wadable coarse-substrate	Age-1 CPE	55	14	5
	8-inch CPE	36	10	4
	14-inch CPE	79	20	6
	RSD	86	22	6

Wisconsin's smallmouth bass streams and rivers that accurately accounts for much of the inherent variation in smallmouth bass populations and fishery potential among waters. Stream size, summer temperature, and location in the state are the three environmental factors that define classes. Statistical analyses of catch data from least-impacted streams and rivers indicate that each class is capable of supporting a significantly different abundance and size/age distribution of smallmouth bass. These differences are inherent to the class, and not the result of human impacts such as pollution, habitat modifications, or over-harvest. Because the classification is based on environmental factors that are known or have been modeled for all waters in the state, the process of classifying all flowing waters in the state is practical and straightforward.

Because the classification is based on inherent "natural" factors, data from the least-impacted sites can be used to estimate the expected abundances and size distributions of smallmouth bass in each class. These estimates (Table 5) provide an objective framework for determining if a particular stream or river is meeting its potential. Field data on smallmouth bass CPE and RSD from a stream in a particular class can be compared with the appropriate expectations to determine the current status of the smallmouth bass population. Significant negative deviations from these expectations indicate where smallmouth bass populations are below their potential and in need of restoration. Further investigations of other factors, including watershed and riparian land use, can help explain why the population is below expectations and suggest management

Table 5. Population criteria for assessing smallmouth bass in Wisconsin's streams and rivers. CPE is expressed as number per mile and RSD as %. NA= not applicable.

Stream class	Population metric	Assessment criteria		
		Below	Acceptable	Exceptional
Non-wadable rivers				
Northern fine substrate	Age-1 CPE	<2	2-5	>5
	8-inch CPE	<1	1-2	>2
	14-inch CPE	<1	1	>1
	RSD	<20	20-40	>40
Northern coarse substrate	Age-1 CPE	<2	2-9	>9
	8-inch CPE	<1	1-5	>5
	14-inch CPE	<1	1	>1
	RSD	<20	20-33	>33
Southern fine substrate	Age-1 CPE	<3	3-9	>9
	8-inch CPE	<2	2-6	>6
	14-inch CPE	<1	1-2	>2
	RSD	<20	20-60	>60
Southern coarse substrate	Age-1 CPE	<10	10-32	>32
	8-inch CPE	<5	5-9	>9
	14-inch CPE	<2	2-3	>3
	RSD	<30	30-50	>50
Wadable streams and rivers				
N. wadable warmwater	Age-1 CPE	<20	20-90	>90
	8-inch CPE	<7	7-50	>50
	14-inch CPE	<1	1-3	>3
	RSD	<5	5-50	>50
N. wadable coolwater	Age-1 CPE	NA	NA	NA
	8-inch CPE	<2	2-4	>4
	14-inch CPE	NA	NA	NA
	RSD	NA	NA	NA
Northern wadable nursery	Age-1 CPE	<2	2-4	>4
	8-inch CPE	NA	NA	NA
	14-inch CPE	NA	NA	NA
	RSD	NA	NA	NA
S. wadable warmwater	Age-1 CPE	<25	25-110	>110
	8-inch CPE	<8	8-50	>50
	14-inch CPE	<2	2-4	>4
	RSD	<5	5-50	>50
S. wadable coolwater	Age-1 CPE	NA	NA	NA
	8-inch CPE	<2	2-4	>4
	14-inch CPE	NA	NA	NA
	RSD	NA	NA	NA
Southern wadable nursery	Age-1 CPE	<30	30-60	>60
	8-inch CPE	NA	NA	NA
	14-inch CPE	NA	NA	NA
	RSD	NA	NA	NA

strategies for restoration. Streams with CPE and RSD well above expectations represent unusually good smallmouth bass populations that may warrant special protection efforts.

Our results emphasize that smallmouth bass are a low-density species in Wisconsin's streams and rivers. Most CPE values are below 10 fish/mile, which is 50-100 times lower than CPE values for trout in least-impacted Wisconsin streams (J.

Lyons, WDNR Fish Research, unpublished data). Smallmouth bass larger than 14 inches are particularly scarce, with the majority of streams and rivers having fewer than two fish of this size per mile. Low densities require that sampling distances be relatively long, much greater than lengths necessary to assess the overall fish community, if an adequate number of smallmouth bass are to be collected for age and length analyses. Low densities also imply that smallmouth bass may be particularly vulnerable to over-harvest in many settings, and that restrictive angling regulations are necessary.

Despite inherently low densities of smallmouth bass in streams and rivers, the classification and sampling recommendations developed here allow an objective evaluation of the response of smallmouth bass populations to human impacts and management activities. Because the CPE and RSD data are relatively imprecise (standard deviation typically 50-100% of mean value; Table 3), if the goal is to detect relatively subtle population responses, then large sample sizes are required, on the order of 50-200 sites (25-100 pairs) each sampled before and after the impact or activity of interest. Clearly such a sampling effort would require a large-scale coordinated project involving many different biologists. However, to detect more dramatic population responses, the sample sizes are much lower, on the order of 10-20 sites, a level of effort realistic for a single biologist to carry out. Because large smallmouth bass are less common and relatively more variable in abundance than smaller bass, efforts to evaluate management activities that focus on larger bass, such as trophy regulations, will require greater sampling effort than more general population evaluations.

ACKNOWLEDGEMENTS

Committee members Al Niebur, Dave Seibel, Bradd Sims, Dave Vetrano, Doug Welch, John Lyons, and Karl Scheidegger should be commended for their efforts. In particular, the team is indebted to John Lyons for his smallmouth bass and statistical analyses expertise. Paul Rasmussen and Brian Weigel provided many fruitful discussions and helpful comments on analyses and conclusions.

REFERENCES

Anderson, R. O., and R. M. Neumann. 1996. Length, weight, and associated structural indices. Pages 447-482 in B. R. Murphy and D. W. Willis, editors. Fisheries techniques (2nd edition). American Fisheries Society, Bethesda, Maryland.

Forbes, A. M. 1985. Summary of survey data for smallmouth bass in Wisconsin streams, 1952-80. Wisconsin Department of Natural Resources Research Report 133.

Forbes, A. M. 1989. Population dynamics of smallmouth bass (Micropterus dolomieui) in the Galena (Fever) River and one of its tributaries. Wisconsin Department of Natural Resources Technical Bulletin 165.

Kanehl, P. D., J. Lyons, and J. E. Nelson. 1997. Changes in the habitat and fish community of the Milwaukee River, Wisconsin, following removal of the Woolen Mills Dam. North American Journal of Fisheries Management 17:387-400.

Lyons, J. 1989. Correspondence between the distribution of fish assemblages in Wisconsin streams and Omernik's ecoregions. American Midland Naturalist 122:163-182.

Lyons, J. 1991. Predicting smallmouth bass presence/absence and abundance in Wisconsin streams using physical habitat characteristics. pp. 96-103 In Jackson, D. C., editor. Proceedings of the First

International Smallmouth Bass Symposium. Mississippi State University, Mississippi State.

Lyons, J. 1992. The length of stream to sample with a towed electrofishing unit when fish species richness is estimated. North American Journal of Fisheries Management 12:198-203.

Lyons, J. 1996. Patterns in the composition of fish assemblages among Wisconsin streams. Environmental Biology of Fishes 45:329-341.

Lyons, J. 1997. Influence of winter starvation on the distribution of smallmouth bass among Wisconsin streams: a bioenergetics modeling assessment. Transactions of the American Fisheries Society 126:157-162.

Lyons, J. 2004. Study SSDM: Effects of flow regulation and restriction of passage due to hydroelectric project operation on the structure of fish and invertebrate communities in Wisconsin's large river systems. Final Report: Phase II: Menominee River. Fish and habitat conditions prior to flow regulation changes. Wisconsin Department of Natural Resources, Federal Aid in Sport Fish Restoration Project WI F-95-P, Study SSDM, Final Report.

Lyons, J. 2005a. Longitudinal and lateral patterns of fish species composition and biotic integrity in the lower Wolf River, Wisconsin, a relatively undegraded floodplain river. Journal of Freshwater Ecology 20:47-58.

Lyons, J. 2005b. Fish assemblage structure, composition, and biotic integrity of the Wisconsin River. Pages 345-363 in R. Calamusso, R. Hughes, and J. Rinne, editors. Historical changes in large river fish assemblages of North America. American Fisheries Society Symposium Number 45, Bethesda, Maryland.

Lyons, J. 2006a. Status and trends in sportfish populations of southwestern Wisconsin warmwater streams. Wisconsin Department of Natural Resources, Bureau of Integrated Science Services, Fisheries and Habitat Research Section, Annual Performance Report for Federal Aid in Sportfish Restoration, Project F-95-P, Study SSOU.

Lyons, J. 2006b. Status and trends in the fish community of the lower Wisconsin River. Wisconsin Department of Natural Resources, Bureau of Integrated Science Services, Fisheries and Habitat Research Section, Annual Performance Report for Federal Aid in Sportfish Restoration, Project F-95-P, Study SSOV.

Lyons, J., P. A. Cochran, and D. Fago. 2000. Wisconsin fishes 2000. Status and distribution. University of Wisconsin Sea Grant, Madison.

Lyons, J., A. M. Forbes, and M. D. Staggs. 1988. Fish species assemblages in southwestern Wisconsin streams, with implications for smallmouth bass management. Wisconsin Department of Natural Resources Technical Bulletin No. 161.

Lyons, J., and P. D. Kanehl. 1993. A comparison of four electroshocking procedures for assessing the abundance of smallmouth bass in Wisconsin streams. U.S. Forest Service, North Central Forest Experiment Station, St. Paul, Minnesota, General Technical Report NC-159.

Lyons, J., and P. Kanehl. 2002. Seasonal movements of smallmouth bass in streams. Pages 149-160 in M. Ridgway and D. P. Philipp, editors. Black bass: ecology, conservation, and management. American Fisheries Society Symposium Number 31, Bethesda, Maryland.

Lyons, J., P. D. Kanehl, and D. M. Day. 1996. Evaluation of a 356-mm minimum-length limit for smallmouth bass in Wisconsin streams. North American Journal of Fisheries Management 16:952-957.

Lyons, J., and M. Mitro. 2006. Development and evaluation of watershed models for predicting stream fishery potential. Wisconsin Department of Natural Resources, Bureau of Integrated Science Services, Fisheries and Habitat Research Section, Annual Performance Report for Federal Aid in Sportfish Restoration, Project F-95-P, Study SSMP.

Lyons, J., R. R. Piette, and K. W. Niermeyer. 2001. Development, validation, and application of a fish-based index of biotic integrity for Wisconsin's large warmwater rivers. Transactions of the American Fisheries Society 130:1077-1094.

Lyons, J., L. Wang, and T. D. Simonson. 1996. Development and validation of an index of biotic integrity for coldwater streams in Wisconsin. North American Journal of Fisheries Management 16:241-256.

Mason, J. W., R. A. Kerr, and J. Lyons. 1993. Smallmouth bass populations. pp. 56-66 In D. J. Gracyzk, editor. Surface-water hydrology and quality, and macroinvertebrate and smallmouth bass populations in four stream basins in southwestern Wisconsin, 1987-1990. U.S. Geological Survey, Water Resources Investigations Report 93-4024.

Osenberg, C. W., R. J. Schmitt, S. J. Holbrook, K. E. Abu-Saba, and A. R. Flegal. 1994. Detection of environmental impacts: natural variability, effect size, and power analysis. Ecological Applications 4:16-30.

Simonson, T. D., and J. Lyons. 1995. Comparison of catch per effort and removal procedures for sampling stream fish assemblages. North American Journal of Fisheries Management 15:419-427.

Underwood, A. J. 1994. On beyond BACI: sampling designs that might reliably detect environmental disturbances. Ecological Applications 4:3-15.

Wang, L., and J. Lyons. 2002. Fish and benthic macroinvertebrate assemblages as indicators of stream degradation in urbanizing watersheds. Pages 227-249 in T. P. Simon, editor. Biological response signatures: Multimetric index patterns for assessment of freshwater aquatic assemblages. CRC Press, Boca Raton, Florida.

Wang, L., J. Lyons, P. Kanehl, and R. Gatti. 1997. Influences of watershed land use on habitat quality and biotic integrity in Wisconsin streams. Fisheries 22(6):6-12.

Wang, L., J. Lyons, P. Rasmussen, P. Seelbach, T. Simon, M. Wiley, P. Kanehl, E. Baker, S. Niemala, and P. M. Stewart. 2003. Watershed, reach, and riparian influences on stream fish assemblages in the Northern Lakes and Forest Ecoregion, USA. Canadian Journal of Fisheries and Aquatic Sciences 60:491-505.

Weigel, B. M., J. Lyons, and P. W. Rasmussen. 2006a. Fish assemblages and biotic integrity of a highly modified floodplain river, the Upper Mississippi, and a large relatively unimpacted tributary, the lower Wisconsin. River Research and Applications 22:923-936.

Weigel, B. M., J. Lyons, P. W. Rasmussen, and L. Wang. 2006b. Relative influence of environmental variables at multiple spatial scales on fishes in Wisconsin's warmwater rivers. Pages 493-511 in R. M. Hughes, L. Wang, and P. W. Seelbach, editors. Influences of landscapes on stream habitats and biological assemblages. American Fisheries Society Symposium Number 48, Bethesda, Maryland.

WDNR (Wisconsin Department of Natural Resources). 1968. Smallmouth bass streams in Wisconsin. Wisconsin Department of Natural Resources, Division of Conservation, Publication 224-68.

APPENDIX 1

STREAM AND RIVER TYPES

Wisconsin streams and rivers that support significant smallmouth bass populations can be classified into the following five types:

1) Wadable warmwater A wadable stream with habitat suitable for a fishable population of adult smallmouth bass, including legal-sized (≥ 354 mm (14") fish. These streams are typically 4th to 6th order with watershed areas of 50-500 square miles (usually less than 350) and mean widths of 11-50 m (35-175 ft). Deeper pools often exceed 1.25 m (4 ft) and may have areas that are un-wadable, but at


Black River, Clark Co.

summer baseflow more than 67% of the surface area of pools and deep runs can be sampled effectively by wading. Maximum instantaneous summer water temperatures usually exceed 28 C (82.5 F), and maximum daily mean temperatures are 24-30 C (75-86 F). Stream gradient is high enough, usually more than 0.8 m/km (4.2 ft/mile), to produce at least some riffles or shallow rocky runs.

Wadable warmwater streams are common and widespread throughout much of the southern two-thirds of the state, particularly the extreme southwest. They


Jump River, Rusk Co.

also occur in the northern third, although there coolwater streams (see below) are more common. In regions that are particularly flat with fine-textured soils, many otherwise suitable warmwater streams may lack sufficient gradient or rocky substrate to produce good smallmouth bass habitat.

Examples of wadable warmwater smallmouth bass streams: The Galena River in Lafayette County, the Little Platte


Otter Creek, Lafayette Co.

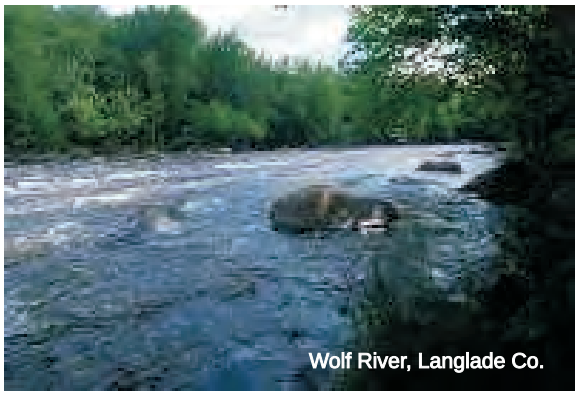
River and Rattlesnake Creek in Grant County, the Milwaukee River in Ozaukee and Washington counties, the Sheboygan River in Sheboygan County, the Black River in Clark County, the Big Rib River in

Marathon County, the Jump River in Rusk and Taylor counties, and the upper South Fork of the Flambeau River (above Fifield) in Price County.

2) Wadable coolwater A wadable stream with habitat suitable for smallmouth bass but summer water temperatures too cold to support a population as large as in a comparable warmwater stream. Maximum instantaneous summer water temperatures


Pecatonica River, Lafayette Co.

rarely exceed 26 C (79 F) and maximum daily mean temperatures are usually 20-25 C (70-77 F). Coolwater streams have a fishable population of adults ($\geq$ age 3, $\geq$ 204 (8") TL), at least during summer months, but they typically have relatively few legal-sized fish, perhaps because of slow growth.


Wolf River, Langlade Co.

Wadable coolwater streams are widespread, especially in the northern third of the state, and vary in their smallmouth bass characteristics. Some coolwater streams may have a permanent resident population and natural reproduction, whereas others have smallmouth bass only because of seasonal movements from nearby warmwater streams or lakes. Coolwater streams often comprise the lower reaches of trout streams, and trout and smallmouth bass may overlap in occurrence.

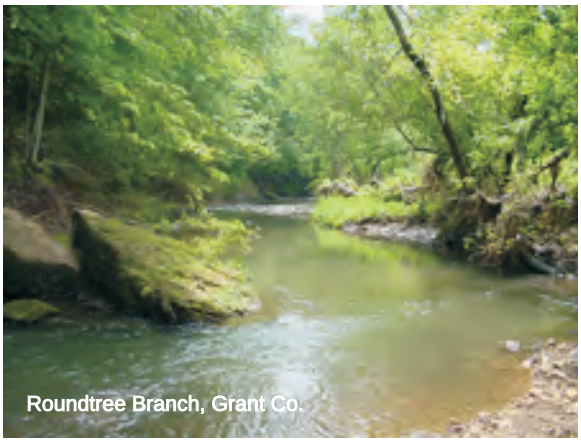
Examples of wadable coolwater smallmouth bass streams: The upper Grant


Namekagon River, Washburn Co.

River (above CTH A) in Grant County, the Wolf River in Langlade County, the Red River in Shawano County, the Namekagon River in Sawyer and Washburn counties, and the Brule River in Florence County.

3) Wadable nursery A warmwater stream too small and/or shallow to support a fishable population of adults, but capable of holding a significant number of juveniles. Adults may use the stream during the spawning period, but are otherwise scarce or absent, and their abundance does not necessarily indicate the quality of the stream. Most nursery streams are large 2nd order, 3rd order, and perhaps smaller 4th

order, with watershed areas of 20-50 square miles. Mean width is usually less than 13 m (40 ft) and few if any pools have a


Roundtree Branch, Grant Co.

maximum depth of more than 1.25 m (4 ft). Gradient is usually greater than 1 m/km (5.3 ft/mile), and rocky riffles and shallow runs are common. A few much larger (4th and 5th order; 15-20 m (50-70 ft) wide) but consistently shallow and relatively high gradient streams may also be categorized as nursery streams. Invariably a nursery stream is the headwaters or a tributary of a larger stream or lake with a large smallmouth bass population.

Most wadable nursery streams occur in extreme southwestern Wisconsin, where small high-gradient warmwater streams


Pensaukee River, Oconto Co.

are common. Only a few occur elsewhere in the state, probably because most small streams are either too low gradient or have summer water temperatures too low to provide good smallmouth bass habitat.

Nursery streams in southwestern Wisconsin include: Pats Creek, Lafayette County, Boice Creek and Pigeon Creek, Grant County, and Mineral Point Branch, Iowa County. Examples from elsewhere are Wedges Creek (above Hwy 10) in Clark County, and the Branch River in Manitowoc County. A relatively large (5th order; 16+ m (53 ft) wide) but shallow (95% of stream surface area less than 1-m deep at summer baseflow) stream that qualifies as nursery water is the Pensaukee River in Oconto County, which empties into Green Bay.

4) Non-wadable coarse-substrate A warmwater river with significant amounts of natural rubble/cobble and boulder


Wisconsin River, Marathon Co.

substrate. More than 10% and often more than 25% of the river surface area consists of habitats where the majority of the substrate is rubble/cobble or boulder (riffles or rapids and shallow runs). Typically these rivers have a largely upland riparian area and a relatively high gradient for a non-wadable river, usually above 0.6 m/km (3.1 ft/mile). They tend to be on the

smaller end of the spectrum for non-wadable rivers, 4th-7th order with drainage areas of 300-3,000 square miles and mean widths of 30-100 m (100-330 ft). Coarse-substrate rivers typically shift to fine-substrate rivers (see below) once they exceed a certain size.

Coarse-substrate rivers occur throughout the state, but because of topography and geology are most common in the northern two-thirds. Examples (impounded reaches excluded) include the LaCrosse River just below Lake Neshonoc in LaCrosse County, the Embarrass River in Outagamie County, the Menominee River above Chalk Hill Reservoir in Marinette and Florence counties, the Wisconsin River in Marathon, Lincoln, and Oneida counties, the Flambeau River in Price, Sawyer, and Rusk counties, the Chippewa River in Sawyer and Rusk counties, and the St. Croix River in Douglas and Burnett counties.

5) Non-wadable fine-substrate A warmwater river dominated by natural


Wisconsin River, Columbia Co.

substrates of gravel, sand, silt, and/or clay. Less than 10% of the river surface area consists of habitats where the majority of the substrate is rubble/cobble or boulder, although these uncommon habitats are important for smallmouth bass spawning and recruitment. Fine-substrate rivers often have relatively wide and flat floodplains with predominantly lowland or wetland riparian areas. Gradients are less


Chippewa River, Eau Claire Co.

than 0.6 m/km (3.1 ft/mile) and are often only 0.2 m/km (1 ft/mile). Sizes vary greatly and include the largest rivers in the state, ranging from 4th-9th order with drainage areas of 300-30,000 square miles and mean widths of 30-800 m (100-2600 ft). Because large rocky substrate is uncommon in these rivers, fallen trees along the banks and large woody debris in the channel are the most important natural smallmouth bass habitats.

Fine-substrate rivers occur throughout the state but because of topography and geology are rare in the north. Examples (impounded reaches excluded) include the Rock River in Rock County, the Wisconsin River, in Juneau, Adams, Sauk, Columbia, Dane, Iowa, Richland, Crawford, and Grant counties, the Yellow River in Juneau County, the Black River in Jackson County, the Fox River in Winnebago County, the Chippewa River in Eau Claire, Dunn, Pepin, and Buffalo counties, the St. Croix River in Polk and St. Croix counties, the Wolf River in

Appendix B.1

Wisconsin Department of Natural Resources

Guidelines for Collecting Macroinvertebrate Samples from Wadable Streams

State of Wisconsin
Department of Natural Resources

Guidelines for Collecting Macroinvertebrate Samples from Wadable Streams

September 2000

Bureau of Fisheries Management and Habitat Protection
Monitoring and Data Assessment Section
101 S. Webster St.
Madison, WI 53707

OBJECTIVES AND DESIGN OF MACROINVERTEBRATE SAMPLING

Data derived from aquatic macroinvertebrate samples provide valuable information on the biological and physical condition of streams, which along with stream habitat and fish community data permits a comprehensive assessment of stream health. Most aquatic macroinvertebrates such as immature insects live for 1 or more years in streams, integrating the effects of environmental stressors over time. Since the majority of aquatic invertebrates have limited mobility (relative to fish), they can be good indicators of local conditions as well as upstream land and water resource influences.

Various metrics and indices are used to interpret macroinvertebrate sample data. Hilsenhoff's Biotic Index (HBI) has been used extensively by the Department as an indicator of reduced dissolved oxygen concentrations in streams resulting from organic pollution. In streams where oxygen stress may not be a problem (e.g. high gradient streams with significant groundwater discharge), other metrics can be applied that assess the impacts of riparian habitat degradation, sedimentation, scouring, etc. 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 taxa.

General Sampling Procedures:

Time of year, sampling site selection, and sampling technique are important factors to be considered when collecting macroinvertebrate samples.

In general:

1. Collect samples in the spring (March – May) or in the fall (September – November).
2. Collect samples in riffles (shallow areas where water breaks over rocks).
3. Sample where the stream flow velocity is at least 0.3 meters per second.
4. Sample substrate composed of coarse gravel to larger rubble ($<$ 0.3 meters diameter).
5. Sample similar environments and standardize protocols (points 1 – 4) at all assessment sites if possible, particularly if comparisons are being made between or among sites, or at sites over time.
6. In the absence of riffles, vegetation caught in logjams, snags, or vegetation overhanging from the stream banks can be sampled. Investigators should be aware that direct comparisons between samples collected from different habitats is undesirable.
7. For baseline monitoring, one sample per stream assessment reach is sufficient. If the macroinvertebrate data will be used in impact assessments or enforcement cases, replicate sampling should be considered. Additional samples can be collected and processed later if additional data is needed.

Equipment:

Net mesh size strongly influences the types and proportions of macroinvertebrate taxa collected. A 600-micron mesh net should be used for collecting baseline macroinvertebrate samples for the Department. Net frames are available in various sizes and shapes. Rectangular or D-shaped net frames are good choices. Smaller triangular-shaped net frames are also available, but their relatively small size miss many of the invertebrates dislodged from the substrate when kick sampling.

METHODS / HANDLING / PRESERVATION

Sampling Methods:

Sample with a kick net by holding the net frame firmly against the stream bottom and disturbing the substrate upstream (approximately a full arm's length) from the net with your feet. Dig deeply into the substrate with the heel or toe to dislodge macroinvertebrates from the streambed. Avoid kicking course debris into the net (let the macroinvertebrates wash downstream into the net). Make sure that the plume of silt that results from disturbing the substrate is flowing into the net, as this plume also contains the dislodged invertebrates.

In streams that lack riffles, vegetation (twigs, leaves, grass) caught in instream snags or logjams, or riparian vegetation overhanging into the stream can be sampled. Sample by jabbing the net into the vegetation to dislodge the clinging invertebrates. The net should be inspected often to make sure the invertebrates that are being dislodged are washing into the net. If two people are collecting the sample, one person can hold the net while the other manually removes vegetation from the snag or logjam and rinses it into the net, or shakes the snags to loosen the vegetation caught so it drifts into the net. Course debris should be removed from the net, while making sure to rinse the macroinvertebrates that are clinging to the vegetation back into the net.

Sampling Effort, Number of Macroinvertebrates / Sample Volume:

More than 100 macroinvertebrates should be collected per sample. A quantity of debris about the size of a softball should contain over 100 macroinvertebrates, and such a sample should take approximately 3 minutes or less to collect. Inspect the net contents to insure that more than 100 macroinvertebrates have been collected. If it is determined that insufficient numbers of macroinvertebrates are captured after the initial sampling effort, sampling should be extended for a second period of equal duration and noted on the field sheet. If an insufficient number of invertebrates are captured upon completion of the second sampling effort, stop collecting and preserve the sample. Low numbers of organisms may be indicative of water quality or habitat problems and should also be noted on the field sheet.

Sample Handling and Preservation:

After the sample is collected, remove large sticks, rocks, and undecomposed leaves from the net after rinsing any clinging macroinvertebrates back into the net. Rinse fine sediment from the net by forcefully swishing the net through the water a few times, being careful not to lose the organisms captured. Removing fine sediment from the net makes lab picking of the sample easier and helps insure adequate preservation of the sample.

Transfer the fine debris and macroinvertebrates into a tightly sealing plastic or glass wide-mouth jar of sufficient size. Inspect the net and transfer clinging macroinvertebrates into the sample jar. The sample debris should occupy less than 1/2 the sample jar's volume. Make sure that the container is properly labeled, with both an internal and external label. Labels should have at least the following information: **sample ID number**, **replicate number**, **waterbody name**, **collector's name** and a **split-sample designation** if needed. If a single sample's contents has to be placed in 2 separate containers

due to large sample quantity, label the container accordingly, e.g. container 1 of 2, sample 19990510-16-05. The internal label should consist of bond paper written in pencil.

Initially preserve the sample with 80-85% alcohol (isopropyl or ethanol). Higher concentrations of alcohol make some invertebrates brittle, making taxonomic identification more difficult or impossible. Fill the sample jar to the top with the alcohol solution. Be sure to tightly seal the sample container and gently invert the container several times to thoroughly mix the sample and preservative. Within 24 hours pour-off the alcohol solution and refill with fresh 80-85% alcohol. Samples containing large amounts of filamentous algae or other organic materials should be preserved and re-preserved several times. Poorly preserved samples are unpleasant to analyze, and decayed organisms make taxonomic identifications difficult or impossible.

ADVANTAGES / DISADVANTAGES OF SPRING OR FALL SAMPLING

Spring sampling:

Advantages Spring samples have more mature insect larvae and nymphs, making identification easier.

Disadvantages Spring samples are more likely to be affected by the catastrophic effects of spring floods. Spring samples are also less likely to reflect localized impacts resulting from organic enrichment (e.g. barnyard runoff) because intolerant macroinvertebrates can recolonize and inhabit impact areas as long as colder water temperatures maintain favorable D.O. concentrations.

Fall sampling:

Advantages Fall samples are more likely to detect the extent of organic enrichment (low D.O.) problems. Fall samples more closely follow the summer and fall stresses of warm water temperatures, low-flows, and low-D.O. associated with point source and nonpoint source pollution sources, while limiting the time that may elapse in which favorable conditions have existed for the imigration of intolerant taxa into impacted areas.

Disadvantages Small or immature larvae and nymphs of some insect taxa make identification to genus or species level difficult or impossible; however, in most cases insects collected in September and October should be identifiable by a qualified taxonomist.

SITE DOCUMENTATION

Macroinvertebrate Field Data Report (Form 3200-081)

This form is used to record the sampling location identifiers, sample and site descriptions, stream and watershed descriptors, and provides special instructions to the laboratory for analyzing or retaining the sample. **One Macroinvertebrate Field Data Report form should be filled-out for each sampling location**. Fields written in **bold** font are mandatory information.

***Location Identifiers*----------**

Waterbody Name The name of the waterbody as shown on the most recent U.S.Geological Survey (USGS) 7.5' topographic quadrangle map. The name reported should be identical to that used for other data sheets that may have been filled-out for habitat or fisheries assessments. Make sure that the waterbody name is identical on all other macroinvertebrate field data report forms filled-out for all other sampling sites on the waterbody.

Waterbody ID Code A unique seven-digit number that identifies each stream; all streams, rivers, and lakes in Wisconsin have an assigned number. These numbers are available on the WDNR Intranet, under the listing for "DNR Tabular Database Service" for the WDNR Register of Waterbodies (ROW). As with stream name, waterbody ID code should be the same for all stations on a stream.

Site Mile The reporting of this parameter is optional. The distance along the stream channel from the mouth of the stream to the sampling site. This distance is a useful shorthand for indicating and identifying the location of the station. Site mile should be measured on the most recent USGS 7.5' topographic map to the nearest 0.1 mile using a map measurer (map wheel).

Sample ID Record a sample identification number using an 8-digit format for the date the sample was collected; the 2-digit county code (e.g. Adams County is 01, and Wood County is 72); and a 2-digit field number that indicates the sample site number for the stream (YYYYMMDD-CY-FD). For example, if 1 site is sampled on a stream that date, the field number is 01 even if multiple (replicate) samples are collected at the site; if a second site is sampled on the same stream, or another stream is sampled by the collector on that date, the field number for the second sampling site is 02. County codes are found in Table 2.

Sampling Location A precise narrative description of the point on the stream where the macroinvertebrate sample(s) was collected. The description should include the exact distance and direction to the sample site from a "permanent" landmark such as a bridge, building, road marker, rock formation, etc. **Avoid using landmarks that might be lost in future years** (e.g., don't use tree or fence lines). Make the description as specific and precise as possible so that someone visiting the sampling site for the first time can easily locate the sample site.

Township, Range, Section, ¼ - ¼ Section, 1/4 Section Legal description for the Sampling Location within the Public Lands System. These can be determined from recent USGS 7.5' topographic maps or a detailed county map. On a topographic map, a "land locator" template is useful for determining the ¼ - ¼ and 1/4 Sections, which are indicated by a compass direction (NW, NE, SW, or SE). Note that in Wisconsin, all Townships are "N" (north), but Range can be either "E" or "W" (east or west). Make sure the appropriate letter is included for both Township and Range.

Latitude and Longitude It is important that geographic coordinates of the sampling site are recorded, along with the method used to determine latitude and longitude (e.g. USGS topo map, mapping software, plat map, global positioning system (GPS) units). If a map, mapping software, or a GPS unit are used, the geodetic datum upon which the coordinates are based (e.g. North American Datum 1983 (NAD 83)), should also be recorded. Datum for USGS topo maps are shown on the map legend.

STORET If water chemistry samples are collected at the macroinvertebrate sampling location, and processed through the Wisconsin State Laboratory of Hygiene, record the 6-digit STORET number assigned to the water chemistry sample.

7.5' Quad Map Name The name of the USGS 7.5' topographic quadrangle map on which the station is found.

Basin Name The name of the basin in which the stream is located.

Watershed Name The name of the watershed in which the stream is located.

County The name of the county in which the sampling site is located.

***Sample and Site Descriptors*----------**

Sample Collector The full names of the person(s) who collected the sample(s).

Project Name The name of the project for which the sample(s) were collected; e.g. baseline monitoring, basin assessment, nonpoint pollution investigation, etc.

Sampling Device Check which type of sampling device was used to collect the sample(s).

Habitat Sampled Check which type of habitat was sampled.

Total Sampling Time Record the number of minutes it took to collect each sample. Some collectors use a set amount of time to collect samples, this standardization technique may be valuable in making comparisons among sites or between sites over time, as differing numbers of macroinvertebrates collected for a given effort may be indicative of differing stream productivity or environmental stress.

Estimated Area Sampled (m^2) Record an estimate of the area of stream bottom disturbed to collect the sample(s). Like total sampling time, some collectors standardize the area of stream bottom disturbed as a way of comparing stream productivity or environmental stress among sites or at a site over time.

Number of Composites in Sample Record the number of discrete samples that are combined into 1 sample to form a composite sample. Macroinvertebrate taxa can have patchy distribution even within a single habitat such as a riffle. Some collectors sample multiple locations within a riffle or multiple stream habitats such as riffles, and overhanging vegetation, and combine into a single sample to get a representation of the entire riffle or stream macroinvertebrate community versus sampling a single location. The collector should be aware that the HBI was developed specifically for riffle samples, and that sampling snags, depositional or other non-riffle habitats may influence diversity measures or water quality indices like the HBI.

Replicate No. __ of __ Record the number of replicate samples collected at a sampling site. If no replicates were collected report 1 of 1.

Reason for Sampling Check the box that indicates why the sample was collected.

Water Color Record whether the water is Clear, Turbid due to suspended sediment, or Stained due to dissolved organic compounds.

Water Temperature If possible measure the water temperature in mid-channel, during the warmest part of the day to estimate maximum values.

Dissolved Oxygen (D.O.) The reporting of this parameter is optional. If reported, measure with a high-quality meter, which should be air-calibrated before every use. Follow the manufacturer's instructions for use and maintenance (e.g. the probe membrane and electrolyte should be replaced frequently during the field season). Record D.O. in milligrams per liter (parts per million).

Dissolved Oxygen % Saturation The reporting of this parameter is optional. If reported, measure with a high-quality meter, which calibrated before every use. Follow the manufacturer's instructions for use and maintenance.

pH (su) The reporting of this parameter is optional. If reported, measure with a high-quality meter, which is routinely calibrated. Follow the manufacturer's instructions for use and maintenance (e.g. the probe membrane and electrolyte should be replaced frequently during the field season).

Turbidity The reporting of this parameter is optional. If reported, measure with a high-quality meter, that is calibrated before every use. Follow the manufacturer's instructions for use and maintenance. Report turbidity in nephelometric turbidity units (NTUs).

Total Dissolved Solids The reporting of this parameter is optional. If reported, measure with a high-quality meter, that is calibrated before every use. Follow the manufacturer's instructions for use and maintenance. Report total dissolved solids in milligrams per liter (parts per million).

Conductivity (umhos/cm) The reporting of this parameter is optional. If reported, measure with a high-quality electronic meter. Most conductivity meters have built-in automatic temperature compensation to 25 °C (77 °F), but this should be confirmed before using the meter. On some older meters the temperature compensation must be set by hand, and on others, there is no compensation. For the latter meters, conductivity at 25 °C can be calculated using procedures outlined in "Standard Methods for the Analysis of Water and Wastewater", a book available at many WDNR offices. Whatever meter is used, it should be calibrated before every use. Report conductivity in umhos/cm.

Stream Order A qualitative measure of stream size, based on the amount of branching of the watershed upstream from the station, using Strahler's modification of Horton's original system. Generally, the higher the order, the larger the stream. Determine from USGS 7.5' topographic maps; usually requires multiple maps because the entire stream network upstream from the station must be examined. In making determinations, all "blue lines" (streams) on the maps, including intermittent streams, are included. The order system is as follows: All streams (including intermittent streams) from their source downstream to their first tributary are **First** order (stream order is "1" on data sheet). When two first order streams meet, the stream below this confluence is

Second order (stream order is "2"). When two second order streams meet, the stream below this confluence is **Third** order (stream order is "3"), and so on. When two streams of unequal order meet, the stream order below this confluence is equal to the higher of the two orders. For example, if a first and a third order stream meet, the stream below this confluence is third order. Stream order increases only when two streams of equal order meet.

Stream Gradient The overall decrease in elevation (on a per kilometer basis) of a 1 km stream reach within which the sample site is located. Determine from USGS 7.5' topographic maps, using a map wheel. First, find the sampling site on the map. Then find the first contour line that **crosses** the stream upstream of the sample site and the first contour line that **crosses** the stream downstream of the sample site. For low gradient streams this may require going to additional maps, covering many miles of stream, and possibly including other streams. With the map wheel, determine the distance along the stream channel between these two contour line crossings. Then determine the elevation drop between these two contour lines. The drop in elevation is equal to the total number of contour lines crossing the stream within the station (often zero), plus the contour line immediately above and the contour line immediately below the station, minus one, times the elevation drop between adjacent contour lines (Note: most topographic maps have 10 ft contours, but some have 20 ft contours; check the legend at the bottom of the map). Divide the elevation drop by the distance measured by the map wheel. This is the gradient for the station. Convert feet/mile to m/km by dividing by 5.3.

Estimated Stream Velocity (mps) Either an **estimated** or **measured** stream-flow velocity should be reported. Ideally, macroinvertebrate samples should be collected from stream sites with flow velocities of $\geq$0.3 meters per second. The estimated flow velocity categories are Slow: $<$0.15 m/s; Moderate: 0.15 m/s – 0.5 m/s, Fast: $>$0.5 m/s.

Measured Velocity (mps) Report the measured velocity of the stream in meters per second.

Average Stream Depth (m) Report the estimated average stream depth upstream of the riffle area sampled encompassing all habitat types present e.g. pools, riffles, and runs. If a habitat assessment was conducted at the site use the measured value from the habitat assessment.

Average Stream Width (m) Report the estimated average stream width upstream of the area sampled encompassing all habitat types such as pools, riffles, and runs. If a habitat assessment was conducted at the site use the measured value from the habitat assessment.

Composition of Substrate Sampled (Percent) Record the percent composition of the substrate where the macroinvertebrate sample(s) were collected, the total percentages should equal 100 percent.

Embeddedness of Substrate at Sample Site (%) Record the percent embeddedness of the substrate sampled. See: "Guidelines for Evaluating Habitat of Wadable Streams in Wisconsin" for guidance on quantifying substrate embeddedness.

Canopy Cover at Sample Site (%) Estimate the percent canopy that is shading the stream at the site where the macroinvertebrate sample(s) were collected (preferably using a concave forestry densiometer). Stream shading reduces instream periphytic growth, which can strongly influence the community of macroinvertebrate scrapers, and grazers that feed on periphyton.

***Stream and Watershed Descriptors*----------**

Categories of Local and Watershed-wide Biological, Physical, and Chemical, stressors that may be influencing the macroinvertebrate community, and Sources of Stream Impacts are listed. Local: land use or water resource factors that may be influencing water resource integrity within the stream reach from which the macroinvertebrate sample was collected. Watershed-wide: land use or water resource factors that may be influencing water resource integrity within the watershed **upstream** of the site where macroinvertebrate sample was collected. For each factor or Sources of Stream Impacts report whether it is certain that it is: (N) Not a problem; (U) Present, but uncertain as to the degree of impact; (P) Present, and probably creating a problem; or (Leave Blank) if uncertain to the potential impacts to the macroinvertebrate community.

Comments Any and all information that is relevant to the macroinvertebrate sample(s) should be recorded here. For example if it was difficult to collect over 100 organisms, or any other information that may be of importance when interpreting the taxonomic results. **If in doubt write it down**.

Special Instructions for Laboratory Provide specific instructions to the laboratory, if for example the lab should retain samples for enforcement cases, scan the entire sample for rare or endangered species, report non-HBI taxa, etc.

TABLE 1: Equipment used for collecting macroinvertebrates and equipment suppliers.

Item	Supplier
Macroinvertebrate Kick Net Used to collect macroinvertebrate samples.	
Turtox – Design, D - Frame Dip Net Stock # 425-A46 (**be sure to order 600 micron mesh size**)	Wildlife Supply Co. 301 Cass St. Saginaw, MI 48602-2097 517 / 799-8100 800 / 799-8301 http://www.wildco.com
Widemouth Sample Jars Used for sample storage.	
16 oz. Or larger plastic or glass widemouth jars	Fischer Scientific* 4500 Turnberry Dr. Hanover Park, IL 60103 800 / 766-7000 http://www.fischersci.com
Alcohol Used to preserve macroinvertebrate samples. Isopropyl or enthanol can be used.	State of Wisconsin Materials Distribution Service* 2102 Wright St. Madison, WI 53704 608 / 245-2910
Waterproof Paper Used for making copies of the macroinvertebrate field form.	J. L. Darling Co. 2212 Port of Tacoma Rd. Tacoma, WA 98421 206 / 383-1714 http://www.riteintherain.com

*The State of Wisconsin has a contract with Fischer Scientific and other vendors for substantial discounts on equipment and supplies purchases. To receive these discounts Regional WDNR staff should set-up an account with Fischer or other vendors by contacting their Region purchasing agent. Along with a discount on equipment and supplies, there are no shipping charges on regular or hazardous materials.

TABLE 2: County Codes:

Adams (01)
Ashland (02)
Barron (03)
Bayfield (04)
Brown (05)
Buffalo (06)
Burnett (07)
Calumet (08)
Chippewa (09)
Clark (10)
Columbia (11)
Crawford (12)
Dane (13)
Dodge (14)
Door (15)
Douglas (16)
Dunn (17)
Eau Claire (18)
Florence (19)
Fond Du Lac (20)
Forest (21)
Grant (22)
Green (23)
Green Lake (24)
Iowa (25)
Iron (26)
Jackson (27)
Jefferson (28)
Juneau (29)
Kenosha (30)
Kewaunee (31)
La Crosse (32)
Lafayette (33)
Langlade (34)
Lincoln (35)
Manitowoc (36)
Marathon (37)
Marinette (38)
Marquette (39)
Menomenee (40)
Milwaukee (41)
Monroe (42)
Oconto (43)
Oneida (44)
Outagamie (45)
Ozaukee (46)

Pepin (47)
Pierce (48)
Polk (49)
Portage (50)
Price (51)
Racine (52)
Richland (53)
Rock (54)
Rusk (55)
St. Croix (56)
Sauk (57)
Sawyer (58)
Shawano (59)
Sheboygan (60)
Taylor (61)
Trempealeau (62)
Vernon (63)
Vilas (64)
Walworth (65)
Washburn (66)
Washington (67)
Waukesha (68)
Waupaca (69)
Waushara (70)
Winnebago (71)
Wood (72)

Appendix B.2

Wisconsin Department of Natural Resources

Introduction to Standardized Collection and Assessment of Macroinvertebrates in Nonwadeable Rivers of Wisconsin

Introduction to Standardized Collection and Assessment of Macroinvertebrates in Nonwadeable Rivers of Wisconsin

Brian Weigel, Research Scientist
Fisheries and Aquatic Research, WDNR
2801 Progress Road
Madison, WI 53716

June 2011

Phone: (608) 221-6326
E-mail: brian.weigel@wisconsin.gov

This document introduces a standardized method for collecting and interpreting macroinvertebrate data from nonwadeable rivers. Most of this work is based upon the background research by Weigel and Dimick (2011) in which they conducted a standardized macroinvertebrate survey at 100 sites on 38 rivers across Wisconsin to construct, test, and apply an index of biotic integrity (IBI) as a bioassessment tool for rivers.

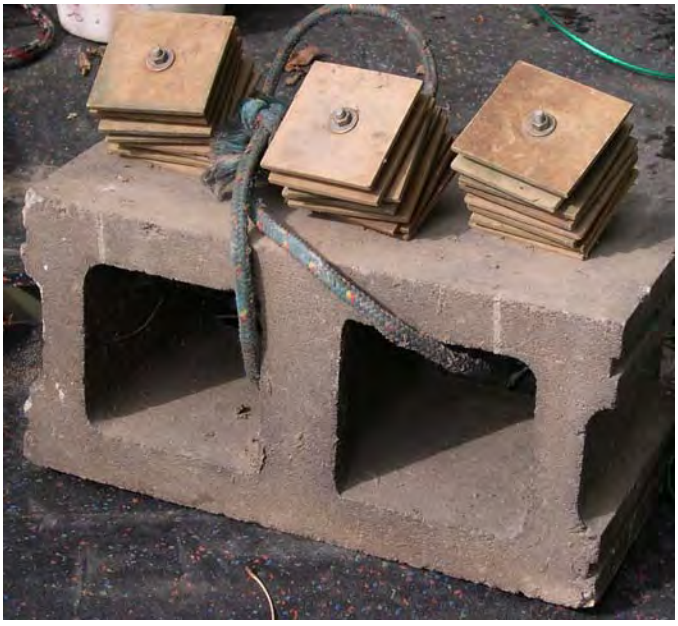
Two applications of the new macroinvertebrate IBI were identified (Weigel and Dimick 2011). The 1st use would be as a rapid bioassessment tool to assess the ecological condition of rivers. Currently Wisconsin uses only standardized fish-based bioassessment on large rivers despite strong evidence that routine assessment should incorporate more than one kind of biota. Adding a macroinvertebrate monitoring component promises to enhance detection of additional stressors, confirm appropriate condition assessment, repudiate inaccurate assessments, or at least lead to further investigation where fish and macroinvertebrate IBI results are ambiguous. A combination of the large river macroinvertebrate and fish IBIs will be used to fulfill Clean Water Act reporting requirements and determine impairment of a water body. Starting in 2011, WDNR Water Division includes river macroinvertebrate monitoring as part of the Tier 1 Monitoring Program (list in Appendix 1). This effort is coordinated to monitor 100 sites over a 5-year cycle, and it includes a trend component where 5 locations are sampled annually. The monitoring locations match those used for IBI development and validation because they represent the variety of rivers, and stressors acting upon those rivers, statewide.

The 2nd use anticipated for the macroinvertebrate IBI is as a tool for evaluating management and regulatory activities. The IBI would determine the efficacy of legislation or programs aimed at river ecosystem protection and restoration (e.g., nutrient criteria or broad-scale protective land management goals). In addition, the IBI could be used as a biocriterion and

would play an integral role in evaluating whether or not a management program achieved its intended restoration objectives for specific river reaches (e.g., post TMDL monitoring and ultimately for delisting 303(d) impaired waters). It is envisioned that this macroinvertebrate IBI is part of an overarching river monitoring program that includes a systematic assessment of macroinvertebrates, fish, and water chemistry among rivers across the region annually, with a trend component to detect temporal trends and responses to management activities.

Standardized Sampling Devices for Collecting River Macroinvertebrate Samples

Hester-Dendy (HD) artificial substrate samplers were selected as collection devices because they are uniformly applicable in a wide variety of rivers, including other habitats where other methods will not work (Ohio EPA 1987). The samplers are deployed and allowed to colonize for a 6-week duration between June and September. Sampler construction and deployment were based upon Ohio EPA (1987) protocols. Each sampler consisted of an eyebolt that held eight 7.6 × 7.6-cm (3 inch x 3 inch) plates made of 3.2-mm-thick (1/8 inch) masonite hardboard. Spacing between the plates was 3.2 mm (1/8 inch) between each of the first 3 plates, 6.4 mm (1/4 inch) between each of the next 3 plates, and 9.6 mm (3/8 inch) between the last 2 plates. I have used washers to acquire the appropriate spacing between the hardboard plates, but since washer thickness varies among washers it can be time consuming



to get accurate spacing. Others have used ½" square hardboard material for spacers, whereas others use bushings made of biologically inert plastic. Stainless steel hardware does not rust so it is easy to disassemble and reusable. HD samplers are available from several vendors (e.g., NKY Environmental at www.hesterdendy.com for $13 each), or you can make them yourself. I estimate one 4' x 8' hardboard sheet, after subtracting for all of the 1/8" saw cuts, yields 56 samplers. Three HD samplers constitute one sampling unit (i.e., for each site, 3 samplers are deployed and the inverts collected from those samplers are combined).

Sampler Deployment

Three HD samplers are fastened to an 18-kg (40-lb) cinder block and either set directly on rocky substrate or suspended from a snag to maintain 0.75 to 1.5 m of water above the sampler at low flow. Placement directly on fine sediment should be avoided where muck or shifting sand will bury the block and samplers. Sampler placement should be consistent with the recommended minimum velocity of 0.09 m/s (Ohio EPA 1987).

Attaching samplers to a cinder block can be accomplished by using zip ties or a strong twine (e.g., 250# net twine borrowed from a FM colleague). I prefer 3/8" x 24" zip ties for efficiency. A length of 3/8" poly-braid rope is tied so it does not contact any sampler. A float 4-6" in diameter can be tied on the rope for easier relocation. I also write my contact information on the float. Setting the float right at the water surface and flagging tape in a nearby tree make the sampler easier for you to find, but it also makes the sampler more obvious to the curious public. A good GPS for identifying sampler coordinates, detailed written description, photos, and hand-drawn map of the exact location can be extremely helpful in retrieving the samplers.

Sampler Retrieval

After 6-wk, retrieve the samplers, scrape off the organisms, combine the sample contents, preserve them in ethanol, and deliver to Aquatic Entomology Lab at UW-Stevens Point. Minimize disturbance and dislodging the invertebrates as the samplers are lifted to the surface. Without delay, set the block on the boat deck or shore, then quickly cut the HDs from the block and place the HDs in a pan. Remove from the pan any remaining rope or zip tie material along with their attached macroinvertebrates. Disassemble the HDs and retain all hardboard squares and hardware in the pan. A plastic putty knife works well to scrape the organisms from the squares, and then a squeeze bottle with water is helpful to spray any remaining animals from the squares or hardware. After scraping all of the squares and hardware into the pan, the contents can be washed into 1-gal container. The water needs to be poured out through a 500-micron mesh screen (e.g., a D-net bag works well). 70% ethanol should be added to preserve the contents, and the alcohol should be refreshed after ~1-d to maintain concentration. Just as with macroinvertebrate samples from wadeable streams, please contact UWSP AEL for sample processing.

Laboratory and Analytical Processing

The laboratory and database processing of macroinvertebrate data from large river samples is similar to that of wadeable streams with some exceptions. The same randomized grid-pan subsampling procedure is employed, but for river samples 500-individuals are targeted (compared to 100 for streams) and a large-rare individual search follows for up to 10 min to include uncommon taxa. The extra effort incurs 3x the lab cost of stream samples. All individuals subsampled are identified to the lowest practical taxonomic level, usually species.

The data are entered into the BugProgram and migrated to SWIMS where a river IBI score is calculated. The IBI is based upon 10 macroinvertebrate metrics that represent the assemblage structure, composition, and function (Table 1). Qualitative ratings are at 20-point increments where <20=very poor, 20-39=poor, 40-59=fair, 60-79=good, and 80-100=excellent.

Table 1. (Taken from Table 3, Weigel and Dimick (2011)). Final IBI metrics and scoring criteria (suffix T=taxa, I=individuals).

Metric	Scoring criteria and rating (points)		
	Poor (0)	Fair (5)	Good (10)
Insect-T	0–21	22–31	>31
Insect-%I	0–89%	90–95%	>95%
EPT-T	0–6	7–15	>15
Dom_3-%I	>66%	41–66%	0–40%
MPTV	>6.440	5.875–6.440	0.000–5.875
$IntolEPT_2$-%I	0%	0.1–3%	>3%
$TolChir_8$-%I	>16.0%	2.5–16.0%	0.0–2.4%
EcoFTN	0–8	9–12	>12
Gath-%I	>54%	16–54%	0–15%
Scr-%I	0.0%	0.1–7.4%	>7.4%

Literature Cited

Ohio EPA (Ohio Environmental Protection Agency). 1987. Biological criteria for the protection of aquatic life. Volume II: users manual for biological field assessment of Ohio surface waters. Ohio Environmental Protection Agency, Columbus, Ohio. (Available from: http://www.epa.state.oh.us/dsw/bioassess/BioCriteriaProtAqLife.aspx).

Weigel, B.M., and J.J. Dimick. 2011. Development, validation, and application of a macroinvertebrate-based index of biotic integrity for nonwadeable rivers of Wisconsin. Journal of the North American Benthological Society 30:665-679.

Quick Reference List

HD samplers

- 3 HD samplers constitute one sample.
- Each HD sampler consists of 8 hardboard squares, 3" x 3".
- Spacing: 1/8" between each of the first 3 squares, ¼" between each of the next 3 squares, and 3/8" between the last 2 squares. Use stainless steel washers, plastic bushings, or board pieces for appropriate spacing.
- Stainless steel hardware: 4" eyebolt with wingnut or locknut.
- Can be purchased retail for ~$13 ea. (e.g., NKY Environmental at www.hesterdendy.com).
- Can be made in bulk by WT staff. One 4' x 8' sheet of hardboard yields 56 HD samplers.

Sampler deployment

- Timeframe: colonize for 6-wk period sometime from mid-June through September.
- Use zip ties (3/8" x 24") or net twine to attach 3 HD samplers to a 40# cinder block.
- Attach 3/8" poly-braid rope to cinder block, avoiding contact with samplers.
- Attach float with contact info. Float should be placed to help you relocate the sampler but not so obvious that it beckons the curious public.
- Attach rope to a snag or rest block on streambed if substrate will not inundate the block or sampler.
- Locate to maintain 0.75 – 1.5 m water above sampler if possible.
- Water velocity at the samplers should be $\geq$ 0.09 m/s.
- Field sheet (Appendix 1): Sample ID number should correspond to the retrieval date. Necessary data include coordinates, water velocity, sampler location notes and map, substrate, and water depth at location. Potentially useful data includes water temperature, DO, conductivity, Secchi or turbidity, snags, and riparian information.
- Gear summary: 3 HDs, zip ties, cinder block, rope, float, utility knife, flagging tape, camera, flow meter w/staff to measure depth, GPS, field sheet, waders or boat, PFDs.

Sampler retrieval

- Locate sampler using written description, hand-drawn map, GPS coordinates, and flagging or float as applicable.
- Raise sampler to minimize dislodging the macroinvertebrates.
- Quickly set block on shore or boat deck, cut off HD samplers, and place samplers in pan.
- Disassemble samplers, retaining all hardware and plates in the pan. May need pliers, 7/16" wrench, or crescent wrench, and utility knife (to cut samplers from block).
- Scrape plates (top, bottom, and all 4 edges) using a plastic putty knife, pick or scrape the hardware, and wash all plates and hardware using water from a squeeze bottle.

- Wash contents from pan into a 1-gal container. Strain off water using D-net bag or similar 500 micron mesh netting. Fill the jar with 70% ETOH, and replace ETOH the next day.
- Retain hardware for reuse.
- Arrange for sample delivery to UWSP AEL.
- Gear summary: Field sheets, GPS, pan, scraper, squeeze bottle, knife, wrenches, 70% ETOH, D-net bag or mesh netting, 1-gal sample jar, waders or boat, PFDs.

Appendix 1. River macroinvertebrate sample field sheet.

River Macroinvertebrate IBI Sampling Notes

River:_____ **Station:**_____

Sample ID (YYYMMDD-CC-FF):_____ **SWIMS no.:**_____

Deployment: Date:_____ **Personnel:**_____

Retrieval: Date:_____ **Personnel:**_____

Lat:_____._____ **Long:**_____._____ **Deployment : riverbed snag**

Time:_____ **Temp.**_____^{o}**C D.O.**_____ **Cond**_____

Secchi_____**mm Turbidity**_____**ntu Water Velocity:**_____**m/s**^{2}

Sampler location & deployment notes: Bank: R L. Dist. from bank_____**m**

_____

_____

_____

_____

_____

_____.

Habitat:

Substrate %: boulder_____. **cobble**_____. **gravel**_____. **sand**_____. **silt**_____. **clay**_____.

Snags: no. / 100m_____. **Avg. size**_____**dbh. Deciduous Coniferous**

Riparian land use, vegetation, and condition_____

_____.

Water depth at sampler location: At deployment_____**m. At retrieval**_____**m.**

Sampler height above substrate_____**m.**

Retrieval notes:_____

_____

_____

_____

_____.

Appendix 2. WT Tier 1 river macroinvertebrate sites. Year 1 = 2011.

STAID	WATERBODY	RIVERSITE	LOCDESCRIP	County	Region	LAT_DD	LONG_DD	SAMPLING YEAR	Biologist	Installed	Retrieved	Comments
81	FOX	FOX1	DEPERE	Brown	NER	44.46150	-88.05710	1				
23	LITTLE WOLF	LITTLEWOLF1	ROYALTON	Waupaca	NER	44.41330	-88.86540	1				
62	PESHTIGO	PESHTIGO2	CRIVITZ	Marinette	NER	45.23230	-88.01370	1				
48	FOX	FOX4	MONTELLO	Marquette	NER	43.75810	-89.28800	2				
86	MENOMINEE	MENOMINEE1	MARINETTE USH 41	Marinette	NER	45.10200	-87.62390	2				
82	WOLF	WOLF4	KOEPKE LANDING	Outagamie	NER	44.48450	-88.57670	2				
21	EMBARRASS	EMBARRASS1	RANGELINE RD	Shawano	NER	44.72430	-88.73670	3				
87	MENOMINEE	MENOMINEE3	MISCAUNO IS	Marinette	NER	45.56840	-87.84060	3				
83	WOLF	WOLF1	GILLS LANDING	Waupaca	NER	44.30620	-88.86480	3				
19	FOX	FOX2	WRIGHTSTOWN	Brown	NER	44.32010	-88.17970	4				
25	OCONTO	OCONTO1	CTH BB	Oconto	NER	44.86500	-88.29990	4				
84	WOLF	WOLF2	SHAW LANDING	Waupaca	NER	44.37240	-88.80780	4				
20	FOX	FOX3	BERLIN	Green Lake	NER	43.95230	-88.95790	5				
24	PESHTIGO	PESHTIGO1	PESHTIGO	Marinette	NER	45.04750	-87.74540	5				
78	WOLF	WOLF5	CTH F DIEMEL'S BAR	Outagamie	NER	44.57480	-88.56450	5				
26	WOLF	WOLF6	CTH A	Shawano	NER	44.82170	-88.62720	5				
50	MENOMINEE	MENOMINEE2	CTH JJ	Marinette	NER	45.32420	-87.66280	TREND				
22	WOLF	WOLF3	NEW LONDON	Waupaca	NER	44.39090	-88.75790	TREND				
43	CHIPPEWA	CHIPPEWA7	TOWNLINE RD SAWYER CO	Sawyer	NOR	45.72870	-91.21200	1				
32	ST CROIX	STCROIX5	STH 35	Burnett	NOR	46.07500	-92.24730	1				
88	WISCONSIN	WISCONSIN14	HAT RAPIDS	Oneida	NOR	45.57030	-89.48070	1				
77	WISCONSIN	WISCONSIN15	OTTER RAPIDS	Vilas	NOR	45.90360	-89.32170	1				
39	FLAMBEAU	FLAMBEAU1	THORNAPPLE DAM	Rusk	NOR	45.40700	-91.21840	2				
46	FLAMBEAU	FLAMBEAU2	BEAVER DAM RAPIDS	Rusk	NOR	45.61590	-90.90630	2				
31	NAMEKAGON	NAMEKAGON2	CTH K	Washburn	NOR	45.95310	-91.89080	2				
33	ST CROIX	STCROIX2	INTERSTATE PARK	Polk	NOR	45.39020	-92.66640	2				
38	CHIPPEWA	CHIPPEWA6	USH 8	Rusk	NOR	45.45270	-91.25980	3				
40	JUMP	JUMP1	SHELDON	Taylor	NOR	45.32020	-90.90420	3				
59	NAMEKAGON	NAMEKAGON1	NAMEKAGON TRAIL RD	Burnett	NOR	46.08300	-92.11430	3				
85	WISCONSIN	WISCONSIN13	GRANDFATHER FALLS	Lincoln	NOR	45.28830	-89.79370	3				
51	MENOMINEE	MENOMINEE4	NIAGARA	Florence	NOR	45.78320	-88.07680	4				
63	RED CEDAR	REDCEDAR2	CTH MW	Dunn	NOR	45.05330	-91.71130	4				
69	ST CROIX	STCROIX3	STH 70	Burnett	NOR	45.77640	-92.77950	4				
30	NF FLAMBEAU	NFFLAMBEAU1	OXBO	Sawyer	NOR	45.86280	-90.70470	5				
60	NF FLAMBEAU	NFFLAMBEAU2	HOLTS LANDING	Ashland	NOR	46.00950	-90.36810	5				
66	SF FLAMBEAU	SFFLAMBEAU1	CTH W	Price	NOR	45.70330	-90.61550	5				
27	WHITE	WHITE1	STH 112	Ashland	NOR	46.49840	-90.90310	5				
89	ST CROIX	STCROIX4	STH 77	Burnett	NOR	46.01110	-92.44380	TREND				
41	BARABOO	BARABOO3	REEDSBURG	Sauk	SCR	43.52910	-90.01120	1				
1	KICKAPOO	KICKAPOO1	STUBEN	Crawford	SCR	43.18270	-90.85920	1				
3	WISCONSIN	WISCONSIN05	PORTAGE	Columbia	SCR	43.53840	-89.48750	1				
18	YAHARA	YAHARA1	CTH D	Rock	SCR	42.82640	-89.17230	1				
65	ROCK	ROCK1	HAPPY HOLLOW	Rock	SCR	42.59750	-89.04310	2				
10	SUGAR	SUGAR1	VAN EYCK RD	Green	SCR	42.61200	-89.39840	2				
71	WISCONSIN	WISCONSIN01	BOSCOBEL	Crawford	SCR	43.15450	-90.70940	2				
118	WISCONSIN	WISCONSIN06	WI DELLS	Sauk	SCR	43.59660	-89.73630	2				
15	CRAWFISH	CRAWFISH1	CTH A	Jefferson	SCR	43.09960	-88.84970	3				
16	ROCK	ROCK3	FORT ATKINSON	Jefferson	SCR	42.92770	-88.84200	3				
71	WISCONSIN	WISCONSIN01a	Bridgeport	Crawford	SCR	43.00076	-91.05308	3				
116	BARABOO	BARABOO1	MOUTH	Columbia	SCR	43.48950	-89.44120	4				
17	BARK	BARK1	PRINCESS POINT	Jefferson	SCR	42.89450	-88.70080	4				
117	WISCONSIN	WISCONSIN03	LONE ROCK	Iowa	SCR	43.15920	-90.18730	4				
74	WISCONSIN	WISCONSIN04	FERRY BLUFF	Sauk	SCR	43.23920	-89.80690	4				
8	BARABOO	BARABOO2	CTH X	Sauk	SCR	43.48150	-89.63620	5				
4	GRANT	GRANT1	CTH N	Grant	SCR	42.72050	-90.81940	5				
5	PECATONICA	PECATONICA1	CTH M	Green	SCR	42.51080	-89.80130	5				
2	WISCONSIN	WISCONSIN02	MUSCODA	Grant	SCR	43.19760	-90.43540	TREND				
12	MILWAUKEE	MILWAUKEE1	CAP-HUMBOLDT PARK LOT	Milwaukee	SER	43.09660	-87.90250	1				
115	FOX_IL	FOX_IL2	CTH I	Waukesha	SER	42.93340	-88.29290	2				
11	FOX_IL	FOX_IL1	CTH JB	Kenosha	SER	42.61110	-88.22530	3				
14	SHEBOYGAN	SHEBOYGAN1	SHEBOYGAN	Sheboygan	SER	43.75480	-87.72110	4				
97	BLACK	BLACK3	OPELT AVE	Clark	WCR	44.51680	-90.62390	1				
96	CHIPPEWA	CHIPPEWA5	CORNELL	Chippewa	WCR	45.14440	-91.19030	1				
6	LEMONWEIR	LEMONWEIR1	CTH M	Juneau	WCR	43.92980	-90.17790	1				
64	RED CEDAR	REDCEDAR1	DOWNSVILLE	Dunn	WCR	44.77340	-91.93380	1				
42	BLACK	BLACK2	Palm Rd	Jackson	WCR	44.39506	-90.74542	2				
45	CHIPPEWA	CHIPPEWA4	BADGER MILLS	Chippewa	WCR	44.88760	-91.45450	2				
73	WISCONSIN	WISCONSIN07	DS LEMONWEIR	Juneau	WCR	43.75610	-89.84490	2				
70	WISCONSIN	WISCONSIN10	BIRON FLOWAGE	Portage	WCR	44.47620	-89.57500	2				
34	BUFFALO	BUFFALO1	CTH F	Buffalo	WCR	44.39090	-91.84930	3				
105	CHIPPEWA	CHIPPEWA3	CTH H NEAR ELK CR	Dunn	WCR	44.76080	-91.68440	3				
68	ST CROIX	STCROIX1	MARINE MN	St. Croix	WCR	45.20480	-92.76320	3				
100	WISCONSIN	WISCONSIN11	DUBAY	Portage	WCR	44.65650	-89.64740	3				
102	CHIPPEWA	CHIPPEWA1	TIFFANY BOTTOMS STH 35	Buffalo	WCR	44.43810	-92.07100	4				
35	EAU CLAIRE	EAUCLAIRE1	CTH K	Eau Claire	WCR	44.81170	-91.28310	4				
37	TREMPEALEAU	TREMPEALEAU1	DODGE	Trempealeau	WCR	44.12610	-91.54960	4				
79	YELLOW	YELLOW1	NECEDAH	Juneau	WCR	44.01640	-90.06280	4				
7	LA CROSSE	LACROSSE1	STH 16	La Crosse	WCR	43.86080	-91.21020	5				
99	WISCONSIN	WISCONSIN08	NECEDAH STH 21	Juneau	WCR	44.04100	-90.01460	5				
101	WISCONSIN	WISCONSIN09	NEKOOSA	Wood	WCR	44.28860	-89.88720	5				
72	WISCONSIN	WISCONSIN12	BROKAW	Marathon	WCR	45.03410	-89.65840	5				
9	BLACK	BLACK1	GALESVILLE	Trempealeau	WCR	44.06150	-91.29200	TREND				
36	CHIPPEWA	CHIPPEWA2	DURAND	Pepin	WCR	44.63100	-91.96980	TREND				

Appendix 2 (cont), locations to be sampled during 2011, included is the assigned SWIMS ID.

WATERBODY	RIVERSITE	LOCDESCRIP	Region	LAT_DD	LONG_DD	SWIMS ID
MENOMINEE	MENOMINEE2	CTH JJ	**NER**	45.32420	-87.66280	10009738
WOLF	WOLF3	NEW LONDON	**NER**	44.39090	-88.75790	693137
FOX	FOX3	BERLIN	**NER**	43.95230	-88.95790	10022818
WOLF	WOLF5	CTH F DIEMEL'S BAR	**NER**	44.57480	-88.56450	10029632
PESHTIGO	PESHTIGO1	PESHTIGO	**NER**	45.04750	-87.74540	10033878
ST CROIX	STCROIX4	STH 77	**NOR**	46.01110	-92.44380	10033694
WISCONSIN	WISCONSIN13	GRANDFATHER FALLS	**NOR**	45.28830	-89.79370	10029618
ST CROIX	STCROIX2	INTERSTATE PARK	**NOR**	45.39020	-92.66640	10029621
NF FLAMBEAU	NFFLAMBEAU1	OXBO	**NOR**	45.86280	-90.70470	583186
WISCONSIN	WISCONSIN02	MUSCODA	**SCR**	43.19760	-90.43540	10029646
WISCONSIN	WISCONSIN01a	Bridgeport	**SCR**	43.00076	-91.05308	10033730
BARABOO	BARABOO2	CTH X	**SCR**	43.48150	-89.63620	573051
ROCK	ROCK3	FORT ATKINSON	**SCR**	42.92770	-88.84200	10033731
SHEBOYGAN	SHEBOYGAN1	SHEBOYGAN	**SER**	43.75480	-87.72110	10017686
CHIPPEWA	CHIPPEWA2	DURAND	**WCR**	44.63100	-91.96980	10033690
BLACK	BLACK1	GALESVILLE	**WCR**	44.06150	-91.29200	10020903
CHIPPEWA	CHIPPEWA5	CORNELL	**WCR**	45.14440	-91.19030	10033691
RED CEDAR	REDCEDAR1	DOWNSVILLE	**WCR**	44.77340	-91.93380	10033692
BLACK	BLACK2	Palm Rd	**WCR**	44.39506	-90.74542	10033693

Appendix B.3

Wisconsin Department of Natural Resources

Macroinvertebrate Field Data Report

State of Wisconsin
Department of Natural Resources
dnr.wi.gov

Macroinvertebrate Field Data Report

Form 3200-081 (R 2/07)

Instructions: Bold fields must be completed.

Station Summary

Waterbody Name	**Waterbody ID Code**	Site Mile	**Sample ID (YYYYMMDD-CY-FD)**

Sampling Location	Township	Range	Section	¼ - ¼	¼

SWIMS Station ID	SWIMS Station Name	**Database Key**

Latitude	**Longitude**	**Lat.-Long. Determination Method Used**	**Datum Used**

Basin (WMU)	**Watershed Name**	**County**

Sample and Site Descriptors

Sample Collector (Last Name, First)	**Project Name**

Sampling Device

☐ Kick Net ☐ Surber Sampler ☐ Eckman
☐ Ponar ☐ Artificial Substrate ☐ Hess Sampler ☐ Other: _____

Habitat Sampled

☐ Riffle ☐ Run ☐ Pool
☐ Microhabitat ☐ Shoreline Composite ☐ Proportionally-Sampled Habitat
☐ Littoral Zone ☐ Profundal Zone ☐ Wetland

Total Sampling Time (min)	**Estimated Area Sampled (m²)**	**Number of Samples in Composite**	**Replicate No. _____ of _____**

Reason for Sampling

☐ Least Impacted Reference ☐ Baseline ☐ Impact / Treatment Site
☐ Control Site ☐ Trend ☐ Other: _____

Water Color ☐ Clear ☐ Turbid ☐ Stained	**Water Temp. (C)**	D.O. (mg/l)	D.O. (% sat.)	pH (su)	Turbidity (NTUs)	TDS (mg/l)

Conductivity (umhos/cm)	**Stream Order**	**Stream Gradient (m/km)**	**Estimated Stream Velocity (m/s)** ☐ Slow (< 0.15 m/s) ☐ Moderate (0.15 m/s - 0.5 m/s) ☐ Fast (>0.5 m/s)

Measured Velocity (mps)	**Average Stream Depth (m)**	**Average Stream Width (m)**

Composition of Substrate Sampled (Percent):

Bedrock:_____ Boulders (261 mm - 4.1 m dia.):_____ Rubble (65 - 260 mm dia.):_____ Gravel (2 - 64 mm dia.):_____

Sand:_____ Clay:_____ Silt:_____ Muck:_____ Overhanging Vegetation:_____

Aquatic Macrophytes:_____ Leaf Snags:_____ Course Woody Debris:_____ Other (_____):_____

Embeddedness of Substrate at Sample Site (%)	**Canopy Cover at Sample Site (%)**

Stream and Watershed Descriptors

N = Not a problem
U = Present, but uncertain as to degree of impact
P = Present, and probably creating a problem
Blank = Uncertain

Factors that may be Influencing Water Resource Integrity	Local	Water-shed	Factors that may be Influencing Water Resource Integrity	Local	Water-shed
Biological			**Chemical**		
Macrophytes			Chlorine		
Filamentous Algae			Organic Toxics		
Planktonic Algae			Inorganic Toxics		
Diatoms / Periphyton			Nutrients		
Slimes			Dissolved Oxygen		
Iron Bacteria			Other - Specify:		
Exotics - Specify:			**Sources of Stream Impacts**		
Other - Specify:			Urban NPS		
Physical			Construction Erosion		
Sludge			Point Source - Specify:		
Thermal			Cropland Erosion		
Turbidity			Pasturing		
Sedimentation / Channel Aggradation			Bank Erosion		
Hydraulic Scour / Channel Incision			Barnyard Run-Off		
Bank Erosion			Tile Drainage - Organic Soils		
Upstream Channelization			Tile Drainage - Mineral Soils		
Local Channelization			Septic Systems		
Low Flow			Tributary(s)		
Upstream Impoundment			Springs		
Downstream Impoundment			Wetland Drainage		
Other - Specify:			Other - Specify:		

Comments

Special Instructions for Laboratory

For Lab Use Only		
Sample Sorter	Taxonomist	Estimated Percent of Sample Sorted
Date Processed	Specimens Saved	

Appendix B.4

Wisconsin Department of Natural Resources

Guidelines for Qualitative Physical Habitat Evaluation of Wadeable Streams

State of Wisconsin
Department of Natural Resources

Guidelines for Qualitative Physical Habitat Evaluation of Wadeable Streams

Bureau of Fisheries Management
Monitoring and Data Analysis Section
101 S. Webster St.,
Madison, WI 53703

Modified from Simonson et al. 1994.
Guidelines for Evaluating Fish Habitat in
Wisconsin Streams. USDA Forest Service
General Technical Report NC-16

June, 2007

Objectives of Qualitative Habitat Assessment of Wadeable Streams

Water quality characteristics, and physical habitat features and conditions are primary factors that influence the biological potential and current conditions of streams. Collection of physical habitat, water quality, and biological data can provide a comprehensive evaluation of stream health, and help identify limiting factors.

Quantitative habitat assessment methods (e.g. Simonson et al. 1994) typically provide more accurate and precise measures than qualitative habitat assessments, but depending upon the specific data needs, qualitative assessments may provide worthwhile and cost-effective habitat information.

General Sampling Procedures

Ideally, habitat assessments should be done at “normal” water levels and during the same time frame and in the same sampling station as the fish surveys. Station length for qualitative habitat assessments like for fish and quantitative habitat surveys are based on a stream reach 35 times the mean stream width (MSW), with a minimum station length of 100 meters (for streams less than 2.9 m MSW), and for Clean Water Act (CWA) sampling, the maximum station length for fish assemblage and habitat surveys is 400 m. Visual observations of habitat conditions can be made while conducting fish assessments and the qualitative habitat form filled out upon completion of the fish survey.

Seven different habitat parameters for streams less than 10 meters wide (E-form 3600 – 532A), and five parameters for streams greater than 10 meters wide (E-form 3600 – 532B) are visually - estimated for a qualitative habitat assessment. The same habitat measures and scoring criteria used in the quantitative habitat assessment in Simonson et al. are used for the qualitative survey. Each habitat parameter is given a rating of excellent, good, fair, or poor, and the associated individual numeric scores are summed to provide an overall rating of stream habitat quality.

Appendix B.5

Wisconsin Department of Natural Resources

Wadable Stream Qualitative Fish Habitat Rating Forms

State of Wisconsin
Department of Natural Resources
dnr.wi.gov

Wadable Stream Qualitative Fish Habitat Rating for Streams < 10 m wide

Form 3600-532A (R 6/07)

Instructions: **Bold** fields must be completed. Record all measurements in metric units.

Station Summary

Stream Name	**Waterbody ID Code**	**SWIMS Station ID**	FH Database ID

Date (MMDDYYYY)	**Station Name**

Latitude - Longitude Determination Method Used	**Datum Used**

Start Latitude	**Start Longitude**	End Latitude	End Longitude	**County**

Water Characteristics

Time (24-hr clock)	Air Temperature (C)	Water Temperature (C)	Conductivity (µs/cm)	Transparency (cm)

Dissolved Oxygen (mg/l)	Dissolved Oxygen % Saturation	pH

Flow (m^3/sec)	**Water Level** (check one - measure distance if Above or Below Normal):	**Water Clarity:**
	☐ Normal ☐ Below: _____(m) ☐ Above: _____(m)	☐ Clear ☐ Turbid ☐ Stained

Channel and Basin Characteristics

Mean Stream Width (m)	**Station Length (m)**

Channel Condition: (check one) ☐ Natural ☐ > 20-year-old Channelization ☐ 10- to 20-year-old Channelization ☐ < 10-year-old Channelization ☐ Concrete Channel

Percent Channelization	Sinuosity	Gradient (m/km)	Stream Order	Basin Area (km^2)

Comments / Notes

Wadable Stream Qualitative Fish Habitat Rating for Streams < 10 m wide

Rating Item	Excellent	Good	Fair	Poor	Score
Riparian Buffer Width (m) Width of contiguous undisturbed land uses; meadow, shrubs, woodland, wetland, exposed rock	Riparian zone well protected; buffer wide (> 10.0 m) 15	Riparian zone protected, but buffer width moderate (5.0 - 10.0 m) 10	Riparian zone moderately disturbed, buffer narrow (1.0 - 4.9 m) 5	Most of the riparian zone disturbed, buffer very narrow or absent (< 1.0 m) 0	
Bank Erosion Width of bare soil on bank, along transects	No significant bank erosion; < 0.20 m of bank is bare soil 15	Limited erosion; 0.20 - 0.50 m of bank is bare soil 10	Moderate erosion; 0.51 - 1.0 m of bank is bare soil 5	Extensive erosion; > 1.0 m of bank is bare soil 0	
Pool Area % of stream length in pools	Pools common; wide, deep, slow velocity habitat, balanced by other habitats; 40 to 60% of station 10	Pools present; not frequent or over-abundant; 30 to 39% or 61 to 70% of station 7	Pools present, but either rare or overly dominant, few other habitats present; 10 to 29% or 71 to 90% of station 3	Pools either absent or dominant, not balanced by other habitats; < 10% or > 90% of station 0	
Width:Depth Ratio Average stream width divided by average thalweg depth in runs and pools	Streams very deep and narrow; width/depth ≤ 7 15	Stream relatively deep and narrow; width/depth 8-15 10	Stream moderately deep and narrow; width/depth 16-25 5	Stream relatively wide and shallow; width/depth > 25 0	
Riffle:Riffle or Bend:Bend Ratio Average distance between riffles or bends divided by average stream width	Diverse habitats; meandering stream with deep bends and riffles common; ratio < 10 15	Diverse habitats; bends and riffles present, but not abundant; ratio 10 to 14 10	Habitat diversity low; occasional riffles or bends, ratio 15 to 25 5	Habitat monotonous; riffles or bends rare; generally continuous run habitat; ratio > 25 0	
Fine Sediments % of the substrate that is < 2 mm (sand, silt, or clay)	Fines rare or absent, < 10% of the stream bed 15	Fines present but limited, generally in stream margins or pools; 10 to 20% of stream bed 10	Fines common in mid-channel areas, present in riffles and extensive in pools; 21 to 60% 5	Fines extensive in all habitats; > 60% of stream bed covered 0	
Cover for Fish % of the stream area with cover	Cover/shelter for fish abundant; > 15% of stream 15	Cover common, but not extensive; 10 - 15% of stream 10	Occasional cover, limited to one or two areas; 5 - 9% of stream 5	Cover rare or absent; limited to < 5% of stream 0	
				Total Score	

State of Wisconsin
Department of Natural Resources
dnr.wi.gov

Wadable Stream Qualitative Fish Habitat Rating for Streams > 10 m wide

Form 3600-532B (R 6/07)

Instructions: **Bold** fields must be completed. Record all measurements in metric units.

Station Summary

Stream Name	**Waterbody ID Code**	**SWIMS Station ID**	FH Database ID

Date (MMDDYYYY)	**Station Name**

Latitude - Longitude Determination Method Used	**Datum Used**

Start Latitude	**Start Longitude**	End Latitude	End Longitude	**County**

Water Characteristics

Time (24-hr clock)	Air Temperature (C)	Water Temperature (C)	Conductivity (µs/cm)	Transparency (cm)

Dissolved Oxygen (mg/l)	Dissolved Oxygen % Saturation	pH

Flow (m^3/sec)	**Water Level (check one - measure distance if Above or Below Normal):**	**Water Clarity:**
	☐ Normal ☐ Below: _____(m) ☐ Above: _____(m)	☐ Clear ☐ Turbid ☐ Stained

Channel and Basin Characteristics

Mean Stream Width (m)	**Station Length (m)**

Channel Condition: (check one) ☐ Natural ☐ > 20-year-old Channelization ☐ 10- to 20-year-old Channelization ☐ < 10-year-old Channelization ☐ Concrete Channel

Percent Channelization	Sinuosity	Gradient (m/km)	Stream Order	Basin Area (km^2)

Comments / Notes

Wadable Stream Qualitative Fish Habitat Rating for Streams > 10 m wide

Rating Item	Excellent	Good	Fair	Poor	Score
Bank Stability % of bank protected by rock or vegetation	No significant bank erosion; ≥ 90% of bank protected; < 10% bare soil	Limited erosion; 70 to 90% of bank protected; 10 - 30% bare soil	Moderate erosion; 50 to 69% of bank protected; 31 - 50% bare soil	Extensive erosion; < 50% of bank protected; > 50% bare soil	
	12	8	4	0	
Maximum Thalweg Depth Average of the four deepest depths recorded	Stream very deep; ≥ 1.5 m	Stream relatively deep; 1 - 1.5 m	Stream moderately deep; 0.6 - 0.9 m	Stream relatively shallow; < 0.6 m	
	25	16	8	0	
Riffle:Riffle or Bend:Bend Ratio Average distance between riffles or bends divided by average stream width	Diverse habitats; meandering stream with deep bends and riffles common; ratio < 10	Diverse habitats; bends and riffles present, but not abundant; ratio 10 to 14	Habitat diversity low; occasional riffles or bends, ratio 15 to 25	Habitat monotonous; riffles or bends rare; generally continuous run habitat; ratio > 25	
	12	8	4	0	
Rocky Substrate % of substrate, by area, that is bedrock, boulder, rubble/ cobble, or gravel	Extensive rocky substrate; ≥ 65% of the stream bed	Moderate rocky substrate; 45 - 65% of stream bed	Limited rocky substrate; 15 - 44% of stream bed	Rocky substrate uncommon; < 15% of stream bed	
	25	16	8	0	
Cover for Fish % of the stream area with cover	Cover/shelter for fish abundant; ≥ 12% of stream	Cover common, but not extensive; 7 - 12% of stream	Occasional cover, limited to one or two areas; 2 - 6% of stream	Cover rare or absent; limited to < 2% of stream	
	25	16	8	0	
				Total Score	

Appendix B.6

Wisconsin Department of Natural Resources

Recommended Baseline Monitoring of Aquatic Plants in Wisconsin: Sampling Design, Field and Laboratory Procedures, Data Entry and Analysis, and Applications

Recommended Baseline Monitoring of Aquatic Plants in Wisconsin:
Sampling Design, Field and Laboratory Procedures, Data Entry and Analysis, and Applications

Jennifer Hauxwell, Susan Knight, Kelly Wagner, Alison Mikulyuk, Michelle Nault, Meghan Porzky and Shaunna Chase

March 2010

Document citation:
Hauxwell, J., S. Knight, K. Wagner, A. Mikulyuk, M. Nault, M. Porzky and S. Chase. 2010. Recommended baseline monitoring of aquatic plants in Wisconsin: sampling design, field and laboratory procedures, data entry and analysis, and applications. Wisconsin Department of Natural Resources Bureau of Science Services, PUB-SS-1068 2010. Madison, Wisconsin, USA.

WISCONSIN
DEPT. OF NATURAL RESOURCES

Photo credits (all photos used with permission):
J. Hauxwell, Wisconsin Department of Natural Resources
B. Korth, University of Wisconsin-Extension
F. Koshere, Wisconsin Department of Natural Resources
A. Mikulyuk, Wisconsin Department of Natural Resources

Recommended Baseline Monitoring of Aquatic Plants in Wisconsin: Sampling Design, Field and Laboratory Procedures, Data Entry and Analysis, and Applications

Jennifer Hauxwell[1], Susan Knight[2], Kelly Wagner[1], Alison Mikulyuk[1], Michelle Nault[1], Meghan Porzky[1], and Shaunna Chase[1]

[1]Wisconsin Department of Natural Resources
Bureau of Science Services
Fisheries and Aquatic Sciences Research Section
2801 Progress Road
Madison, WI 53716

[2]University of Wisconsin – Madison
Trout Lake Station
10810 County Hwy N
Boulder Junction, WI 54512

Last Updated:
March 2010

EXECUTIVE SUMMARY

We outline a baseline monitoring protocol designed to quantitatively assess the distribution and abundance of aquatic plants in lake ecosystems. This protocol employs a point-intercept sampling design, with sites located on a geo-referenced sampling grid placed over the entire lake. At each site, the aquatic plant community is surveyed from a boat with a rake sampler to characterize species presence and rake fullness. In addition, a qualitative survey is recommended to map obvious species and augment the species list generated through quantitative sampling. Application of this methodology allows: 1) assessment of the frequencies of occurrence of different plant species, as well as estimates of species richness, abundance, and maximum depth of plant colonization; and 2) comparisons of aquatic plant variables over time and among lakes. This document contains complete instructions for conducting a baseline aquatic plant survey, including details on obtaining an electronic file of site coordinates, uploading site coordinates into a Global Positioning System (GPS) receiver, conducting field work, entering data, working with data summaries, processing voucher specimens, and provides example applications of the collected data. Final products from each baseline survey will include: 1) raw data from the quantitative survey which provides individual site-by-site species distribution and rake fullness data, 2) summary statistics useful in characterizing and comparing populations, 3) additional species observations from the general qualitative survey, and 4) voucher specimens cataloguing species presence. All electronic data should be sent for long-term record-keeping to the WDNR (DNRBaselineAquaticPlants@wisconsin.gov).

CONTENTS

INTRODUCTION

In lake ecosystems, the aquatic plant community serves as critical habitat and nursery for fish and other animals, a source of oxygen for all organisms, a refuge for prey as well as a foraging area for predators, a buffer against erosion and sediment resuspension from both waves and shoreline inputs, and can significantly contribute to overall lake primary productivity. Over the past several decades, losses of or changes in assemblages of native submersed aquatic vegetation has been a reoccurring phenomenon due to a relatively limited number of factors. Repeatedly, changes in landscapes and atmospheric conditions as a result of human activities have increasingly affected the ecology of adjacent aquatic systems, including aquatic plant communities. In addition, in-lake aquatic plant management activities have increased due to the increasing spread of invasive exotic plants[1].

The Wisconsin Department of Natural Resources (WDNR) is charged with protecting and enhancing the state's natural resources, including lake ecosystems. Given the many ecosystem services associated with aquatic plant communities as well as the recent threats to native species, it has become increasingly important to develop monitoring techniques to support science-based decision-making for effectively managing lake ecosystems. In this document, we present a quantitative, replicable monitoring protocol. Standardized, quantitative and replicable data are an essential part of strategic lake management for three reasons. First, good data allows us to better understand each individual lake; we can use survey data to produce detailed lake maps that show the locations of native, rare, or exotic plant species. Data can then be used as a baseline against which any changes in a lake associated with water clarity, exotic species introduction, water level, or lake management activity can be compared. Second, good data helps direct management by taking the conflict and guesswork out of planning. Aquatic plant management requires weighing a number of potential management options, some of which can be very costly or extensive. Baseline data allows lake groups to identify the most appropriate management options and design the best possible management plan. Additionally, by conducting quantitative comparisons between the aquatic plant communities before and after management actions, lake groups and managers may evaluate whether or not management goals were achieved. Third, by compiling and comparing survey information on lakes statewide, we are able to identify regional trends and refine our understanding of aquatic plant populations on a broader scale in both space and time.

SURVEY OBJECTIVE

In this document, we outline a baseline monitoring protocol designed to assess aquatic plant communities on a whole-lake scale. We recommend a formal quantitative survey conducted at pre-determined sampling locations distributed evenly throughout the lake, accompanied by a general qualitative survey to map obvious species and augment the species list generated through the quantitative survey. Our primary goals in adopting this methodology are to:

[1] Knight, S., and J. Hauxwell. 2009. Distribution and abundance of aquatic plants- human impacts. *In*: G. Likens (editor-in-chief), *Encyclopedia of Inland Waters*. Elsevier, Oxford, United Kingdom.

1) Collect quantitative data describing the frequencies of occurrence of different plant species, as well as estimates of species richness, abundance, and maximum depth of plant colonization for use in developing various management plans; and

2) Use the data to statistically compare aquatic plant variables over time and among lakes.

The importance of a statewide standardized protocol is that observed differences in a lake's plant community can be attributed to actual changes in the community over time, without the confounding variation that results from different field workers employing different sampling techniques.

The quantitative survey employs a point-intercept sampling design, adapted from terrestrial methods, with sites located on a geo-referenced sampling grid placed over the entire lake. At each site, the aquatic plant community is surveyed from a boat with a rake sampler to characterize species presence and rake fullness ratings. Although the presence/absence data cannot be used to estimate biomass or percent cover, it is less sensitive to interannual or seasonal variations in plant abundance[2]. The method is also relatively rapid and cost-effective and can be used on the large scale to collect baseline data and statistically compare communities over time[2,3]. In summary, it has the following attributes for estimation of aquatic plant distribution and abundance:

- Systematic, quantitative, and replicable
- Appropriate for lakes that vary in depth, size, region, shoreline complexity, and vegetation distribution
- Evenly spaced distribution of sites results in a good coverage of the entire lake, precluding the random exclusion of niche habitats
- Procedural simplicity
- Inexpensive implementation
- Results are easily analyzed with scientifically rigorous statistical methods
- Spatial data preserved and can be mapped for both the managers' use and for clearly communicating distributional data with the public

These guidelines are intended to work on most lakes. However, modifications may be required if a lake is uniquely shaped so that a uniform distribution of points isn't representative (long, skinny lake shape), or if obtaining rake samples is difficult due to substrate (rocky/cobble bottom).

Please note that these are "baseline" recommendations. Additional monitoring activities may be warranted if the goal is to assess a specific management activity. For example, to gauge the ability of chemical spot-treatments to control relatively small stands of an exotic species in a

[2] Madsen, J.D. 1999. Point intercept and line intercept methods for aquatic plant management. Aquatic plant control technical note MI-02. Army Engineer Waterways Experiment Station, Vicksburg, MS.

[3] Dodd-Williams, L., G.O. Dick, R.M. Smart and C.S. Owens. 2008. Point Intercept and Surface Observation GPS (SOG): A Comparison of Survey Methods – Lake Gaston, NC/VA. ERDC/TN APCRP-EA-19. Vicksburg, MS: U.S. Army Engineer Research and Development Center

relatively large lake, we recommend additional mapping of the beds following the pre- and post-treatment protocol available in Appendix D of the Aquatic Plant Management guide[4].

Unlike the procedures used by the Citizen-Based Lake Monitoring Network, this protocol is not designed for most volunteers. The protocol requires at least one of the field workers be an experienced plant taxonomist and able to identify most plant species in the field. Less experienced volunteers may be able to help with data recording and navigation, but without the help of a professional aquatic ecologist, volunteers may not be able to conduct an entire plant survey without a significant degree of training or study.

SURVEY OVERVIEW

Sampling Sites

This method employs a point-intercept design in which a grid of sampling sites is distributed evenly over the entire lake surface (Figure 1). Lake organizations or individuals can request an electronic file of survey sites by contacting the WDNR Lake Coordinator from their region (see Appendix 1) with the lake name and county, as well as the town, range and section (TRS) or water body identification code (WBIC). Please make requests well in advance of planned field work to allow WDNR staff sufficient time for map creation (recommend at least 1 month). WDNR staff will determine the number of sites and grid resolution based on the estimated size of the littoral zone (the area in which plants grow) and shape of the lake. Grids will be scaled to produce a greater number of sites on lakes that are larger and have more complex shorelines. Lakes with a narrow littoral zone may be assigned a comparatively high number of sampling sites to achieve sufficient survey coverage. Once created, the sampling map (Figure 1) and an associated GPS text file containing the latitude and longitude information associated with each sample site will be provided electronically by the WDNR.

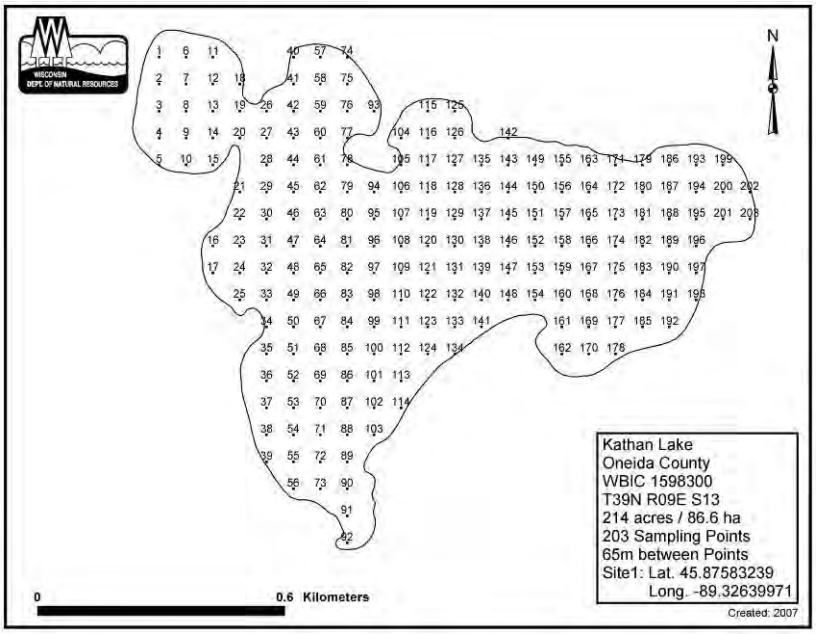


Figure 1: The point-intercept grid for Kathan Lake, Oneida County, WI, with 203 sampling sites.

Timing of Sampling

Surveys should be conducted between early July and mid August. Although certain plant community parameters (such as rake fullness and biomass) can change over the course of the

[4] Aquatic Plant Treatment Evaluation. http://www.uwsp.edu/cnr/uwexlakes/ecology/APM/Appendix-D.pdf

growing season, presence/absence data is less sensitive to seasonal variation[2]; presence can often be detected throughout the season. For many species, including Eurasian water milfoil (EWM), plant biomass and density may increase as the season progresses, whereas some species like curly-leaf pondweed (CLP), senesce much earlier in the sampling season. Rake fullness data for these species must be interpreted carefully with the sampling date in mind. If early-senescing species such as CLP are targets of management actions, please contact the WDNR Lake Coordinator in your region to coordinate the best possible sampling time.

Time Spent Sampling

Depending on the size of the lake, a survey may be completed in a few hours, or it may take several days. Ideally, a crew spends one-half to three minutes per sample site; however, this may vary depending on the following factors:

- Distance between sample sites
- Weather (i.e. wind, rain, etc.)
- Rake fullness
- Ease of navigation
- Experience; less experienced field workers may take longer to identify unfamiliar plants. However, most field workers have found that the time spent per site drops dramatically with experience. Others have reported their speed increasing greatly with a few hours of training.

PREPARING FOR FIELD WORK

Field Gear

Necessary equipment:

- Appropriate watercraft and all equipment required by state law
- Double-sided sampling rake attached to a 15-ft (4.6m) pole
- Weighted sampling rake attached to a 40-ft (12m) rope
- Handheld GPS receiver with WDNR sample sites loaded
- Print-out of lake map with WDNR sample sites
- Print-out of WDNR field datasheets on waterproof paper
- Pencils
- Sealable storage bags for voucher specimens
- Waterproof voucher sample labels
- Cooler(s) with ice for storing voucher specimens
- Depth finder

Helpful, but not required:

- Trolling motor for reaching shallow sites
- Bathymetric map
- Plant ID references or guides to aid in plant identification
- Hand lens to aid in plant identification
- Digital camera for plant specimens or field pictures
- Underwater video camera for viewing the maximum depth of plant colonization

Loading Sample Site Locations onto the GPS Receiver

Detailed instructions on loading sample site locations onto the GPS receiver depend greatly on the type of GPS receiver as well as the software used to translate site location from the text file to "waypoints" in the receiver. The WDNR commonly utilizes Garmin 76 model GPS receivers and the WDNR Garmin GPS Standalone Tool software. The WDNR Standalone Tool is only available to WDNR employees, and only works with Garmin GPS receivers. The Minnesota Garmin GPS Tool and appropriate guidance documents are available to the public and can be found online at the Minnesota DNR internet site[5]. The two programs are similar; their chief difference is that the Minnesota tool requires the GPS text file to be comma-delimited instead of tab-delimited. Procedures for other GPS models with a Wide Area Augmentation System (WAAS-capability) may be used; please refer to the manufacturer's instructions for details on uploading site locations.

Please note that storage capability varies by GPS model. Some GPS receivers are unable to store the large numbers of data sites required in some surveys. In the event that the number of sampling sites exceeds your receiver's storage capacity, the text file containing the survey site information can be split into smaller text files. You will then be able to upload successive files of sites as needed or work from multiple receivers in the field.

The instructions below describe how WDNR employees can use the WDNR Garmin Standalone Tool software to load sample site locations, or "waypoints," onto a Garmin 76 model GPS receiver.

To upload waypoints from a GPS text file to the GPS receiver, you will need:

- **PC/laptop with WDNR Garmin GPS Tool.** Your IT administrator can help you obtain and install the software.

- **GPS text file (.txt extension).** A tab-delimited text file containing the sample sites and their geographical information.

- **A Garmin 76 model GPS receiver with external data port.**

[5] Available online at: http://www.dnr.state.mn.us/mis/gis/tools/arcview/extensions/DNRGarmin/DNRGarmin.html (accessed September, 2009)

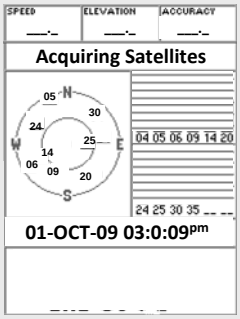
- **PC interface cable (with USB or 9-pin serial connector).** Can be purchased online at http://www.garmin.com

Step 1: Set GPS to the "Simulating GPS" Mode

Operating the receiver in "Simulating GPS" mode prevents the GPS receiver from trying to acquire a satellite signal indoors.

1. Press and hold the red [ON/OFF] button for two seconds to turn the GPS receiver on.

2. Press [PAGE] to navigate through the welcome screens until the "Acquiring Satellites" page is visible.



3. Press the [MENU] button, select "Start Simulator", and press [ENTER]; the screen heading should now read "Simulating GPS."

Step 2: Set Serial Data Format (this setting will **not** have to be re-set upon each use)

Set the serial data format on the Garmin 76 receiver to GARMIN prior to transferring data. Failure to set the serial data format to GARMIN will cause a communication error.

1. Press the [MENU] button twice to reach the main menu, use the rocker key to select "Setup", and then press [ENTER].

2. Use the rocker key to scroll left or right until the "Interface" tab is highlighted. Use the rocker key to scroll down to highlight the drop-down box and press [ENTER].

3. A menu will appear; select "GARMIN" and press [ENTER]. Press [QUIT] twice to exit the menu.

Step 3: Plug in the PC Interface Cable

1. The GPS receiver should be on and in simulation mode.

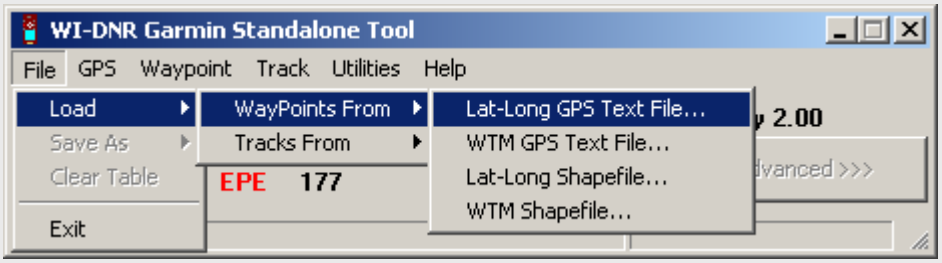
2. Plug the 9-pin serial connector cable into COM port #1 on your PC. If port #1 is in use, plug into the next available port and note the port number. The newest version of the WDNR Garmin GPS Tool (ver. 8.2.8) supports USB connectivity as an alternate to COM port connection.

3. Plug the round end of the PC interface cable into the external data/auxiliary power port under the rubber panel on the back of the GPS receiver.

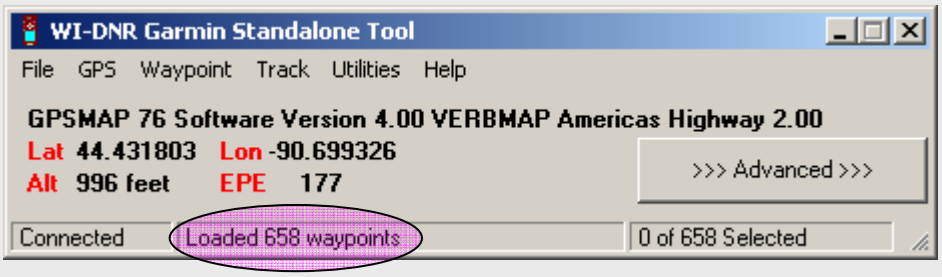
Step 4: Load the GPS text file into the WDNR Garmin Standalone Tool

1. Open the WDNR Garmin GPS Tool file on your computer. Select:

   File > Load > Waypoints From > Lat-Long GPS Text File.



2. Navigate to and select the appropriate GPS text file and select OK. The waypoints will be visible in the Tool's status bar.



3. If necessary, you can view and edit waypoints by clicking the [Advanced] button on the WDNR Garmin GPS Tool.

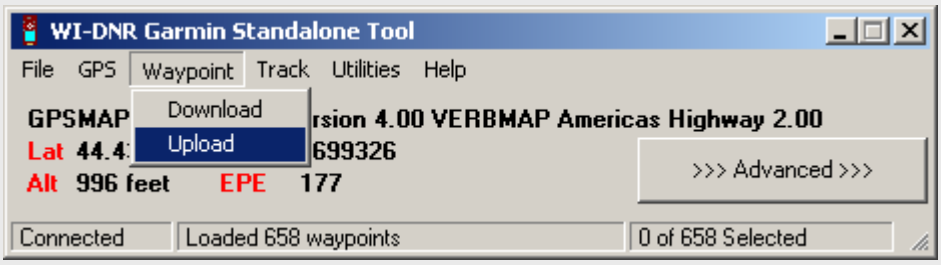
4. Troubleshooting COM-enabled setups
   - **a.** Check that the correct COM port is selected in the WDNR Garmin GPS tool.
     - **i.** GPS > Assign Port > select correct port #
   - **b.** Check that the baud rate matches that of the GPS receiver.
     - **i.** GPS > Assign Port >Baud Rate > 9600
     - **ii.** A Garmin 76 receiver will transfer at 9600 bits per second

c. Check that the serial data format is set to "GARMIN" (see Step 2).

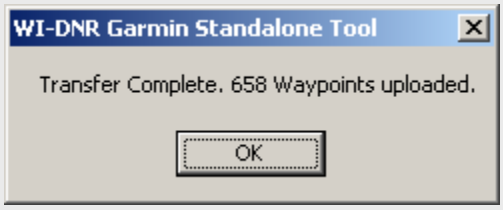
d. If your problem persists, please consult your GPS unit's user's manual.

Step 5: Upload Waypoint Data from the WDNR Garmin GPS Tool to the GPS receiver

1. In the menu bar, select: Waypoint > Upload



2. A pop-up window will indicate the completion of a successful upload. Click OK.



3. Check that the uploaded waypoints are visible on the GPS receiver: press [MENU] twice to get to the main menu, select "Points", press [ENTER], select "Waypoints", and press [ENTER].

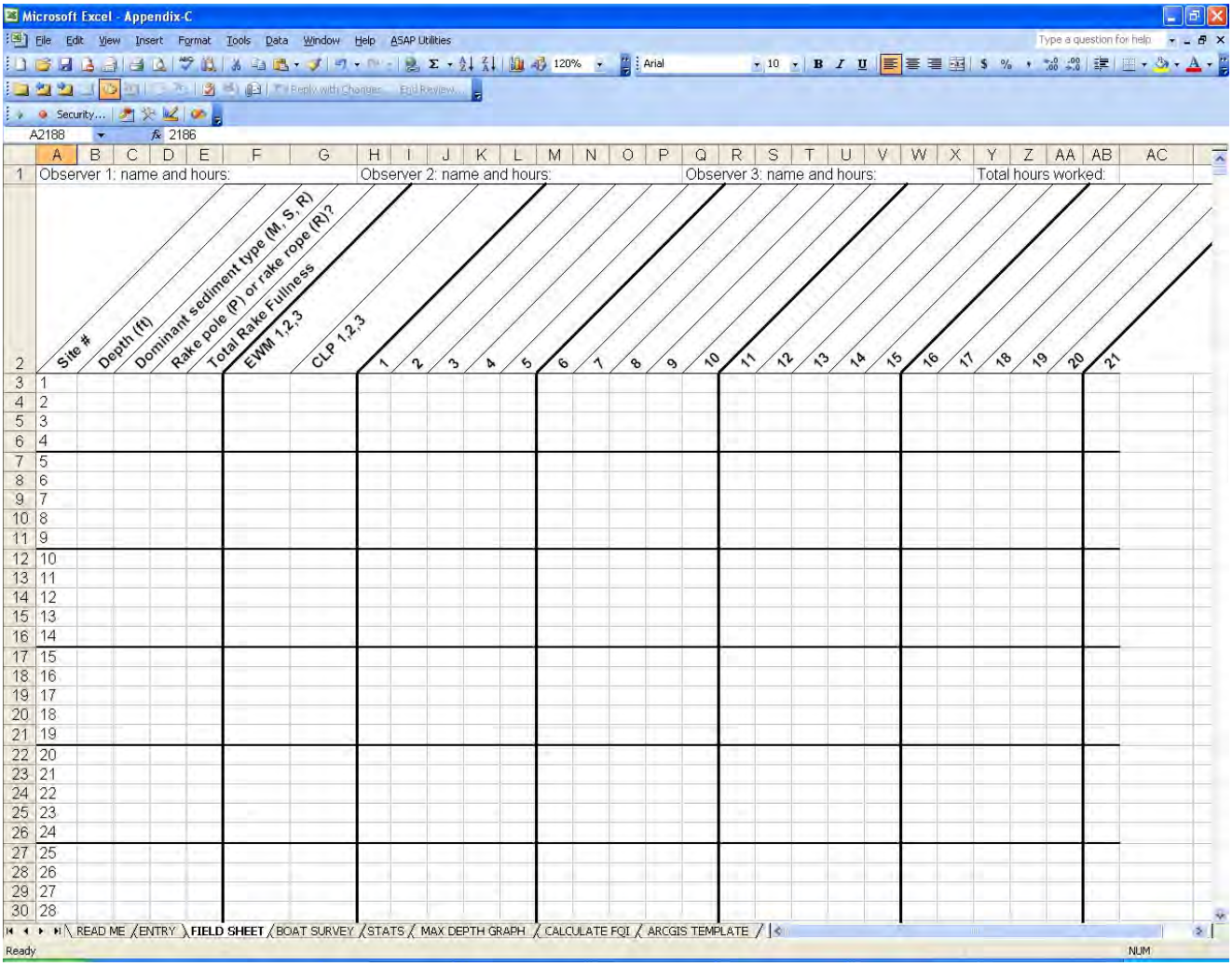
4. Troubleshooting

a. Storage capability varies by GPS model. In the event that the number of sampling sites exceeds your receiver's storage capacity, the text file containing the survey site information can be split into smaller text files. You will then be able to upload successive files as needed or work from multiple receivers in the field.

b. For more help, please refer to the appropriate online documentation or user's manuals.

Printing Datasheets

The form used for recording data can be found on the tab labeled “FIELD SHEET” in the Aquatic Plant Survey Data Workbook, downloadable from the University of Wisconsin Extension website (http://www.uwsp.edu/cnr/uwexlakes/ecology/APM/Appendix-C.xls). Print the field sheet (waterproof paper recommended), using the “Print Area > Set Print Area” function under the “File” menu to set the appropriate number of rows to print. Under Header (View > Header and Footer > Custom Header) record lake name, Waterbody Identification Code (WBIC), county and survey date.



Constructing the Rake Samplers

The rake samplers are each constructed of two rake heads welded together, bar-to-bar, to form a double-sided rake head. The rake head is 13.8 inches (35 centimeters) long, with approximately 14 tines on each side. For use in shallow waters, mount a double-sided rake head to a pole that has the capability to extend to 15 feet (4.6 meters). For use in deeper waters, attach a second double-sided rake head to a rope; this rake head should also be weighted (Figure 2).

Pole Sampler

To make the pole samplers shown in the photographs, we removed the handles from 2 standard bow rakes (available at most hardware stores), and welded the rake heads together bar-to-bar. We mounted the rake head to an 8-foot (telescoping to 15.5 feet) pool skimmer handle purchased from a supply store (left, $50). For an even sturdier sampler we purchased an aluminum Co-Handle from Duraframe Dipnet and designed a rake pole which attaches and detaches into 3 sections (right, $200). For depth recording, mark the rake handle in one-foot increments. Electrical tape marked with permanent marker, then covered with a length of clear packing tape works well and holds up over time.

Rope Sampler

A similar rake head should be constructed and attached to a 40-foot-long rope or anchor line. In order to ensure a quick vertical descent to the lake bottom, attach a light weight (~5 lb) to the rake head, away from the tines. The rope sampler pictured here has a short piece of steel tubing welded to the rake head to serve as a handle through which 45 feet of rope is attached.

Figure 2: Examples of sampling rakes used during surveys.

COLLECTING AND RECORDING FIELD DATA

Using the Rake Samplers

Collect one rake sample per sample site.

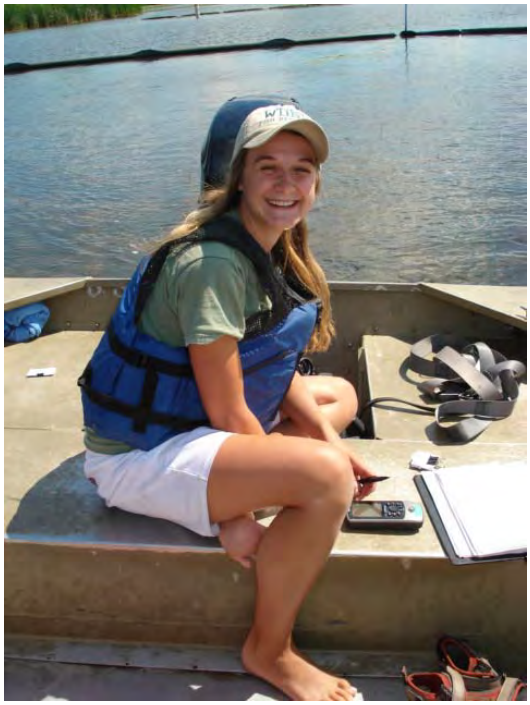
In water shallower than 15 feet deep, use the pole sampler. At each sample site, lower the rake straight through the water column to rest lightly on the bottom, twist the rake around twice, and then pull the rake straight out of the water.

In water deeper than 15 feet, drop the rope sampler straight into the water alongside the boat, drag the rake along the sediment surface for approximately one foot (0.3 m), and then pull the rake to the surface.

A large tray or bin may be used to aid in processing the entire sample.

Navigating to Sites

Accuracy



The location reported by the GPS receiver has an element of error that varies under different conditions. The total error from the GPS and your navigational error *combined* should not exceed half of the sampling resolution. Therefore, when sampling with a Garmin 76 receiver, navigate at no greater than an 80-foot zoom level and aim to completely cover the sampling site with the arrow. At 80-foot zoom, the locator arrow shown on the screen represents approximately 25 feet in length. In order to sample with acceptable accuracy, the arrow must completely cover the sample site on screen. At coarser zoom levels, because the size of the arrow remains constant, the boat may be more distant from the site even though the arrow completely covers the site. You can use a lower zoom level (120-feet is appropriate) in order to travel from site to site, but as you approach the target site, you must confirm your location at using at least the 80-ft zoom resolution to ensure you are sampling with acceptable accuracy.

Determining Maximum Depth of Plant Colonization

When sampling, you will have to determine the maximum depth at which the plants are rooted. The maximum depth of colonization (MDC) can vary greatly among lakes, from just a few feet to as deep as the physiological requirements of a species will allow. When sampling a line of sites heading from shore out to deep water, take samples until plants are no longer found on the

rake. Continue sampling at least two sites deeper to ensure you sampled well over the maximum depth of colonization. If no plants are found at these sites, simply record the depth, sampling tool used, and dominant sediment type. Leave the rake fullness and species information blank. Depending on the lake bathymetry, you may choose to continue down the same row to the other side of the lake. Use a depth finder and begin sampling again when the depth reaches that of the last (no plant) site sampled. Alternatively, if the rows are very long, you may choose to move over to the next row and sample sites back into shore, working back and forth along the shoreline and around the lake. However, if the second row is shallower than the first, be sure to start sampling sufficiently far from shore so that the depth is similar to that at which you stopped sampling in the first row. By sampling in this way, over time you will begin to hone in on the maximum depth of plant colonization.

After working several rows crossing the edge of the littoral zone, estimate the maximum depth of colonization (e.g. 20 feet) and only continue to sample deeper sites within 6 feet of this estimation (all sites ≤ 26 feet). As you complete more rows and gain confidence in your estimation, you can then begin to gradually omit sampling depths that are too deep for plants to grow. Once you have sampled the deep end of your estimated maximum depth of colonization (i.e. 26 feet) at least three times and have not found any plants, then you can discontinue sampling at anything deeper, but continue to sample any sites shallower (≤ 25 feet). If you then sample a shallower depth three times (i.e. 25 feet) and find no plants at any of those sites, you may now discontinue sampling at these deeper sites and only sample sites shallower than this new sampling depth (≤ 24 feet). Continue to successively eliminate shallower depths in sequence until you establish the maximum depth of colonization. To account for patchiness and other sources of variation, never narrow the sampling window to less than 1.5 feet of the estimated maximum depth of colonization. Use your best judgment when eliminating depths, and remember that plant distribution may be uneven and that different areas of a single lake may have plants growing relatively deeper or shallower. It is good practice to err on the side of oversampling.

Recording Data

Completing the Field Sheet

1. General site information
   Complete the top portion of the "Field Sheet" with the lake name, county, WBIC, date, names of observers, and how many hours each person worked during the survey.

2. Site number

   Each site location is numbered sequentially. Each site number will have one row of data on the "Field Sheet."

3. Depth

   Measure and record the depth to the nearest half-foot increment at each site sampled, regardless of whether vegetation is present. The pole mounted rake and rope sampler should be marked to measure the depth of water at a sample site. However, a variety of options exist for taking depth measurements, including sonar handheld depth finders (trigger models) and boat-mounted depth finders. If you are using a depth finder, it is useful to know that the accuracy may decrease greatly in densely vegetated areas. Depth finders sometimes report the depth to the top of the vegetation instead of to the sediment surface. In most cases, it is best to use depth markings on a pole-mounted rake for shallow sites.

4. Dominant sediment type

   At each sample site, record the dominant sediment type based on how the rake feels when in contact with the sediment surface as: mucky (M), sandy (S), or rocky (R).

5. Pole vs. Rope

   Record whether the pole (P) mounted rake or the rake-on-a-rope (R) was used to take the sample.

6. Rake fullness

   At each site, after pulling the rake from the water record the overall rake fullness rating that best estimates the total coverage of plants on the rake (1 - few, 2 - moderate, 3 - abundant; see Figure 3). Also identify the different species present on the rake and record a separate rake fullness rating for each. Account for plant parts that dangle or trail from the rake tines as if they were fully wrapped around the rake head. The rake may dislodge plants that will float to the surface, especially short rosette species not easily caught in the tines. Include the rake fullness rating for plants dislodged and floating but not collected on the rake. Record rake fullness ratings for filamentous algae, aquatic moss, freshwater sponges, and liverworts, but do not include these ratings when determining the overall rake fullness rating. While at a site, perform a brief visual scan. If you observe any species within 6 feet (2m) of the sample site, but not collected with the rake, record these species as observed visually ("V") on the field sheet. These species will be included in total number of species observed.

Fullness Rating	Coverage	Description
1		Only few plants. There are not enough plants to entirely cover the length of the rake head in a single layer.
2		There are enough plants to cover the length of the rake head in a single layer, but not enough to fully cover the tines.
3		The rake is completely covered and tines are not visible.

Figure 3: Illustration of rake fullness ratings used during the survey.

7. Species names

Note that the field datasheet does not include any species names, except for EWM (Eurasian water milfoil) and CLP (Curly-leaf pondweed). The sampling crew must write the species name in subsequent columns the first time that species is encountered. Names must be re-written on successive field sheets as they are encountered. You may use common or Latin names, but be sure there is no ambiguity in the name that will present problems during data entry. The use of standard abbreviations can greatly shorten this process. It is generally safe to shorten the names to include the first three letters of the genus name followed by the first three letters of the species name (i.e. *Ceratophyllum demersum* = CerDem).

8. Inaccessible sites

It may be impossible or unsafe to reach some sample sites. Where the water is very shallow, rocks are present, or dense plant growth prevents navigation, field workers should attempt to access the site as long as doing so is safe and relatively practical. It is often possible to reach difficult sites by using oars or poling; however, keep safety in mind and practice good judgment. Do not get out and drag the boat through mucky sediment to reach a site. If the sampling site is shallow but the substrate is firm, you may be able to walk to the site from shore or from the boat. If you cannot access a site, leave the depth blank and record the appropriate comment on the field datasheet from the list below. Remember to also transfer these to the "Comments" column of the ENTRY sheet (see data entry section):

a. NONNAVIGABLE (PLANTS)

1. Sample site cannot be accessed due to thick plant growth.
2. Aquatic plants that are visible within 6 feet of a non-navigable sample site (e.g. water lilies, cattails, bulrushes, etc.) should be recorded as visuals (V) on the datasheet.

b. TERRESTRIAL

1. Sample site occurs on land (including islands).
2. Aquatic plants visible within 6 feet of a terrestrial sample site (e.g. water lilies, cattails, bulrushes, etc.) may be included in the general boat survey list, but should not be marked as visuals (V) on the datasheet.
3. Only species rooted in water should be recorded as present or as part of the boat survey.

c. SHALLOW

1. Sample site is in water that is too shallow to allow access.
2. Aquatic plants that are visible within 6 feet of a shallow sample site should be recorded as visuals (V) on the datasheet.

d. ROCKS

1. Sample site is inaccessible due to the presence of rocks.

e. DOCK

1. Sample site is inaccessible due to the presence of a dock or pier.

f. SWIM AREA

1. Sample site is inaccessible due to the presence of a designated swimming area.

g. TEMPORARY OBSTACLE

1. Sample site is inaccessible due to the presence of a temporary obstacle such as a boater, swimmer, raft, loon, etc.
2. If possible, try to revisit this site later on during the survey once the temporary obstacle has moved.

h. NO INFORMATION

1. No information is available about the sample site because it was not traveled to (inaccessible channel, accidently omitted during survey, skipped due to time constraints, etc.).

i. OTHER

1. Site was not sampled for another reason; please provide a brief description.

9. Filling Out the Boat Survey Datasheet

Often there will be localized occurrences of certain species (e.g., floating-leaf or emergent species) that are missed by the point-intercept grid. For areas that are outside the grid or in between sampling sites, record the name of the plant and the closest site to the plant. This information will be entered into the "BOAT SURVEY" section of the data entry file. Emergent near-shore vegetation should only be recorded if it's rooted in water.

Collecting and Identifying Voucher Samples

Voucher each plant species for verification and identification. You can often use plants collected on the rake as vouchers. However, if the sample is of poor quality or lacks reproductive structures, attempt to collect a better specimen. If a better specimen is unavailable, voucher and press what you are able to collect. Remember that the more material collected, the easier identification will be. Whenever possible, collect at least two specimens, and include reproductive material such as seeds, flowers, fruit, roots, etc. Place the voucher plant into a re-sealable plastic bag with a waterproof voucher label. The voucher label should include the species name, or in the case of unknown species, a unique identifier, the lake name, county, sample site, sediment type, collector's name, and the date. Additional information about habitat or co-occurring species may also be included on the tag. Place all specimens in a cooler for transport to the lab. See below, "Pressing Plants" for instructions once back at the laboratory.

Plant Identification and Troublesome Taxa

1. Plants should be identified to species whenever possible. Certain genera, including *Carex*, *Sparganium*, and *Sagittaria* must be flowering and/or fruiting to confirm identification and may not be identifiable to species without these parts.

2. Non-angiosperms such as *Chara* or *Nitella* are identified to genus only. Often, *Isoetes* can be identified to species by looking at spores, if present. Filamentous algae, aquatic moss, and freshwater sponge can be referred to simply as algae, moss, and sponge.

3. If a plant cannot be identified in the field, place the two voucher specimens in a re-sealable bag with a separate voucher label. Take these specimens back to the lab to verify the identity. The label should include a unique identifier, lake, county, the sample site number, and sediment type. The presence and fullness of the species should be recorded on the field datasheet under the same unique identifier name listed on the voucher label.

4. In the lab, try to identify the plant using plant identification keys and a stereo microscope. If you are still uncertain of the identity of the plant, contact a DNR biologist in your region to help with identification. Do not send specimens to an expert until you notify them of your intended shipment and they have instructed you to do so. Once the plant is identified, record this information so that the correct identification is used during data entry.

ENTERING DATA ELECTRONICALLY

Worksheet Descriptions and Instructions

The Aquatic Plant Survey Data Workbook
(http://www.uwsp.edu/cnr/uwexlakes/ecology/APM/Appendix-C.xls) contains eight worksheets:

1. READ ME

   Provide a brief description of the six other worksheets included in the workbook.

2. FIELD SHEET

   The FIELD SHEET should be printed on waterproof paper for recording the field data.

3. ENTRY

   a. There are many formulas embedded in the ENTRY sheet that allow for the statistical calculations on the STATS sheet. Thus, **DO NOT add or delete columns or rows on the ENTRY or STATS sheets**.

   b. Data collected in the field is recorded on the FIELD SHEET and afterwards transferred to the electronic ENTRY sheet.

   c. Copy latitude and longitude information for the sample sites from the GPS text file and paste into the appropriate columns of the ENTRY sheet.

   d. Record the lake and county name, WBIC, survey date, and the names of the field workers.

   e. There is a column for comments on the ENTRY sheet. Please use the standardized comments discussed on page 18 of this protocol.

   f. Species' Latin names appear alphabetically in the first row of the spreadsheet. Species such as aquatic moss, freshwater sponge, filamentous algae, and liverworts are listed separately at the end of the alphabetical list.

   g. Additional species not already listed should be added in the columns at the end of the alphabetical list (sp1, sp2, etc.). Any vouchered specimens that are awaiting ID confirmation should be entered here as well. You should use the same unique voucher identifier established in the field to for ease of updating the information.

h. We strongly recommend double-checking the electronically entered data against the original field datasheets to ensure that no errors or omissions occurred during the entry process.

4. BOAT SURVEY

a. Enter information on plants observed during the survey that were observed more than 6 feet away from a sample site.

b. Additional comments about field conditions, known management activities, or other observations can also be recorded in this worksheet.

5. STATS

The STATS worksheet automatically calculates summary statistics using the data entered into the ENTRY worksheet (see Appendix 2, Table 1). There are several summary calculations including:

a. Individual Species Statistics:

- **i. Frequency of occurrence within vegetated areas (%)**: Number of sites at which a species was observed divided by the total number of vegetated sites. Frequency of occurrence is sensitive to the number of sample sites included. Including non-vegetated sites will lower the frequency of occurrence.
- **ii. Frequency of occurrence at sites shallower than maximum depth of plants**: Number of sites a species was observed at divided by the total number of sites shallower than maximum depth of plants.
- **iii. Relative frequency (%)**: This is a proportional value that reflects the degree to which an individual species contributes to the sum total of all species observations. The sum of the relative frequencies of all species is 100%. Relative frequency is not sensitive to whether all sampled sites, including non-vegetated sites, are included. Relative frequency does not take into account aquatic moss, freshwater sponges, filamentous algae, or liverworts.
- **iv. Relative frequency (squared)**: This value is only part of a calculation and is not used directly.
- **v. Number of sites where a species was found**: This is the sum of the number of sites at which a species was recorded on the rake.
- **vi. Average rake fullness**: Mean rake fullness rating, ranges from 1-3.
- **vii. Number of visual sightings**: This is the total number of times a plant was seen within 6 feet of the boat, but not collected on the rake.
- **viii. Present (visual or collected)**: Automatically fills in “present” if the species was observed at a sample site.

b. Summary Statistics:

i. **Total number of sites visited**: Total number of sites where depth was recorded, even if a rake sample was not taken.

ii. **Total number of sites with vegetation**: Total number of sites where at least one plant was found on the rake.

iii. **Total number of sites shallower than maximum depth of plants**: Total number of sites where the depth was less than or equal to the maximum depth at which plants were found. This value is used for frequency of occurrence at sites shallower than maximum depth of plants.

iv. **Frequency of occurrence at sites shallower than maximum depth of plants**: Number of times plants were recorded at a site divided by the total number of sites sampled that were shallower than the maximum depth of plants.

v. **Simpson's Diversity Index**: A nonparametric estimator of community heterogeneity. It is based on relative frequency and thus is not sensitive to whether all sampled sites (including non-vegetated sites) are included. The closer the Simpson Diversity Index is to 1, the more diverse the community.

vi. **The maximum depth of plants**: This is the depth of the deepest site sampled at which vegetation was present. Please note that this value does not take into account aquatic moss, freshwater sponges, filamentous algae, or liverworts. See "MAX DEPTH GRAPH" below for more information.

vii. **Number of sites sampled using rake on rope (R)**

viii. **Number of sites sampled using rake on pole (P)**

ix. **Average number of all species per site (shallower than max depth)**: Mean number of species found at sample sites which were less than or equal to the maximum depth of plant colonization.

x. **Average number of species per site (vegetated sites only)**: Mean number of species found at sample sites were vegetation was present.

xi. **Average number of native species per site (shallower than maximum depth)**: This does not include Eurasian water milfoil, Curly-leaf pondweed, Purple loosestrife, Spiny naiad, or Reed canary grass.

xii. **Average number of native species per site (vegetated sites only)**

xiii. **Species richness**: Total number of species observed not including visual sightings. Please note that this value does not include aquatic moss, freshwater sponges, filamentous algae, or liverworts.

xiv. **Species richness (including visuals)**: Total number of species observed including visual sightings recorded within 6 feet of the sample site (but does not include additional species found during the boat survey).

6. MAX DEPTH GRAPH

The maximum depth of colonization is an important metric to characterize accurately, as it can indicate changes in water clarity and water quality over time. This worksheet automatically displays a histogram of plant occurrences by water depth. Occasionally, unrooted plants floating in the water column are snagged by the rake, which can sometimes result in an inaccurate estimation of the maximum depth of colonization. It is

important to examine the reported maximum depth of plant colonization in order to detect potential outliers. As a general rule, a single plant occurrence reported at a site which is 2 or more feet deeper than the next shallowest site with plants is considered an outlier, and should be excluded when determining the maximum depth of plant colonization (see Figure 4).

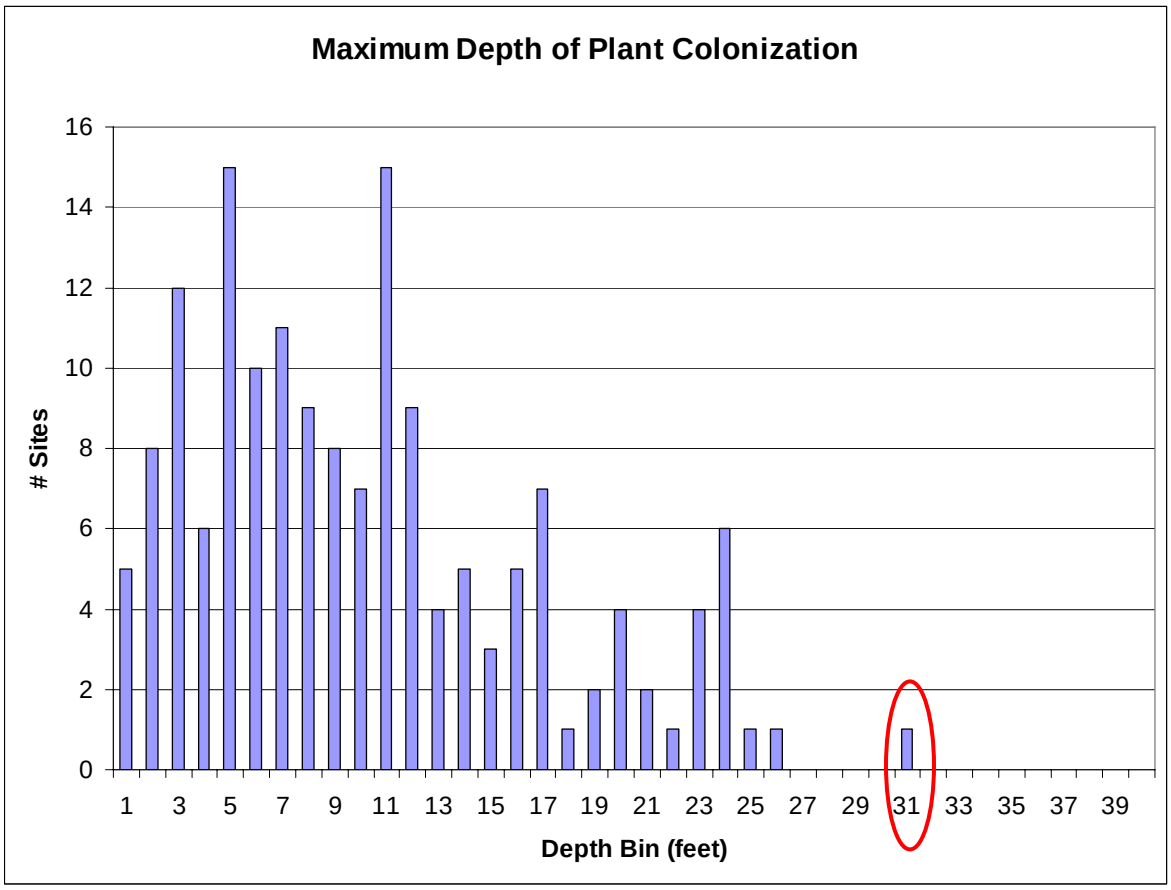


Figure 4: Distribution of plant occurrences versus water column depth. The value circled in red is more than 2 feet deeper than all other plants found during the survey, and is considered an outlier. Outliers should be omitted when determining the maximum depth of plant colonization.

It is necessary to delete the occurrence of this outlier from the ENTRY spreadsheet so that the automatically-calculated statistics will reflect the revised maximum depth of colonization. To do this, locate the sampling point number on the ENTRY worksheet where the outlier was found. Scroll across the row until you find the outlier to omit. Once you've located the cell with the outlier, press delete to clear the cell. Right click on the cell and select "Insert Comment". Briefly describe the occurrence of the outlier and the reason for omitting it. Follow the same steps with the overall rake fullness column, deleting out the contents of the cell and including a brief comment. Please also include information regarding any omissions of outliers and revised MDC directly on the STATS spreadsheet, typing all comments in the space below "See Max Depth Graph Worksheet to Confirm".

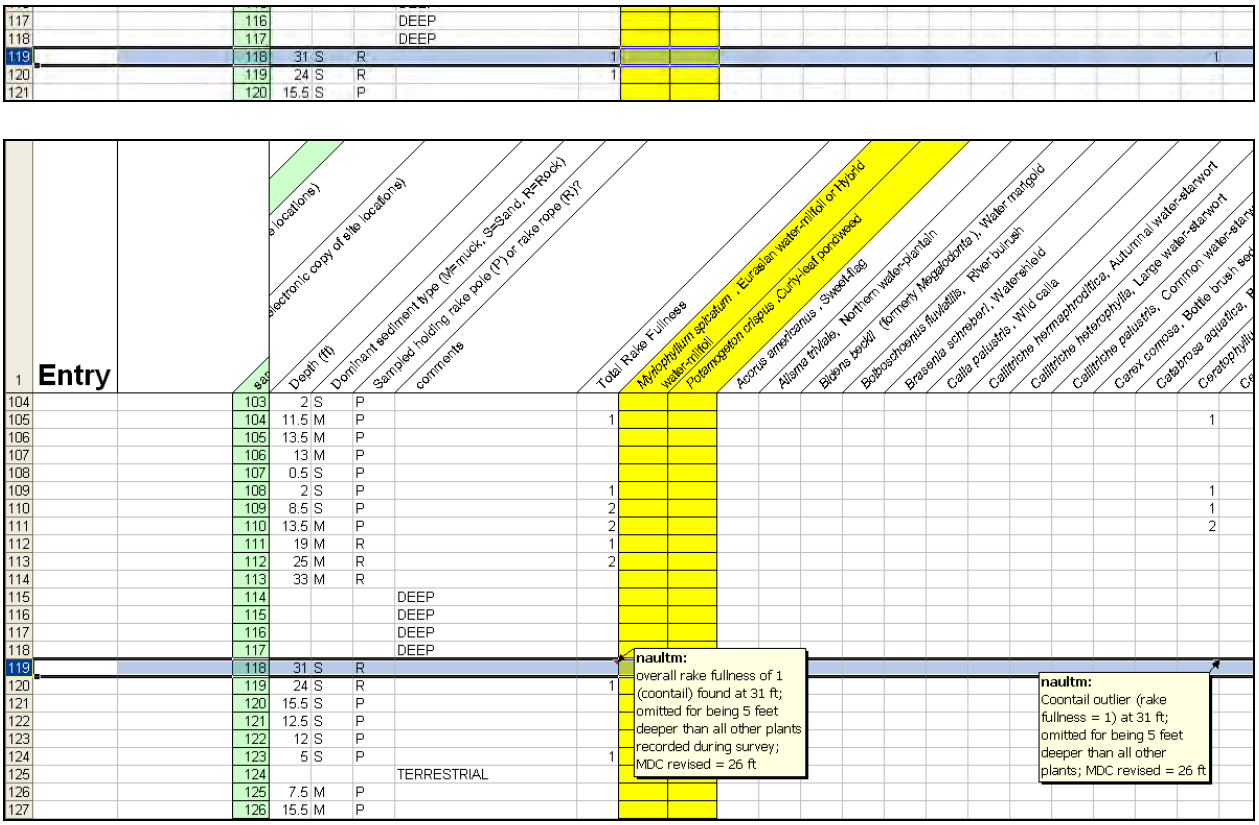


Figure 5: ***Top*** **- Ceratophyllum demersum outlier at 31 feet (sampling point #118).** ***Bottom*** **- C. demersum outlier at 31 feet deleted from both C. demersum and total rake fullness columns. Brief descriptive comments should be inserted in cells where outliers have been deleted.**

7. CALCULATE FQI

This worksheet automatically calculates the Floristic Quality Index (FQI) based upon the data entered into the ENTRY worksheet. The FQI metric is designed to evaluate the closeness of the flora in an area to that of undisturbed conditions[6]. The species list considered in this calculation is that which Nichols[6] originally considered, and the "C values" used in this spreadsheet reflect those currently accepted by the Wisconsin State Herbarium[7]. Species are counted as being present only if they are collected on the rake at some point during the baseline survey.

8. ARCGIS TEMPLATE

This worksheet of truncated species names is used when creating plant distribution maps using ArcGIS 9.3. See Appendix 3 for more information.

[6] Nichols, S.A. 1999. Floristic Quality Assessment of Wisconsin Lake Plant Communities with Example Applications. Journal of Lake and Reservoir Management, 15(2):133-141.

[7] University of Wisconsin-Madison, 2001. Wisconsin Floristic Quality Assessment (WFQA). Retrieved October 27, 2009 from: http://www.botany.wisc.edu/WFQA.asp

Saving the File

Once the data is electronically entered into the Aquatic Plant Survey Data Workbook (http://www.uwsp.edu/cnr/uwexlakes/ecology/APM/Appendix-C.xls), please save the file with a name indicating the lake, county, WBIC, and year sampled. The format we recommend is: Lake_County_WBIC_(year).xls. For example, Lake Mendota sampled in 2009 would be named: Mendota_Dane_805400_(2009).xls

Double-Checking the Data

We strongly recommend double-checking the electronic data against the field sheet to catch any errors made during the entry process.

Sending the Data

Send the final electronic file to the WDNR via email (DNRBaselineAquaticPlants@wisconsin.gov). There should be one file for each completed lake survey.

Creation of Plant Distribution Maps

Aquatic plant distribution maps can be easily created using the point-intercept data collected during the survey. Instructions on how to create these maps can be found in Appendix 3 and 4.

Statistical Analysis of Data

Statistical comparisons of datasets can easily be analyzed between pre- and post-management activities or between two survey years by using a simple chi-square analysis. The chi-square analysis is commonly used to examine whether or not there was a statistically significant change in the occurrence of a plant species between the survey years or after management activities have occurred. The "Compute Pre-Post Data" worksheet (available at: http://www.uwsp.edu/cnr/uwexlakes/ecology/APM/Apendix-D1.xls), allows users to enter in the number of sites at which a species was recorded during each survey, and provides an output indicating whether or not differences reflect a statistically significant change in the plant community.

PRESSING PLANTS – PREPARATION OF VOUCHER SPECIMENS

"Floating" Specimens

Because most aquatic plants, especially finely dissected specimens, tend to stick to paper as they dry, it is usually better to "float" the plant directly onto herbarium paper. However, if the plant is large and robust, or not entirely aquatic (such as bulrushes, emergent sedges or pickerelweed) you can press the plant in newsprint.

1. Use a pencil to label the mounting paper with the plant name, geographic location, date collected, and serial code (a unique identifier in a series that identifies all specimens you have pressed; we use the initials of the presser followed by the year and a sequential number; i.e. AM2009-01). Mount only one species per sheet, and do not cut herbarium sheets in half.

2. Carefully rinse the plant so it is free of epiphyton, silt, and other debris.

3. Fill a sink or tray with about one inch of water. Slip the labeled mounting paper into the water.

4. Float the plant in the water and arrange it onto the sheet.

5. If the plant has fine leaflets, such as water milfoil or bladderwort, cut off one leaf and display it floated out onto the paper so that leaflet characteristics can be readily observed.

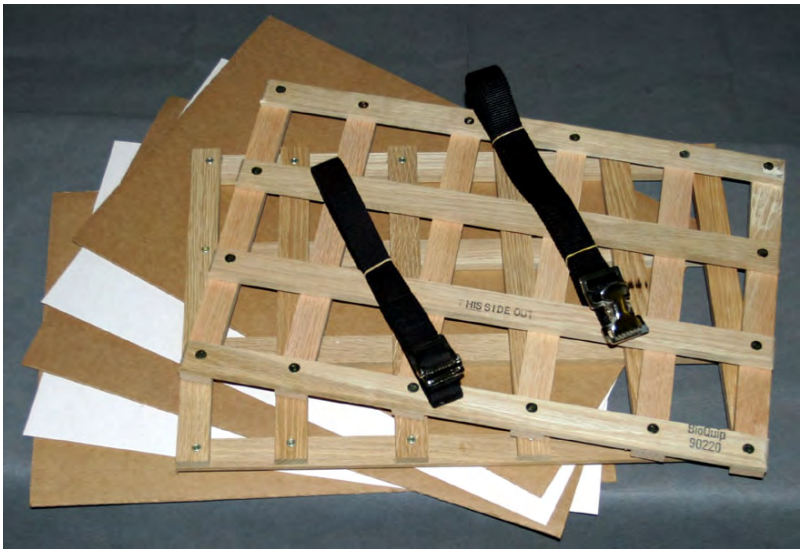
6. The plant may be bent into a "V" or "W" or curled shape to fit on the sheet.

7. Slowly lift the paper out of the water by one end. Keeping the plant in place, let the water slowly drain off.

8. Use a toothpick or probe to spread out plant parts for better display, making sure to expose identifiable characteristics such as stipules, sheaths or seeds.

Pressing Specimens

- Cover the plant with a sheet of waxed paper or plastic wrap if it is especially delicate (we recommend this technique especially for bladderworts and other fine, delicate species).
- Place the specimen sheet inside folds of newspaper.
- Place the newspaper between two sheets of blotting paper, and the blotting paper between two sheets of corrugated cardboard.
- Place multiple specimens in a plant press. Use rope or straps to compress plants to keep specimens flat as they dry.



- Place the press somewhere warm and dry. Placing the press on its long edge on top of a ventilated aluminum or aluminum-lined box containing incandescent light bulbs allows for quick drying. Remove plants after several days when they are thoroughly dry.

Suggested Herbarium Materials

Herbarium and science supply businesses such as the Herbarium Supply Company (www.herbariumsupply.com; 800-348-2338) sell many herbarium products including mounting paper, plant presses, blotting paper, and cardboard spacers. When ordering herbarium mounting paper, look for acid-free, non-glossy, 100% rag, and heavy or standard weights.

Preparing Dried Specimens for Shipment to an Herbarium

1. **Package specimens**. Place each dried specimen with unique identifier clearly marked on the newsprint or mounting paper in the fold of a single sheet of newspaper and place all of the newspaper/specimens between two pieces of cardboard. Tie or rubber band the cardboard bundle together, and put it into a padded envelope or a box. As long as the package is going to or from an educational institution, a special 4th class mailing rate called "Library Rate" can be used.

2. **Label information**. Both of the herbaria utilized by the WDNR label the dried plant specimens themselves. Prepare an electronic spreadsheet with the relevant information for each specimen. Send the file to Mark Wetter (mawetter@wisc.edu) for the Madison herbarium or to Robert Freckmann (rfreckma@uwsp.edu) for the Stevens Point herbarium. Each row (i.e. each specimen) in the file will need a unique identifier such as the collector's initials followed by a specimen number. Use the same identifier on the specimen so the herbaria can match the label to the specimen. Each row of the spreadsheet should include columns for the following (column heading in **bold**, example in plain text):

    a. **Specimen Identifier** CD2009-01
    b. **Collector Name** Isabel Velez
    c. **Preparer's Name** (If different from collector) Chad Douwe
    d. **Lake Name** Little John Jr.
    e. **County** Vilas
    f. **Date collected** 7 July 2009
    g. **Specimen ID** *Potamogeton spirillus*, Spiral-fruited pondweed
    h. **Habitat** muck over sand
    i. **Associated species (if known)** *Najas gracillima*, *Potamogeton friesii*
    j. **TRS** T41N R07E S29
    k. **WBIC** 1861700
    l. **More detailed location** (if known) SW edge of lake, 1 m depth
    m. **GPS lat/long coordinates** (if known) N 46°15.037' W090°01.804'
    n. **Herbarium of deposition** UWSP

3. **Send pressed plants** to Mark Wetter or Ted Cochrane (UW- Madison), or to Dr. Robert Freckmann (UW-Stevens Point). **Please notify the herbarium of your intention and wait for confirmation before sending plants**:

Mark Wetter or Ted Cochrane
University of Wisconsin-Madison Herbarium
Department of Botany, Birge Hall
430 Lincoln Drive
Madison, WI 53706-1381
tel.: (608) 262-2792
FAX: (608) 262-7509
www.botany.wisc.edu/herbarium/

Dr. Robert Freckmann
Robert Freckmann Herbarium
0310 CNR Addition
1900 Franklin Street
Stevens Point, WI 54481
rfreckma@uwsp.edu

4. **Send electronic record to the WDNR.** Please send a copy of the electronic herbarium file along with the plant data to DNRBaselineAquaticPlants@wisconsin.gov.

CONCLUSIONS

There will be four products from each plant survey. First, there will be the raw data from the quantitative survey which provides a lakewide plant species list and distribution and rake fullness data for each species observed. Second, there will be summary statistics useful in characterizing and comparing populations. Third, there will be observations from the general boat survey. Fourth, voucher specimens will provide a catalog of plant species present in the lake and will bolster the state collections. All electronic data should be sent by email to the WDNR (DNRBaselineAquaticPlants@wisconsin.gov).

ACKNOWLEDGEMENTS

We would like to extend our sincere thanks to the WDNR Lake Coordinators and Aquatic Plant Management staff for recommendations and comments in the design, implementation, and applications of the data and the survey methodology. The many hours the field staff put into testing this methodology was integral to its successful development, and we are very grateful for all of their hard work.

Appendix 1

Current (02/2010) contact information for regional WDNR aquatic plant management (APM) and lake coordinators

Northern Region (NOR)

(Ashland, Barron, Bayfield, Burnett, Douglas, Florence, Forest, Iron, Langlade, Lincoln, Oneida, Polk, Price, Rusk, Sawyer, Taylor, Vilas, & Washburn Co.)

Frank Koshere
APM Coordinator
715-392-0807
frank.koshere@wisconsin.gov

Kevin Gauthier, Sr.
Florence, Forest, Langlade, Lincoln, Oneida, & Vilas Co.
715-365-8937
kevin.gauthiersr@wisconsin.gov

Pamela Toshner
Barron, Bayfield, Burnett, Douglas, Polk, & Washburn Co.
715-635-4073
pamela.toshner@wisconsin.gov

Jim Kreitlow
Ashland, Iron, Price, Rusk, Sawyer, & Taylor Co.
715-365-8947
james.kreitlow@wisconsin.gov

Southeast Region (SER)

(Kenosha, Milwaukee, Ozaukee, Racine, Sheboygan, Walworth, Washington, & Waukesha Co.)

Heidi Bunk
: Ozaukee, Sheboygan, Walworth, Washington, & Waukesha Co.
262-574-2130
heidi.bunk@wisconsin.gov

Craig Helker
Kenosha, Milwaukee, & Racine Co.
262-884-2357
craig.helker@wisconsin.gov

South Central Region (SCR)

(Columbia, Dane, Dodge, Green, Grant, Iowa, Jefferson, Lafayette, Richland, Rock, & Sauk Co.)

Susan Graham
Lake & APM Coordinator
608-275-3329
susan.graham@wisconsin.gov

Northeast Region (NER)

(Brown, Calumet, Door, Fond du Lac, Green Lake, Kewaunee, Manitowoc, Marinette, Marquette, Menominee, Oconto, Outagamie, Shawano, Waupaca, Waushara, & Winnebago Co.)

Mary Gansberg
Kewaunee, Door, Manitowoc, & Menominee Co.
920-662-5489
mary.gansberg@wisconsin.gov

Ted Johnson
Green Lake, Marquette, Waupaca, & Waushara
920-787-4686 ext. 3017
tedm.johnson@wisconsin.gov

Mark Sesing
Fond du Lac, Outagamie, & Winnebago Co.
920-485-3023
mark.sesing@wisconsin.gov

Jim Reyburn
Brown, Oconto, & Shawano Co.
920-662-5465
james.reyburn@wisconsin.gov

Greg Sevener
Marinette Co.
715-582-5013
gregory.sevener@wisconsin.gov

West Central Region (WCR)

(Adams, Buffalo, Chippewa, Clark, Crawford, Dunn, Eau Claire, Jackson, Juneau, La Crosse, Marathon, Monroe, Pepin, Pierce, Polk, Portage, St. Croix, Trempealeau, Vernon, & Wood Co.)

Scott Provost
APM Coordinator
715-421-7881 ext. 3017
scott.provost@wisconsin.gov

Buzz Sorge
Lake Coordinator
715-839-3794
patrick.sorge@wisconsin.gov

Appendix 2

This appendix contains examples of statistical outputs created through the point-intercept sampling method for Kathan Lake, Oneida County. The data was collected during a survey conducted August 21-22, 2007.

Table 1. Summary Statistics

Total number of sites set-up	**203**
Total number of sites visited	**171**
Total number of sites with vegetation	**149**
Total number of sites shallower than maximum depth of plants	**165**
Frequency of occurrence at sites shallower than maximum depth of plants	**90.30**
Simpson Diversity Index	**0.94**
Maximum depth of plants (ft)	**9.50**
Number of sites sampled using rake on Rope (R)	**0**
Number of sites sampled using rake on Pole (P)	**171**
Average number of all species per site (shallower than max depth)	**3.96**
Average number of all species per site (veg. sites only)	**4.39**
Average number of native species per site (shallower than max depth)	**3.56**
Average number of native species per site (veg. sites only)	**3.95**
Species Richness	**37**
Species Richness (including visuals)	**38**
Species Richness (including visuals & boat survey)	**40**

Table 2. Individual species frequency of occurrences

Common Name	Scientific Name	% Frequency (Littoral)	% Frequency (Whole lake)	% Frequency (in vegetated areas)	Relative Frequency (%)
Bushy pondweed	*Najas flexilis*	41.2	39.8	45.6	10.4
Common waterweed	*Elodea canadensis*	40.6	39.2	45.0	10.2
Eurasian water milfoil*	*Myriophyllum spicatum**	40.0	38.6	44.3	10.1
Filamentous algae	*Algae* spp.	26.1	25.1	28.9	6.6
Coontail	*Ceratophyllum demersum*	23.0	22.2	25.5	5.8
Stoneworts	*Nitella* spp.	21.8	21.1	24.2	5.5
Watershield	*Brasenia schreberi*	20.6	19.9	22.8	5.2
Small bladderwort	*Utricularia minor*	17.6	17.0	19.5	4.4
Small pondweed	*Potamogeton pusillus*	17.0	16.4	18.8	4.3
Common bladderwort	*Utricularia vulgaris*	16.4	15.8	18.1	4.1
Wild celery	*Vallisneria americana*	15.2	14.6	16.8	3.8
Flat stem pondweed	*Potamogeton zosteriformis*	13.9	13.5	15.4	3.5
Stiff pondweed	*Potamogeton strictifolius*	11.5	11.1	12.8	2.9
Ribbon leaf pondweed	*Potamogeton epihydrus*	9.1	8.8	10.1	2.3
White water lily	*Nymphaea odorata*	7.9	7.6	8.7	2.0
Muskgrasses	*Chara* spp.	7.3	7.0	8.1	1.8
Freshwater sponge	Sponge spp.	6.1	5.8	6.7	1.5
Moss	Moss spp.	6.1	5.8	6.7	1.5
Large-leaf pondweed	*Potamogeton amplifolius*	5.5	5.3	6.0	1.4
Spiny-spored quillwort	*Isoetes echinospora*	4.9	4.7	5.4	1.2
Waterwort	*Elatine minima*	4.2	4.1	4.7	1.1
Creeping spikerush	*Eleocharis palustris*	4.2	4.1	4.7	1.1
Water horsetail	*Equisetum fluviatile*	4.2	4.1	4.7	1.1
Northern water milfoil	*Myriophyllum sibiricum*	4.2	4.1	4.7	1.1
Thin floating-leaf bur-reed	*Sparganium* sp.	4.2	4.1	4.7	1.1
Spatterdock	*Nuphar variegata*	3.6	3.5	4.0	0.9
Spiral-fruited pondweed	*Potamogeton spirillus*	3.6	3.5	4.0	0.9
American bur-reed	*Sparganium americanum*	3.6	3.5	4.0	0.9
Shoreweed	*Littorella uniflora*	3.0	2.9	3.4	0.8
Brown-fruited rush	*Juncus pelocarpus* f. *submersus*	2.4	2.3	2.7	0.6
Variable pondweed	*Potamogeton gramineus*	2.4	2.3	2.7	0.6
Twin-stemmed bladderwort	*Utricularia geminiscapa*	1.8	1.8	2.0	0.5
Pipewort	*Eriocaulon aquaticum*	0.6	0.6	0.7	0.2
Clasping leaf pondweed	*Potamogeton richardsonii*	0.6	0.6	0.7	0.2
Broad-leaved arrowhead	*Sagittaria latifolia*	0.6	0.6	0.7	0.2
Thin-leaved pondweed	*Potamogeton* sp.	0.6	0.6	0.7	0.2
Flat-leaved bladderwort	*Utricularia intermedia*	0.6	0.6	0.7	0.2
Cattail	*Typha* sp.	Visual	Visual	Visual	Visual
Needle spikerush	*Eleocharis acicularis*	Boat Survey	Boat Survey	Boat Survey	Boat Survey
Three-way sedge	*Dulichium arundinaceum*	Boat Survey	Boat Survey	Boat Survey	Boat Survey

Table 3. Number of sites where species was found and average rake fullness rating

Common Name	Scientific Name	# sites where species was found	# sites where species was found (including visuals)	Average rake fullness rating
Bushy pondweed	*Najas flexilis*	68	68	1.28
Common waterweed	*Elodea canadensis*	67	67	1.28
Eurasian water milfoil*	*Myriophyllum spicatum**	66	71	1.47
Filamentous algae	*Algae* spp.	43	43	1.00
Coontail	*Ceratophyllum demersum*	38	38	1.37
Stoneworts	*Nitella* spp.	36	36	1.00
Watershield	*Brasenia schreberi*	34	58	1.68
Small bladderwort	*Utricularia minor*	29	29	1.10
Small pondweed	*Potamogeton pusillus*	28	28	1.14
Common bladderwort	*Utricularia vulgaris*	27	27	1.30
Wild celery	*Vallisneria americana*	25	26	1.36
Flat stem pondweed	*Potamogeton zosteriformis*	23	25	1.22
Stiff pondweed	*Potamogeton strictifolius*	19	19	1.16
Ribbon leaf pondweed	*Potamogeton epihydrus*	15	18	1.27
White water lily	*Nymphaea odorata*	13	42	1.69
Muskgrasses	*Chara* spp.	12	12	1.25
Freshwater sponge	Sponge spp.	10	11	1.00
Moss	Moss spp.	10	10	1.20
Large-leaf pondweed	*Potamogeton amplifolius*	9	10	1.33
Spiny-spored quillwort	*Isoetes echinospora*	8	11	1.00
Waterwort	*Elatine minima*	7	8	1.00
Creeping spikerush	*Eleocharis palustris*	7	9	1.14
Water horsetail	*Equisetum fluviatile*	7	15	1.43
Northern water milfoil	*Myriophyllum sibiricum*	7	7	1.00
Thin floating-leaf bur-reed	*Sparganium* sp.	7	7	1.00
Spatterdock	*Nuphar variegata*	6	22	1.17
Spiral-fruited pondweed	*Potamogeton spirillus*	6	6	1.00
American bur-reed	*Sparganium americanum*	6	11	1.50
Shoreweed	*Littorella uniflora*	5	5	1.00
Brown-fruited rush	*Juncus pelocarpus* f. *submersus*	4	5	1.25
Variable pondweed	*Potamogeton gramineus*	4	5	1.00
Twin-stemmed bladderwort	*Utricularia geminiscapa*	3	3	1.00
Pipewort	*Eriocaulon aquaticum*	1	2	1.00
Clasping leaf pondweed	*Potamogeton richardsonii*	1	1	2.00
Broad-leaved arrowhead	*Sagittaria latifolia*	1	1	1.00
Thin-leaved pondweed	*Potamogeton* sp.	1	1	1.00
Flat-leaved bladderwort	*Utricularia intermedia*	1	1	1.00
Cattail	*Typha* sp.	Visual	3	n/a
Needle spikerush	*Eleocharis acicularis*	Boat Survey	Boat Survey	n/a
Three-way sedge	*Dulichium arundinaceum*	Boat Survey	Boat Survey	n/a

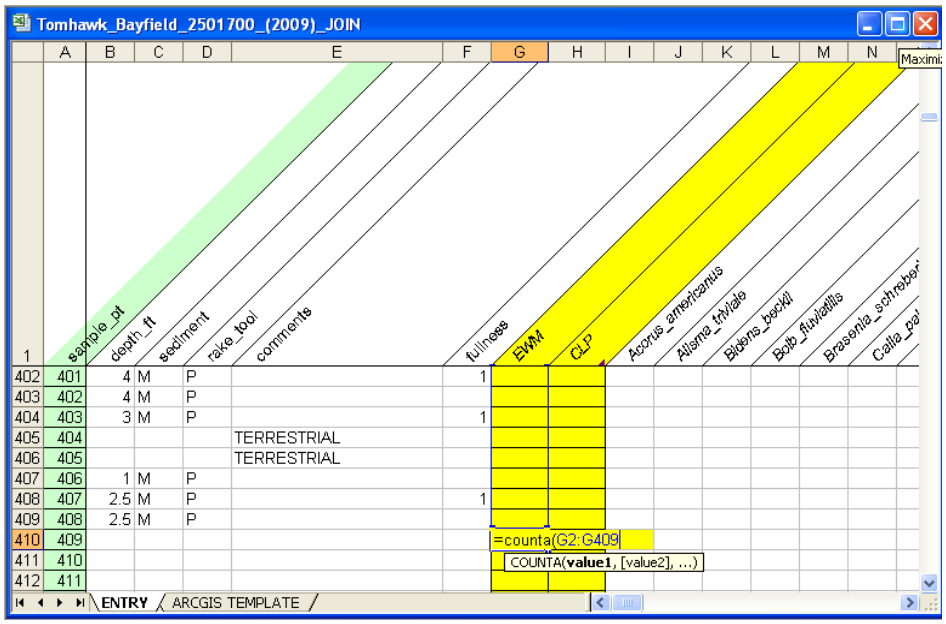
Appendix 3

Creating a Plant Distribution Map Using Point Intercept Data in ArcGIS 9.3

This is a protocol for making a plant distribution map using ArcGIS 9.3 and the Excel (2003 version) file of data from the point intercept (PI) survey. This protocol can be changed in a number of different ways and still produce a similar product. The best way to make PI-based maps depends on the particular dataset; however, this procedure works well in most cases. Similar images may be created in PowerPoint or in photo editing software if the dataset is not large or complex.

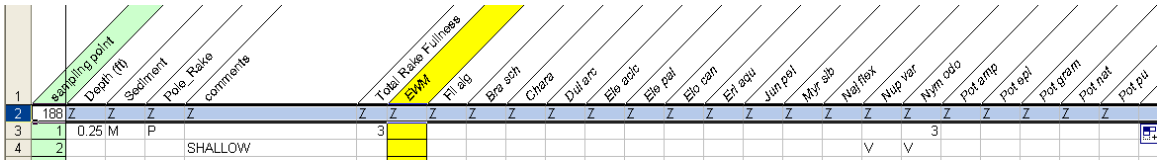
1. After entering the PI survey data into the Aquatic Plant Survey Data Workbook (Appendix-C.xls), save the file using a unique name. We recommend the convention: Lake_County_WBIC_(YYYY).xls

2. Prepare <Lake_County_WBIC_(YYYY).xls> For Join
    a. Open file in Excel
    b. File → Save As → Lake_County_WBIC_(YYYY)_JOIN.xls (DO NOT MODIFY ORIGINAL FILE)
    c. Delete all worksheets except for ENTRY and ARCGIS TEMPLATE (make sure to scroll left and delete the README sheet)
        i. Click on worksheet tab; Edit → Delete Sheet → Delete
    d. Delete the following columns
        i. Entry columns (A & I) and calculated columns (B-H)
            1. Columns B-H are normally hidden. To "unhide" them, cursor over the column heading (A) at the top of the sheet and click/drag to highlight it and the adjacent column (I). Right click the highlighted region, then select unhide. Columns B-H are colored blue. Now delete all columns A-I.
        ii. Latitude, Longitude columns (possibly hidden, located between sampling point and depth columns)
        iii. Replace first row of ENTRY with ARCGIS TEMPLATE
            1. Copy the entire first row of truncated species names from the ARCGIS TEMPLATE worksheet
            2. Highlight the first row on the ENTRY worksheet and replace with the template (Edit → Paste)
        iv. Species columns with no data
            1. Add a count row to identify empty columns to delete
                a. Select all cells and remove any validation
                    i. Select All (Ctrl-A)
                    ii. Data → Validation → OK → Allow Any Value → OK
                b. In the row below the last sampled point, and in the first column under a plant species, enter the formula =counta(
                c. Then highlight the column up to the first sampling point. The beginning of this procedure is depicted below.



d. Finally, add a closing) and hit enter. The final formula will be similar to this: =counta(G2:G500)
e. Point the cursor over the bottom right corner of the cell until cursor turns into a "+". Click/Drag this formula all the way across to the end of the species list.
f. Delete any columns where the sum row is equal to 0
g. Then delete the sum row

e. Delete any rows after the last applicable sample point
   i. The "sample_pt" column is usually populated up to 4000 points; delete any rows where the sampling point column is numbered, but these sample points are greater than the number of points set-up in the lakewide grid, and therefore the row doesn't contain any information.
f. Add a "dummy" row so all data imports into ArcGIS as "text"
   i. Add a row directly above the first sampled point
   ii. In this newly created row, under the Sampling Point column, enter the number equal to the total number of sample points plus 1 (i.e. total sampling points in example image is 187. The number 188 would be entered into the "dummy" row under the sampling point)
g. Enter "Z" in all other cells in all columns that contain any information



h. Save the file and close Excel

3. Save the lake specific polygon and point shapefiles to a folder on a local drive
   a. We'll refer to this folder as "MapFolder"

4. Open ArcMap
   a. Select to Start using ArcMap with "a new empty map" and click "OK"

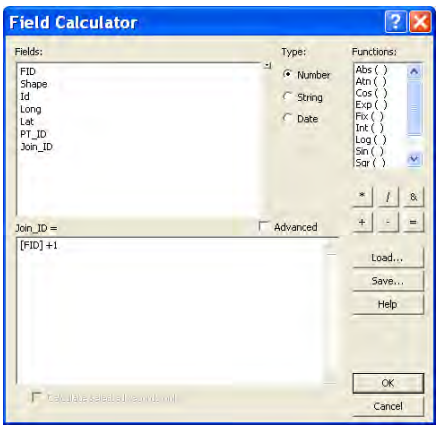
5. Add Data (either method “a” or “b”)
    a. Using Add Data Button
        i. Select the “Add Data” button; or File → Add Data 
        ii. Navigate to MapFolder
        iii. Highlight both the lake polygon (lake_country_WBIC_poly.shp) and point (lake_county_WBIC_XXmpts.shp) shapefiles
        iv. Click on ‘Add’
    b. Directly from ArcCatalog
        i. Situate ArcMap and ArcCatalog windows so that you can see both
        ii. Navigate to MapFolder in ArcCatalog
        iii. Highlight both the lake polygon (lake_county_WBIC_poly) and point (lake_county_WBIC_XXmpts) shapefiles
        iv. Drag and drop these shapefiles into ArcMap
        v. Note: Shapefiles should only be saved, deleted, moved, etc. in ArcCatalog. Using Windows Explorer with shapefiles can result in accidental deletion of individual shapefile files (i.e. *.shp, *.dbf, *.sbn, *.shx, *.sbx, and *.sbn files must all be stored together. ArcCatalog packages these files together so nothing gets lost)

6. Defining Shapefile Projections
    a. If after adding in your shapefiles a warning message regarding “Unknown Spatial Reference” appears, the shapefiles coordinate system is not defined
        i. To define and verify projection, please contact DNRBaselineAquaticPlants@wisconsin.gov
        ii. Alternatively, the shapefile projection can be defined manually by using the Define Projection Tool located in ArcToolbox
            1. ArcToolbox → Data Management Tools → Projections and Transformations → Define Projection
            2. Input Dataset or Feature Class
                a. Select the shapefile that needs a defined projection
            3. Click on the browse button (right side of dialog box)
            4. In the Spatial Reference Properties dialog box, click on the “Select” button
            5. Browse for the correct coordinate system
                a. Projected Coordinate System → State Systems → NAD 1983 HARN Wisconsin TM.prj; Click Add.
                    i. Do not use the US Feet system
                    ii. The coordinate system name may also be displayed as NAD 1983 HARN Transverse Mercator
                    iii. Coordinate system parameters:
                        1. Projection → Transverse Mercator
                        False Easting → 520000.00000000
                        False Northing → -4480000.000000
                        Central Meridian → -90.00000000
                        Linear Unit → Meter

6. Select “OK” on Spatial Reference Properties dialog box, and “OK” on define projection tool

7. Edit Attribute Table for point shapefile
    a. Open Attribute Table
        i. Right click on point shapefile in ArcMap table of contents
        ii. Select “Open Attribute Table”
    b. Add a Field
        i. Select the “Options” button → “Add Field”
        ii. Name: Join_ID
        iii. Type: Double
        iv. Precision: 10
        v. Scale: 3

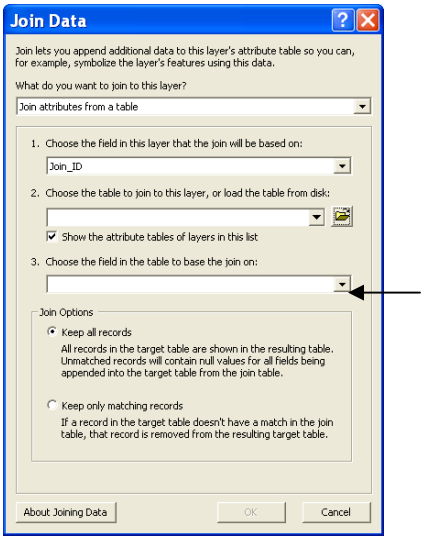
    c. Populate Join_ID Column
        i. Right click on “Join_ID” column heading
        ii. Select “Field Calculator”
        iii. If Field Calculator warning message pops up, click “Yes”
        iv. Set expression by double-clicking FID in the “Fields:” box and typing +1. The white box under “Join_ID =” should now read **[FID] +1**
        v. Click “OK”
        vi. Your Join_ID column should now be populated in sequential order, starting with point #1 at the top
        vii. Close the attribute table
        viii. Note: This expression is assuming that each unique ID was based off of the calculation [FID] +1 when creating the initial point file. If the unique ID’s were not created in sequential order based on the FID field, then calculate Join_ID field accordingly (example: Truncate a unique ID such as ‘Como001’ so that it just reads ‘001’ in the Join_ID field.)



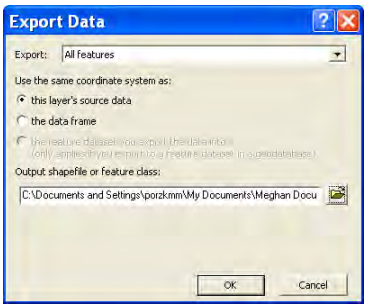
8. Join shapefile to <Lake_County_WBIC_(YYYY)_JOIN.xls>
    a. Right click on point shapefile in ArcMap table of contents
    b. Select Joins and Relates → Join…
    c. Set the following options:
        i. Join Attributes from a table
        ii. Join will be based on “Join_ID”
        iii. Choose the table to join to this layer
            1. Click on Window Folder (See arrow)

2. Navigate to and double-click on the Excel file saved in step 2
3. Double-click on the 'ENTRY $' sheet
4. Click "Add"

iv. Base the join on "sample_pt"
v. Join Options: Keep All Records (If using ArcGIS 9.2, these options can be viewed by clicking the "Advanced" button)
vi. Click "OK"
vii. If prompted to create index, select "Yes"



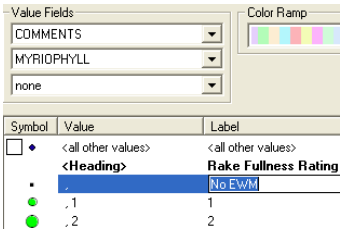
9. Export joined shapefile to make it permanent
    a. Right click on joined point shapefile in ArcMap table of contents
    b. Select Data → Export Data
    c. Set the following options:
        i. Export: All Features
        ii. Use the same coordinate system as: this layer's source data
        iii. Output shapefile or feature class: Save in MapFolder as **Lake_County_WBIC_ XXpts_YEAR_JOIN.shp**



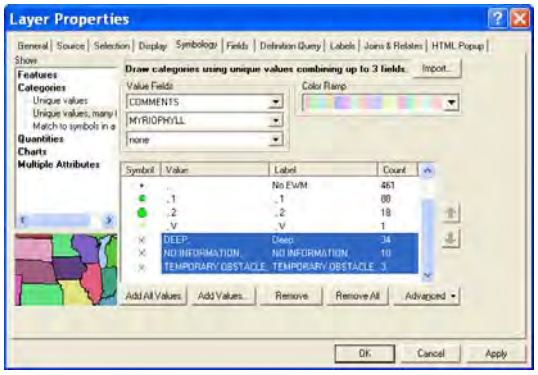
    d. Click "OK"
    e. When asked if you want to add the exported data to the map as a layer, select "Yes"
        i. This final joined shapefile will now be referred to as "Joined Point Shapefile"
    f. Remove the Join from the original point shapefile
        i. Right click on point shapefile in ArcMap table of contents
        ii. Select Joins and Relates → Remove Join(s) → Remove All Joins
    g. In the table of contents, uncheck or remove the original point shapefile that was used to create the Joined Point Shapefile.

10. Check Join Results
    a. Right click on the Joined Point Shapefile in the table of contents
    b. Select "Open Attribute Table"
    c. Verify that Join was successful
        i. All data present in Excel file should now be located in the Joined Point Shapefile attribute table, and the Join_ID and Sample_Pt columns will be identical

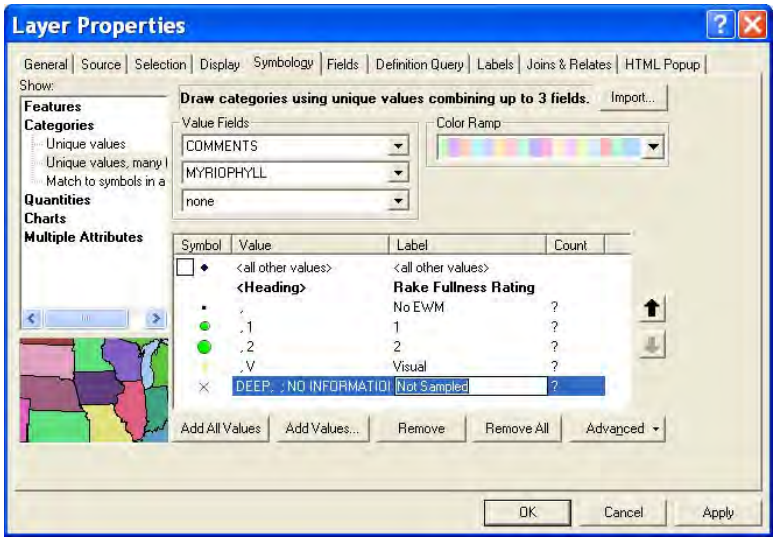
11. Display Plant Distribution Data
    a. Right click on the Joined Point Shapefile in the table of contents
    b. Select “Properties”
    c. Select “Symbology” tab
    d. On left side of dialog box under “Show:”, select “Categories – Unique Values, Many Fields”
    e. Value Fields should be “Comments”. Be sure to select the appropriate Comments field, as there may be two that appear similar.
    f. You will then choose additional Value Fields to display species information (i.e. If you want to display both EWM and CLP species information, then both EWM and CLP need to be chosen as Value Fields)
    g. Select “Add All Values”
        i. All possible values are now displayed, separated by a comma. Each position indicates the unique values for each Value Field you designated in steps e & f, in the order entered. That is, if you selected ‘comments’, ‘EWM’, and ‘CLP’ as your value fields, the first value might read: ‘ , , ‘ indicating points that were sampled, but had neither a comment, EWM, nor CLP present. The next value might read ‘ , ,1’, which includes points with no comments, no EWM, and fullness rating of 1 for CLP.
        ii. Points with information for the ‘comments’ value field were likely not sampled; the comment listed should clarify how to work with these points.
    h. Un-check <all other values> box
    i. Double-click on symbol next to each value to set symbology
        i. You must now choose appropriate symbols and colors for the different variables being expressed.
        ii. Typically we use increasing sizes of a green circle for EWM density ratings (values: 1, 2, 3), a small light green circle for visuals (V), a small black dot for sites sampled that had no relevant plant data, and a small “x” symbol for all sites not sampled
    j. You can change the label name of the symbol being represented by clicking on the respective space under “Label”. (e.g. change “ , ” to “No EWM”; “ ,1” to “1”; “ ,V” to “Visual”; “Deep, ” to “Not Sampled”)



    k. You can also group values together (e.g. No Information, Deep, Shallow, etc)
        i. Hold down the Shift key and highlight all rows that should be grouped



ii. Right click on highlighted rows and select “Group Values”
iii. The final Layer Properties dialog box should look similar to this: Note: If you want to change the order that these will appear in the legend, highlight a row and use the arrows on the right side to move.
iv. Click “Apply” then “OK” to update symbols on map

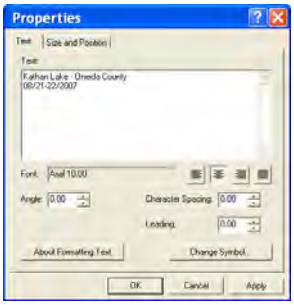


v. The polygon shapefile fill color and outline may also be modified similarly under the “Symbology” tab

12. Map Page Layout
    a. Verify that the coordinate system is defined correctly for the Data Frame
        i. Select View → Data Frame Properties → Coordinate System Tab
        ii. If the coordinate system is incorrectly defined, browse for the correct coordinate system
            1. Predefined → Projected Coordinate System → State Systems → NAD 1983 HARN Wisconsin TM.prj
    b. View → Layout View
    c. File → Page and Print Setup → Select Landscape or Portrait
    d. Modify size/shape of data frame to fit on entire page and serve as map border
        i. Right click data frame, select Properties, under the ‘Frame’ tab, change border to a thickness of 2 and select OK.
    e. Insert → North Arrow
        i. Size and position appropriately
    f. Insert → Scale Bar
        i. Select “Alternating Scale Bar 1” and click “OK”
        ii. Double-click on Scale Bar in Layout view to edit properties
        iii. Set the following properties:
            1. Number of divisions: 2
            2. Number of subdivisions: 1
            3. Set units to kilometers

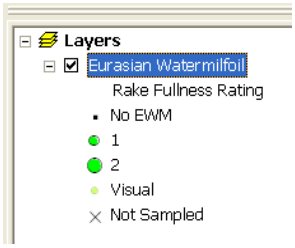
4. Click “OK”

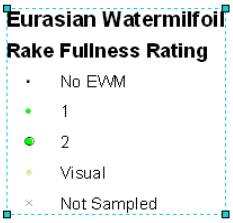
g. Insert → Text
   i. Double-click on Text Box to edit information
      1. Create text box with the following information:
         a. Lake Name, County, Date Sampled, etc.
      2. Format text as appropriate using “Change Symbol…” button



h. Insert → Picture → Navigate to WDNR Logo (Black & White)
   i. Size and position appropriately

i. Legend
   i. In the table of contents, modify the displayed name of your shapefile as you would like it to appear in your legend by single clicking on the text
   ii. Insert → Legend
   iii. Choose which layers you want to include in your legend
      1. Include the layer that has the plant distribution symbology information
      2. You may have to remove the polygon layer by highlighting it under “Legend Items” and clicking the single left angle bracket (<), then select “Next”
   iv. Remove the word “Legend” from the Legend Title and select “Next”
   v. Continue selecting “Next” and then “Finish”
   vi. Format legend text
      1. Right click on Legend and select “Properties”
   vii. Size and position legend as appropriate





j. If you’re going to be switching between maps quickly to look at comparisons between years or species, we suggest making and refining the layout first, then saving it as an ArcMap Template so you can use the same one each time
   i. File → Save As → Save As Type: ArcMap Template

k. Check printed map for color accuracy before you export (Step 13). Sometimes the colors may look different on screen, but may print with the same hue and value, making interpretation impossible. You can set a custom color if necessary.

13. Saving Map as JPEG
   a. File → Export Map
      i. Save as type: JPEG
      ii. Set Resolution: 300 dpi
      iii. Navigate to appropriate folder and Save

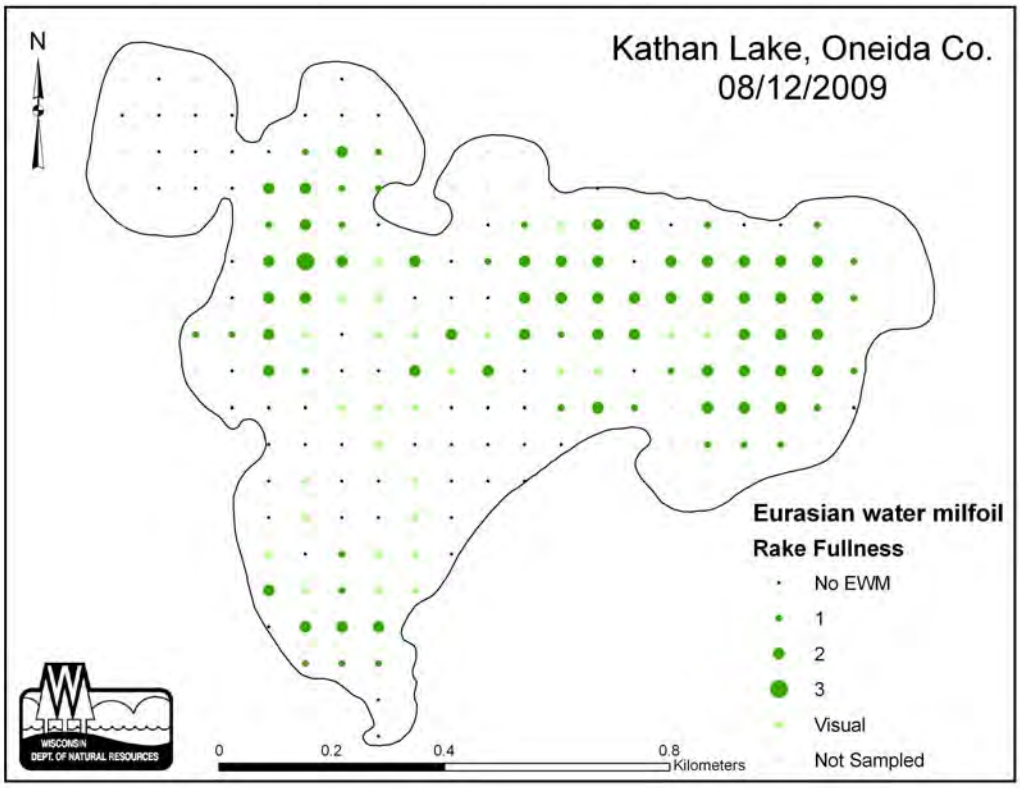


Figure 6: Example plant distribution map created using point-intercept data and ArcGIS 9.3 software for Kathan Lake, Oneida County.

Appendix 4

Creating a Plant Distribution Map Using Point Intercept Data in ArcGIS 3.3

This is a protocol for making plant maps using ArcView GIS 3.3 and the Aquatic Plant Survey Data Workbook Excel file <Appendix-C.xls.>. This protocol can be changed in a number of different ways and still produce a similar product. The best way to make PI-based maps depends on the particular dataset; however, this procedure works well in most cases. Similar images may be created in PowerPoint or in photo editing software if the dataset is not large or complex.

1. Save the ArcView shapefiles (*.shp, *.dbf, *.sbn, *.shx, *.sbx, *.sbn) to a folder on a local drive.
    a. We'll refer to this folder as "MapFolder"

2. Open ArcView and create a new project with a new view.
    a. Click "yes" to add data

3. Add shapefiles from MapFolder
    a. You can add multiple files at once by holding down "shift" while you click the individual files

4. View window: select the point file
    a. Make sure both themes have the box checked in order to view them
    b. Click once on the point layer to activate that theme (raised box around that item)
    c. If necessary, drag the activated point layer above the polygon layer in order to see the sample points

5. Open theme table
    a. Theme > Table or
    b. The open theme table shortcut button

6. Start editing, add variable column
    a. Table > Start Editing
    b. Edit > Add Field
        i. Enter the name of the field (e.g. EWM_2009)
        ii. Specifications 'type', 'width', and 'decimal places' do not need to be changed
        iii. Click "OK"

7. Stop editing, save edits
    a. Table > Stop Editing, 'Yes' to save edits

8. Export point file

a. File > Export
b. Select ‘dBASE’
c. Select MapFolder to save file
d. Default will be named <table1.dbf>
e. Close table

9. Set-working directory
    i. File > Set Working Directory
    ii. Change working directory to MapFolder

10. Save project, exit ArcView
    a. File > Save Project As > save in MapFolder (for ease of reference, lets call the file EWM_Map.apr)
    b. Exit ArcView

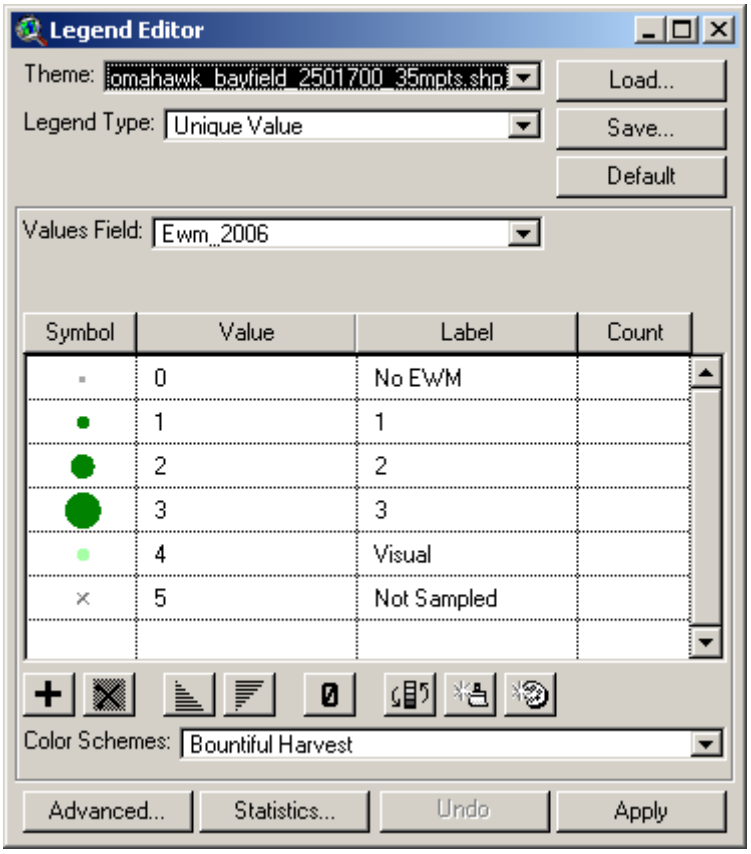
11. Open file saved in step 8 with Excel
    a. Open excel; Open a file, when prompted to find the file, navigate to MapFolder
    b. In “Files of type” option bar select “All files”
    c. Open <table1.dbf>

12. List information under data field created (EWM_2009)
    a. Open PI data entry excel file (WiAPMS.xls)
    b. Copy columns “Sample point, Depth, Comments, & EWM”
    c. Paste special “values” into new excel workbook
        i. Edit > Paste Special > Values
    d. Highlight all data, sort by comments
        i. Data > Sort > Comments
    e. Enter the number 5 into EWM column for all unsampled sites (deep, terrestrial, non-navigable, etc) (this is so the legend can code these sites)
    f. Highlight EWM data column and replace all blanks with 0 (zero), and V (visuals) with 4
        i. Edit > Replace, replace all
    g. Highlight all data, re-sort by sampling site
        i. Data > Sort > Sampling Point
    h. Copy EWM column, excluding header, paste into the .dbf file (already open, originally created in step 8)
    i. “Save as” this file as the **original dbf** file’s name (the copy you placed in MapFolder, not the original file, obviously)
        i. i.e. overwrite the ISS original (e.g. Kathan_Oneida_1598300_65mpts.dbf) with the new file you just modified in excel. The name must be EXACTLY the same!!
        ii. Close excel

13. Reopen project in ArcView
    a. Open existing project

b. Open MapFolder and click on EWM_Map.apr (or whatever you chose to name it in step 9)

14. Create legend
    a. Double-click point symbol in the View frame to open the legend window
    b. In "Legend Type" option bar, choose "Unique Value"
    c. In "Values Field" option bar select "EWM_2009" column (or whatever column you want this map to show)
    d. Apply
    e. You must now choose appropriate symbols and colors for the different variables being expressed by the legend. You can change the symbol by double clicking on it
    f. Typically we use increasing sizes of a green circle for EWM density ratings (values: 1, 2 , 3), a small light green circle for visuals (value: 4), a small black dot for sites sampled, but without EWM, (value: 0), and a small "x" symbol for sites not sampled (value: 5).



    g. You can change the label name of the symbol being represented by clicking on the respective cell under "Label". (e.g. change "5" to "Not Sampled", change "4" to Visual)
    h. The color or shading of the polygon can also be changed by double clicking on the theme

15. Set units
    a. View > Properties
    b. Change map units to "meters" and distance units to "kilometers"

16. Layout
    a. View > Layout
    b. Select Landscape or Portrait
    c. Double-click 'View1' to change map title
    d. Double-click scale bar to adjust range or units
    e. If you're going to be switching between maps quickly to look at comparisons between years or species, we suggest making and refining the layout first, then saving it as a Template (Layout > Store as Template) so you can use the same one each time.

f. Check printed map for color accuracy before you export (step 17). Sometimes the colors may look different on screen, but may print with the same hue and value, making interpretation impossible. You can set a custom color if necessary.

17. Save as JPEG
    a. Have the final layout window active
    b. Select File > Export
    c. In "List Files of Type" option bar, select JPEG
    d. Click 'Options' button
        i. Set resolution to highest number
        ii. Likely 144 DPI and Quality = 100
    e. Type file name, choose location in which to save the JPEG
    f. Click OK

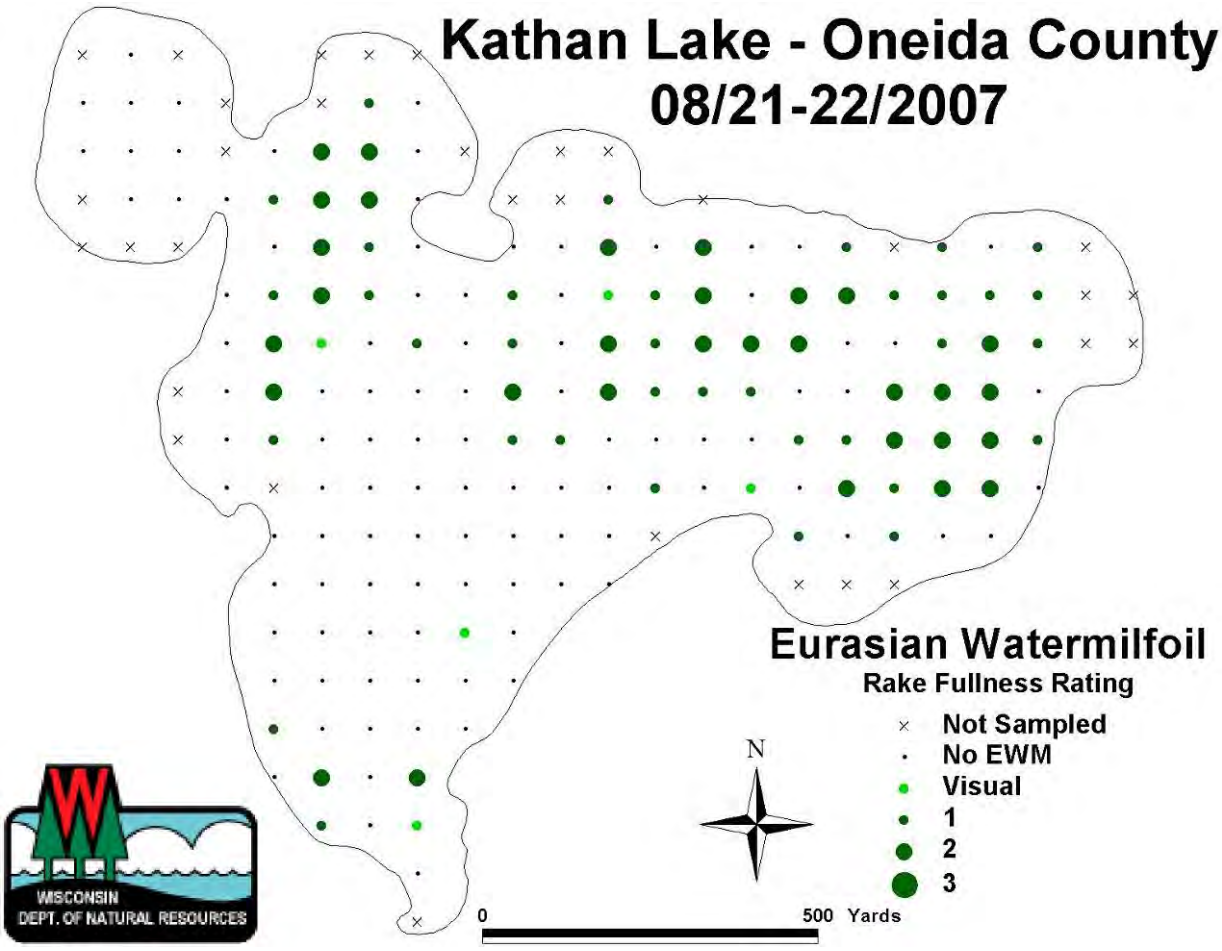


Figure 7: Example plant distribution map created using point-intercept data and ArcGIS 3.3 software for Kathan Lake, Oneida County.

Document citation:

Hauxwell, J., S. Knight, K. Wagner, A. Mikulyuk, M. Nault, M. Porzky and S. Chase. 2010. Recommended baseline monitoring of aquatic plants in Wisconsin: sampling design, field and laboratory procedures, data entry and analysis, and applications. Wisconsin Department of Natural Resources Bureau of Science Services, PUB-SS-1068 2010. Madison, Wisconsin, USA.


WISCONSIN
DEPT. OF NATURAL RESOURCES

Science Services

Center for Excellence –
providing expertise for science-based decision-making

We develop and deliver science-based information, technologies, and applications to help people make well-informed decisions about natural resource management, conservation, and environmental protection.

Our Mission: The Bureau of Science Services supports the Wisconsin Department of Natural Resources and its partners by:

- conducting research and acquiring original knowledge.
- analyzing new information and emerging technologies.
- synthesizing information for policy and management decisions.
- applying the scientific method to the solution of environmental and natural resources problems.
- providing science-based support services for department initiatives.
- collaborating with local, state, regional, and federal agencies and academic institutions in Wisconsin and around the world.

Appendix B.7

Aquatic Plant Monitoring Data Sheet

Sheboygan River AOC Sheboygan County WBIC 50700 **Date:**

Observer 1: name and hours:							Observer 2: name and hours:										Observer 3: name and hours:										Total hours worked:										
Site #	Depth (ft)	Dominant sediment type (M, S, R)	Rake pole (P) or rake rope (R)?	Total Rake Fullness	EWM 1,2,3	CLP 1,2,3	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
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																											

Appendix B.8

Boat Survey Data Sheet

Boat Survey	
Lake	
County	
WBIC	
Date of Survey	
Field Crew	
Nearest Point	Species seen, habitat information
	ADDITIONAL COMMENTS

Appendix B.9

UW-Stevens Point Aquatic Biomonitoring Laboratory

Quality Assurance Program Plan for Macroinvertebrate Sample Processing

QUALITY ASSURANCE PROGRAM PLAN

FOR

MACROINVERTEBRATE SAMPLE PROCESSING

AQUATIC BIOMONITORING LABORATORY
College of Natural Resources
University of Wisconsin – Stevens Point
Stevens Point, WI 54481-3897
Laboratory Phone (715) 346-3868
Building FAX (715) 346-3624

5 April 2010

QUALITY ASSURANCE PROGRAM PLAN

FOR

MACROINVERTEBRATE SAMPLE PROCESSING

5 April 2010

Prepared by
Jeffrey J. Dimick, Laboratory Supervisor
and
Dr. Stanley W. Szczytko, Principal Investigator

for the

Aquatic Biomonitoring Laboratory
College of Natural Resources, University of Wisconsin – Stevens Point

---

Laboratory Supervisor Date
Jeffrey J. Dimick

Principal Investigator Date
Dr. Stanley W. Szczytko

1. INTRODUCTION

1.1 Commitment to Quality Assurance

Quality assurance (QA) is a continuing procedure and essential component of the Aquatic Biomonitoring Laboratory (ABL) in the College of Natural Resources (CNR) at the University of Wisconsin – Stevens Point (UWSP). The principal investigator (PI – Dr. Stanley W. Szczytko), and laboratory supervisor (LS – Jeffrey J. Dimick) are involved with all aspects of quality assurance and control in the laboratory and will serve as QA project supervisors. They will be responsible for monitoring laboratory records to ensure that methods and standards operating procedures (SOP) are followed closely. All current laboratory SOP's are available as appendices to this document. The laboratory supervisor will communicate closely with the PI regarding QA and any problems which arise in the laboratory. The PI assumes the ultimate responsibility for adherence to the practices described in this document.

1.2 Purpose and Scope of Quality Assurance Plan

The main objective of the QA plan is to guarantee high quality benthic macroinvertebrate sample processing, and laboratory data analysis, to meet the requirements of aquatic biomonitoring programs for the Wisconsin Department of Natural Resources (WDNR) and other cooperators. ABL depends on agency personnel for quality control of field data, labeling, sample collection, and adherence to established agency bioassessment guidelines.

The scope of this plan covers all work and activities conducted by ABL for aquatic biomonitoring programs. Detailed SOP's are available for all phases of work performed in ABL.

2. ABL DESCRIPTION AND ACTIVITIES

2.1 Function and Activities of ABL

ABL is a multipurpose benthic laboratory in the CNR at UWSP. The laboratory is involved with general undergraduate education and a variety of graduate student research projects including basic and applied research on lotic ecosystems. The laboratory is also responsible for macroinvertebrate sample processing and biological water quality assessments for Wisconsin streams as part of various WDNR biomonitoring programs, and upper midwestern aquatic systems by request of other cooperators.

Aquatic macroinvertebrate surveys are used to characterize lotic populations at specific locations in rivers and streams. Data generated from these surveys are important in assessing temporal and spatial changes which may be related to water quality changes.

The biotic index (BI – Hilsenhoff 1979, 1982, 1987) has been used by WDNR since 1979 in bioassessments of water quality as part of their Surface Water Monitoring Program. In 1983, WDNR established standardized protocols for sampling and laboratory procedures involved with applying the BI for water quality assessments. The BI was originally designed to detect dissolved oxygen problems caused by putrescible wastes, and it appears to work well for that purpose. The BI is used widely by other states and agencies due to its ease of application and ability to infer environmental condition. Bioassessments utilizing the BI, and also additional aquatic macroinvertebrate community metrics, have gained wide acceptance because the community of organisms used are continually exposed throughout their life cycles to extremes in environmental conditions, theoretically serving as effective indicators of environmental stress and change.

2.2 ABL Organizational Structure

The LS of ABL reports directly to the PI. The PI reports directly to the Dean of CNR, who reports to the Vice Chancellor for Academic Affairs (UWSP), and ultimately to the Chancellor of the University (UWSP). ABL has one director (PI), one laboratory supervisor (LS), one assistant laboratory supervisor (ALS) and variable numbers of laboratory personnel including graduate and undergraduate students. Other CNR and Biology faculty may become involved with ABL on specific projects.

3. QUALITY ASSURANCE MANAGEMENT

3.1 General Organization of QA Personnel

The QA Director or PI works closely with the LS in overseeing the QA activities related to each project and interacts directly with laboratory personnel on a regular basis.

3.2 Responsibilities of QA and Project Personnel

Principal Investigator

- Develop QA plan
- Design research proposals
- Oversee the activities and job responsibilities of the laboratory supervisor (LS)
- Maintain records and monitors QA of the laboratory
- Work with the LS in developing SOPs and project methodologies
- Review QA reports from the LS
- Financial management of the laboratory
- Prepare final project reports
- Determine project priorities in consultation with agencies

Laboratory Supervisor

- Supervise laboratory personnel and day-to-day laboratory activities
- Select new laboratory personnel

- Authorize payroll for laboratory staff
- Work with the PI in developing the QA plan and SOPs
- Monitor daily QA activities
- Train laboratory personnel in all phases of laboratory work
- Assist the PI in maintaining project databases
- Track sample processing and assign work to laboratory personnel
- Maintenance of laboratory inventory
- Maintenance of laboratory equipment
- Ensure timely processing of samples

Assistant Laboratory Supervisor
- Supervise laboratory personnel and day-to-day laboratory activities
- Work with the LS in developing the QA plan and SOPs
- Monitor daily QA activities
- Train laboratory personnel in all phases of laboratory work

4. QUALITY ASSURANCE PLAN COMPONENTS

4.1 General Quality Assurance Practices

The specific QA protocols for each research or monitoring project will be dictated by the methods used and expected results. The general QA practices common to most of the research projects conducted in ABL are discussed below.

4.1a Sample logs

Sample logs to record sample processing effort data are developed in electronic format using Excel® software. Hard copy sample logs will be developed to allow laboratory personnel to record efforts applied to samples. Effort data will be ultimately entered to the electronic sample logs. Effort data entries will include date of sample processing, the initials of laboratory personnel performing sample processing, percent of sample processed to obtain project-specified minimum subsort, and time expenditure for the subsort. Any errors to hard copy sample log entries will be cancelled with a single line drawn through the erroneous record and initialed with an explanation provided in the margin. Data entries shall be legible and detailed to prevent misinterpretation. The LS will routinely review all sample logs and sign and date the logs. The PI will also review logs on a regular basis.

4.1b Methodology

All laboratory methods will be available in written form (SOPs) to all laboratory personnel. SOPs will have a unique method number, title, description of applicability, basic principles involved, equipment required, safety precautions, procedures, calculations, and quality assurance. SOPs will be retained in a loose-leaf binder. Methods which are considered standard (state and/or government approved) will be used

when possible and applicable. Methods and standard operation procedures should be detailed in project proposals and contracts.

4.1c Reagents

Only reagents and preservatives in sufficient quality to meet project requirements will be used. As a general rule, 80% isopropyl alcohol will be used as the standard preservative for benthic macroinvertebrate samples. Purchased reagents such as mounting media will be labeled with the date received, the date opened, and the expiration date when appropriate.

4.1d Sample handling

All macroinvertebrate samples will be checked in by the LS and then logged into the laboratory system. All samples will be correlated with their respective agency field sheets, and any discrepancies noted will be rectified with the responsible agency personnel for corrections. The logging system will include a unique 12-digit sample identification number, agency title, contract year, rep count, sample type, waterbody name, agency field person and date of receipt. These data will be captured in an Excel spreadsheet. One hard copy log will be generated for in-lab effort recording, and two electronic log copies will be stored in different locations. Samples will be checked for integrity and level of preservative; represervation will be performed when necessary. All samples from a project will be stored, and processed, together.

4.1e Instrument Calibration and Maintenance

Any calibration or maintenance work done on instruments or laboratory equipment will be documented. Records of calibration and maintenance will be maintained in an Excel® spreadsheet, with two copies stored in different locations. Records will include: identification of the equipment and problem; date of work; person completing the work; and any other pertinent information considered important. Each piece of equipment will be listed to its own worksheet within the Excel® spreadsheet.

4.1f Data Processing

The procedures for data processing should be detailed in the project proposal and contract. All WDNR data will be generated using the computer DNRBUG program which was developed at the ABL. This program, a compiled Visual dBase program, calculates 26 benthic macroinvertebrate community metrics including the BI. A standard hard copy report for each sample will be generated using the DNRBUG program including a header and taxalist sheets. The header sheet includes all specific site information completed by a WDNR field person and the unique 12-digit sample identification number. The taxalist sheet includes the unique 12-digit sample identification number, taxonomic determinations, number of organisms/taxon, tolerance value for each taxon, unique 8-digit organism identification number, BI determination and state threatened/endangered ranks where appropriate. Additionally, an Excel®

spreadsheet is developed listing fourteen community metrics (biotic index, family-level biotic index, Max-10 biotic index, index of biotic integrity, species richness, generic richness, percent individuals in the orders Ephemeroptera-Plecoptera-Trichoptera, percent generic taxa in the orders Ephemeroptera-Plecoptera-Trichoptera, Shannon diversity, percent scrapers, percent filterers, percent gatherers, percent shredders and percent individuals in the family Chironomidae) will be calculated for routine WDNR biomonitoring samples, and will be forwarded to WDNR Surface Water Information Management System. Additional community metrics can be developed as requested by WDNR. Non-WDNR projects will be reported in a format agreed to in the project proposal and contract for same, usually DNRBUG-generated reports or Excel spreadsheet per United States Environmental Protection Agency format.

Accuracy of data entry will be hand checked by comparing the computer generated reports against the hand written bench and field sheets. Additional data validity checks are made within the DNRBUG computer program. All data checks are executed prior to performing metric calculations and subsequent database storage. The agency providing the samples is ultimately responsible for the accuracy of the field collected data. After hard copy reports are generated and checked, they will be sent to the appropriate agency personnel.

Master computer database files containing all field and laboratory data will be updated as new samples are processed. All data developed by ABL will be stored in the master database unless specified by the agency providing the samples. Electronic copies of the master database will be stored on three different hard drives and two different forms of external media storage (CD and flash drive). Hard drive storage will be accessible to only the PI and LS. External media storage is secured by the LS. At regular intervals, the updated master database will be made accessible through the ABL website (available at http://www.uwsp.edu/water/biomonitoring/index3.htm). Data files will also be sent electronically forwarded to agency personnel upon request.

Taxonomic information for Wisconsin aquatic macroinvertebrates is stored within the DNRBUG program in the file TAXAMAST.DBF. This file contains the taxon-unique 8 digit organism identification number, taxonomic nomenclature, BI tolerance value (if applicable) and Wisconsin Threatened/Endangered rank listing of the aquatic macroinvertebrates in Wisconsin. This file will be updated as new taxonomic classifications and information become available. Periodic literature reviews, and consultations with colleagues, will be conducted to ensure that TAXAMAST.DBF data are current.

4.2 Personnel Training

The PI and LS will ensure that all laboratory personnel are familiar with the importance of the QA plan as it applies to their work efforts. The QA plan will be posted in the laboratory and readily available for review. All SOPs will also be posted and available for review in the laboratory. The LS will ensure that each laboratory personnel has read, and has a clear understanding of, each SOP as it applies to their work duties.

The LS and/or ALS will train each new employee on all phases of laboratory work and review each SOP with the employee.

Each new employee will initially train in an assigned SOP only under direct supervision of the LS and/or (ALS) until SOP is performed to ABL standards. Once trained in SOP, each employee will be subject to spot check QA/QC review by LS and/or ALS. Any noted deficiencies or errors will be addressed as soon as they arise. If deficiencies/errors are noted, the employee will be retrained under direct supervision of the LS and/or ALS until SOP is performed to ABL standards.

Literature Cited

Hilsenhoff, W. L. 1977. Use of arthropods to evaluate water quality of streams. WDNR Tech. Bull. No. 100: 1-15.

Hilsenhoff, W. L. 1982. Using a biotic index to evaluate water quality in streams. WDNR Tech. Bull. No. 132: 1-22.

Hilsenhoff, W. L. 1987. An improved biotic index of organic stream pollution. Great Lakes Entomol. 20: 31-39.

Appendix B.10

UW-Stevens Point Aquatic Biomonitoring Laboratory

Subsorting Procedures for Semi-Quantitative Hilsenhoff Biotic Index (HBI) Samples

Procedure No. 7-94 (Revised 08/2010)

SUBSORTING PROCEDURES FOR SEMI-QUANTITATIVE HILSENHOFF BIOTIC INDEX (HBI) SAMPLES

INTRODUCTION

Benthic macroinvertebrates are sorted from Hilsenhoff Biotic Index (HBI) samples by water-rinsing the sample, placing the sample in a sorting tray and removing the organisms, using forceps, from the sample debris. Using a 2X magnifying sorting lamp facilitates organism recognition. The sample is grid-sub sorted according to procedures outlined by Hilsenhoff (1987) and modified as presented below. All non-plant organisms visible with the aid of the 2X sorting lamp are removed from the selected grid(s) and preserved with 80% isopropyl alcohol in secure receiving containers for subsequent identification. Any unsorted sample remnant is re-preserved, using 99% isopropyl alcohol, in the original sample container and retained until sample report is accepted by the agency providing the sample.

EQUIPMENT LIST

► Protective laboratory gear (Protective goggles, vinyl gloves, laboratory apron)
► Waste preservative container
► Nitex® mesh piece, #30 mesh, 8” x 8”
► Large funnel
► Magnifying sorting lamp, 2X
► Pyrex® sorting pan, 28 cm x 18 cm x 4 cm
► Gridded sorting pan tray, grid dimensions 5 cm^2, grids numbered 1 – n
► Misting bottle with distilled water
► Distilled water
► Petri dish, 9 cm diameter
► Petri dish, 11.5 cm diameter
► Baby food jar
► Fine-tipped forceps, 2
► Isopropyl alcohol, 99%
► Isopropyl alcohol, 80 %
► 20 ml scintillation vial
► Random grid generator
► Hand-held tally
► Black stage plate
► Dissecting microscope and light source
► White labeling tape
► Bond paper, 20% rag content
► Permanent black marker
► HB (#2) pencil or India ink pen

SUBSORTING PROCEDURES FOR SEMI-QUANTITATIVE HILSENHOFF BIOTIC INDEX (HBI) SAMPLES

EQUIPMENT LIST (continued)

- ► Sample debris container
- ► Scissors
- ► Single-edge razor blade, 2
- ► Teaspoon
- ► Large serving spoon
- ► Plastic putty knife, 1.5" wide blade

PROCEDURE

1) Don all laboratory protective gear.

2) Examine the Laboratory Sample Log Sheet and select the next sample for sorting.

3) Remove the sample jar(s) and associated field sheet from the laboratory sample storage area. The field sheet will indicate the number of jars containing the selected sample. Samples contained in multiple jars must have the sample contents from all jars combined in the sorting pan prior to commencing the gridded subsort.

4) Each Rep of a multi-rep sample is treated as a separate sample. One person will be responsible for all Reps associated with a specific SampleIDnum.

5) Laboratory personnel sorting the sample will initial and date the Laboratory Sample Log Sheet at the entry for the sample's unique SampleIDnum and Rep designation, and also sign onto the field sheet where indicated.

6) Drain preservative from the selected sample into the waste preservative container, pouring off the preservative through the 8" x 8" #30 Nitex® mesh piece installed in the large funnel. Waste preservative is subsequently delivered to the University of Wisconsin – Stevens Point laboratory waste collection program.

7) Transfer the sample into the mesh-lined funnel and water rinse the sample. The sample is rinsed under a gentle stream of running water until the water passing through the mesh is free of turbidity. Large sample debris is rinsed, inspected for organisms and discarded into the sample debris container when free of organisms. Large sample volumes may require splitting to avoid losing sample debris over the top of the rinsing device. Water rinsing the sample reduces laboratory personnel exposure to preservative fumes and removes fine particles from the sample, reducing turbidity in the sorting pan and facilitating sorting procedures.

Procedure No. 7-94 (Revised 09/2008)

SUBSORTING PROCEDURES FOR SEMI-QUANTITATIVE HILSENHOFF BIOTIC INDEX (HBI) SAMPLES

PROCEDURE (continued)

8) Transfer the sample to the Pyrex® sorting pan. Examine the sample container and washing mesh to ensure that all sample components are transferred to the sorting pan. If the sample is contained in more than one sample container, all sample containers are to be water-rinsed and all contents combined in the sorting pan.

9) Relocate the sample, in the sorting pan, to a sorting station. Turn on the 2X magnifying lamp. Place the sorting pan in a gridded sorting tray.

10) Keep all aspects of the sample, its original container and labels, and field sheet associated at all times. Bring these to the sorting station along with the sample.

11) Distribute the sample debris evenly in the sorting pan. Maintain moist sample debris, utilizing the misting bottle as necessary.

12) Fill the 9 cm diameter Petri dish 1/3 full with 80% isopropyl alcohol. This dish will serve as the initial receiving container for the sorted organisms. Fill the baby food jar with distilled water. The jar will serve as a tweezers rinse following alcohol contact by the tweezers.

13) Select initial sorting grid using the random grid generator. Select one letter (A – E) and one number (1 – 3). Record the selection on the field sheet under the "**For Lab Use Only**" section.

	A	B	C	D	E
1					
2					
3					

14) Delineate boundaries of the selected grid. Every other organism lying on the grid boundaries is included in the selected grid, starting with the first encountered organism. Non-included grid line organisms are moved to the adjacent grid. Grid boundary delineation is facilitated

SUBSORTING PROCEDURES FOR SEMI-QUANTITATIVE HILSENHOFF BIOTIC INDEX (HBI) SAMPLES

PROCEDURE (continued)

14) (continued) by physical separation of debris. Large debris is cut along grid boundaries using the scissors or a single-edge razor blade.

15) Transfer debris from the selected grid into the 11.5 cm diameter Petri dish. The teaspoon is used to transfer larger volumes and sizes of debris to the Petri dish. Using two single-edge razor blades as "dustpans" will facilitate transfer of smaller debris components. Transferring debris into the 11.5 cm Petri dish allows greater spreading of debris, facilitating location of organisms within the debris.

16) Examine the cleared grid in the sorting tray, using the 2X magnifying lamp. Using tweezers, remove all non-plant organisms from the grid, placing them in the 9 cm diameter Petri dish containing 80% isopropyl alcohol. Tally the HBI organisms (organisms with an established tolerance value per Hilsenhoff 1987) as they are removed from the sample. Rinsing the tweezers after alcohol contact in the baby food jar containing water will facilitate grasping the next selected specimen.

17) Begin sorting the debris in the 11.5 cm diameter Petri dish. Using tweezers, remove all non-plant organisms from the 11.5 cm diameter Petri dish, placing them in the 9 cm diameter Petri dish containing 80% isopropyl alcohol. Tally the HBI organisms (organisms with an established tolerance value per Hilsenhoff 1987) as they are removed from the sample. Viewing the selected debris through the 2X sorting lamp will aid in locating organisms. Rinsing the tweezers after alcohol contact in the baby food jar containing water will facilitate grasping the next selected specimen. After sorting through the Petri dish on the lab counter top background, sort through the Petri dish again while the Petri dish is on the black stage plate. Placing the Petri dish on a black stage plate will facilitate seeing small larvae and worms.

18) A grid, when selected for sorting, will be sorted to completion. Sorting of the selected grid is complete when all non-plant organisms are removed from the grid debris.

19) Record the tally value of HBI organisms on the field sheet next to the grid designation recorded in Step 12 (above). If 125+ organisms were sorted from the grid, the subsort is halted and the tally value is verified per Steps 19 & 20 below. If less than 125 HBI organisms were sorted from the selected grid, the sort and HBI organism tally continue in the next selected grid, and other grids as necessary until125+ HBI organisms are sorted from the sample. Additional grids are selected using the random number table. Apply Steps 13 - 18 to successively selected grids. Remove all non-plant organisms from a grid in progress

SUBSORTING PROCEDURES FOR SEMI-QUANTITATIVE HILSENHOFF BIOTIC INDEX (HBI) SAMPLES

PROCEDURE (continued)

19) (continued) before advancing to the next grid. Continue sorting until either 125+ HBI organisms are removed from the sample, or the sample is sorted in its entirety, whichever situation first arises. Sorting Tip – If the tally indicates a count of 115 – 124, halt the subsort and verify the tally under the dissecting microscope. Sometimes, specimens uncounted during the subsort will be recognized as countable under the dissecting microscope.

20) Select a 20 ml scintillation vial for the sorted organisms. Fill the vial with 80% isopropyl alcohol. A subsort containing large quantity of organism mass may be split into as many vials as necessary to contain the subsort.

21) Relocate to a dissecting microscope station. Zero the tally and, using the dissecting microscope, verify the count of HBI organisms in the Petri dish. Using tweezers, transfer the sorted organisms from the Petri dish to the scintillation vial while conducting the verification tally. Check the baby food water rinse jar for specimens that were intended for inclusion in the subsort! A verification tally value of 125+ HBI organisms is indicative of a completed subsort. If the verification tally value is less than 125 HBI organisms, the sort is continued as per Step 18 (above) until either 125+ HBI organisms are removed from the sample, or the sample is sorted in its entirety, whichever situation first arises. A subsort continuing to additional grid(s) per this step (Step 20) must be count re-verified per this step before proceeding to the next step.

22) Upon achieving a completed sort, record the percent of sample sorted to complete the subsort on the field sheet where indicated. The percent is calculated by dividing the number of grids sorted to complete the subsort by the total number of grids in the sorting tray, then multiplying the result by 100. **Example:**

A) 2 grids sorted to achieve 125+ HBI organisms
B) 2 grids sorted/15 grids in the sorting tray
C) 2/15 = 0.13333333…
D) 0.1333333… x 100 = 13.3%
E) Round to nearest whole number value - 13%

23) Label the scintillation vial(s) with an internal paper label, written in HB pencil or India ink. Labels **must** express the following: **SampleIDnum; Rep designation; Waterbody name; Sample type; Percent of sample sorted; Sorter's initials, Split designation**. **Example:**

20031008-42-01 R1
Kickapoo River
HBI 7% KCL
Vial 1 of 1

SUBSORTING PROCEDURES FOR SEMI-QUANTITATIVE HILSENHOFF BIOTIC INDEX (HBI) SAMPLES

PROCEDURE (continued)

23) (continued) The internal label will not exceed 38 mm in length, and will be written with a firm hand, allowing label reuse in AEL organism archives. Close the receiving container securely.

24) Place the scintillation vial(s) and associated field sheet in the laboratory storage area designated for sorted samples.

25) Enter onto the Laboratory Sample Log Sheet, at the entry for the unique SampleIDnum and Rep designation, the percent of sample sorted and the time expended to complete the subsort.

26) Discard sorted debris from the selected grid(s) into the sample debris container.

27) Transfer unsorted debris into the original sample container. The large funnel and plastic putty knife facilitate transfer of large debris volumes. Debris clinging to the Pyrex® sorting pan is rinsed into the original sample container with a directed stream of 80% isopropyl alcohol. Place the original interior sample label in the container.

28) Re-preserve unsorted debris, in original sample container, with 99% isopropyl. Volume of 99% alcohol must be at least triple the volume of the unsorted debris.

29) Place a piece of label tape across the original container lid. Using the permanent ink pen, write "**UNSORTED REMNANT**" on the tape label.

30) Place original sample container in designated sample storage area.

31) Clean and dry all laboratory equipment used to perform the gridded subsort. Return the equipment to the equipment storage area.

32) The sample debris container is emptied into an exterior waste receptacle upon completion of a work session.

33) Laboratory personnel are advised to see the project manager, laboratory supervisor, assistant laboratory supervisor or principal investigator immediately if any questions arise throughout the entire SOP.

Appendix B.11

UW-Stevens Point Aquatic Biomonitoring Laboratory

Procedures for Multiple-Plate Artificial Substrate Samples

SORTING PROCEDURES FOR MULTIPLE-PLATE ARTIFICIAL SUBSTRATE SAMPLES (SAMPLER DISASSEMBLED AND CLEANED)

INTRODUCTION

Aquatic macroinvertebrates were sorted from multiple-plate artificial substrate samples to allow calculation of selected macroinvertebrate community metrics. The resultant data will be analyzed for development of a macroinvertebrate-based, large river Index of Biotic Integrity (IBI).

Three multiple-plate artificial substrate samplers were employed at each sample location, and allowed to colonize for 6 weeks. Upon sampler retrieval, samplers were: disassembled; placed into 1 gallon wide-mouth plastic containers; and preserved using 80% ethanol. The three samplers from each sample location were combined into one sample.

In the lab, all sampler surfaces from one sampling location were cleaned of macroinvertebrates and debris. The resultant sample was placed in a sample container and preserved using 80% isopropyl alcohol, maintaining sample integrity for subsequent sorting procedures.

Samples were sorted by: water rinsing the sample; placing the sample in a sorting tray; and removing the specimens, using forceps, from the sample debris. Organism recognition was facilitated with the aid of a 2X magnifying sorting lamp. The sample was grid subsampled according to procedures outlined in Hilsenhoff (1987) and modified as presented below. All non-plant specimens visible with the aid of the sorting lamp were removed from the selected grid and preserved with 80% isopropyl alcohol in a secure receiving container for subsequent identification to project-specified taxonomic unit. Unsorted sample debris was re-preserved in the original sample container and retained until sample provider accepted sample results.

A two-phase sorting routine was applied to the sample. Phase One was a gridded subsample of the sample, targeting a minimum of 500 individuals. Phase Two was a large/rare organism subsample of ten-minute duration, applied after the Phase One subsample was complete. Criteria used to determine Large/Rare status was minimum specimen size of 12 mm, and present in less than 1/8 of the grids in the sample sorting pan. The subsample resulting from each sorting phase was secured separately.

SORTING PROCEDURES FOR
MULTIPLE-PLATE ARTIFICIAL SUBSTRATE SAMPLES
(SAMPLER DISASSEMBLED AND CLEANED)

EQUIPMENT LIST

- Protective laboratory gear (protective goggles, vinyl gloves)
- Waste preservative container
- Nitex® mesh piece, #30 mesh, 20 cm x 20 cm
- Large funnel
- Magnifying sorting lamp, 2X
- Pyrex sorting pan, 28 cm x 18 cm x 4 cm
- Gridded sorting pan tray, grid dimensions 5 cm^2, grids numbered 1 – n
- Distilled water
- Misting bottle with distilled water
- Petri dish, 9 cm diameter
- Petri dish, 11.5 cm diameter
- Baby food jar
- Fine-tipped forceps, 2
- Isopropyl alcohol, 99%
- Isopropyl alcohol, 80%
- 20 ml scintillation vials
- Random number table, 1 – n
- Hand-held tally
- Heavy-duty shears
- Dissecting microscope and light source
- Small funnel
- White labeling tape
- Bond paper, 20% rag content
- Permanent black marker
- HB pencil
- Sample debris container

PROCEDURE

1) Don laboratory protective gear.

2) The sample and associated field sheet for a given sample location are removed from the laboratory sample storage area.

3) Laboratory personnel processing the sample will enter their name, and processing date, at the entry for the sample's unique Sample ID Number on the Laboratory Sample Log Sheet, and also on the field sheet where indicated.

SORTING PROCEDURES FOR
MULTIPLE-PLATE ARTIFICIAL SUBSTRATE SAMPLES
(SAMPLER DISASSEMBLED AND CLEANED)

4) Keep all aspects of the sample, container, labels, and field sheet associated at all times.

5) Drain preservative from the sample, pouring off the preservative through the #30 Nitex® mesh (20 cm X 20 cm) stretched taut over sample container mouth. Original sample preservative is drained into the waste preservative container. Waste preservative is subsequently delivered to the UWSP recycling program.

7) Water rinse the sample in the sample container, utilizing the piece of #30 Nitex® mesh stretched taut over sample container mouth while pouring off the rinse water. If the rinse water pours off clear, continue rinsing in the original sample container. If the rinse water pours off turbid, place the 8" x 8" #30 Nitex® mesh piece into the large funnel and transfer the sample debris to the mesh-lined funnel. Rinse the sample with a gentle stream of distilled water until the rinse water passing the sample debris is not turbid. Large sample debris may be rinsed, inspected for specimens and discarded into the sample debris container when free from specimens. Water washing the sample reduces laboratory personnel exposure to preservative fumes and removes fine particles from the sample, reducing turbidity in the sorting pan to facilitate sorting procedures.

8) Transfer the sample to the sorting pan. Examine sample container and all washing apparati to ensure that all sample components are transferred to the sorting pan.

9) Place the sorting pan in a gridded sorting pan tray. Distribute the sample components evenly in the sorting pan. Use the misting bottle as necessary to maintain moist condition of sample debris.

10) Fill the 9 cm diameter petri dish 1/3 full with 80% isopropyl alcohol as the initial receiving container for Phase One sorted specimens. Fill the baby food jar with distilled water to dip-rinse the tweezers after alcohol contact.

11) Perform the Phase One gridded subsample. The minimum enumeration target of the Phase One subsample is 500 specimens.

12) Select the initial sorting grid[a], using a random number table. Establish the grid boundaries, using tweezers to manipulate debris and expose grid boundaries. Specimens encountered on the grid boundaries are distributed in the following manner: The first organism encountered on a grid boundary is included in the grid subsample; the second organism encountered on the grid boundary is moved to the adjacent grid. Alternate the placement of specimens encountered on the grid boundaries until the grid boundaries are clearly established. The heavy-duty shears are used to cut large organic debris crossing the selected grid boundaries where necessary

SORTING PROCEDURES FOR
MULTIPLE-PLATE ARTIFICIAL SUBSTRATE SAMPLES
(SAMPLER DISASSEMBLED AND CLEANED)

a: Due to curvature of the Pyrex sorting pan, the outer grids of the sorting pan (Grids 1 – 13, 24 & 25, 36 & 37, 48 & 49, 60 & 61, 72 & 73, 84 – 96) contain less sample debris than the inner grids. Due to this unevenness of sample debris positioning, a sort will not begin in an outer grid. If the initial grid chosen is an outer grid, reselect until an inner grid is selected.

13) Begin sorting in the selected grid. Using tweezers, remove all non-plant specimens from the grid, sorting them into the 9 cm diameter petri dish containing 80% isopropyl alcohol. Tally the specimens as they are removed from the sample. Viewing the sample through the sorting lamp will aid in locating specimens. Sample debris may be removed from the selected grid and placed into the 11.5 cm diameter petri dish to facilitate sorting.

14) Note the number of specimens upon completion of the selected grid. If 500+ specimens have been sorted from the grid, the sort is paused, and the count is verified under a dissecting microscope as per Step 15 (below). If less than 500 specimens have been sorted from the grid, proceed to the next highest successively numbered grid[b] (and others as necessary) until 500+ specimens are subsampled from the sample. Delineate all grid boundaries of each grid selected for sorting prior to sorting that grid. Remove all specimens from the grid in progress prior to advancing to the next grid. Continue sorting until: A) 500+ specimens are sorted from the sample, or B) the sample has been sorted in its entirety, whichever comes first.

b: Due to curvature of the Pyrex sorting pan, the outer grids of the sorting pan (Grids 1 – 13, 24 & 25, 36 & 37, 48 & 49, 60 & 61, 72 & 73, 84 – 96) contain less sample debris than the inner grids. Due to this unevenness of sample debris positioning, an outer grid cannot be the selected grid, nor will two outer grids be sorted consecutively. If the sort proceeds into an outer grid and the sort must continue past the outer grid, a new, inner grid is randomly selected, and the sort continues from the new selected grid. The only situation in which consecutive outer grids are sorted is when all inner grids have been sorted, and the sort must continue. Only in this situation are outer grids sorted consecutively.

15) Working in the 9 cm petri dish under the dissecting microscope, verify the count of sorted specimens. Zero the tally and re-enumerate the specimens in the petri dish. A verification tally value of 500+ indicates the Phase One sort is complete. If the verification tally value is less than 500, the sort is continued as per Step 14 (above). A sort continued from this step will be re-verified as per this step before proceeding to Step 16.

16) Record the percent of sample sorted to complete the Phase One subsample on the field sheet where indicated. The percent is calculated by dividing the number of grids

SORTING PROCEDURES FOR
MULTIPLE-PLATE ARTIFICIAL SUBSTRATE SAMPLES
(SAMPLER DISASSEMBLED AND CLEANED)

16) (continued) sorted to completion by the total number of grids in the sorting tray, multiplying this result by 100.

17) The Phase One subsample is placed in a 20 ml scintillation vial upon completion of the Phase One sort and preserved using 80% isopropyl alcohol, maintaining specimen integrity for subsequent identification to project-specified taxonomic unit.

18) Label the Phase One 20 ml scintillation vial with an internal paper label, written in HB pencil. The labels will express the following: Sample ID Number; Waterbody Name; Split A; Phase One gridded subsample; sorter initials. **Example –**

20030728-22-03
Wisconsin River
Split A
Phase One gridded subsample
JJD

Close the Phase One 20 ml scintillation securely, associating it with all other aspects of the sample.

19) Enter to the Laboratory Sample Log Sheet the percent of sample sorted to achieve the Phase One gridded subsample, and the time elapsed to develop the subsample.

20) Prepare for the Phase Two Large/Rare subsample. The Large/Rare subsample targets organisms larger than 12 mm in length, and present in less than 1/8 of the grids in the sorting pan. Maximum time duration of the Large/Rare subsample is 10 minutes.

21) Fill the 9 cm diameter petri dish 1/3 full with 80% isopropyl alcohol as the initial receiving container for the Phase Two sorted specimens.

22) Perform the Large/Rare subsample. Scan the sample carefully for organisms meeting the requirements of Large/Rare classification. Using tweezers, remove selected specimens from the sample and place in the Petri dish prepared in step 21 (above).

23) The Large/Rare subsample is complete when either 10 minutes of examination have elapsed, or all Large/Rare specimens have been sorted from the sample, whichever comes first.

24) The Phase Two, Large/Rare subsample is placed in a 20 ml scintillation vial upon completion of the Phase Two subsample and preserved using 80% isopropyl alcohol, maintaining specimen integrity for subsequent identification to project-specified

SORTING PROCEDURES FOR
MULTIPLE-PLATE ARTIFICIAL SUBSTRATE SAMPLES
(SAMPLER DISASSEMBLED AND CLEANED)

24) (Continued) taxonomic unit.

25) Label the Phase Two 20 ml scintillation vial with an internal paper label, written in HB pencil. The labels will express the following: Sample ID Number; Waterbody Name; Split A; Phase Two Large/Rare subsample; sorter initials. **Example –**

20030728-22-03
Wisconsin River
Split B
Phase Two Large/Rare subsample
JJD

Close the Phase Two 20 ml scintillation vial securely, associating it with all other aspects of the sample.

26) Indicate on the Laboratory Sample Log Sheet the presence/absence of a Phase Two Large/Rare subsample from the selected sample.

27) Place the unsorted remainder of the sample into the original sample container. Preserve the unsorted remnant of sample with 99% isopropyl alcohol. Volume of alcohol will be at least triple the volume of sample remnant. With permanent black marker, write "**UNSORTED REMNANT**" on exterior label of original sample container. Place the sample container in specified sample storage area.

28) Place the resulting 20 ml scintillation vial(s) and field sheet in the laboratory storage area designated for sorted samples.

29) Laboratory personnel are advised to see the laboratory supervisor immediately if any questions arise throughout the entire SOP.

Appendix B.12

UW-Stevens Point Aquatic Biomonitoring Laboratory

Taxonomic Procedures for Macroinvertebrates

TAXONOMIC PROCEDURES FOR MACROINVERTEBRATES

INTRODUCTION

Macroinvertebrates are identified to project-specified taxonomic unit and enumerated. Data including taxonomic designation, life stage, enumeration by taxon and life stage and taxonomic reference(s) used to make the designation are recorded to a sample-specific bench sheet.

Dissections and/or slide preparations are made as necessary to facilitate viewing of physical characteristics examined to make the taxonomic designation.

Specimens missing key physical characteristics due to sampling stress, immature specimens, and specimens representing obscure groups are identified as far as possible, usually to order or family level.

OBJECTIVES

Specimens are identified to the lowest possible taxonomic level when possible.

Taxonomic literature reviews are performed regularly and collegial discussions are engaged to discuss recent advancements in taxonomic literature.

The Integrated Taxonomic Information System (ITIS), available at http://www.itis.gov/index.html, is the taxonomic standard applied to almost all taxonomic designations. Occasional non-ITIS taxonomic designations are applied (slash taxonomy, species "complex" and species "group" designations) when the listing of these non-ITIS designations represent distinct taxa not already recorded from the sample.

EQUIPMENT LIST

- Dissecting microscope, 8 – 130 X
- Light source, incident light
- Light source, backlight
- Compound microscope, 40 – 1000X
- Petri dish, 9 cm diameter
- 80% isopropyl alcohol
- Fine-tipped forceps
- Manipulating probes
- Bench sheet
- Black ink pen
- Hand-held tally
- Taxonomic keys and references
- 1 dram vials with snap top closure
- 0.6 ml microvials with locking lid
- HB pencil
- Bond paper, 20% rag bond content
- Microscope slides, 3" X 1" X 1 mm

TAXONOMIC PROCEDURES FOR MACROINVERTEBRATES

EQUIPMENT LIST(Continued)

- Cover slips, #2, 22 mm X 22 mm
- Microscope slide labels
- CMC-10 mounting media

PROCEDURE

1) Perform microscope set-up procedures. Turn on microscope light sources at low levels, providing time to warm-up the light sources.

2) Select sample from sample storage area. The sample is a project-specified subsample of the original sample, stored in 80% isopropyl alcohol to maintain sample integrity for subsequent taxonomic procedures.

3) Develop a bench sheet for the selected sample. The bench sheet must display the unique project-specified identifier(s), waterbody name, and entry locations for taxonomic designation, life stage, taxonomic reference and enumeration.

4) Transfer the sample to the 9 cm diameter Petri dish. Examine the sample container, cap, and label to ensure all sample components are transferred to the 9 cm diameter Petri dish.

5) Add sufficient amount of 80% isopropyl alcohol to the sample in the 9 cm diameter Petri dish to cover the specimens and prevent sample desiccation during the identification procedure.

6) Adjust microscope light sources to working illumination levels.

7) Place the 9 cm diameter Petri dish on the dissecting microscope stage.

8) Sort specimens into like groups.

9) Select groups (Chironomidae larvae, Oligochaeta) are placed in separate 1 dram snap top vials. These vials are labeled with all pertinent unique sample identifiers and vial contents. Labels are developed on 20% rag content bond paper, written in HB pencil. Specimens vialed in this step receive additional processing, development of microscope slide mounts, prior to identification of these specimens.

10) Groups not vialed in Step 9 (above) are examined. Specimens are identified to project-specified taxonomic level. Specimens are manipulated with fine-tipped forceps and manipulating probes to aid viewing of physical structures. Dissections are made as necessary. Alcohol-wet mounts of dissections are viewed under a compound microscope, the dissection(s) and source specimen subsequently contained in 80% isopropyl in a 0.6 ml microvial, the 0.6 ml microvial contained in the original sample container. Permanent microscope slide mounts, if developed, are made using CMC-10 mounting media and subsequently viewed under a compound microscope. The developed permanent slide is labeled with all pertinent unique sample identifiers and dissected structure identifiers, and stored with the original sample

TAXONOMIC PROCEDURES FOR MACROINVERTEBRATES

PROCEDURE (Continued)

10) (Continued) vial. Specimens are enumerated by life stage by taxonomic level. The hand-held tally facilitates enumeration.

11) The resultant taxonomic designations, life stages, taxonomic references used, and enumerations are listed to the bench sheet developed in Step 3 (above) using the black ink pen.

12) All specimens identified using the dissecting microscope are returned to the original sample container and stored in sufficient volume 80% isopropyl alcohol to maintain sample integrity.

13) The original sample container, containing the identified specimens, is placed in the laboratory storage area for identified samples and retained per project-specified protocol.

14) Permanent microscope slide preparations of specimens vialed in Step 9 (above) are examined using a compound microscope. Specimens are identified to project-specified taxonomic level.

15) The resultant taxonomic designations, life stages, taxonomic references used, and enumerations are listed to the bench sheet developed in Step 3 (above) using the black ink pen.

16) The permanent microscope slides, displaying the identified specimens, are placed in the laboratory storage area for identified samples and retained per project-specified protocol.

17) The bench sheet is examined to ensure all data entries are complete.

18) The bench sheet is placed in the laboratory storage area for completed bench sheets.

19) Microscope light sources are turned down to minimal settings for a short time prior to turning them off, allowing them to cool down.

20) Laboratory personnel are advised to consult the project manager, laboratory manager or principal investigator if any questions arise throughout the entire SOP.

Appendix B.13

UW-Stevens Point Aquatic Biomonitoring Laboratory

Developing Sample Processing Microscope Slide Mounts of Chironomidae Larvae – Head Slicing Technique

Procedure No. 7-94 (Rev. 09/2008)

DEVELOPING SAMPLE PROCESSING MICROSCOPE SLIDE MOUNTS OF CHIRONOMIDAE LARVAE HEAD SLICING TECHNIQUE

INTRODUCTION

Chironomidae larval head mounts (squashes) are developed by removing the head from the larval body and mounting the head, ventral side up, under cover slips on microscope slides with the aid of mounting media. The mounting media "clears" the larval head, allowing the viewing of physical characteristics necessary to identify the specimen.

OBJECTIVE

The objective of mounting Chironomidae larval heads on microscope slides is to produce head capsule squashes, arrayed ventral side up, to allow viewing of the various head structures used in identification. The viewing of the head structures is facilitated with the use of a mounting media that "clears" the head capsule, dissolving the interior muscle tissue. This allows for easier viewing of interior head structures and also allows better light transmission when using backlighting techniques during identification. Speed in mounting must be considered secondary to producing quality mounts, as poor mounts will yield as little information as un-mounted specimens. Strive to produce the best mounts that you can!

ABOUT MOUNTING MEDIA

Mounting media allows you to array Chironomidae larvae on the slide and, when applied in a sufficient amount, will displace the air under the cover slip, resulting in the larval heads being totally surrounded by the media. Mounting media used in mounting Chironomidae larvae should be of a clearing type.

Mounting media may be resinous or non-resinous. Resinous mounting media (i.e. Canada balsam, Harleco's Coverbond for xylene) provide optimal resolution and longevity of mounted materials. These media require dehydration of specimens through the alcohol series before using the media and produce the best permanent mounts, but the procedures are time-consuming and yield few mounted specimens per unit time spent processing.

Non-resinous mounting media (i.e. Aquamount, Hoyers, or CMC-10) do not require the use of alcohol series to prepare the specimens; therefore, specimen processing involves less time. Non-resinous media mounts are semi-permanent (at least 10 years- JJD), but permanence of the prepared slide can be enhanced by ringing the cover slip with clear fingernail lacquer. Large numbers of specimens can be processed in a short period of time using non-resinous media.

Procedure No. 7-94 (Rev. 09/2008)

DEVELOPING SAMPLE PROCESSING MICROSCOPE SLIDE MOUNTS OF CHIRONOMIDAE LARVAE - HEAD SLICING TECHNIQUE

The UWSP Aquatic Biomonitoring Laboratory uses CMC-10. This media clears all but the largest specimens in a short time. Also, CMC-10 takes a fair length of time to set up. This allows for extended "open slide time," during which the media is exposed on the slide and specimens are being arrayed. A quicker-setting media would force the procedures to be rushed, possibly introducing a larger number of poor mounts. The ability to have the media exposed for 5-6 minutes if necessary without worrying about the media beginning to set up aids the process, enhancing the quality of the finished slide.

THE "ROARK PROCEDURE"

Microscope slide manufacturers often apply a coating to their finished product. This coating can cause the media to "pool up" after it is distributed on the slide working area. Media pooling interferes with the array of specimens in the media, possibly altering the orientation and ordination of specimens under the cover slip.

The pooling of media can be reduced by applying a coating to the working surface of the slide prior to introducing media to the slide. Trevor Roark, after trying many substances, determined that a dried film of Cherry Coke reduced media pooling.

A drop of Cherry Coke is smeared over the working surface of the slide, using a finger. As the Cherry Coke dries, the resulting film is re-smeared as needed to develop a relatively even distribution of the film over the working surface of the slide.

EQUIPMENT AND MATERIALS

- Cherry Coke
- Dissecting microscope (10-25 X) and associated light sources
- Watch glass
- Hand-held tally
- Micro-rehydrator (2)
- Fine tipped forceps (2)
- Slicing pins (2) (No. 1 insect pins mounted into wood sticks or glass tubing)
- Re-used isopropyl alcohol
- Distilled water
- Blotting paper
- Microscope slides, 3" x 1" x 1.0 mm
- Cover slips, No. 2, 22mm x 22mm
- Mounting media
- Dropper

Procedure No. 7-94 (Rev. 09/2008)

DEVELOPING SAMPLE PROCESSING MICROSCOPE SLIDE MOUNTS OF CHIRONOMIDAE LARVAE - HEAD SLICING TECHNIQUE

EQUIPMENT AND MATERIALS (continued)

- Slide labels or labeling tape
- Single edge razor blade
- Ball-point pen, medium point, black ink

PROCEDURES

1) Apply the Roark Procedure (Page 2) to microscope slides.

2) Select sample vial of Chironomidae larvae from vial storage rack. Only one sample is mounted at a time.

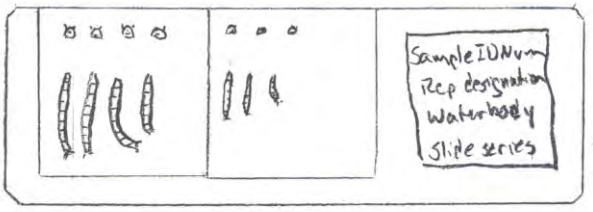
3) The vial and cap, watch glass, micro-rehydrators, tweezers and blotting paper are thoroughly inspected to insure removal and transfer of all larvae during each step of SOP.

4) Transfer Chironomidae larvae from vial to watch glass. Four or five rinses of re-used isopropyl alcohol facilitate the transfer. Retain the vial label, containing all pertinent sample tracking information, for slide labeling purposes.

5) Using the hand-held tally, verify the specimen count listed to the vial label. Re-examine the vial, vial cap and label if the verification tally is short the vial label count. List any discrepancy between the vial label count and the verification tally to a second slide label, the second label applied directly over the main slide label.

6) Two cover slips fit on each microscope slide (Fig. 1). The first cover slip will be located near the left margin of the slide. Leave 1/4" (6mm) left-hand margin to allow subsequent storage in slide box. The second cover slip will butt up to the right-hand edge of the first cover slip. This will allow for one label bearing sample tracking information on the right 1/3 of slide. The top of the cover slips will flush with the top of the slide.

Figure 1. Layout, sample processing slide



Procedure No. 7-94 (Rev. 09/2008)

DEVELOPING SAMPLE PROCESSING MICROSCOPE SLIDE MOUNTS OF CHIRONOMIDAE LARVAE - HEAD SLICING TECHNIQUE

7) Fill micro-rehydrators with distilled water.

8) With the watch glass under the microscope, sort larvae into groups based on size. The number of larvae per group is the same as the number of specimens placed under a cover slip. This number is dependent on the size of the specimens and the skill of the mounting personnel.

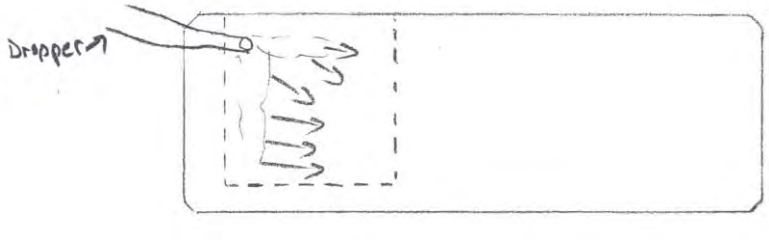
9) Transfer a group of larvae to each micro-rehydrator.

10) Place microscope slide on stage of dissecting microscope, locking slide down with a stage clip placed on the right hand edge of the slide, and a drop of water placed at the left had margin of the slide. The slide is "locked" to the stage to prevent slide movement while processing the larvae.

11) Place selected micro-rehydrator on stage of dissecting microscope. Collect larvae using tweezers and touch group to blotting paper, removing excess water. Retain larvae on tweezers after blotting.

12) Introduce mounting media to the slide, using dropper. Distribute the media evenly, using the tip of the dropper, spreading it out over an area approximately the size of a cover slip (Fig. 2). The amount of media to use is dependant on the size of the larvae. The larvae, when placed in the media, should not be completely covered with media; instead, there should be a raised meniscus of media about each larva. This amount of media will come with practice!

Figure 2. Media distribution to the first working area of the slide.



13) Place the group of blotted larvae on the right half of the media.

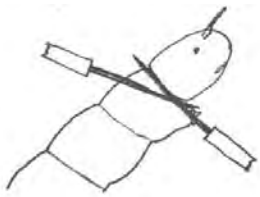
14) Selected the largest larvae from the group and pull it near to the left-hand edge of the media using a slicing pin.

Procedure No. 7-94 (Rev. 09/2008)

DEVELOPING SAMPLE PROCESSING MICROSCOPE SLIDE MOUNTS OF CHIRONOMIDAE LARVAE - HEAD SLICING TECHNIQUE

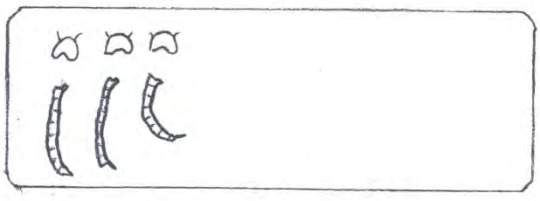
15) Place the point of a slicing pin in the gap between the anterior prolegs and the ventral side of the head, making sure the pin remains posterior to the head capsule. Place the point of the second slicing pin where the dorsum of the head capsule joins the thorax, again making sure the pin remains posterior to the head capsule (Fig. 3). Pull the pin points together, creating a "scissoring action" with the pin points. This will slice the head from the larval body.

Figure 3. Slicing pin placement relative to larva.



16) Array the larval body towards the "bottom" of the cover slip, making sure that the posterior end of the body remains under the cover slip when the slip is applied. Array the head to the "top" of the cover slip with the ventral side up (Fig. 4). The ventral side is up when you can view the inverted "U"-shaped notch of the postero-ventral edge of the head capsule (Fig. 5). On large larvae, separate the head capsules as far as possible from the bodies. Medium and small larvae do not require as much separation of heads and bodies. Extremely large larvae may not completely fit under a cover slip. In this situation, slice the head and the three posterior segments from the body, retaining the three posterior segments as one piece (Fig. 6). Discard the middle body segments.

Figure 4. Array of larval heads and bodies on the slide.



17) Continue selecting larvae from the group, moving them to the left, slicing the head capsules and arraying the parts as you work left-to-right. Maintain the respective ordination of heads and bodies.

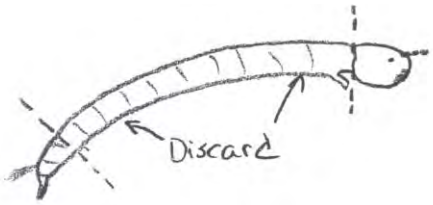
18) When all larvae in the group have been processed, examine the heads one final time to ensure that they are ventral side up.

Procedure No. 7-94 (Rev. 09/2008)

DEVELOPING SAMPLE PROCESSING MICROSCOPE SLIDE MOUNTS OF CHIRONOMIDAE LARVAE - HEAD SLICING TECHNIQUE

Figure 5. Diagrammatic Chironomidae larval heads, showing correct ventral view (left) and incorrect dorsal view (right).

Figure 6. Diagrammatic Chironomidae larval heads, showing slicing technique for large specimens.



19) Select a cover slip and introduce it to the left-hand edge of the media at a low angle. "Drop" the slip to the media and immediately apply light downwards pressure to the corners of the cover slip with your fingers and thumbs. This starts squashing the head capsules and also begins setting them in their respective locations.

20) Apply direct downwards pressure over the head capsules with the points of the forceps. Do not apply so much pressure that the head capsules fragment or that the cover slip fractures. (This amount of pressure will come with practice!)

21) At this point, with light pressure maintained on the cover slip with the tweezers, examine the area under the cover slip.

    A) If there are air bubbles extending from the edge of the slip towards the larvae, introduce media, using the dropper, along the margins of the cover slip. Capillary action will draw the introduced media under the cover slip. Once the air has been displaced by the media, release the pressure. If more air bubbles are drawn under the slip, reapply light pressure to "chase" the air bubbles from under the slip. Reapply more media to the margin of the cover slip at the point where the air bubbles form and release pressure from the slip. Continue until no air bubbles remain under the cover slip.

Procedure No. 7-94 (Rev. 09/2008)

DEVELOPING SAMPLE PROCESSING MICROSCOPE SLIDE MOUNTS OF CHIRONOMIDAE LARVAE - HEAD SLICING TECHNIQUE

21) (continued) B) If no air bubbles extend under the cover slip, release the pressure. If air bubbles begin to form, utilize the technique described in 21A (above) until no air bubbles remain under the cover slip.

22) Flush the top margin of the cover slip with the top margin of the slide. The left-hand margin of the cover slip will be 1/4" (6mm) from the left margin of the slide.

23) Repeat steps 11 through 21 if necessary to the immediate right of the first cover slip (Fig. 1) with the next group of larvae. The top margin of the second cover slip will flush the top margin of the slide. The left-hand margin of the second cover slip will butt against the right hand margin of the first cover slip.

24) Label the slide, using labeling tape and a ballpoint pen. The label **must** express the following: **Sample ID Number, Rep Designation, Waterbody Name, Split designation, Split enumeration, slide series numbering for the sample (i.e. 1 of 4, 2 of 4, etc.) and mounter's initials.** The slide series numbering is dependant on the number of slides developed to mount all larvae from the sample. **Example:**

20060911-66-01 R1
Yellow River
Split A2
42 specimens
3/4 HM

25) Set the mount aside to dry (set up) and to allow time for the head capsules to clear. Clearing time for the head capsules will range from 3 days for small specimens to 10 days for large specimens. While the mount is setting up, the media will want to shrink (suck up) under the cover slip. This will cause formation of air bubbles under the cover slip, resulting in a mount that is virtually impossible to identify. This media shrinkage is combated by applying more media to the margins of the cover slip in the area of air bubble formation. Add media as necessary to prevent the formation of the bubbles. Slides will be checked twice daily for media shrinkage. Shrinkage is more prevalent on mounts of large larvae.

26) At the end of a work session, clean and dry all laboratory equipment used to develop the slide mounts subsort. Return the equipment to the equipment storage area.

27) Laboratory personnel are advised to see the project manager, laboratory manager or principal investigator immediately if any questions arise throughout the entire SOP.

Appendix B.14

UW-Stevens Point Aquatic Biomonitoring Laboratory

Taxonomic Bench Sheet

BillBob6

SAMPLENUM Page 1 of ______________________ **WATERBODY** ______________________

Taxa	Life Stage	Taxonomic Key Code	Organism ID #	Organism Count		
				Rep 1	Rep 2	Rep 3

Appendix B.15

Process for Loading Macroinvertebrate Data into SWIMS

Process for loading Macroinvertebrate Data into SWIMS

What happens now is:

- Jeff Dimick at UWSP sends the samples via email in several files (DBF and XLS format). The files we use are:
  - One with information about the sample
  - One with counts by species
  - One with their calculated metrics
  - One with "top 5" rankings
- I load those from the 4 files into work tables in production SWIMS:
  - WT_SWIMS_UWSP_HEADINFO_WORK
  - WT_SWIMS_UWSP_TAXALIST_WORK
  - WT_SWIMS_UWSP_METRICS_WORK
  - WT_SWIMS_UWSP_RANKING_WORK
- Then, I run a program, PK_SWIMS_UWSP_LOAD.P_LOAD which:
  - Validates the data
  - Converts latitude, longitude, location description, etc. to standard formats
  - Identifies the fieldwork ID and monitoring station so we can match it to data we already have about the sampling event
  - Converts their taxonomy codes to our codes, creating a "lab" sample for each "rep" and a set of sample results for each of the taxa counts.
  - Creates a "computed" sample for each set of metrics and rankings for each rep
  - Data which does not pass validation is held in the work tables until we can correct it or get it resubmitted. Usually, this is because the sample can't be geolocated. Currently, there are 721 such samples out of 11784, or about 6.1%. Of those 721, only 17 are from 2000 or later, and only 1 of those is from 2005 or later.

Enhancements:

- Currently, we're using their calculated metrics. At some point this year, we'll switch over to using our calculations for the metrics. Our programs have been validated against the existing data and give the same results. This will become significant when we start processing data from other sources, like UW Superior, where UWSP won't be providing metrics.
- We're also hoping to get UWSP to convert the data to a standard format so they can directly submit the information, without me having to process the spreadsheet and DBF files for them. That would cut out a couple steps which should improve timeliness and reduce the chance of any errors.

Appendix B.16

Hydrolab User Manual

Catalog Number 003078HY

Hydrolab DS5X, DS5, and MS5 Water Quality Multiprobes

USER MANUAL

February 2006, Edition 3

Table of Contents

Section 1 Specifications

Specifications are subject to change without notice.

DS5 and DS5X Transmitter	
Outer Diameter	8.9 cm (3.5 in.)
Length	58.4 cm (23 in.)
Weight (typical configuration)	3.35 kg (7.4 lb)
Maximum Depth	225 m
Operating Temperature	–5 to 50 °C
Battery Supply (optional)	8 C batteries
Computer Interface	RS232, SDI-12, RS485
Memory (optional)	120,000 measurements
MS5 Transmitter	
Outer Diameter	4.4 cm (1.75 in.)
Length	53.3 cm (21 in.) 74.9 cm (29.5 in.) with battery pack
Weight (typical configuration)	1.0 kg (2.2 lb) 1.3 kg (2.9 lb) with battery pack
Maximum Depth	225 m
Operating Temperature	–5 to 50 °C
Battery Supply (optional)	8 AA batteries
Computer Interface	RS232, SDI-12, RS485
Memory (optional)	120,000 measurements
Temperature Sensor	
Range	–5 to 50 °C
Accuracy	± 0.10 °C
Resolution	0.01 °C
Specific Conductance Sensor	
Range	0 to 100 mS/cm
Accuracy	± 1% of reading; ± 0.001 mS/cm
Resolution	0.0001 units
pH Sensor	
Range	0 to 14 units
Accuracy	± 0.2 units
Resolution	0.01 units

Clark Cell Dissolved Oxygen Sensor	
Range	0 to 50 mg/L
Accuracy	± 0.2 mg/L at ≤ 20 mg/L ± 0.6 mg/L at > 20 mg/L
Resolution	0.01 mg/L
ORP	
Range	–999 to 999 mV
Accuracy	± 20 mV
Resolution	1 mV
Depth Vented Level	
Range	0 to 10 m
Accuracy	± 0.01 m
Resolution	0.001 m
Depth 0–25 m	
Range	0 to 25 m
Accuracy	± 0.05 m
Resolution	0.01 m
Depth 0–100 m	
Range	0 to 100 m
Accuracy	± 0.05 m
Resolution	0.01 m
Depth 0–200 m	
Range	0 to 200 m
Accuracy	± 0.1 m
Resolution	0.1 m
Hach LDO® Sensor	
Range	0–30 mg/L
Accuracy	± 0.01 mg/L for 0–8 mg/L; ± 0.02 mg/L for greater than 8 mg/L
Resolution	0.01 or 0.1 mg/L
Salinity	
Range	0 to 70 ppt
Accuracy	± 0.2 ppt
Resolution	1 mV
4-beam Turbidity (DS5 Only)	
Range	0 to 1000 NTU
Accuracy	± 5% of reading; ± 1 NTU
Resolution	0.1 NTU (<100 NTU); 1NTU (≥ 100 NTU)

Self-cleaning Turbidity	
Range	0 to 3000 NTU
Accuracy	± 1% up to 100 NTU, ± 3% up to 100–400 NTU, ± 5% from 400–3000 NTU
Resolution	0.1, up to 400 NTU; 1.0, 400–3000 NTU
Ammonium/Ammonia	
Range	0 to 100 mg/L-N
Accuracy	Greater of ± 5% of reading or ± 2 mg/L-N (typical)
Resolution	0.01 mg/L-N
Nitrate	
Range	0 to 100 mg/L-N
Accuracy	Greater of ±5% of reading or ±2 mg/L-N (typical)
Resolution	0.01 mg/L-N
Chloride	
Range	0.5 to 18,000 mg/L
Accuracy	Greater of ± 5% of reading or ± 2 mg/L (typical)
Resolution	0.0001 units
Total Dissolved Gas	
Range	400 to 1300 mmHg
Accuracy	± 0.1% of span
Resolution	1.0 mmHg
Ambient Light	
Range	0 to 10,000 µmol s^{-1} m^{-2}
Accuracy	± 5% of reading or ± 1 µmol s^{-1} m^{-2}
Resolution	1 µmol s^{-1} m^{-2}
Chlorophyll *a*	
Range	0 to 500 µg/L, 0 to 50 µg/L, 0 to 5 µg/L
Accuracy	± 3% for signal level equivalents of 1 ppb Rhodamine WT dye
Resolution	0.01 µg/L
Rhodamine WT	
Range	0 to 1000 ppb, 0 to 100 ppb, 0 to 10 ppb
Accuracy	± 3% for signal level equivalents of 1 ppb Rhodamine WT dye
Resolution	0.01 ppb
Blue-green Algae	
Range	100 to 2,000,000 cells/mL, 100 to 200,000 cells/mL, 100 to 20,000 cells/mL
Accuracy	± 3% for signal level equivalents of 1 ppb Rhodamine WT dye
Resolution	0.01 cells/mL

2.1 Safety Information

Please read this entire manual before unpacking, setting up, or operating this instrument.

Pay particular attention to all danger and caution statements. Failure to do so could result in serious injury to the operator or damage to the equipment.

Do not use or install this equipment in any manner other than that which is specified in this manual.

2.1.1 Use of Hazard Information

If multiple hazards exist, this manual will use the signal word (Danger, Caution, Note) corresponding to the greatest hazard.

DANGER
Indicates a potentially or imminently hazardous situation which, if not avoided, could result in death or serious injury.

CAUTION
Indicates a potentially hazardous situation that may result in minor or moderate injury.

Important Note: *Information that requires special emphasis.*

Note: *Information that supplements main points in the text.*

2.1.2 Precautionary Labels

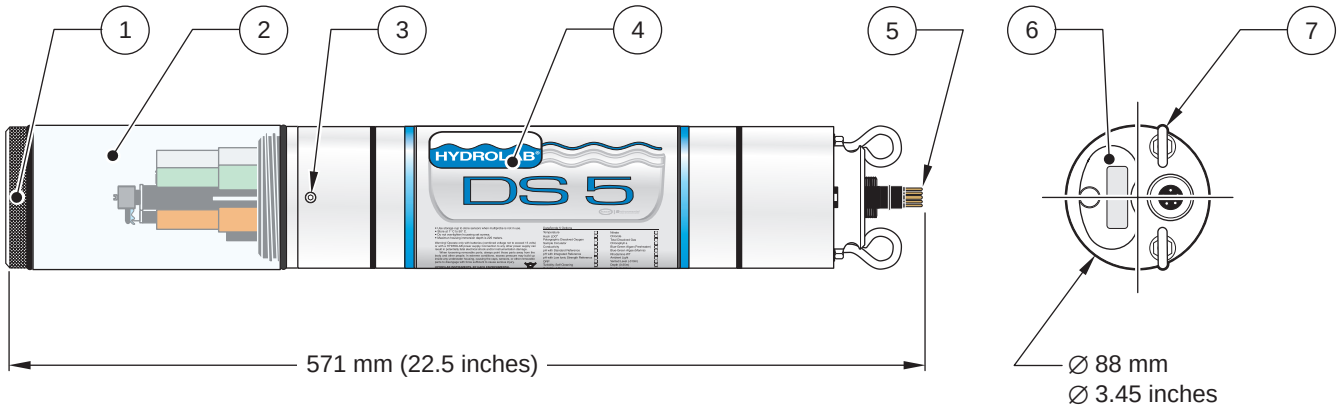
Read all labels and tags attached to the instrument. Personal injury or damage to the instrument could occur if not observed.

	This symbol, when noted on the instrument, references the instruction manual for operation and/or safety information.
	This symbol, when noted on a product enclosure or barrier, indicates that a risk of electrical shock and/or electrocution exists and indicates that only individuals qualified to work with hazardous voltages should open the enclosure or remove the barrier.
	This symbol, when noted on the product, identifies the location of a fuse or current limiting device.
	This symbol, when noted on the product, indicates that the marked item can be hot and should not be touched without care.
	This symbol, when noted on the product, indicates the presence of devices sensitive to Electro-static Discharge and indicates that care must be taken to prevent damage to them.
	This symbol, when noted on the product, identifies a risk of chemical harm and indicates that only individuals qualified and trained to work with chemicals should handle chemicals or perform maintenance on chemical delivery systems associated with the equipment.
	This symbol, when noted on the product, indicates the need for protective eye wear.
	This symbol, when noted on the product, identifies the location of the connection for Protective Earth (ground).

2.2 DS5, DS5X Multiprobe

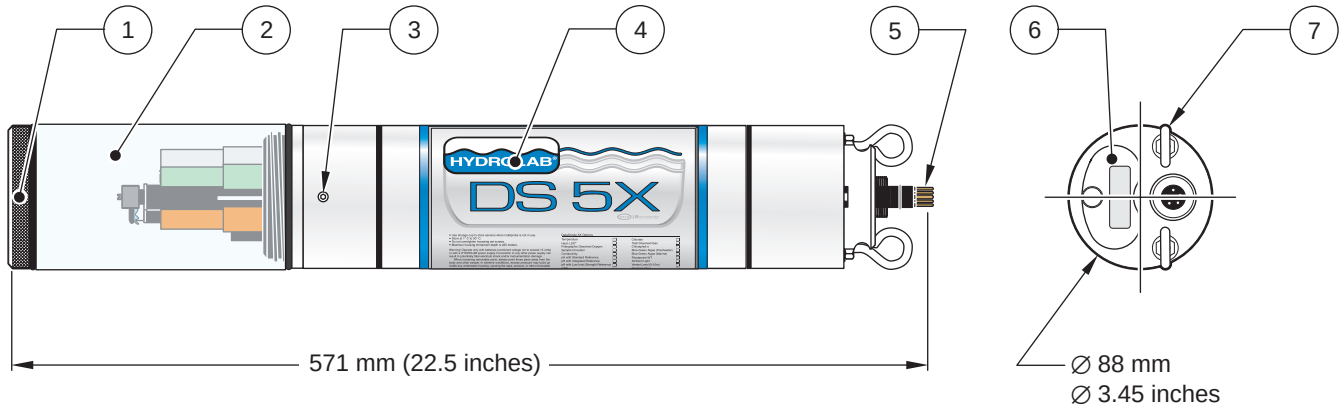
The DS5 and DS5X are designed for in-situ and flow-through applications, and can measure up to 15 parameters simultaneously. The DS5 and DS5X have seven configurable ports that can include up to ten of the following sensors: ambient light, ammonia, chloride, chlorophyll *a*, rhodamine WT, conductivity, depth, dissolved oxygen, nitrate, ORP, pH, temperature, total dissolved gas, turbidity, and blue-green algae.

Figure 1 DS5 Multiprobe



1.	Calibration Cup	**5.**	Bulkhead Connector
2.	Storage Cup	**6.**	Battery Compartment (optional)
3.	Locking Screw	**7.**	Bail Attachment
4.	Housing		

Figure 2 DS5X Multiprobe



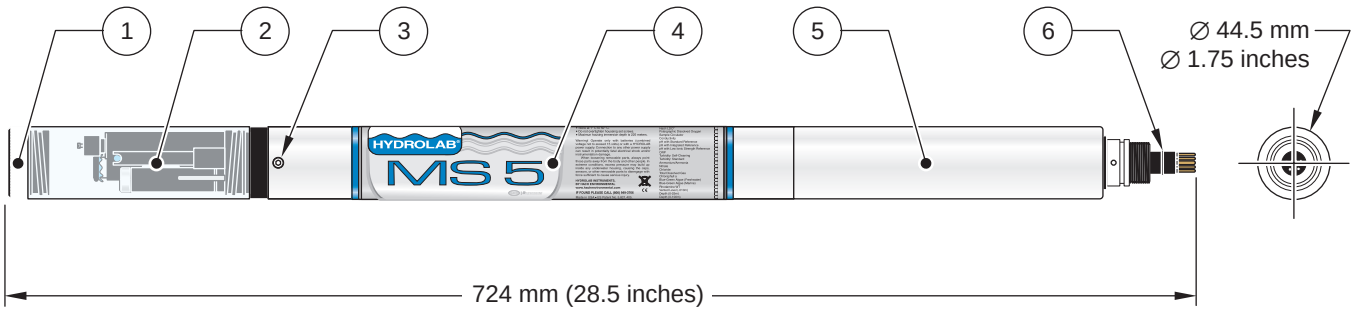
1.	Calibration Cup	**5.**	Bulkhead Connector
2.	Storage Cup	**6.**	Battery Compartment (optional)
3.	Locking Screw	**7.**	Bail Attachment
4.	Housing		

2.3 MS5 Multiprobe

The MS5 is a portable instrument used for long-term monitoring or profiling applications. The MS5 has four configurable ports that can include a combination of the following sensors: ammonia, chloride, chlorophyll a, rhodamine WT, conductivity, depth, dissolved

oxygen, nitrate, ORP, pH, temperature, total dissolved gas, turbidity, and blue-green algae.

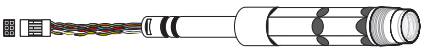
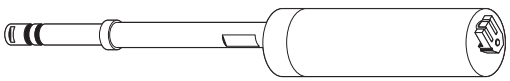
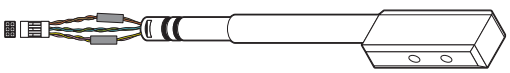
Figure 3 MS5 Multiprobe



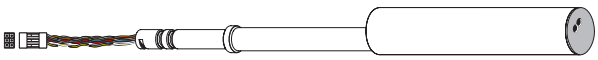
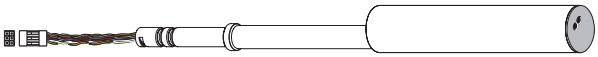
1. Calibration Cap	**4.** Housing
2. Calibration Cup	**5.** Battery Compartment (optional)
3. Locking Screw	**6.** Connector

2.4 Sensor Options

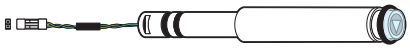
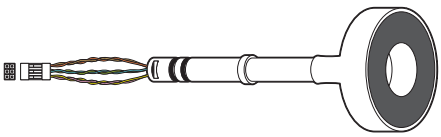
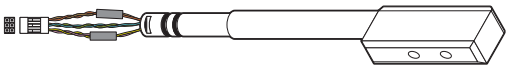
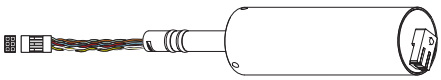
MS5 Sensor Options

Sensor	Description	Cat. No.
	Hach LDO® Sensor	007455
	Clark Cell Dissolved Oxygen/Conductivity	004467
	Clark Cell Dissolved Oxygen	004470
	Conductivity with pH Return	004468
	Total Dissolved Gas	004469
	pH and ORP, with Integrated Reference	004454
	pH with Integrated Reference	004446
	Low Ionic Strength Reference	004445
	pH	004461
	pH and ORP	004462
	Chloride	004496
	Nitrate	004494
	Ammonium/Ammonia	004492
	Standard Reference	004463
	Circulator	007245
	Self-cleaning Turbidity	007280
	Standard Turbidity	004466

MS5 Sensor Options

Sensor	Description	Cat. No.
	Chloropyll a	007284
	Rhodamine WT	007285
	Blue-green Algae	007293

2.4.1 DS5 Sensor Options

Sensor	Description	Cat. No.
	Hach LDO® Sensor	007455
	Clark Cell Dissolved Oxygen/Conductivity	004467
	Clark Cell Dissolved Oxygen	004470
	Conductivity with pH Return	004468
	Total Dissolved Gas	004469
	pH and ORP, with Integrated Reference	004454
	pH with Integrated Reference	004446
	Low Ionic Strength Reference	004445
	pH	004461
	pH and ORP	004462
	Chloride	004496
	Nitrate	004494
	Ammonium/Ammonia	004492
	Standard Reference	004463
	Circulator	007245
	4-beam Turbidity (DS5 only)	004524
	Standard Turbidity (DS5 only)	004466
	Self-cleaning Turbidity	007140
	Chlorophyll a	007202
	Rhodamine WT	007204

2.4.1 DS5 Sensor Options (continued)

Sensor	Description	Cat. No.
	Blue-green Algae	007291

Section 3 Installation

DANGER
Only qualified personnel should conduct the tasks described in this section of the manual.

3.1 Unpacking the Instrument

Remove the multiprobe from its shipping carton and inspect it for any visible damage. Contact Customer Service at 1-800-949-3766 if any items are missing or damaged.

Note: *It is normal for a small amount of solution to be in the cup.*

3.2 Instrument Assembly

There are many ways to connect a multiprobe to a display or a personal computer.

DANGER
An electrical shock hazard can exist in a wet or outdoor environment, if the multiprobe is powered via the external 115 VAC power supply. The safest and preferred method of powering this equipment in wet or outdoor environments is with battery or solar power (with a combined voltage not to exceed 15 volts). If it is necessary to power the multiprobe with the 115 VAC power supply in a wet or outdoor environment, a Ground Fault Interrupt (GFI) circuit is required. The installation of the GFI device must be done by a licensed electrician.

1. Remove all protective plugs and keep them in a safe place, they will be used again for moving and storage.

2. Connect the calibration cable (Cat. No. 013470), or detachable cable (015XXX) to the multiprobe. The connectors are keyed for proper assembly. Align the bigger pin on the multiprobe male connector to the indicator dots on the female cable connector. Do not rotate the cable or force or twist the pins into the connectors to prevent damage to the connector pins.

3. Power the instrument by connecting the power receptacle from the calibration cable and external power adapter to an approved battery or power supply (see Figure 4 on page 16).

4. Connect the other end of the calibration cable, detachable cable, fixed cable, or external power adapter to the computer serial port.

5. Start the communications program (Hydras 3 LT).

6. The software will automatically scan for Sondes. All detected Sondes are displayed in the ‘Connected Sondes’ list in the Main window displayed below. If a Sonde is not found, reattach the data cable and press **RE-SCAN FOR SONDES**. Retry until the Sonde(s) are found.

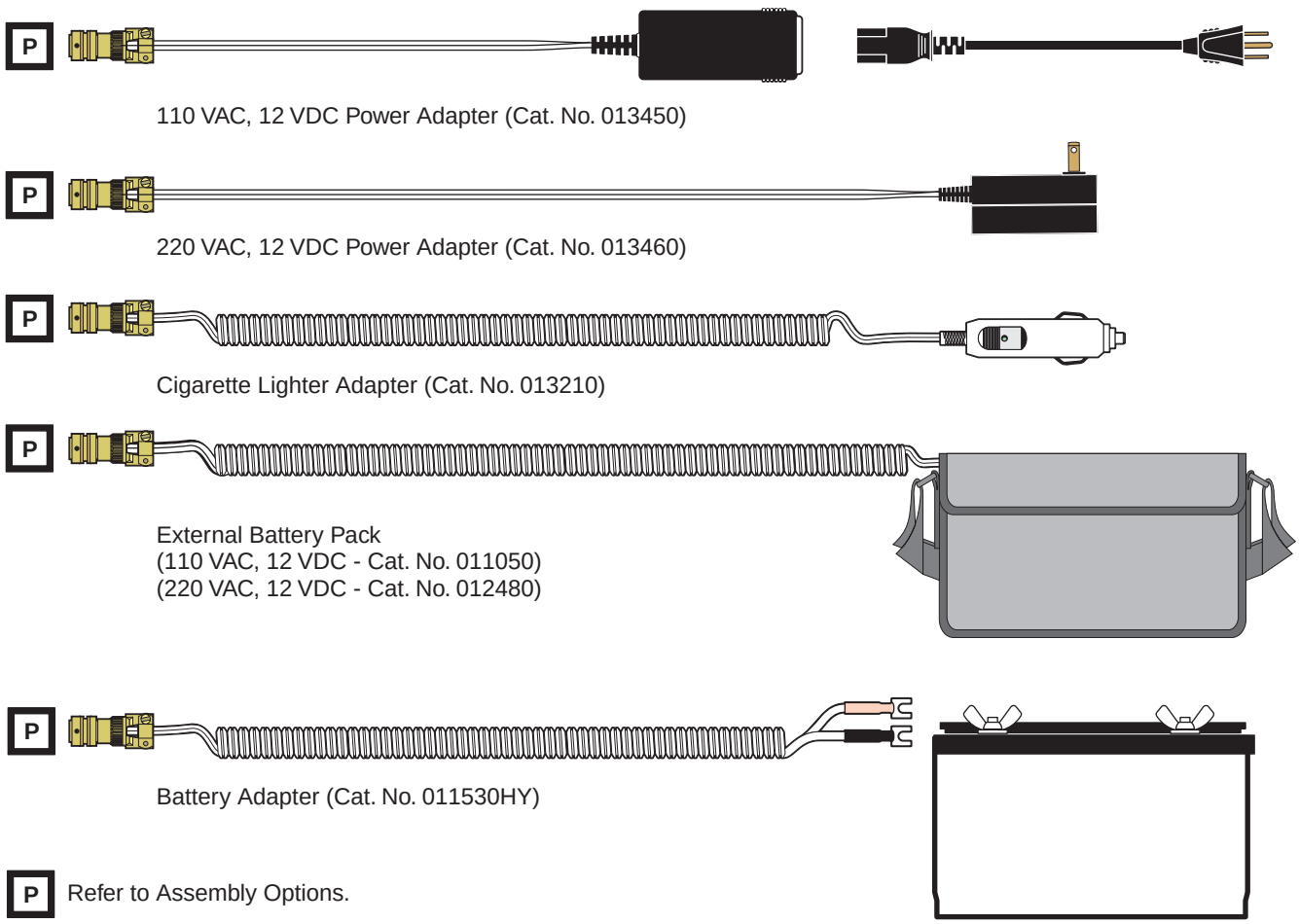
3.3 Power Options

A logging multiprobe can be powered by several sources:

- The DS5 and DS5X have an optional internal battery pack (IBP) holding 8 size C alkaline batteries.
- The MS5 has an optional internal battery pack holding 8 size AA alkaline batteries.
- Both multiprobes can use the external battery pack (EBP).
- Both multiprobes can use either of the following power supplies: the 110 VAC 12 VDC power adapter or the 220 VAC 12 VDC power adapter.
- Both multiprobes can use a customer-supplied 12-volt deep cycle battery with appropriate amp-hour capacity connected via the battery adapter or a cable with a 4-pin female metal shell connector.
- Both multiprobes can be powered using a Surveyor Datalogging Display, which is equipped as a standard with a 7.2V, 3.5 Ah NiMH rechargeable battery.

Note: *Multiprobes configured with the Self-cleaning Turbidity and one or more fluorescence sensors (Chlorophyll a, Rhodamine WT, Blue-green Algae) require either an internal battery pack or an external power source other than the Surveyor.*

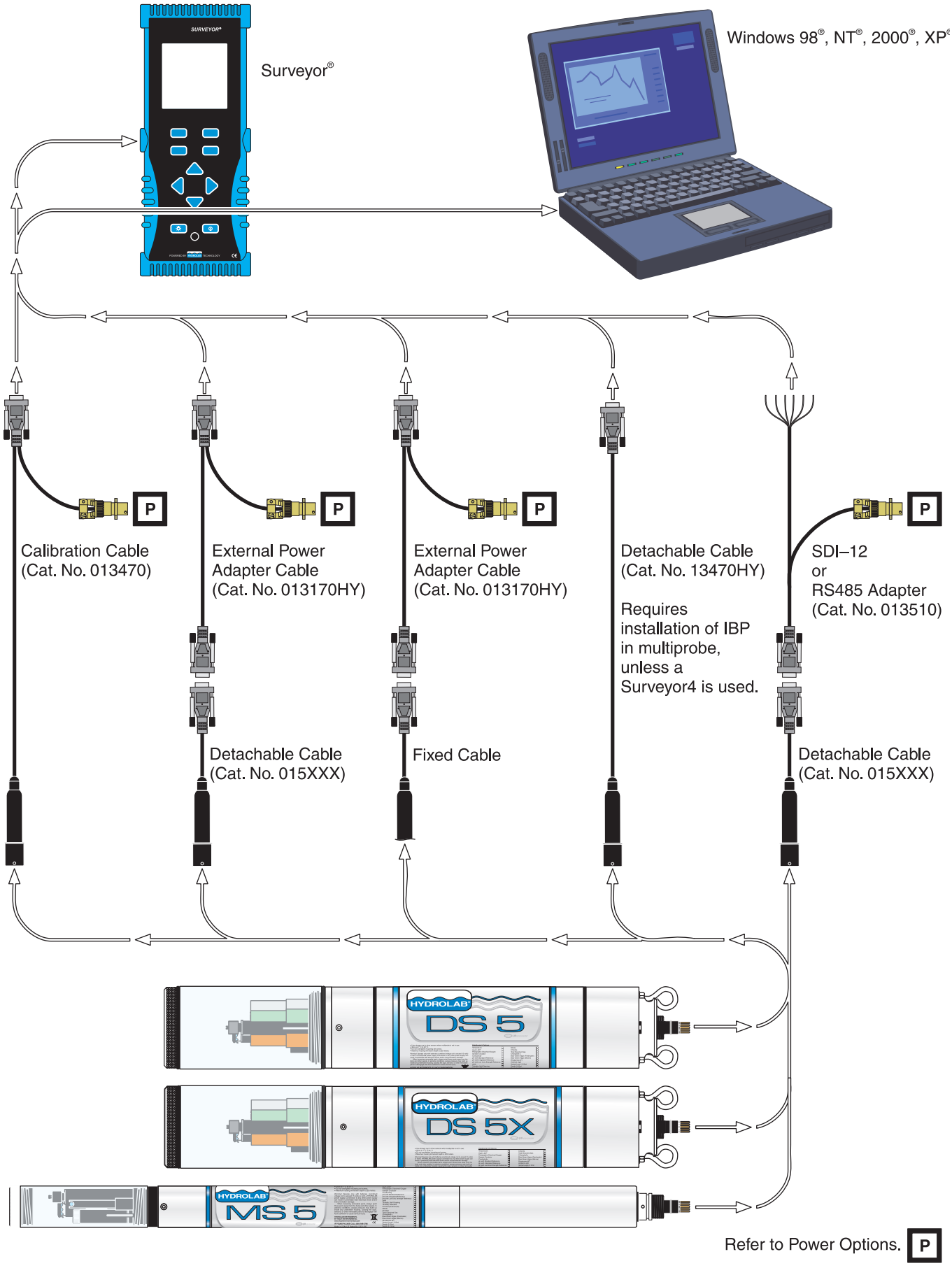
Figure 4 Power Options



*Use the correct power cord with the IEC 320 connector.

**To prevent damage, use a regulated 12 VDC adapter. An unregulated 12 VDC adapter may exceed the instrument voltage limit rating.

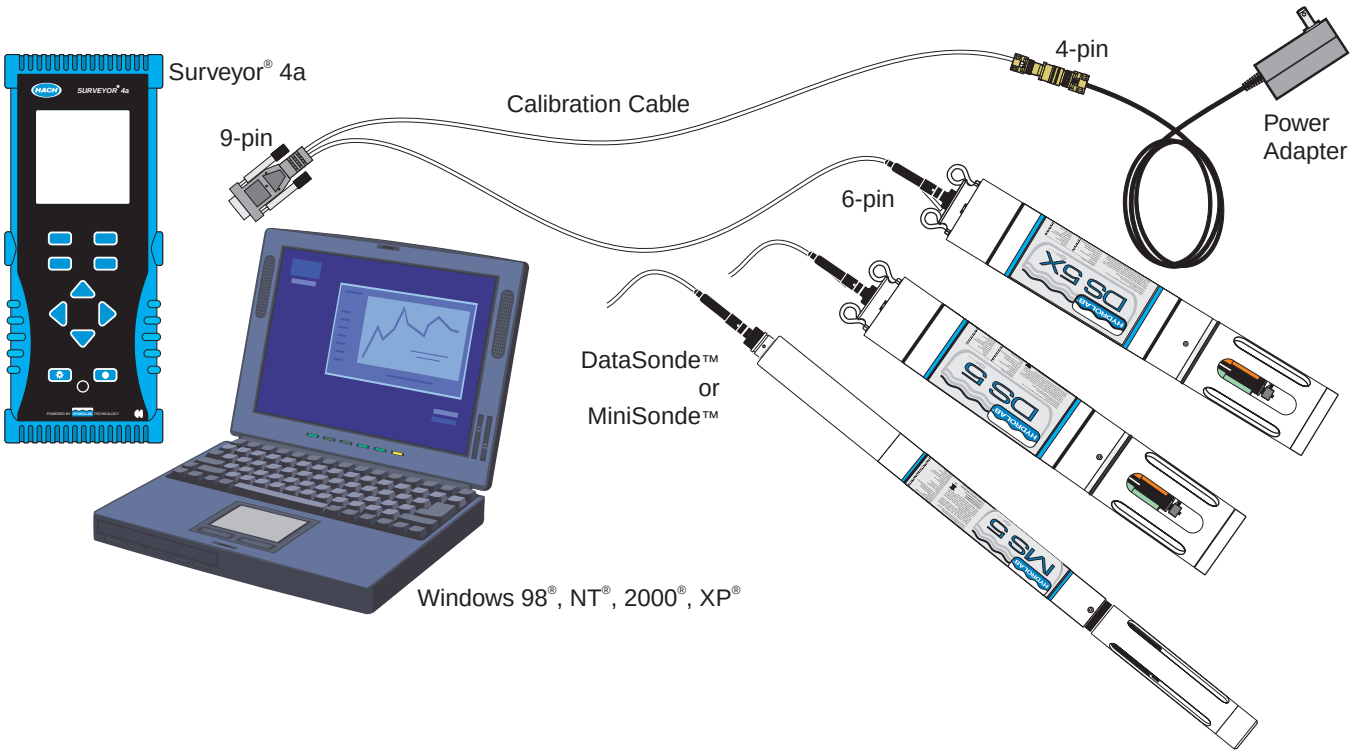
Figure 5 Assembly Options



CAUTION
When loosening removable parts from a multiprobe, always point those parts away from your body and other people. In extreme conditions, excess pressure may build-up inside any underwater housing, causing the caps, sensors, or other removable parts to disengage with force which may cause serious injury.

The Sondes use Hydras 3 LT or a Surveyor to set up parameters and calibrate the sensors.

Figure 6 Operations Setup



4.1 Parameter Setup

4.1.1 Using the Surveyor for Parameter Setup

For more information on the Surveyor, refer to the User Manual (Cat. No. 00719618).

1. Attach the power and data cable to the Sonde. Attach the 9-pin connector to the Surveyor.
2. Turn on the Surveyor. Wait approximately 10 seconds for initialization.
3. Press **SETUP/CAL**. Press **SETUP**. Press **SONDE**.
4. Highlight Parameters and press **SELECT**.
5. Use the **ARROW** keys to highlight the appropriate parameter and press **SELECT**.
6. Highlight the appropriate function and press **SELECT**. A configuration screen will be displayed. Depending on the application, use the **ARROW** keys to change the function, press **SELECT** and **DONE** to finish.

4.1.2 Using Hydras 3 LT for Parameter Setup

For more information on Hydras 3 LT, refer to the Quick Start Guide (Cat. No. 6234289) or press the **F1** key while Hydras 3 LT is active.

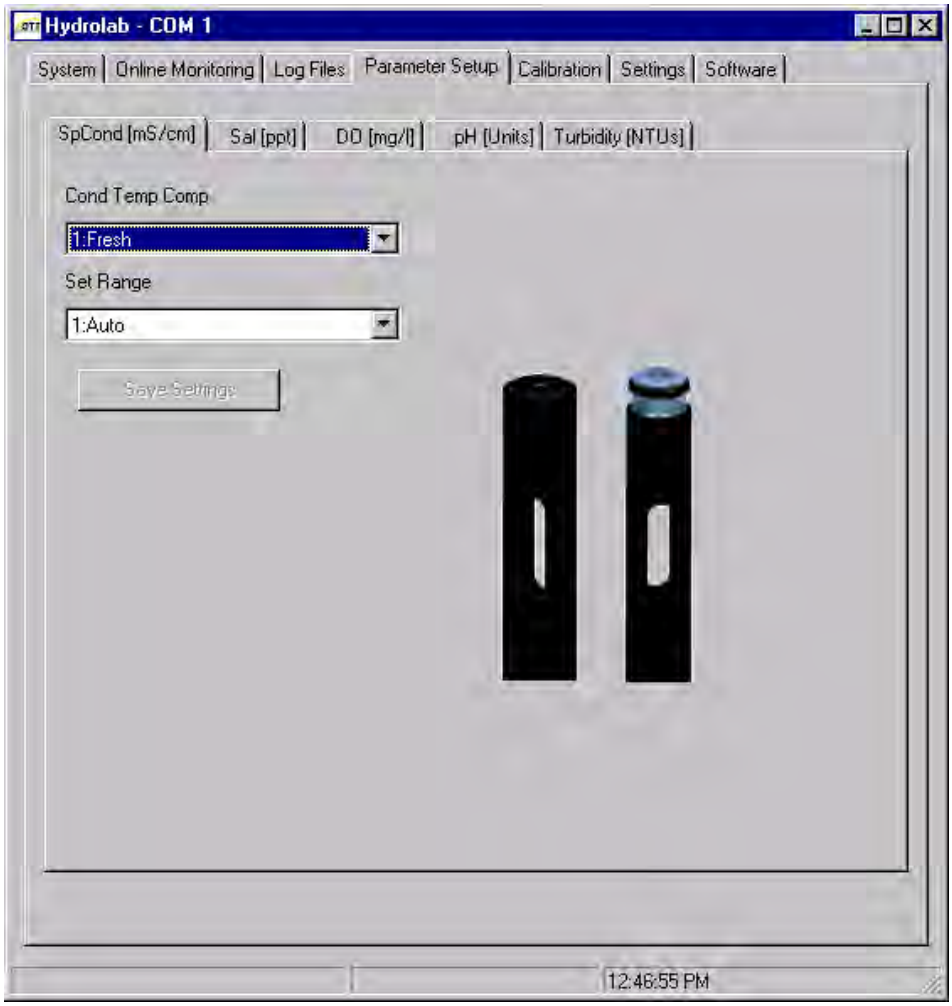
1. Attach the power and data cable to the Sonde. Attach the 9-pin connector to a PC.

2. Start Hydras 3 LT. Wait for the software to scan for connected Sondes. Highlight the multiprobe and press **OPERATE SONDE**.

Note: *If the Sonde appears to be connected and the software does not recognize the Sonde connection, remove and replace the connector cable and press RE-SCAN FOR SONDE. Repeat until Hydras 3 LT recognizes the Sonde.*

3. Click on the Parameter Setup tab and select the parameter tab to be configured.

4. Enter the appropriate values and press **SAVE SETTINGS**.



4.1.3 Specific Conductance Parameter Setup

For specific conductance set the following functions using Hydras 3 LT or the Surveyor:

- Select the specific conductance function (Fresh, Salt, StdMth, None, or Custom).
  - **Fresh** (default) is based on the manufacturer's freshwater temperature compensation. This function is derived from

0.01N KCl: $f(T) = c_1T^5 + c_2T^4 + c_3T^3 + c_4T^2 + c_5T + c_{6,}$

where:

$c_1 = 1.4326 \times 10^{-9}$, $c_2 = -6.0716 \times 10^{-8}$, $c_3 = -1.0665 \times 10^{-5}$, $c_5 = -5.3091 \times 10^{-2}$, $c_6 = 1.8199$.

- **Salt** is based on the manufacturer's saltwater compensation.

$f(T) = c_1T^7 + c_2T^6 + c_3T^5 + c_4T^4 + c_5T^3 + c_6T^2 + c_7T + c_8$

where:

$c1 = 1.2813 \times 10^{-11}$, $c_2 = -2.2129 \times 10^{-9}$, $c_3 = 1.4771 \times 10^{-7}$, $c_4 = -4.6475 \times 10^{-6}$, $c_5 = 5.6170 \times 10^{-5}$, $c_6 = 8.7699 \times 10^{-4}$, $c_7 = -6.1736 \times 10^{-2}$, $c_8 = 1.9524$.

- **StdMth** will remove any temperature compensation, so the readings are equivalent to conductivity: f(T)=1.
- **Custom** will provide a compensation function that the user can define according to the following function:

$f(T) = aT^7 + bT^6 + cT^5 + dT^4 + eT^3 + fT^2 + gT + h$.

- Select the Set Range (1:Auto, 2:High, 3:Mid, or 4:Low).
  - **Auto** (default) allows the multiprobe to automatically select the most appropriate range to measure conductivity. The multiprobe will dynamically change the range based on the current measurement conditions over 0–100 mS/cm. The resolution of the displayed data will also change to accommodate the current range in use.
  - **High**, **Mid**, and **Low** force the multiprobe to measure conductivity using a fixed range. If low range is selected, the readings will indicate an over-ranged condition for values above 1.5 mS/cm. The Mid range will over-range at 15 mS/cm. These choices also force the displayed readings to a fixed point or constant resolution format primarily only needed for certain SDI-12 data loggers. Otherwise, it is best to always select the Auto choice as this gives the best accuracy and performance for the conductivity sensor.
- Select the computation method for salinity (1:2311 or 2:StdMth).
  - **2311** (default): salinity is computed using an algorithm adapted from the United States Geological Survey Water-Supply Paper 2311 titled "Specific Conductance: Theoretical Considerations and Application to Analytical Quality Control". This salinity function is only defined from salinities in the 30 to 40 ppt range (mild concentrations and dilutions of sea water). This salinity function uses specific conductance values C in mS/cm compensated.

$\text{Salinity} = c_1C^4 + c_2C^3 + c_3C^2 + c_4C + c_5$

where:

$c_1 = 5.9950 \times 10^{-8}$, $c_2 = -2.3120 \times 10^{-5}$, $c_3 = 3.4346 \times 10^{-3}$, $c_4 = 5.3532 \times 10^{-1}$, $c_5 = -1.5494 \times 10^{-2}$.

- **StdMth**: salinity will be computed using the Practical Salinity Scale (1978). This algorithm is defined for salinities ranging from 2 to 42 ppt and uses conductivity values corrected to 15 °C, regardless of the compensation function selected for specific conductance. This algorithm is described in section 2520B of "Standard Methods for the Examination of Water and Wastewater", 18th edition.

4.1.4 Clark Cell Dissolved Oxygen Parameter Setup

For dissolved oxygen, set the following functions using Hydras 3 LT or the Surveyor: Enable or Disable Salinity Compensation.

4.1.5 pH Parameter Setup

For pH, set the following functions using Hydras 3 LT or the Surveyor: Select either 2 or 3 calibration points.

4.1.6 Other Parameter Setup

Refer to the sensor specific instruction sheet for more information.

4.2 Calibration

Sensors are checked for calibration before they leave the factory, however calibration needs to be specific for a site and application. Check the calibration prior to the first use.

Calibrate the sensors when:

- Fouling has occurred or is noticeable (site-specific).
- Parameter measurements do not match those of a known calibrated standard.
- Adding or removing certain components for different applications (e.g., the circulator) or when replacing components (e.g., the Teflon junction of the pH reference electrode).

Some system components are affected by time, usage, and the environment. To ensure instrument accuracy, it is recommended to perform routine tests of the system under standard conditions. The multiprobe can be calibrated in the field or at a facility. Equipment checks and adjustment made before going to the field tend to be more precise than those made under field conditions.

4.2.1 Calibrating the Sensors Using the Surveyor

For more information on the Surveyor, refer to the User Manual (Cat. No. 00719618).

1. Attach the power and data cable to the Sonde. Attach the 9-pin connector to the Surveyor.
2. Turn on the Surveyor. Wait approximately 10 seconds for initialization.
3. Press **SETUP/CAL**. Press **CALIBRATION**. Press **SONDE**.
4. Use the **ARROW** keys to highlight the appropriate parameter and press **SELECT**.
5. Highlight the function to program and press **SELECT**. A calibration screen will be displayed. Depending on the application, use the **ARROW** keys to change the function, press **SELECT**, and **DONE** to finish the calibration.

4.2.2 Calibrating the Sensors Using Hydras 3 LT

For more information on Hydras 3 LT, refer to the Quick Start Guide (Cat. No. 6234289) or press the F1 key while Hydras 3 LT is active.

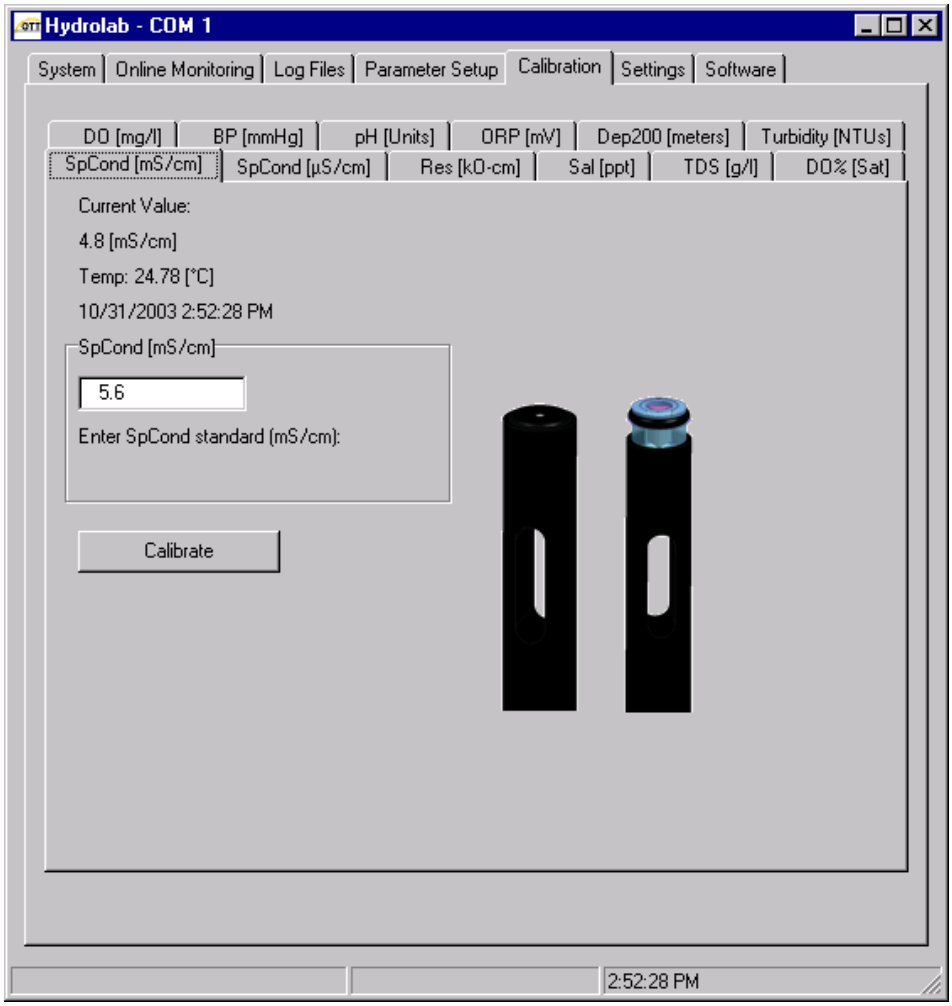
1. Attach the power and data cable to the Sonde. Attach the 9-pin connector to a PC.

2. Start Hydras 3 LT. Wait for the software to scan for connected Sondes. Highlight the multiprobe and press **OPERATE SONDE**.

Note: *If the Sonde appears to be connected and the software does not recognize the Sonde connection, remove and replace the connector cable and press RE-SCAN FOR SONDE. Repeat until Hydras 3 LT recognizes the Sonde.*

3. Click on the Calibration Tab and click on the parameter to be calibrated.

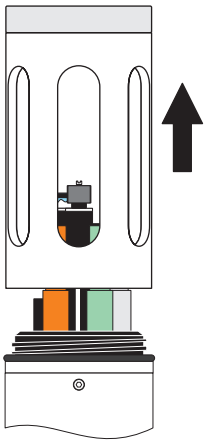
4. Enter the calibration values and click **CALIBRATE**.



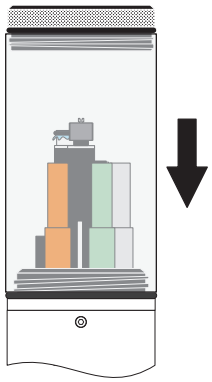
4.2.3 Calibration Preparation

The following is a general outline of the steps required to calibrate all the sensors.

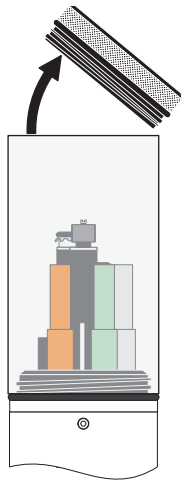
- Select a calibration standard whose value is near the field samples.
- Clean and prepare the sensors.
- To ensure accuracy of calibration, discard used calibration standards appropriately. Do not reuse calibration standards.



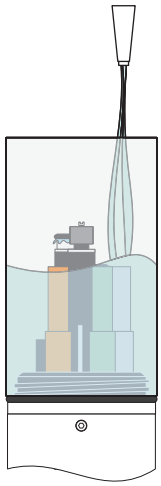
1. Remove Sensor Guard.



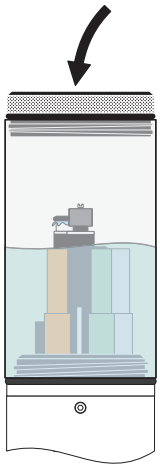
2. Attach the Calibration Cup.



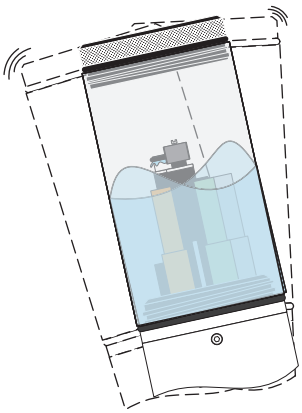
3. Unscrew and remove the cap from the Calibration Cup.



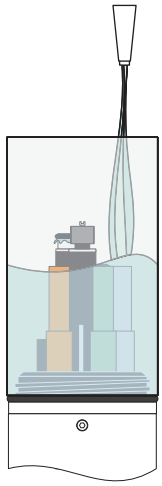
4. Fill the Calibration Cup half-full with deionized water.



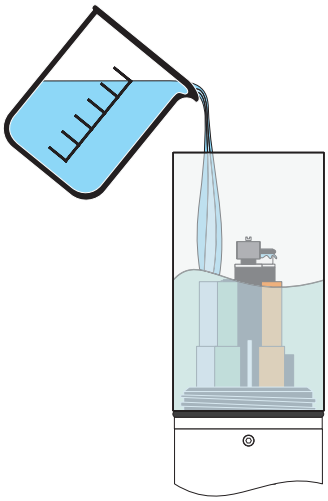
5. Place the Cap on the Calibration Cup.



6. Shake the Sonde to make sure each sensor is free from contaminants that might alter the calibration standard. Repeat several times.



7. In a similar manner, rinse the sensors twice with a small portion of the calibration standard, each time discarding the rinse.



8. Complete the calibration.

4.2.4 Temperature Sensor Calibration

The temperature sensor is factory-set and does not require recalibration.

4.2.5 Specific Conductance Calibration

Note: *TDS measurements are based on specific conductance and a user-defined scale factor. The factory default scale is 0.64 g/L / mS/cm.*

This procedure calibrates TDS, raw Conductivity, and Salinity. Specific conductance requires a two-point calibration. Calibrate the sensor to zero and then to the slope buffer.

1. Pour the specific conductance standard to within a centimeter of the top of the Calibration Cup.

2. Make sure there are no bubbles in the measurement cell of the specific conductance sensor.

3. Enter the SpCond standard for mS/cm or µS/cm using Hydras 3 LT software or a Surveyor.

4.2.6 Clark Cell Dissolved Oxygen Sensor Calibration

Dissolved oxygen calibrations can be performed using water-saturated air or using a water sample with a known dissolved oxygen concentration.

Note: *Dissolved oxygen can also be calibrated in a well stirred bucket of temperature-stable, air-saturated water. This situation resembles the actual field measurement conditions, but is more difficult to accomplish reliably.*

4.2.6.1 Water-Saturated Air Dissolved Oxygen Calibration

CAUTION
The saturated-air method is valid only for the Clark Cell dissolved oxygen sensor. If calibrating the Hach LDO sensor, refer to the Hach LDO Instruction Sheet (Cat. No. 00745589).

Note: *Calibration of D.O. % Saturation also calibrates D.O. mg/L.*

1. Fill the Calibration Cup with deionized or tap water (specific conductance less than 0.5 mS/cm) until the water is just below the membrane O-ring. Do not allow water to contact the membrane or the O-ring.

2. Carefully remove any water droplets from the membrane with the corner of a tissue.

3. Turn the black cap upside down (concave upward) and lay it over the top of the Calibration Cup. This stops the exchange of air and allows the local environment to equilibrate. Wait for the reading to stabilize.

4. Determine the true barometric pressure for entry as the calibration standard. Barometric pressure information can be obtained from a local weather station or airport or the Surveyor (if equipped with BP). Some facilities calibrate BP at sea level, an elevation correction will need to be made.

Local Barometric Pressure, BP, in mmHG can be estimated using:

$BP' = 760 - 2.5(A_{ft}/100)$ or $BP' = 760 - 2.5(A_m/30.5)$

where:

BP' = Barometric Pressure at altitude

BP = Barometric Pressure at sea level

A_{ft} = Altitude in feet

A_m = Altitude in meters

If using the local weather bureau BP, remember these numbers are corrected to sea level. To calculate the uncorrected atmospheric pressure BP', use on the following equations:

$BP' = BP - 2.5(A_{ft}/100)$ or $BP' = BP - 2.5(A_m/30.5)$

where:

BP' = Barometric Pressure at altitude

BP = Barometric Pressure at sea level

A_{ft} = Altitude in feet

A_m = Altitude in meters

Local barometric pressure in mbar (*BPmbar*) can be converted to local barometric pressure in mmHG (*BPmmHg*) using:

BPmmHG = 0.75 x *BPmbar*

5. Enter the barometric pressure in millimeters of Mercury (mmHg) at the site using Hydras 3 LT software or a Surveyor.

4.2.6.2 Known Concentration Dissolved Oxygen Calibration

Note: *Calibration of D.O. mg/L also calibrates D.O. % Saturation.*

1. Immerse the sensor in a water bath for which the D.O. concentration in mg/L is known (for instance, by Winkler titration). This calibration method is more difficult to perform than the saturated-air method but can yield a higher accuracy if the "known" D.O. concentration is highly accurate.

2. Enter the barometric units (mmHg) using Hydras 3 LT or a Surveyor.

3. Enter the D.O. units in mg/L using Hydras 3 LT or a Surveyor.

Note: *If there is a change in barometric pressure after calibration (for instance, if barometric pressure drops as you move the calibrated Transmitter to a higher elevation for deployment), the readings for D.O. % Saturation will not be correct. You must enter a new barometric pressure. However, the readings for D.O. mg/L will be correct regardless of changes in barometric pressure.*

4.2.7 Pressure Sensor Calibration

Note: *The density of water varies with specific conductance. Pressure readings are corrected for specific conductance.*

1. Remove water from the calibration cup.
2. Point sensors down.
3. Enter zero for the standard using Hydras 3 LT or a Surveyor.

4.2.8 pH/ORP Calibration

1. Pour the pH or ORP standard to within a centimeter of the top of the cup.
2. Enter the units for pH or ORP using Hydras 3 LT or a Surveyor.

Note: *pH is a two-point or three-point calibration. A pH standard between 6.8 and 7.2 is treated as the "zero" and all other values are treated as the "slope". First calibrate "zero", then calibrate "slope".*

After the sensors have been properly maintained, the sensors can be calibrated. Always allow sufficient time for thermal stabilization of the standards. To reduce the time for stabilization, try to keep all calibration standards and equipment stored at the same temperature before parameter calibration. Always use fresh standard and do not tamper with standards.

4.2.9 Other Sensor Calibrations

Refer to the sensor specific instruction sheet for more information.

4.3 Using the DS5/MS5 for Short Term Deployments

4.3.1 Gathering Data Using the Surveyor

Refer to the Surveyor Manual (Cat. No. 003070).

4.3.2 Gathering Data Using a PC and Hydras 3 LT

For online monitoring and real-time monitoring information, refer to the Hydras 3 LT Quick Start Guide (Cat. No. 6234289).

4.3.3 Using the DS5/DS5X/MS5 for Unattended Monitoring

4.3.3.1 Creating Log Files

Note: *A log file must be created and then enabled before data can be collected.*

1. Connect the Data Cable to a computer and to the Sonde.
2. Start Hydras 3 LT. The software will automatically scan for Sondes. All detected Sondes are displayed in the 'Connected Sondes' list in the Main window displayed below. If a Sonde is not found, reattach the data cable and press **RE-SCAN FOR SONDES**. Retry until the Sonde(s) are found.
3. Click on the Log Files tab.
4. Click the **CREATE** button.

5. Enter the name for the new log file. The empty log file is now created.

6. Enter the start and end time of the logging, the logging interval, the sensor warm-up time before logging, and how long before logging the circulator will be turned on, and if audio signals will be used while logging.

7. Select the parameters in the ‘Parameter in Sonde’ list and click the **ADD** button to place them into the ‘Parameters in log file’ list. Change the order of the parameters using the **ARROW** buttons.

8. Click **UPDATE SETTING** to send the configuration to the Sonde.

9. Click **ENABLE** to start collecting data. Click **DISABLE** to stop collecting data during logging. A fully completed logging run will automatically disable at the end of the run.

10. Click **DOWNLOAD** to download and display the log file. Select Printable or Spreadsheet format.

Note: *To delete a log file, select the log file in the Log File drop-down menu and click the* ***DELETE*** *button.*

4.3.3.2 Downloading Log Files

After a log file is created in the Log Files tab, the files can be downloaded by checking the appropriate Log File box and clicking **DOWNLOAD SELECTED FILES**. Multiple files can be downloaded at once. The downloaded log files are stored in the ‘LogFiles’ subdirectory of the HYDRAS 3 LT directory on the hard drive.

Section 5 Deployment

5.1 Deployment Considerations

5.1.1 Pressure Extremes

Note: *The multiprobe maximum immersion depth is 225 meters (738 feet).*

Note: *The ion specific sensors (Nitrate, Ammonia, and Chloride) maximum deployment depth is 15 meters.*

Important Note: *The 0–10 meter vented depth sensor should be protected from depths over 15 meters (49 feet) by installing the seal screw (provided in the basic maintenance kit) in the face of the multiprobe sensor cap. Likewise, the 0–25 depth sensor should be protected from depths over 35 meters (164 feet) by installing the same seal screw. However, the 100- and 200-meter depth sensors do not require installation of the seal screw.*

The mulitprobe may be equipped with one of the following depth options:
0–10 meters (33 feet), 0 to 25, 0 to 100, and 0 to 200 meters (82, 328, and 656 feet). The first option is used to detect water level changes that are automatically compensated for barometric pressure changes. Applications include tidal waters, rivers, stream, lakes, reservoirs, and groundwater. The vented level option must have a fixed cable with a vent tube. The second, third, and fourth options are usually used to determine the depth in the water column at which the other parameter readings are made.

5.1.2 Temperature Extremes

The multiprobe storage temperature range is 1 to 50 °C (34 to 122 °F), non-freezing, when going or coming back from a deployment site, or when storing the multiprobe.
The mulitprobe operating temperature range is –5 to 50 °C (23 to 122 °F), non-freezing. Exposing the multiprobe to temperatures outside this range may result in mechanical damage or faulty electronic performance.

To prevent sensors from freezing, store the multiprobe where freezing will not occur.
To prevent the sensors from dehydrating, fill the DS storage cup or MS cup with one inch of clean tap water.

Always rinse the multiprobe with clean tap water after deployment.

5.1.3 Data Transmission Lines

When adding a transmission cable to the multiprobe, the cable must be large enough to carry the operating current and transfer data without distortion. For up to a total of 305 m (1000 ft) of cable, three 26 AWG wires are suitable for data transmission, but two 18 AWG must be used for the power wires. Alternatively, smaller power wires can be used if the power supply is placed closer to the multiprobe. A cable extension kit is available for above-ground applications (Replacement Parts and Accessories on page 47).

5.1.4 Minimum Depth Requirements

Sensors must be immersed. The Standard Turbidity Sensor minimum deployment depth is 1 meter.

5.2 Deployment in Open Waters

5.2.1 Minimum Clearance Requirements

Two inches of clearance is required from the longest probe and two inches around if a Turbidity sensor is supplied.

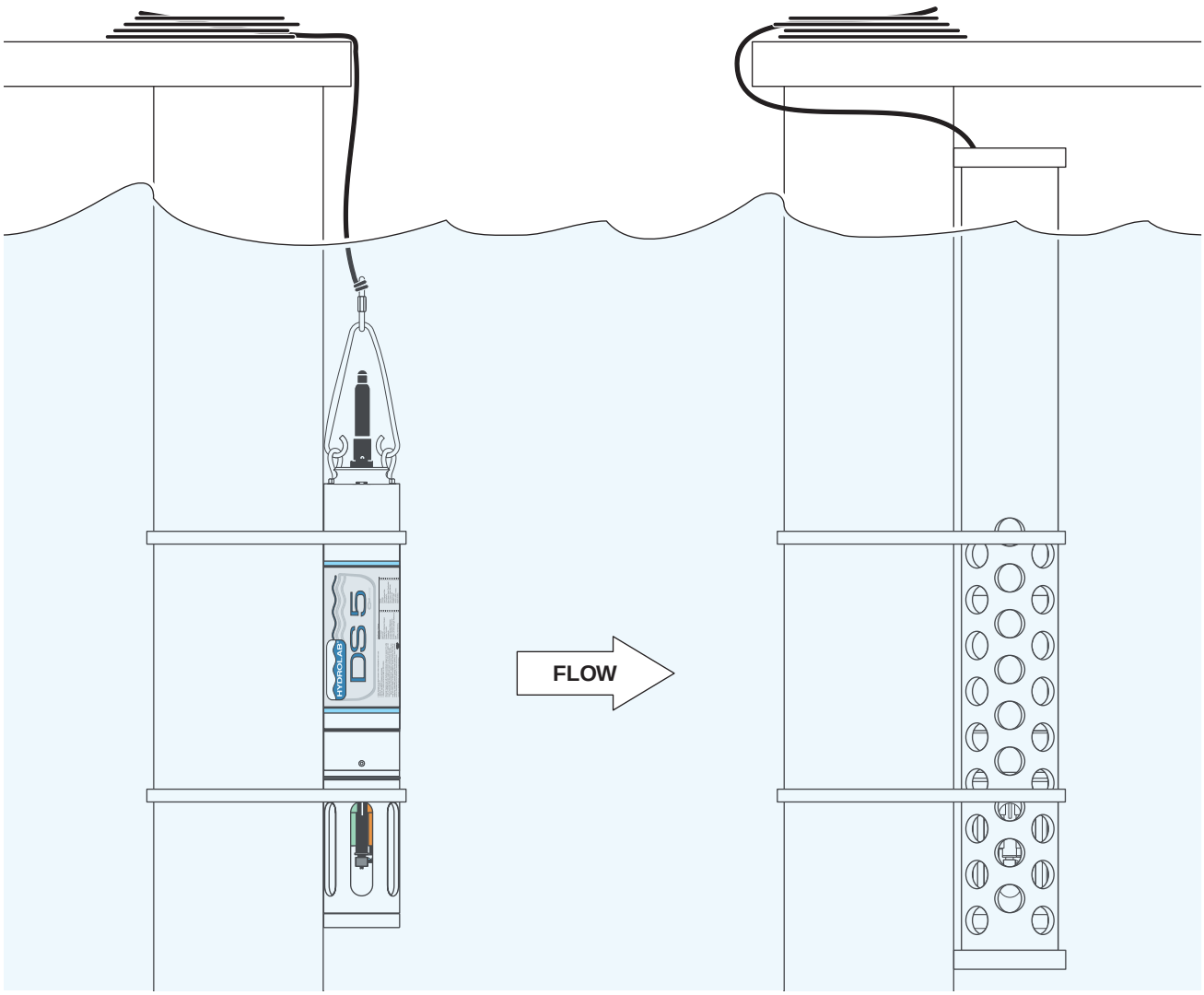
5.2.2 Long-term Deployment in Open Waters

Important Note: *The use of pipe clamps to secure the Sonde can cause serious instrument damage.*

When using the multiprobe in open water, place the multiprobe where it will not get damaged. For example, to protect the multiprobe from being hit by floating debris in moderate-to high-flow levels, anchor the multiprobe to the downstream side of a bridge piling (Figure 7). The protection kit can also be used to protect the multiprobe. In a recreational lake deployment, use a marking buoy that will not attract vandalism.

Place the multiprobe in an upright or on-side position, and avoid areas with deposits of sand, gravel, or silt during heavy rainfall. Avoid deploying in location where ice will form around the sensors or Sonde.

Figure 7 Securing the Multiprope to a Structure



When securing the multiprobe to a structure, carefully place straps such as web belts and large plastic Ty-wraps on both ends of the mulitiprobe housing (Figure 7). Do not use clamps to secure the multiprobe to a structure. Also, secure the cable in the same manner to protect it from floating debris, navigation, and vandalism.

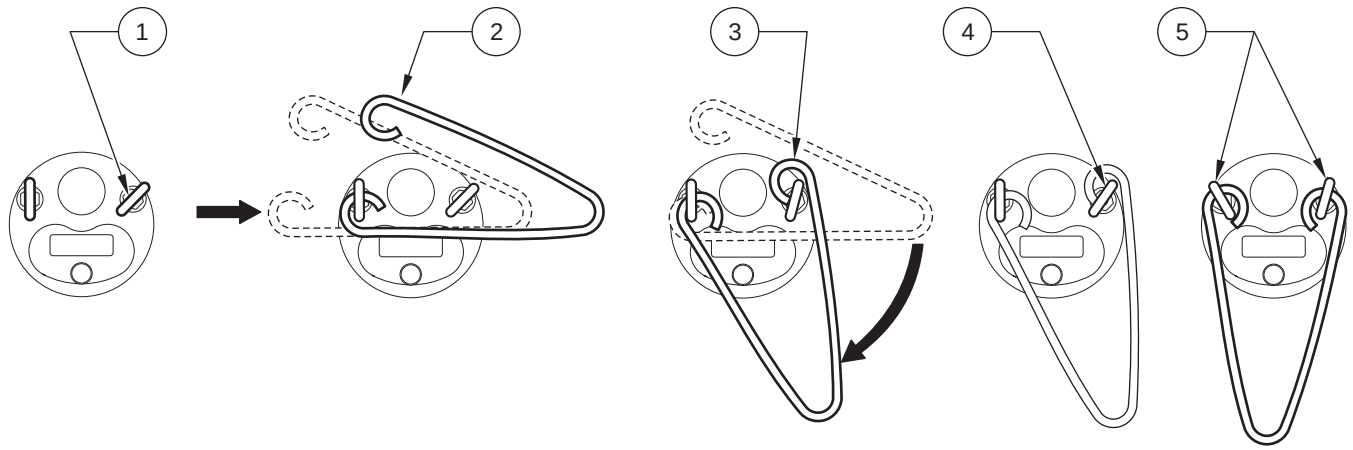
Always make sure the weighted sensor guard is installed to protect the sensors and provide additional sinking weight to the multiprobe.

Calibration stability is dependent on the environmental conditions in which the sonde is deployed. For example, a D.O. sensor on a DS5 or MS5 may become fouled if deployed in a warm, shallow, biologically active lake. However, deployment length can be increased by a factor of 5 by using a DS5X which periodically cleans the fouling from the sensors. On the other hand, the same sonde deployed in a clean water environment, or a sonde configured with sensors impervious to fouling (i.e., Temperature, Conductivity) can be left unattended for months without the need to recalibrate. Optimal deployment time for a specific environment can be determined by making periodic measurements of sensitive parameters with another instrument.

5.2.2.1 Anchoring the DS5 or DS5X using the Support Bail

1. Run a rope or chain through the bail, if the Sonde is equipped with a support bail.
2. Fix the bail into the two eyebolts on the top of the Sonde by first loosening the lock-nuts and turning the eyebolt 90°, and then back, so that the bail can be looped through.
3. Securely tighten the lock-nut on each eyebolt. If the mulitprobe is not equipped with internal batteries, it may not have a bail but can be secured using the locking sleeve.

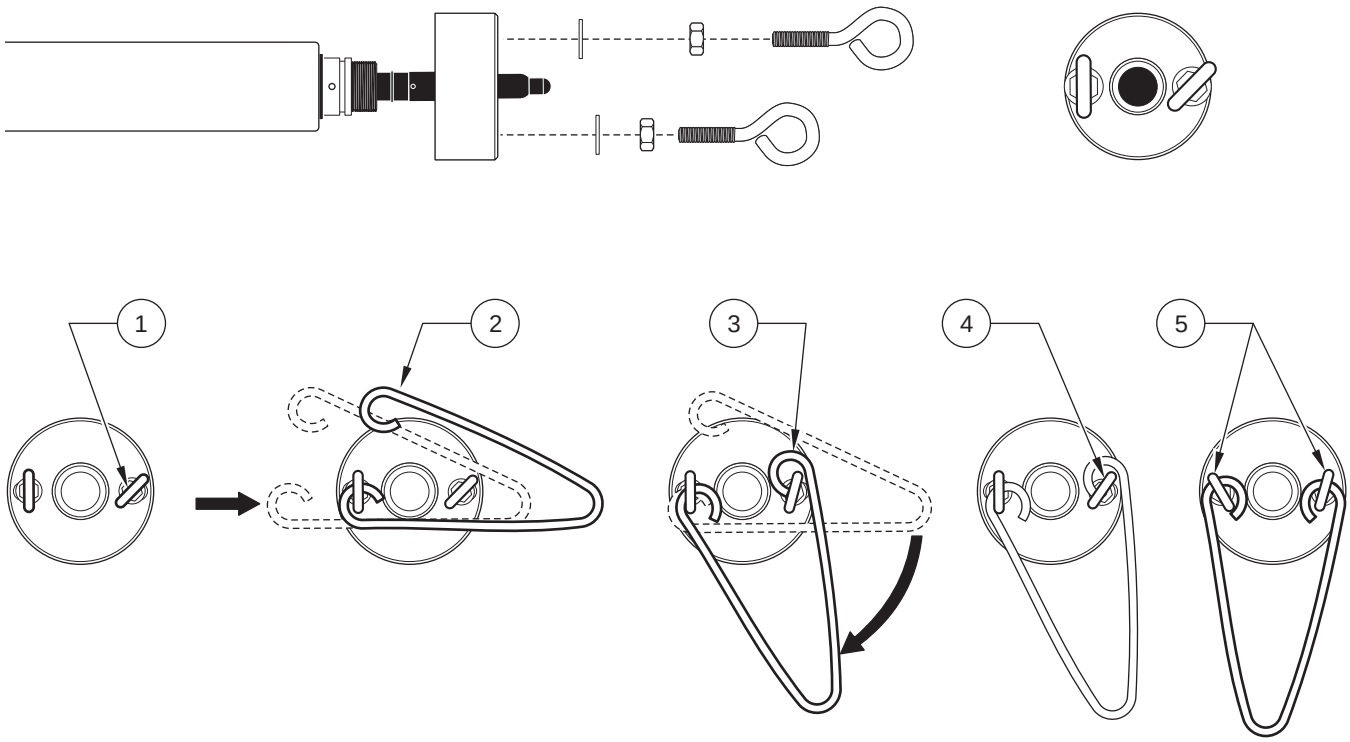
Figure 8 DS5 Support Bail Installation



1.	Turn the eye bolts to the positions shown.	**4.**	Rotate the eye bolt around the bail as shown.
2.	Hook the bail into the eye bolt as shown.	**5.**	Finish rotating the eye bolts until the bail cannot be removed.
3.	Rotate the bail into the other eye bolt as shown.		

5.2.2.2 Anchoring the MS5 using the Bail Kit

Figure 9 MS5 Support Bail Installation

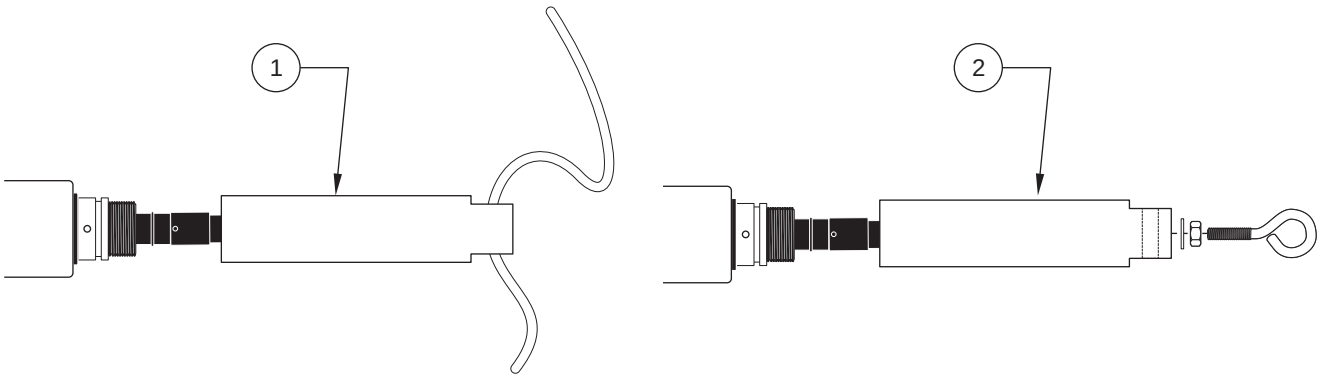


1.	Turn the eye bolts to the positions shown.	**4.**	Rotate the eye bolt around the bail as shown.
2.	Hook the bail into the eye bolt as shown.	**5.**	Finish rotating the eye bolts until the bail cannot be removed.
3.	Rotate the bail into the other eye bolt as shown.		

5.2.2.3 Anchoring the MS5 using the Mooring Fixture

A MS5 equipped with an internal battery pack requires the MS5 mooring fixture which screws onto the mulitprobe bulkhead connector and provides an eyelet for rope or wire when no cable is used during deployment.

Figure 10 MS5 Mooring Fixture



1.	Mooring fixture used with rope or chain.	**2.**	Mooring fixture used with eye bolt.

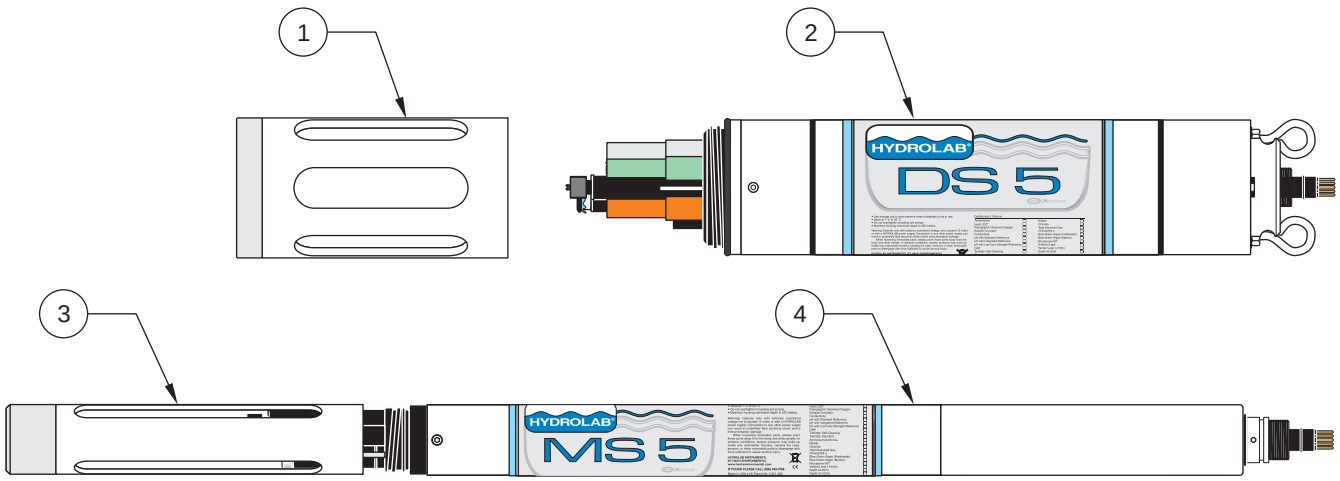
5.2.3 Short-term Deployment in Open Waters

Generally, short-term deployment implies hand-held operation.

Important Note: *Do not pull Sonde from a moving boat or instrument damage may occur and voids the instrument warranty.*

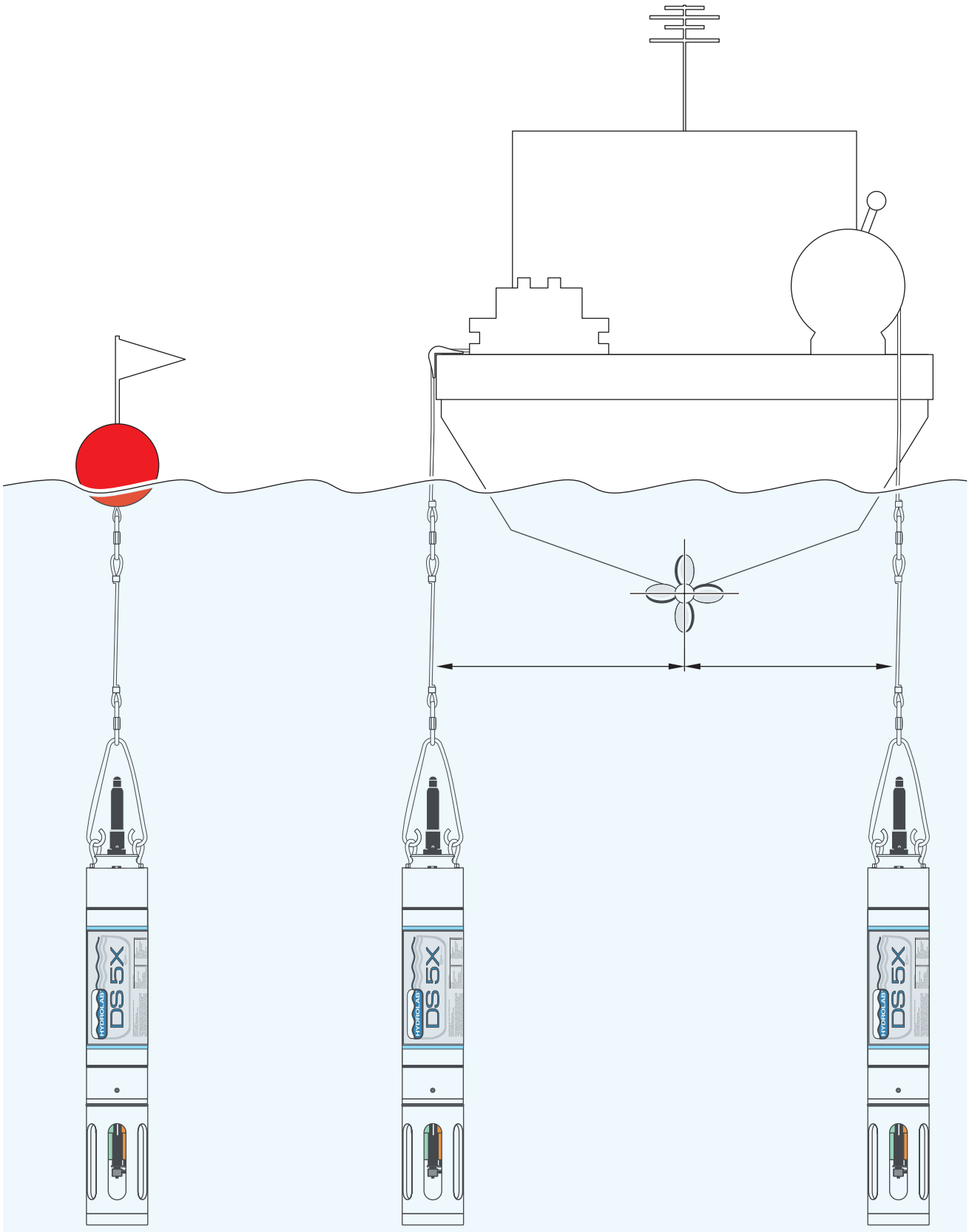
- Do not lower the mulitprobe into the water without screwing on the weighted sensor guard.
- Secure the underwater cable prior to deployment.
- Do not place the instrument where the cable might be severed or damage by boat propellers or any moving parts on a monitoring system.
- Protect all cables from abrasion, unnecessary tension, repetitive flexure, or bending over sharp radii (boat gunwale or a bridge railing).
- Do not bend or run the cable over the sheave or pulleys with less than 3-inch radius or 6 inches in diameter.
- Use the V-shaped support bail to lift and lower the multiprobe, if so equipped. This ensures that the weight of the mulitprobe is suspended from the bail. If the multiprobe is equipped with a locking sleeve instead of a support bail, make sure the locking sleeve or the MSS mooring fixture are properly screwed on the multiprobe 6-pin marine bulkhead connector before deployment.
- Extra weight, up to 5 kg (10 lb) maximum, can be attached to the Sonde (Figure 11). If more weight is needed, use a wire line to support the instrument by its bail, if so equipped.
- Use a battery-powered or hand-cranked reel with electrical slip-rings to lower and raise the instrument, if the cables are very long. A lighter reel without slip-rings for shorter cables can also be used (Figure 12).
- If sufficient deck space permits, mount the reel horizontally with the instrument and a battery installed in the hub (Figure 12). The manufacturer's cable reel can also be used to store up to 150 m (490 ft) of underwater cable (the reel must be ordered with the initial underwater cable purchase.
- In deep deployments currents, in conjunction with the sensor guard, can put extreme strains on the cabling.

Figure 11 Using the Weighted Sensor Guard



1. DS5 Sensor Guard	**3.** MS5 Sensor Guard
2. DS5	**4.** MS5

Figure 12 Open Water Deployment



5.2.4 Minimum Flow Requirements

When deploying the multiprobe in waters flowing at less than one foot per second (0.333 mps), a circulator option may be used for additional flow in order to achieve reliable Clark Cell dissolved oxygen sensor readings. The circulator is activated via Hydras 3 LT software or the Surveyor.

Turning the circulator on or off will help during profiling and logging D.O. with a Clark Cell Sensor, depending on the flow rate of the water at the site. If insufficient flow rate is noticeable, turn the circulator on. Turn the circulator off to extend battery life when data is not needed for an extended period of time. Turn the circulator on when logging data in unattended mode and need to have sufficient flow for accurate measurements, note that this will reduce multiprobe battery life.

When the multiprobe is powered, it takes time to warm-up. The warm-up time refers to the time a sensor will be ready to record accurate data. Warm-up time will vary according to the sensors being used and field conditions (e.g. temperature).

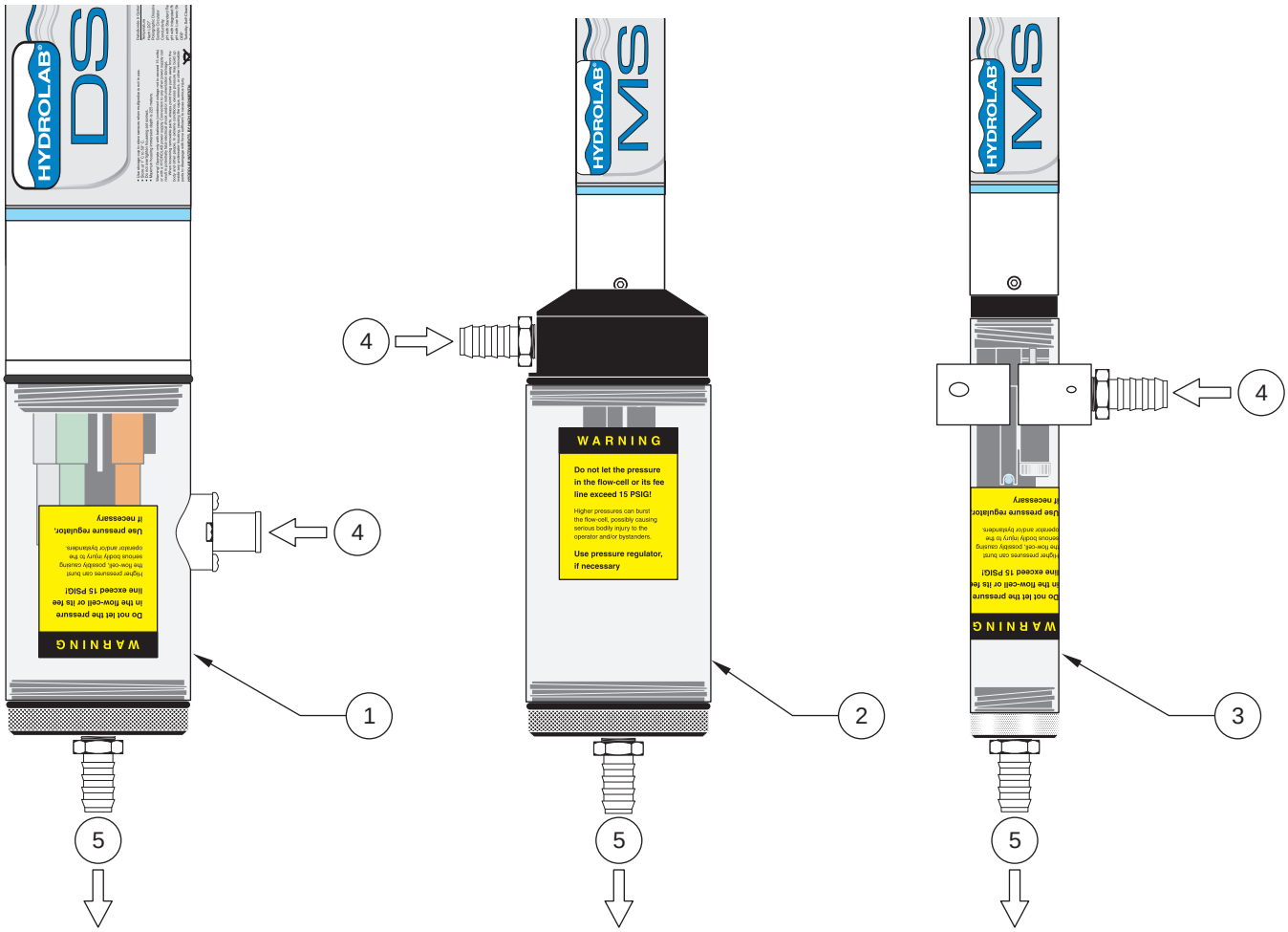
5.2.5 Non-submersible Deployment

DANGER
Do not let the flow cell pressure exceed 15 psig. Higher pressure can burst the flow cell, possibly causing serious bodily injury to oneself and/or others.

For process or pump-through situations, attach the low-pressure flow-cell to the mulitprobe. This configuration allows studying the water without submerging the multiprobe. The flow cell replaces the DS storage cup or MS cup (Figure 13). When measuring D.O. with a Clark Cell Sensor inside a flow cell, the manufacturer recommends using a circulator in conjunction with the sensor. For use without a circulator, use a flow rate of more than 4 liters per minute. A ½-inch hose is required for the MS flow cell and a ¾-inch hose for the DS flow cell.

Filter debris from the feed line, if necessary. If possible, invert the multiprobe, so that bubbles will float away from the sensors and out the port on the bottom of the flow cell.

Figure 13 Flow Cells



1.	DS flow cell	**4.**	Sample flow IN
2.	MS flow cell (with standard turbidity sensor)	**5.**	Sample flow OUT
3.	MS flow cell		

DANGER
Only qualified personnel should conduct the maintenance tasks described in this section of the manual.

To ensure continued and reliable operation of the water quality monitoring system, we recommend scheduling a thorough and regular maintenance program. To determine the appropriate maintenance intervals required for a deployment site, periodically conduct a visual inspection of the equipment and sensors, compare the pre- and post-calibration results, and monitor the sensor response time.

A contaminated, worn-out, or damaged sensor will not produce reliable readings. It is recommended to service all sensors and allow them to equilibrate in tap water overnight before calibration.

Maintenance kits are available for the DS5, DS5X, and MS5. See Replacement Parts and Accessories on page 47.

6.1 Multiprobe and Accessory Maintenance

6.1.1 Cleaning the Multiprobe Housing

Clean the outside of the multiprobe housing using a clean brush, soap, and water. Always use the DS storage cup or the MS cup (filled with one inch of tap water) to protect the sensors from damage, and especially from drying out, whenever the multiprobe is not deployed.

Do not expose equipment to extreme temperatures below 1 °C (34 °F) or above 50 °C (122 °F).

Always rinse the multiprobe with clean tap water soon after returning from deployment.

6.1.2 Dryer Maintenance

Important Note: *Do not submerge the dryer in water.*

The in-line vented level dryer is a part of the cable and penetrator assembly, if the Sonde has the vented depth sensor (0–10 meters).

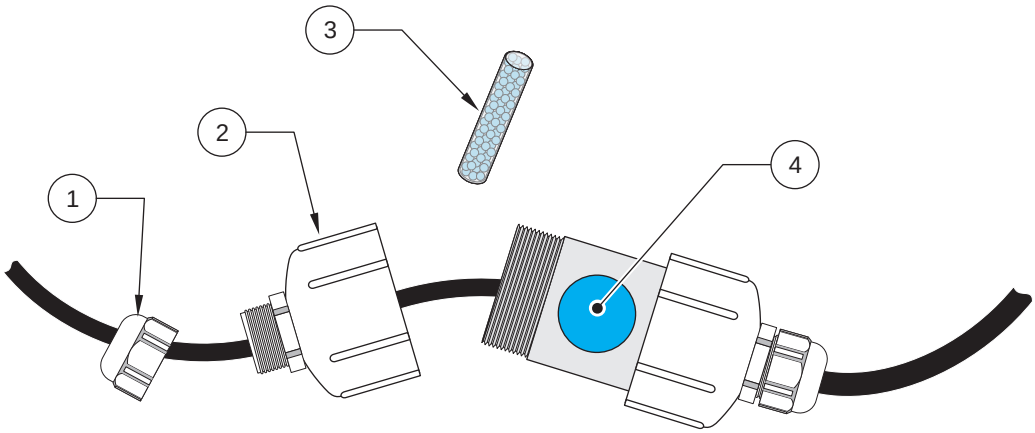
The GORE-TEX® patch (round patch on dryer) allows gases to come into the dryer and is splash proof, but not submersible. Any water leaks inside the dryer can block the tube which goes into the multiprobe. If water leaks are detected, contact Technical Support.

The dryer contains desiccant bag(s) (white bag) to keep condensation from forming inside the vented tube which goes from the dryer to the multiprobe. If moisture is detected inside the dryer, replace the bags (Figure 14).

To replace the desiccant bag(s):

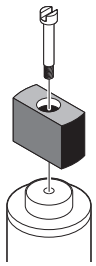
1. Unscrew both dryer nuts and unscrew the dryer cap.
2. Inspect the indicator strip. If the stripe is dark blue, the bag does not need replacement. If the stripe is light pink or purple, remove, discard, and replace the old bag.
3. Reassemble the dryer.

Figure 14 Dryer Maintenance



1. Dryer Nuts (2)	**3.** Desiccate Packet
2. Dryer Cap	**4.** Gore-tex Patch

6.1.3 FreshFlow™ Miniature Sample Circulator Maintenance



1. If the circulator is clogged with twigs or other small debris, clean the impeller with some tap water using a soft bristle brush. Use a pair of plastic tweezers to help remove debris. Rinse with tap water.

2. If there is excessive build-up on the impeller, remove the retaining screw to clean the build-up. After cleaning the impeller, and before inserting the retaining screw, apply a very small amount of Loctite™ 242 threadlock (or equivalent) on the tip of the screw. Do not overtighten.

6.2 Battery Replacement

If the multiprobe is equipped with an internal battery pack, the following batteries are customer-replaceable. The Sondes are also equipped with a customer-replaceable Lithium clock battery.

- 8 size C alkaline batteries for the DS5 and DS5X
- 8 size AA alkaline batteries for the MS5

Important Note: *To keep internal components dry, avoid replacing batteries near a water source.*

Important Note: *If water leaks into the multiprobe battery compartment, remove the batteries, pour the water out, and thoroughly dry the compartment with a towel.*

6.2.1 DS5 and DS5X Battery Replacement

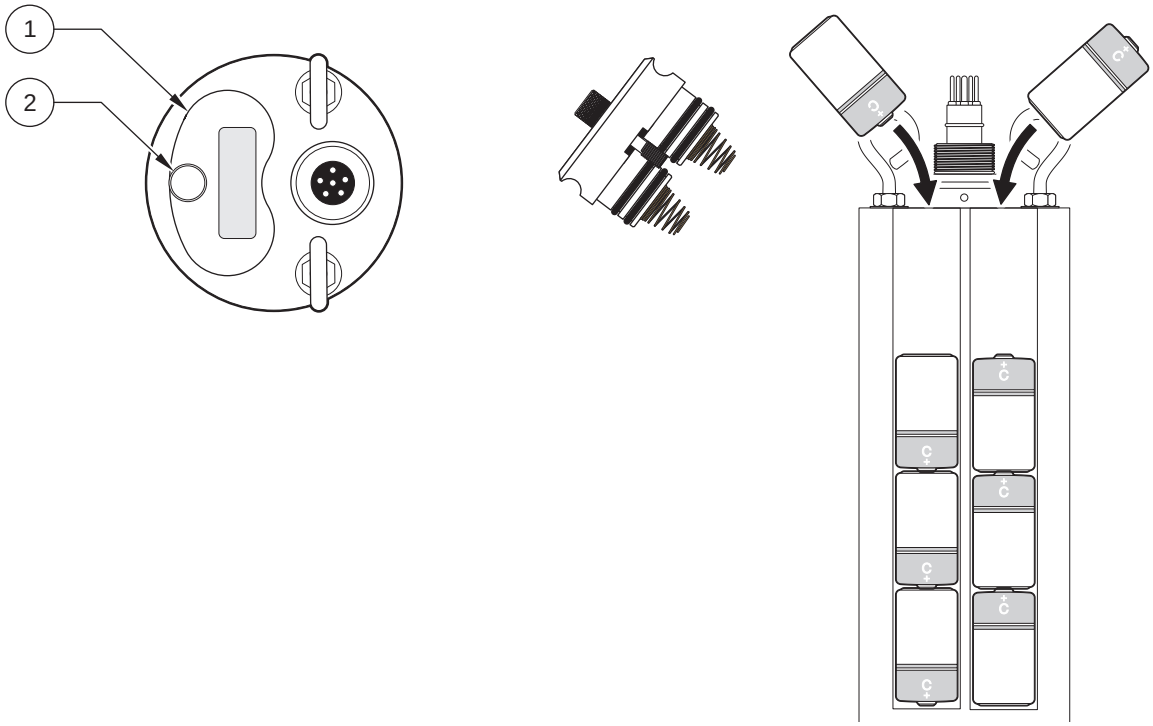
DANGER
If the thumbscrew is difficult to remove, there may be pressure built up inside the housing. To avoid serious injury, use extreme caution when loosening the battery cap thumbscrew.

DANGER
Batteries must be installed in the correct orientation or serious injury and instrument damage may occur. Do not mix depleted and fresh batteries together or serious injury and instrument damage may occur.

Use only high quality, non-rechargeable batteries in the DS5 or DS5X multiprobe. Refer to Figure 15 and the following directions for DS5 and DS5X battery replacement.

1. Set the multiprobe horizontally on the work surface to prevent water leaking into the mulitprobe battery compartment.
2. Unscrew the battery cap thumbscrew, counterclockwise.
3. Pull the cap out of its housing and slide the old batteries out.
4. Discard the old batteries. Insert the new batteries, **observing polarity markings located on the inside label**. Failure to install the batteries in the correct orientation may cause serious injury and instrument damage.
5. Coat the battery cap O-rings sparingly with silicone grease. Insert the cap back into the multiprobe housing. Tighten the thumbscew, clockwise. Finger-tighten only.

Figure 15 DS5 and DS5X Battery Replacement



1. Battery Cap	**2.** Battery Cap Screw

6.2.2 MS5 Battery Replacement

DANGER
Batteries must be installed in the correct orientation or serious injury and instrument damage may occur.

DANGER
Do not mix depleted and fresh batteries together or serious injury and instrument damage may occur.

1. Set the multiprobe horizontally on the work surface to prevent water leaking into the mulitprobe battery compartment.

2. Unscrew the battery sleeve. Slide the battery sleeve off of the Sonde.

3. Discard the old batteries. Insert the new batteries, **observing polarity markings located on the inside label**. Failure to install the batteries in the correct orientation may cause serious injury and instrument damage.

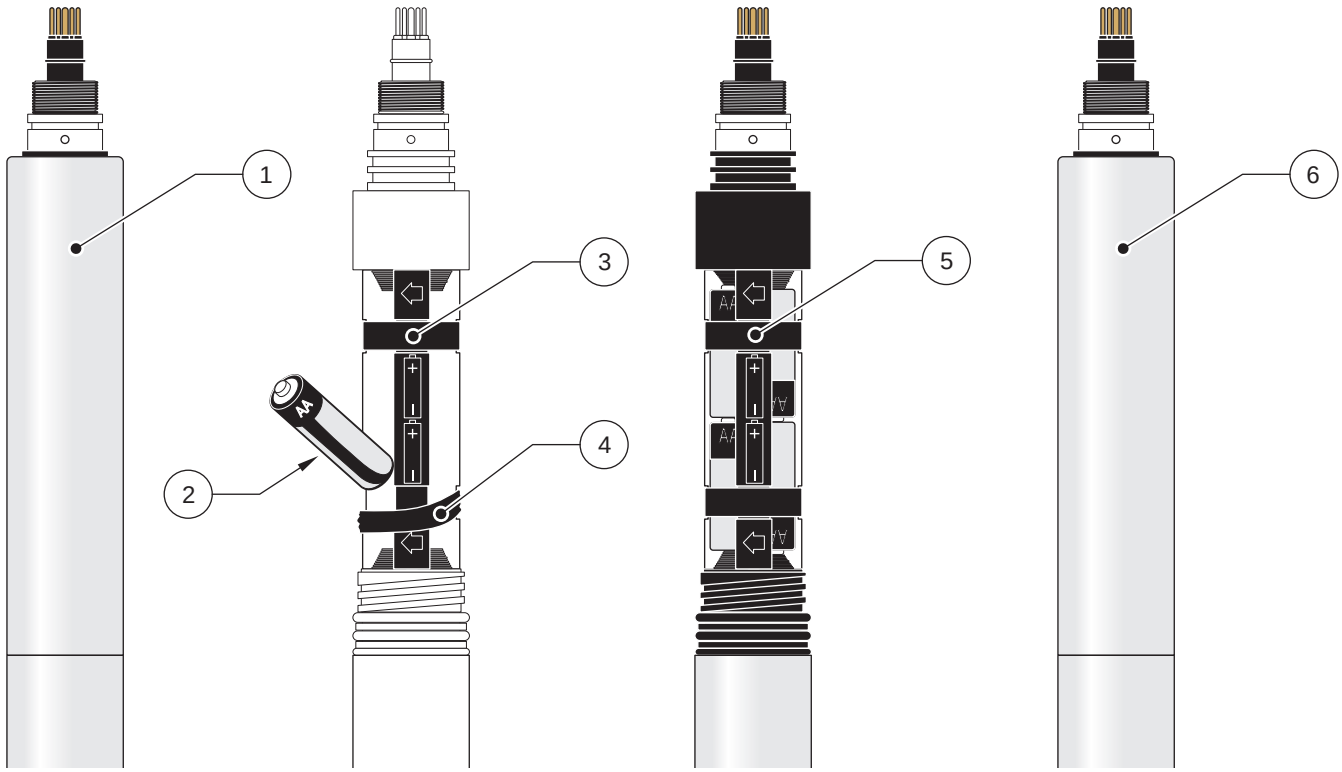
4. Secure the new batteries with the top and bottom rubber bands (Figure 16).

5. Coat the battery sleeve O-rings sparingly with silicone grease.

6. Screw the battery sleeve back on the Sonde. Do not overtighten or instrument damage will occur.

7. Discard batteries according to local regulations.

Figure 16 MS5 Battery Replacement



1.	Remove the Battery Sleeve	**4.**	Bottom Retaining Rubberband
2.	Remove depleted AA Batteries	**5.**	Proper Battery Placement
3.	Top Retaining Rubberband	**6.**	Replace the Battery Sleeve

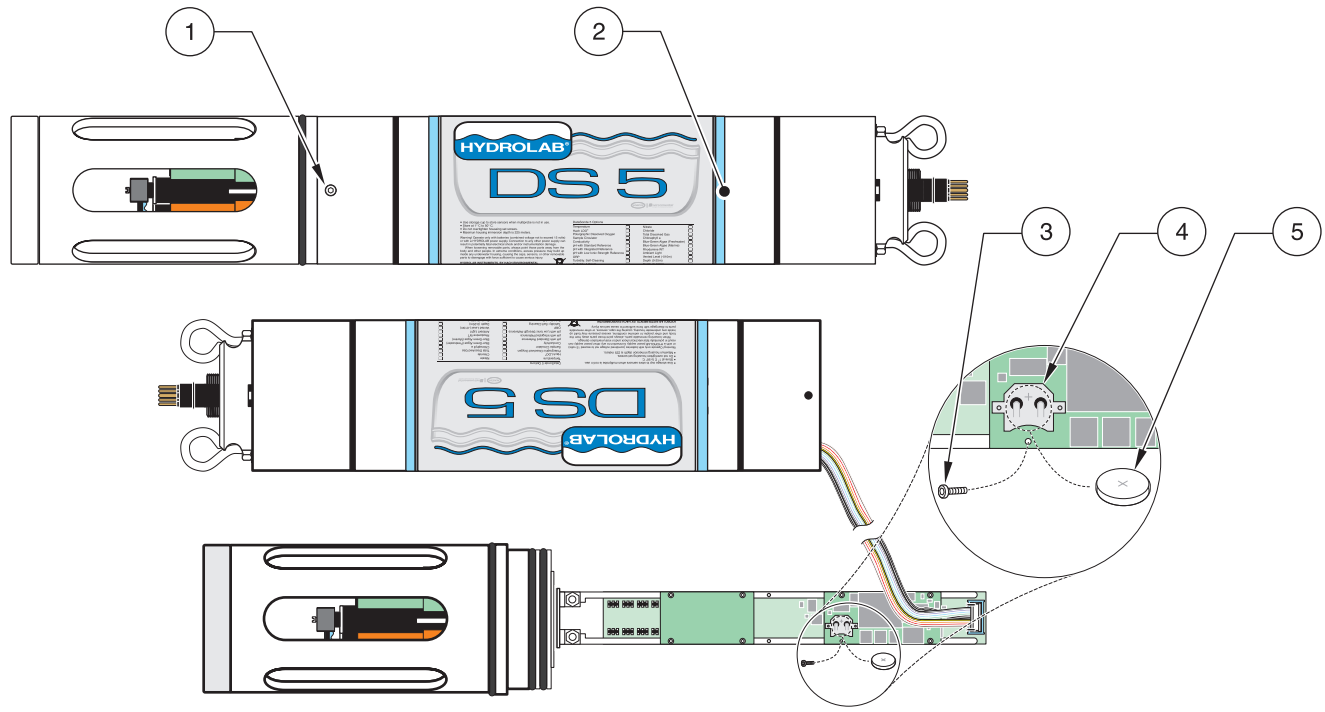
6.2.3 Lithium Battery Replacement

The typical replacement period for the lithium battery is once every two years. The lithium battery powers the real-time clock that provides accurate time readings during datalogging. Refer to Figure 17 and Figure 18 and the following instructions for proper lithium battery replacement.

1. Remove the Allen screws with the Allen wrench to remove the multiprobe sensor cap. To help remove the Sonde sensor cap, insert the screwdriver head into the notches on the mulitprobe housing at the bottom of the sensor cap.

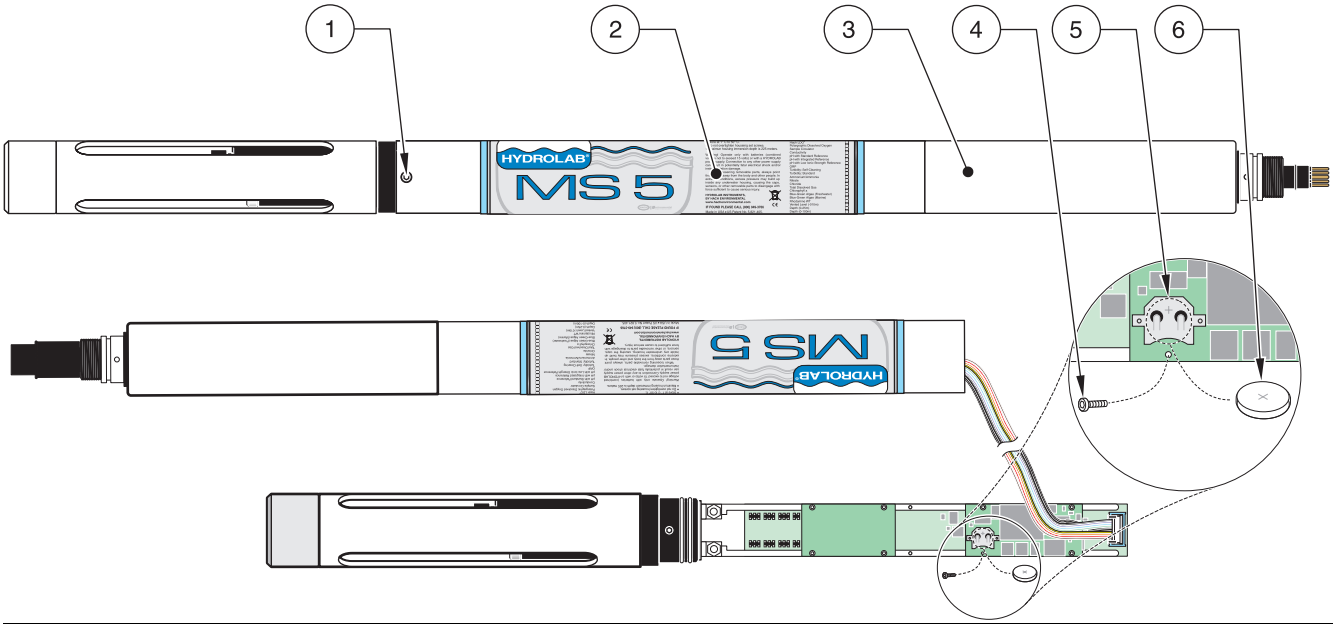
2. Remove the Sonde body, then slide the sleeve off of the Sonde. Avoid damage to the circuit board.
3. Carefully remove the foil shield.
4. Detach the 10-pin connector ribbon cable.
5. Remove the retaining screw next to the battery clip (Figure 17 and Figure 18).
6. Push the battery out of the battery clip using a small screwdriver.
7. Insert the new battery (Panasonic reference: CR 2032, or equivalent) with the positive sign facing up. Replace and tighten the retaining screw.
8. Reattach the 10-pin connector and replace the foil shield.
9. Apply silicone grease to the sensor cap O-rings.
10. Carefully insert the circuit board and sensor cap assembly.
11. Tighten the Allen screws. **Do not overtighten**.
12. Reset the time and date after replacing the lithium battery. Then enter the time of the location and press **ENTER**.
13. Discard batteries according to local regulations.

Figure 17 Replacing the Lithium Battery on the DS5 or DS5X



1.	Allen Screw	**4.**	Battery Clip
2.	Housing	**5.**	Lithium Battery
3.	Retaining Screw		

Figure 18 Replacing the Lithium Battery on the MS5



1.	Allen Screw	**4.**	Retaining Screw
2.	Housing	**5.**	Battery Clip
3.	Sonde Body	**6.**	Lithium Battery

6.3 Storage and Care Recommendations

6.3.1 Mulitprobe and Sensor Storage

- Fill the DS storage cup or MS cup with one inch of clean tap water and screw the cup on the multiprobe. To prevent sensors from freezing, store the multiprobe where freezing will not occur.
- Remove batteries for long-term storage. (8 size C alkaline batteries for the DS5 or DS5X or 8 size AA alkaline batteries for the MS5). Do not remove the lithium battery which powers the mulitprobe internal clock.
- Store equipment in a carrying case (Cat. No. 011780) or a large plastic container with a circular piece of foam rubber for shock protection.
- Lay the cable in coils of at least 15 cm (6 in.) diameter at the bottom of the plastic container.

6.3.2 Electrical Cable Care

- Protect all non-waterproof cables (i.e., all cables except the waterproof underwater cable) from any water source during operation in the field. Keep connectors dry at all times.
- Properly lubricate the sealing surface of all underwater connectors using silicone grease.
- Use protective plugs when the connectors (for underwater and calibration cables) are not connected to any instrument.
- Keep all cables clean, dry, and stored (neatly coiled), in a large plastic container.
- Do not coil cables any tighter than 6 inches in diameter or cable will be damaged.

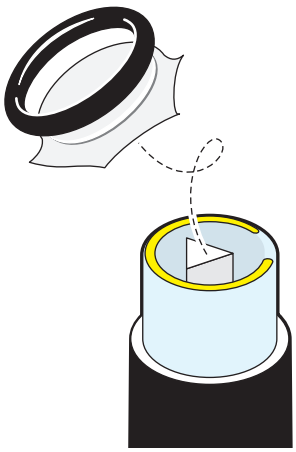
- Do not knot the cables or use clips to mark a certain depth.
- Do not place the instrument where the cable might be severed or damaged by boat propellers or other moving parts.
- Protect all cables from abrasion, unnecessary tension, repetitive flexure, or bending over sharp radii (e.g., the side of a boat or of a bridge). Do not bend or run the cable over the sheave or pulleys with less than a 6-inch diameter.
- If cables are long, use a battery-powered or hand-cranked reel with electrical slip-rings to lower and raise the instrument. Also, a lighter reel without slip-rings for shorter cables can be used. A last option, is to mount the reel horizontally with the instrument and battery installed in the hub.
- Use the V-shaped support bail to lift and lower the multiprobe.
- Do not apply more than 5 kilograms (10 lb) of sinking weight to the multiprobe. This can increase the possibility of cable breakage due to stress on the attachment points. If more weight is needed, use a wire line to support the instrument by its bail.

6.4 Sensor Maintenance

Important Note: *If a sensor is not in use, insert an optional sensor expansion port plug in the vacant expansion port to prevent any contamination or damage during maintenance, operation, or storage.*

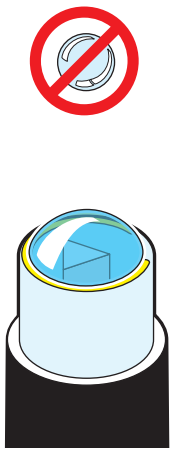
6.5 Clark Cell Dissolved Oxygen Maintenance

Dissolved oxygen sensor maintenance is required when the membrane covering the cell becomes wrinkled, bubbled, torn, dirty, fouled, or otherwise damaged.

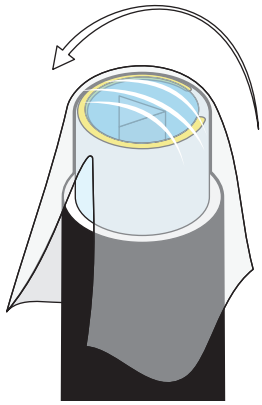


1. Remove the O-ring securing the D.O. membrane. Remove the old membrane. Shake out the old electrolyte and rinse with fresh D.O. electrolyte.

2. Refill with fresh D.O. electrolyte until there is a perceptible meniscus of electrolyte rising above the entire electrode surface of the sensor.

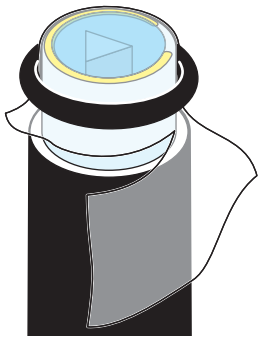


3. Make sure there are no bubbles in the electrolyte.

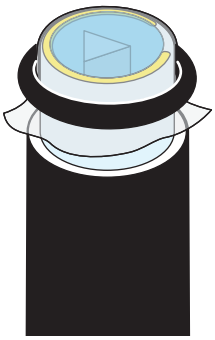


4. Hold one end of a new membrane against the body of the D.O. sensor with your thumb and with a smooth, firm motion, stretch the other end of the membrane over the sensor surface and hold it in place with your index finger.

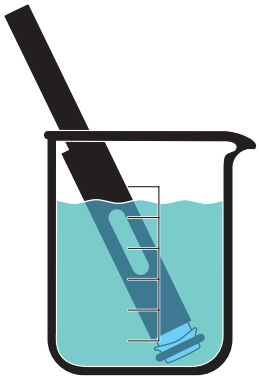
5. Secure the membrane with the O-ring. Make sure there are no wrinkles in the membrane or bubbles in the electrolyte.



6. Trim away the excess membrane extending below the O-ring.



7. Proper membrane assembly.



8. Let the sensor soak a minimum of 4 hours (90% relaxed). Ideally, the sensor should soak for 24 hours.

Note: *Readings may initially drift if calibrated before the membrane is fully relaxed.*

6.6 Specific Conductance, Salinity, and TDS Maintenance

Clean the oval measurement cell on the specific conductance sensor with a small, non-abrasive brush or cotton swab. Use soap to remove grease, oil, or biological growth. Rinse with water.

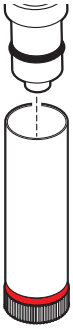
6.7 ORP Sensor Maintenance

If the platinum band or stud of the ORP sensor gets dirty and/or discolored, polish it with a clean cloth and a very mild abrasive, such as toothpaste; or use a fine polishing strip. Rinse with water. Soak the sensor overnight in tap water to allow the platinum surface to restabilize.

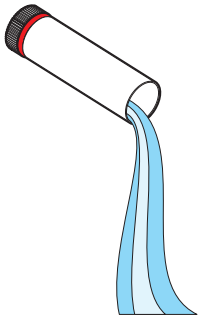
6.8 pH Electrode Maintenance

If the pH sensor is coated with oil, sediment, or biological growth, clean the glass with a very clean, soft, wet non-scratching cloth or cotton ball with mild soap. Rinse with tap water. If the pH sensor becomes dehydrated, soak for 24 hours in a pH 4 buffer solution.

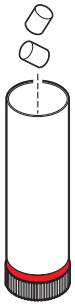
6.8.1 Standard Reference Electrode



1. Gently pull the entire reference sleeve away from the Transmitter.

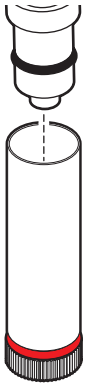


2. Discard the old electrolyte from the reference sleeve.

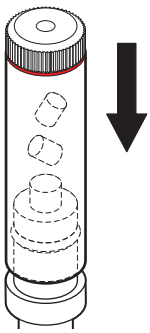


3. Drop two KCl salt pellets (Cat. No. 005376HY) into the reference sleeve.

4. Refill the sleeve to the top with reference electrolyte.



5. With the Transmitter sensors pointed down, push the full reference sleeve back onto its mount until the sleeve has just covered the O-ring located on the mount (just behind the silver electrode).

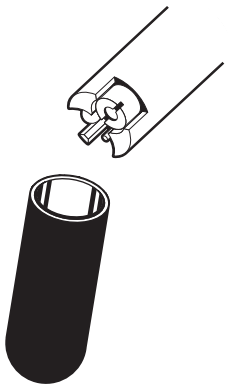


6. Turn the Transmitter so that the sensors point up and push the sleeve the rest of the way onto its mount. Rinse with tap water.

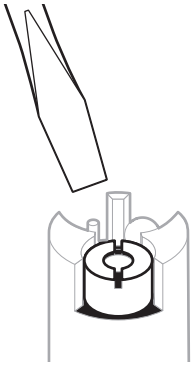
Note: *The porous Teflon® Reference Junction is the most important part of the pH and ORP performance. Make sure it is clean and passes electrolyte readily. If not, replace it with the spare provided in the maintenance kit. Replacement Reference Junctions are Cat. No. 000548HY.*

Note: *When seating the reference sleeve, trapped air and excess electrolyte are purged. This purging flushes and cleans the porous Teflon® Reference Junction.*

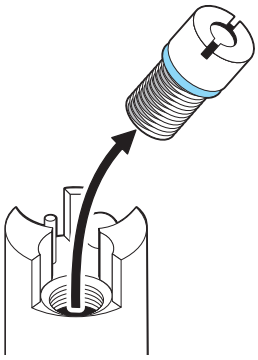
6.8.2 pH Integrated Sensor



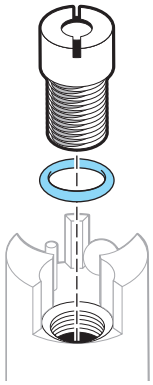
1. Remove the plastic soaking cap. Save the cap for reuse.



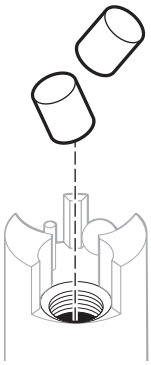
2. Use the supplied screwdriver to loosen the Teflon® Reference Junction.



3. Remove the Teflon Reference Junction and discard if dirty or clogged.

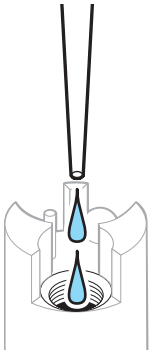


4. Replace the blue O-ring located below the Teflon Reference Junction if it is damaged or loose.

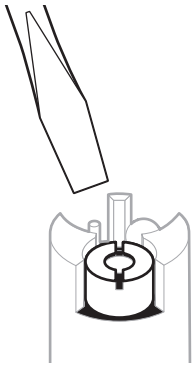


5. Drop two KCl salt pellets (Cat. No. 00537HY) into the reference opening.

6. Inject the pH reference electrolyte into the supplied plastic syringe.



7. Refill the reference opening with electrolyte.



8. Use the supplied screwdriver to install the new Teflon Reference Junction (Cat. No. 002770HY).

6.9 Temperature Sensor Maintenance

Use soap or rubbing alcohol to remove grease, oil, or biological growth and rinse with water. Do not use any objects to poke the sensor or the transducer membrane will rupture.

6.10 Pressure Sensor Maintenance

1. If calcium deposits are forming in the port, squirt vinegar into the pressure sensor port with a syringe and soak overnight.

2. Soap or rubbing alcohol may be used to remove grease, oil, or biological material. Rinse with water. Do not use any objects to poke the sensor or the transducer membrane will rupture.

6.11 Other Sensor Maintenance

Refer to the sensor specific instruction sheet for more information.

Section 7 Replacement Parts and Accessories

Replacement Parts

Description	Catalog Number
110 VAC Power Adapter	013450
220 VAC Power Adapter	013460HY
110 VAC External Battery Pack w/Charger	011050
220 VAC External Battery Pack w/Charger	012480
Battery Adapter	011530HY
Battery Plug	004164HY
Cable, 5 meter	015005HY
Cable, 10 meter	015010
Cable, 15 meter	015015HY
Cable, 25 meter	015025
Cable, 30 meter	015030
Cable, 50 meter	015050HY
Cable, 75 meter	015075HY
Cable, 100 meter	015100
Cable, 150 meter	015150
Cable, 200 meter	015200
Cable Reel	013730
Carrying Case	011780
Calibration Cable	013470
Calibration Stand	013910
Hydras 3 LT Software, CD Kit	6234200
Cigarette Lighter Adapter	013210
Conductivity Standard—0.1 mS/cm, 1 L	013610HY
Conductivity Standard—1.413 mS/cm, 1 L	013620HY
Conductivity Standard—12.856 mS/cm, 1 L	013640HY
Conductivity Standard—47.6 mS/cm, 1 L	013650HY
Conductivity Standard—0.5 mS/cm, 1 L	013770HY
DS5, DS5X Bail Kit	013850HY
DS5, DS5X Flow Cell	014120
DS5, DS5X Pipe Kit	013540
DS5, DS5X Basic Maintenance Kit	014680HY
DS5, DS5X Storage Cup (Large)	003608
DS5, DS5X Storage Cup Cap (Large)	003609HY
Clark Cell Dissolved Oxygen Maintenance Kit	013430HY
pH Maintenance Kit	013410HY
DS5, DS5X Weighted Sensor Guard (Large)	014110HY
D.O. Electrolyte, 59 mL	000537HY
D.O. Membrane Pack	002589HY
D.O. Membrane O-ring	000498HY
External Power Adapter Cable	013170HY
Macintosh Adapter Cable	013740
MS5 Bail Kit	013950HY

Replacement Parts

Description	Catalog Number
MS5 Flow Cell	013520HY
MS5 Flow Cell (for use with turbidity sensor)	014610
MS5 Basic Maintenance Kit	013280
MS5 Weighted Sensor Guard, black (extended)	013760
MS5 Weighted Sensor Guard, black (standard)	013330
MS5 Weighted Sensor Guard, white (extended)	014910
MS5 Weighted Sensor Guard, white (standard)	014920
MS5 Mooring Fixture	013530
MS5 Pipe Kit	013550
MS5 Storage Cup Cap O-ring	002811
MS5 Storage Cup	003306
MS5 Storage Cup (extended)	003395HY
MS5 Weighted Sensor Guard	014130
Modem Adapter	012650
pH Reference Electrolyte, 100 mL	005308HY
Potassium Chloride Pellets (99% KCl), 20 pellets	005376HY
Profiler Data Analysis Software	013350
Retaining Band	005363
SDI-12 and RS485 Adapter Cable	013510
SDI-12 and RS232 Adapter Cable	013790
Small Teflon Junction	002770HY
Thumbscrew for Battery Plug	003301

Section 8 How to Order

U.S.A. Customers

By Telephone:
6:30 a.m. to 5:00 p.m. MST
Monday through Friday
(800) 949-3766

By Fax:
(970)461-3921

By Mail:
Hach Environmental
P.O. Box 389
Loveland, Colorado 80539-0389 U.S.A.
Ordering information by e-mail: sales@hachenvironmental.com

Ordering Information Required

- Account number (if available)
- Your name and phone number
- Purchase order number
- Brief description or model number
- Billing address
- Shipping address
- Catalog number
- Quantity

International Customers

Hach Environmental maintains a worldwide network of dealers and distributors. To locate the representative nearest you, send an e-mail to: sales@hachenvironmental.com or contact:

Hach Environmental; Loveland, Colorado, U.S.A.
Telephone: (970) 669-3050; Fax: (970) 669-2932

Technical and Customer Service (U.S.A. only)

Hach Environmental Technical and Customer Service Department personnel are eager to answer questions about our products and their use.

Call 1 (800) 949–3766 or e-mail techsupport@hachenvironmental.com

Section 9 Repair Service

Authorization must be obtained from Hach Company before sending any items for repair. Please contact the Hach Service Center serving your location.

In the United States and Outside Europe:
Hach Company
Hach Environmental Product Repair
North Dock
5600 Lindbergh Drive
Loveland, CO 80539-0389
Telephone: (800) 227-4224 ext 2080
Fax: (970) 461-3924

In Europe:
OTT Messtechnik GMBH & Co. KG
Ludwigstrasse 16
87437 Kempten
Germany
Telephone: +49/(0)831/5617-0
Fax: +49/(0)831/5617-209

Section 10 Limited Warranty

Hach Company warrants the Hydrolab Series 5 Sondes to the original purchaser against any defects that are due to faulty material or workmanship for a period of two years from date of shipment unless otherwise noted.

In the event that a defect is discovered during the warranty period, Hach Company agrees that, at its option, it will repair or replace the defective product or refund the purchase price excluding original shipping and handling charges. Any product repaired or replaced under this warranty will be warranted only for the remainder of the original product warranty period.

This warranty does not apply to consumable products such as chemical reagents; or consumable components of a product, such as, but not limited to, lamps and tubing.

Contact Hach Company or your distributor to initiate warranty support. Products may not be returned without authorization from Hach Company.

Limitations

This warranty does not cover:

- Damage caused by acts of God, natural disaster, labor unrest, acts of war (declared or undeclared), terrorism, civil strife or acts of any governmental jurisdiction
- Damage caused by misuse, neglect, accident or improper application or installation
- Damage caused by any repair or attempted repair not authorized by Hach Company
- Any product not used in accordance with the instructions furnished by Hach Company
- Freight charges to return merchandise to Hach Company
- Freight charges on expedited or express shipment of warranted parts or product
- Travel fees associated with on-site warranty repair

This warranty contains the sole express warranty made by Hach Company in connection with its products. All implied warranties, including without limitation, the warranties of merchantability and fitness for a particular purpose, are expressly disclaimed.

Some states within the United States do not allow the disclaimer of implied warranties and if this is true in your state the above limitation may not apply to you. This warranty gives you specific rights, and you may also have other rights that vary from state to state.

This warranty constitutes the final, complete, and exclusive statement of warranty terms and no person is authorized to make any other warranties or representations on behalf of Hach Company.

Limitation of Remedies

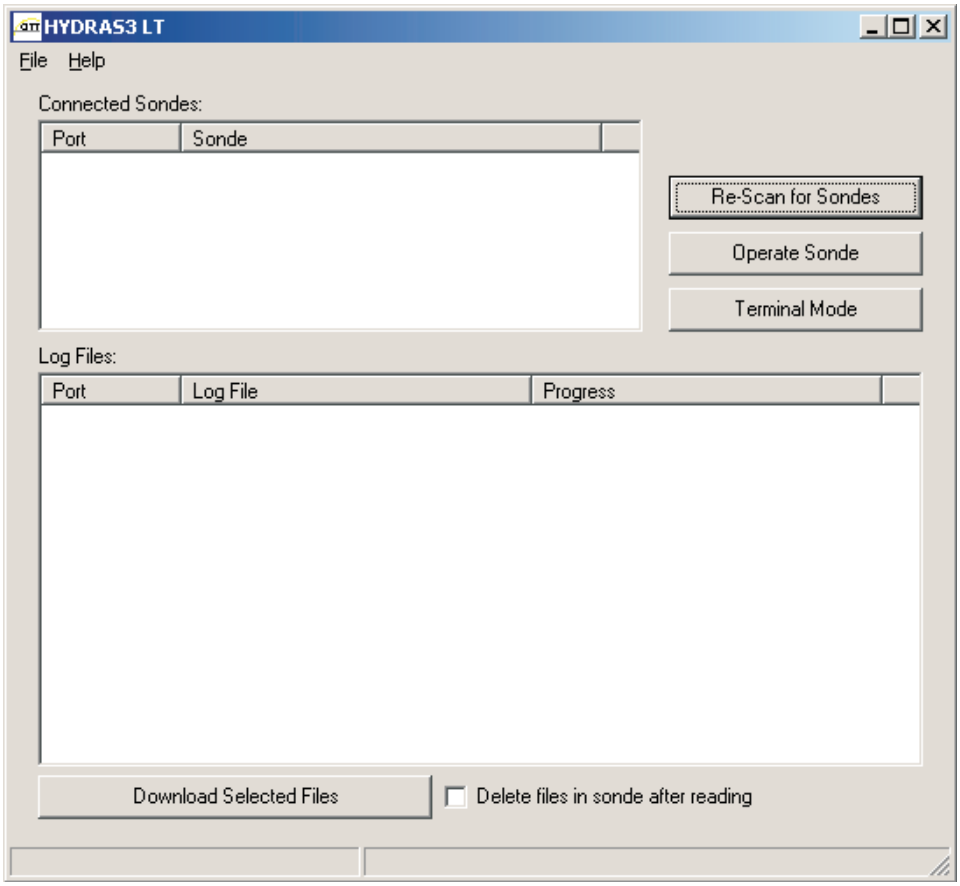
The remedies of repair, replacement or refund of purchase price as stated above are the exclusive remedies for the breach of this warranty. On the basis of strict liability or under any other legal theory, in no event shall Hach Company be liable for any incidental or consequential damages of any kind for breach of warranty or negligence.

Troubleshooting Communications

If the first screen does not appear, after booting up the communications software and connecting the multiprobe to the computer, please check the following items:

Troubleshooting Hydras 3LT
If Hydras 3LT does not automatically detect the Sonde when launched:

Press the **RE-SCAN FOR SONDES** button.

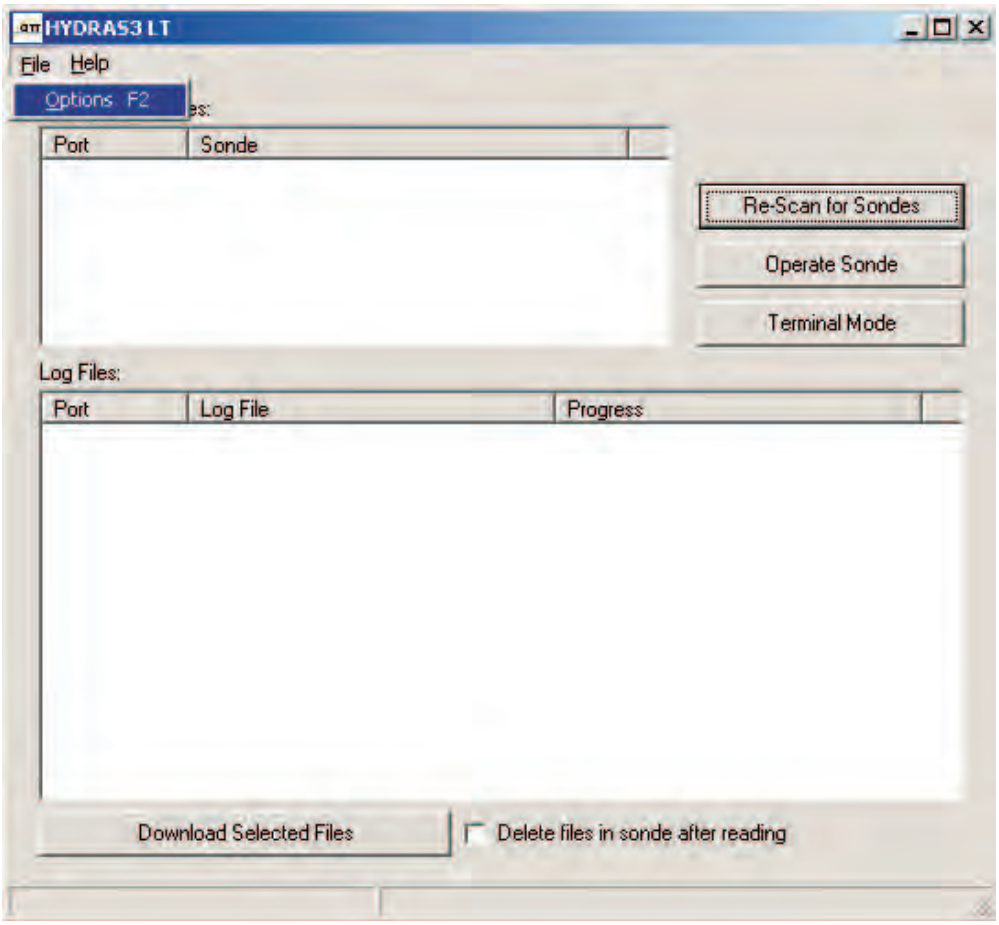


If communication is still not established after several attempts, try the following:

1. Verify that the instrument is powered on and functioning.
2. Check power cables and connections. Verify that your PC and multiprobe are properly connected to the wall outlet or external battery if used.
3. Verify that the input voltage to the multiprobe is between 7V and 18V.
4. If your multiprobe is equipped with an internal battery pack, check the batteries' polarity and voltages.

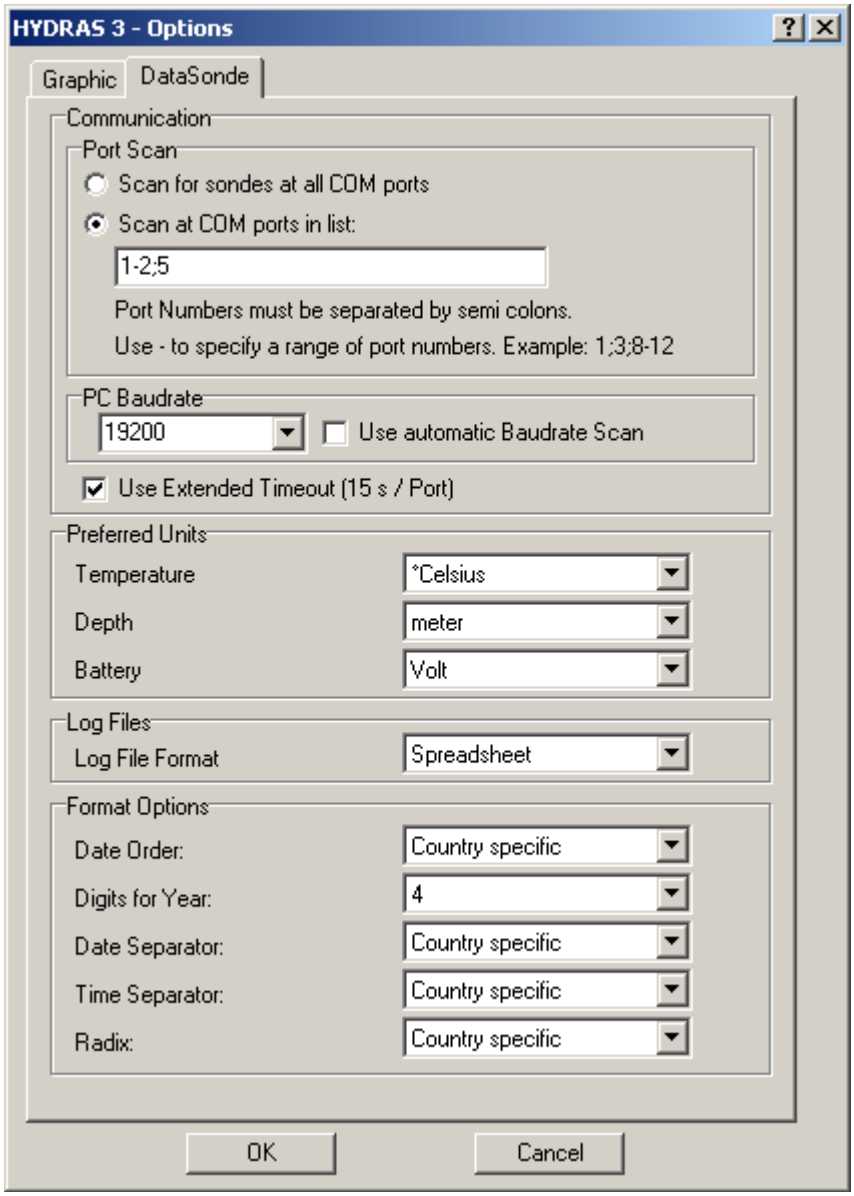
Verify the Hydras 3 LT Communication Settings.

1. Select File>Options from the Hydras 3 LT Connection Screen.



2. If the Sondes/PC baud rate and COM port are known, disable the COM Port and baud rate auto scans and set Hydras3LT to use the known values.

3. In addition, the connection timeout may be extended from 10 to 15 seconds, which will allow time for additional retries.



Troubleshooting using Terminal Mode

Check the PC and terminal emulation or communication software:

- Start the communications software before connecting the instrument.
- Verify that the PC is on and that communication software is running.
- Verify that the correct communication port was selected (COM 1, 2, 3, 4).
- Verify that the terminal was set to ANSI terminal emulation, and that the correct baud rate (19200), eight bits, no parity, and one stop bit (19200, 8, N, 1) was selected.
- If using a 100 meter or longer cable with the Surveyor, make sure the terminal baud rate is set to 9600 and the Sonde baud rate is 9600.

Check the power cables and connections:

- Verify that the PC and multiprobe are properly connected to the wall power outlet or external battery is used.
- Verify that the input voltage to the multiprobe is between 10 and 15 volts.
- Verify that the battery pack is installed correctly, if applicable. Check the battery polarity and voltages.

Check the internal components:

- Make sure all internal connections are securely seated.
- Check for the presence of water in the unit. If damp or wet, dry out thoroughly with a lint-free cloth or towel or let it sit out opened in a dry room overnight. Determine where the leak occurred and repair appropriately. Notify Technical Support for help on preventing further leaks.

If these checks do not reveal the problem, try to substitute other instruments, cables, and terminals to determine the failing component.

Troubleshooting Sensor Issues

The following list is not an extensive account of the problems encountered. If the following solutions do not reveal the problem, try to substitute other sensors to determine the failing component.

Table 1 Sensor Troubleshooting

Problem	Solution
D.O. readings are too low to calibrate and/or pH and/or Redox are very high or very low	Check the value of the sample solution
	Ensure the sensors are properly maintained.
D.O. readings seem wrong	Ensure the D.O. sensor has been properly maintained and calibrated.
Conductivity, Temperature, and/or Depth readings seem wrong	Ensure the sensors are properly maintained and calibrated.
	Ensure the readings displayed are accurate (e.g., for Depth: meters, feet, or psi).

Table 2 Multiprobe Software Symbols

Symbol	Description
#	Data Out of Sensor Range
?	User Service Required or Data Outside Calibrated Range but Still within Sensor Range
*	Parameter is Not Calibrated
~	Temperature Compensation Error
@	Non Temperature Parameter Compensation Error

Appendix B External Communications

B.1 SDI-12 Interface

SDI-12 is an industry-originated, serial digital interface bus designed to allow an operator to connect a wide variety of sensors (meteorological, hydrological, water quality, etc.) to a single SDI-12 datalogger with a single cable bus.

The multiprobe is compatible with SDI-12 V1.2. A copy of the specification can be found at www.sdi-12.org. The optional SDI-12 Interface Adapter Cable is required to operate the multiprobe with an SDI-12 Datalogger.

Note: *All three wires (one ground) must be connected for correct SDI-12 operation.*

A label on the SDI-12 Interface Adapter Cable shows the pinout in Figure 19.

1. Connect the data cable to the SDI-12 Interface Adapter Cable connector.
2. Disconnect power from the multiprobe.
3. Connect the bare wires at the end of the SDI-12 Interface Adapter Cable to the appropriate connections on the SDI-12 datalogger. Follow the label on the SDI-12 Interface Adapter.

Figure 19 SDI-12 Cable

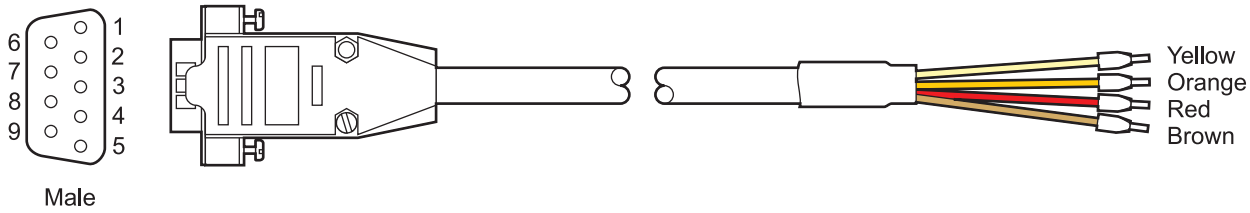


Table 3 SDI-12 Pinouts

Pin Number	Wire Color[1]	SDI-12 Function
4	Brown	+12 VDC
5	Red	Ground
8	Orange	SDI-12 Data
9	Yellow	SDI-12 Return

[1] Wire color is valid only for this cable (Cat. No 007139). Use of other cables or cable modifications may result in instrument damage.

Consult the SDI-12 datalogger manual for information on how to connect the SDI-12 Interface Adapter.

Note: *SDI-12 parameters can be setup through Hydras 3 LT.*

Table 4 is a summary of the SDI-12 user commands supported by the multiprobe. For more details on correct use, consult the SDI-12 V1.2 specification.

Table 4 SDI-12 Commands

Command[1]	Response	Description
a!	a<crlf>	Address Acknowledge
aI!	aXXHydrolab*YYYYYYZZZZserialnumber* <crlf>	Identify XX: SDI–12 Support Version YYYYYY: Instrument ID ZZZZ: Software Version
aAb!	b<crlf>	Change address from a to b
aM!	adddn<crlf>	Measure n values in ddd seconds
aDx!	aSvalueSvalue...<crlf>	Report Data
aRx!	aSvalueSvalue...<crlf>	Report Continuous Data
aC!	adddnn<crlf>	Concurrent Measure: nn values in ddd seconds
aXC!	aXC<crlf>	Initiate a cleaning cycle in units equipped with a wiper
aX1!	aX1<crlf>	Enable Continuous Mode
aX0!	aX0<crlf>	Disable Continuous Mode
aXSS1!	aXSS1<crlf>	Circulator On
aXSS0!	aXSS0<crlf>	Circulator Off

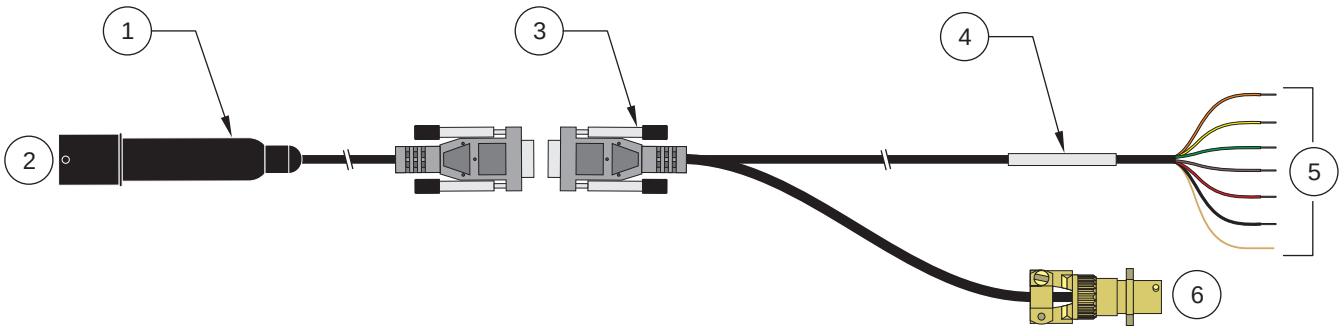
[1] The 'a' used in the SDI-12 commands is the SDI-12 address. The Transmitter's factory default SDI-12 address is '0'.

B.2 Connection to an External Device

The Series 5 Multiprobe can communicate with an external device using an RS232, RS485, or SDI-12 interface. The RS232 interface is always available. The Series 5 Multiprobe must be programmed for communication via an RS485 or SDI-12 interface.

Two cables are available for external communication: a 6-pin marine to DB-9 cable (Cat. No. 015xxx), and a DB-9 to external device cable (Cat. No. 013510) for SDI-12 or RS485 interfaces (Figure 20). Wiring diagrams for these cables are shown in Figure 22 and Figure 23. Wiring for the 6-pin Sonde connector is shown in Figure 21. Wiring to the external device is detailed in Table 5.

Figure 20 Communication Cables for the DS5, DS5X, and MS5 Water Quality Sondes



1.	Cable, 6-pin marine to DB-9 (Cat. No. 015XXX)	**4.**	Label, wire connections
2.	Connection to DS5, DS5X or MS5	**5.**	Connections to external device
3.	Cable for SDI-12/RS485 (Cat. No. 013510)	**6.**	Connection to power

Figure 21 Wiring for 6-Pin Marine Connector on Multiprobe

1. External Power	**3.** RS232 TXD	**5.** RS485 + or SDI-12 DATA
2. Ground	**4.** RS232 RXD	**6.** RS485 - or SDI-12 RETURN

Figure 22 Wiring Diagram for Cable 015xxx, 6-Pin Marine to DB-9

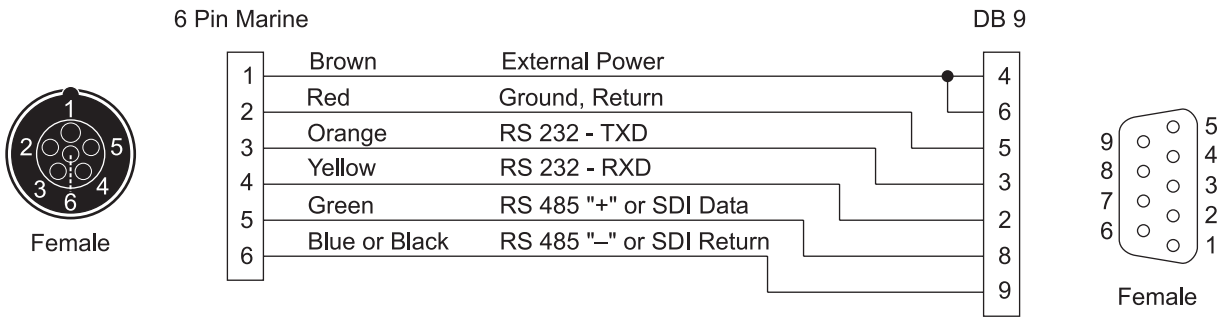


Figure 23 Wiring Diagram for Cable 013510, DB-9 to External Data Device

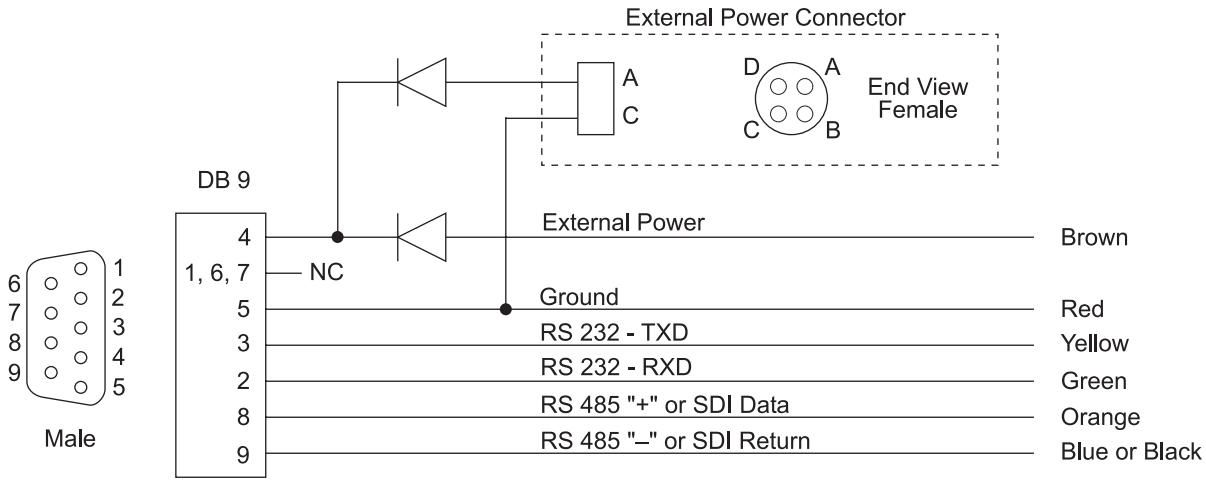


Table 5 Wiring Connections for External Device (Cable 013510)

Wire Color	RS232	RS485	SDI-12
Brown	+12VCD IN	+12VDC IN	+12VCD IN
Red/Shield	Ground	Ground	Ground
Green	RS232 RX	—	—
Yellow	RS232 TX	—	—
Orange	—	RS485 +	SDI DATA
Blue or Black	—	RS485 -	SDI RETURN

B.3 RS485 Interface

Series 5 Multiprobes are compatible with RS485 interfaces. RS485 is a standard that specifies a particular method to transmit and receive digital signals. This standard is maintained by the Electronic Industries Association in a document titled "Standard for Electrical Characteristics of Generators and Receivers for Use in Balanced Digital Multipoint Systems."

RS485 involves sending an inverted or out-of-phase copy of the signal simultaneously on a second wire. This is called a balanced transmission. Any outside electrical noise adds coherently to both signal copies. The receiver electrically subtracts the two signals to reproduce the original signal. The advantage in the subtraction is that only the intended signal gets reproduced since they are out-of-phase. The in-phase noise on the two wires are also subtracted from each other to produce a net zero noise component in the reproduced signal. This noise immunity allows the RS485 interface to transmit digital signals at faster rates over longer distances than the RS232/SDI-12 interface. The RS232/SDI-12 interface does not use balanced transmission and is therefore susceptible to noise interference which limits the transmission distance and speed.

Connections
RS485 can use two wires to both transmit and receive data. A common software protocol must be shared between devices to prevent data collisions on the wires. RS485 also allows for multiple transmitters and receivers to be easily connected together.

Be sure to connect the signal grounds of all devices on the network together. The connection can be made using a conductor in the transmission cable or each device can be connected to a good earth ground. This connection keeps the common mode voltage (the voltage which the signal must overcome to be reproduced) low. The network devices may operate without the signal ground connection, but may not be reliable.

Some RS485 applications require impedance termination because of fast data rates or long cables. The most popular termination involves installing a ½ watt resistor across the receiver at each end of the network. See the RS485 interface user manual for termination requirements on the PC being used.

Alternatively, the preferred network may be AC terminated by placing a 0.01 µF capacitor in series with the terminating resistor. The capacitor appears as a short circuit during signal transitions but appears as an open circuit to any DC loop current. This will reduce the power supply current required to operate the network and still provide the proper terminating impedance.

Do not add a terminating resistor to every receiver in the network. For networks with more than about four nodes, the transmitters will be unable to drive the cable. Only terminate both ends of the main cable.

B.4 Modbus Interface

The multiprobe is configured to respond to Modbus function 3 (Read Holding Registers) in RTU mode through the RS232 or RS485 communications port at 19200 baud, 8 data bits, Even Parity, and 1 stop bit (19200:8:E:1).

All data values are returned in IEEE Floating Point Format (4 bytes). Each data value is returned in two 16 bit words with the low word being transmitted first. Within each word, the high byte is transmitted first. Each byte is transmitted Most Significant Bit first. For example, the floating point value 1.56 = 0x3FC7AE14 would be transmitted as 0xAE 0x14 0x3F 0xC7.

All available data parameters are stored in Holding Registers within the multiprobe, which start counting at 40001. They are addressed in the Modbus message structure with addresses starting at 0. Modbus function 03 is used to request one or more holding register values from the multiprobe. Each Holding Register is 16 bits (2 bytes) in size, therefore two Holding Registers (4 bytes) are required to represent a single floating point value. Only one slave device can be addressed in a single query. The query structure is detailed in Table 6. A complete register list can be found at www.hachenvironmental.com.

Table 6 Function 03 Query Structure

Byte	Value	Description
1	1–247	Slave device address
2	3	Function code
3	0–255	Starting address, high byte
4	0–255	Starting address, low byte
5	0–255	Number of registers, high byte
6	0–255	Number of registers, low byte
7	0–255	CRC, high byte
8	0–255	CRC, low byte

After processing the query, the multiprobe will return the 16 bit Holding Register values that were requested. The Holding registers will be transmitted as High Byte first, followed by the Low Byte. The Modbus response starts with the multiprobe address and the function code 03. The next byte is the number of data bytes that follow. This value is two times the number of registers returned. The two byte CRC is appended at the end.

Example: The multiprobe stores pH information at holding registers 40007 and 40008. These registers are addressed as 0x0006 and 0x0007. The following sequence of bytes request pH from a multiprobe with slave address 1.

pH Query Example:

Byte 1[1]	Byte 2[2]	Byte 3[3]	Byte 4[3]	Byte 5[4]	Byte 6[4]	Byte 7[5]	Byte 8[5]
0x01	0x03	0x00	0x06	0x00	0x02	0x24	0x0A

[1] Slave Address
[2] Command 3—Read Holding Register
[3] Address of the first Holding Register to read (0x0006)
[4] Number of Holding Registers to Read (2 registers—4 bytes)
[5] CRC

Response:

Byte 1[1]	Byte 2[2]	Byte 3[3]	Byte 4[4]	Byte 5[4]	Byte 6[4]	Byte 7[4]	Byte 8[5]	Byte 9[5]
0x01	0x03	0x04	0xA8	0xC9	0x41	0x06	0xBA	0x3F

[1] Slave Address
[2] Command 3—Read Holding Register
[3] Number of Data Bytes (4 bytes—2 registers)
[4] pH
[5] CRC

Result: The data is sent Low Word First, High Byte First, therefore the IEEE Floating Point Formatted value representing the pH is: 0x4106A8C9 = 8.416 Units.

B.5 Using a Modem with Multiprobes

Field Modem

All multiprobes require a modem adapter to enable communications with a commercial telephone modem. The modem adapter provides the necessary handshaking and connections to allow a modem to properly answer the incoming call and power down the multiprobe when the call is terminated. The Modem Adapter has a connector (labeled modem) that connects to the RS232 connection of the modem. The other connector (labeled multiprobe) on the Modem Adapter connects to the multiprobe using a Interface cable and Underwater cable, or a Calibration cable. A 25 to 9 pin adapter is also required for the cables.

The Modem Adapter does not require a power supply, however, the multiprobe and the modem will require power. Usually, commercial modems are supplied with a wall-cube power supply that converts ac mains voltage to 9–12 volts DC. Most modem can use the multiprobe power supply by making a cable with the corrector connector for the modem power input.

The modem, Modem Adapter, power supply, and associate cabling are not water-proof, and should be installed in a water-tight enclosure. If AC power is used, then a GFI (ground fault interrupt) device should be installed in the ac wiring to prevent electrocution. Program the modem as follows:

Table 7 Field Modem Commands[1]

Command	Function
AT&C1	Enable carrier detection
AT&D3	Enable DTR detection
AT&K4	Enable XON/XOFF handshaking
AT&Q0	No error correction or buffering
AT&S0	Force continuous DSR
AT%C0	Disable data compression
ATS0=1	Answer on first ring
AT&W0	Save current settings

[1] The modem will automatically use this setup every time it is powered on.

Office Modem Installation

The office modem is connected to the computer serial port using a standard RS232 connection. Program the modem as follows:

Table 8 Office Modem Commands[1]

Command	Function
ATW1	Report connection speed & protocol
AT&C1	Enable carrier detection
AT&K4	Enable XON/XOFF handshaking
AT&Q0	No error correction or buffering
AT%C0	Disable data compression
AT&W0	Save current settings

[1] The modem will automatically use this setup every time it is powered on.

The computer will need a terminal emulation program to communicate with the remote multiprobe. Setup the program to provide ANSI terminal emulation, 19200 baud, no parity, 8 data bits, and 1 stop bit for Series 5 Multiprobes. Setup the software to provide a direct connection to the COM port connected to the modem.

Operating the Modem

Check if the modem is communicating with PC by typing AT and pressing **ENTER**. The modem should respond with OK. To access a remote site from the office, type ATD followed by the phone number, for example, ATD15122558841. Add the proper prefixes to the phone number such as 9 (for PBX office systems) or 1 (for long distance). Press **ENTER**. The modem should starting dialing the number and negotiating the connection. Series 5 Mulitprobes may require as long as 15 seconds to show the startup screen. If the startup screen is not displayed, send a Break by typing **ALT-B**. Telephone noise and delay may prevent the Series 5 Multiprobe from properly determining the presence of an ANSI terminal. You will not be able to access a Series 5 Multiprobe if logging event is currently active. This can be avoided by setting the interval to no less than 2 minutes to allow enough time to call the modem between logging events (a logging event occurring during a call will not cause a problem).

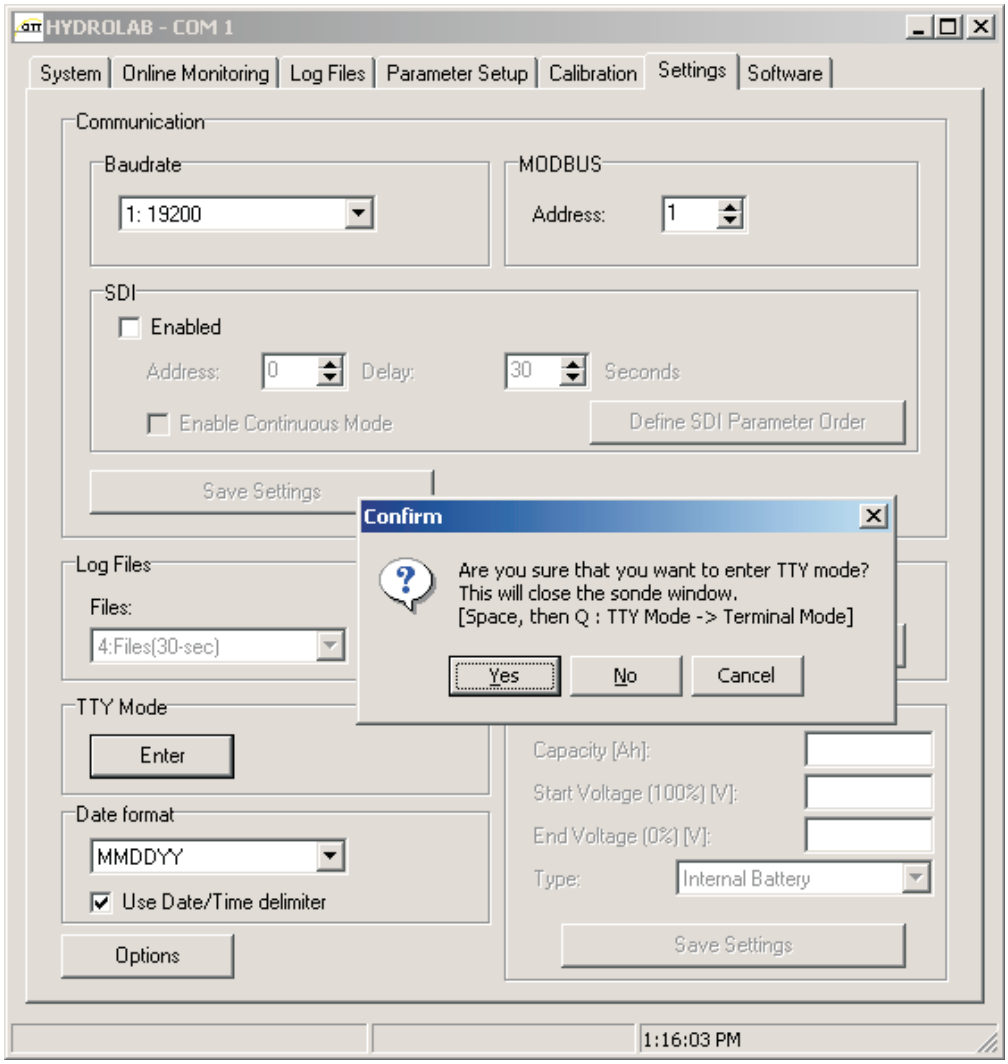
B.6 TTY Mode

The sonde is equipped with a TTY Communication Mode, which enables the Sonde to send an ASCII string of characters, representing selected parameter values, once per second. Parameters and Parameter Order may be selected using the "Define SDI Parameter Order" button in the SDI section of the Settings Tab.

To enter TTY Mode:

1. Press **ENTER** in the TTY Mode section of the Settings Tab.

2. Press **YES** to verify the entry in mode.



Once the Sonde has been placed in TTY mode, it will no longer communicate with Hydras3LT except in terminal mode. The Sonde will retain its current baud rate. Any ANSI terminal emulator may be used to communicate with the Sonde at 8 data bits, No Parity, and 1 stop bit.

B.6.1 TTY Menu and Commands

The TTY menu is accessed by first pressing the spacebar (or sending an ASCII space character). The multiprobe will finish any line in progress and then start a new line (by sending <cr><lf>) followed by the menu:

<cr><lf>**HM?:**<sp>

The multiprobe will echo the user entry if it is listed in the menu, otherwise an ASCII BEL character is sent. An ASCII escape character will abort the menu after displaying a cancel message.

Responding with a **?** will produce a verbose version of the menu:

<cr><lf>**Main Menu**<cr><lf>
(H)eader<cr><lf>
(M)easure<cr><lf>
(Q)uit TTY Mode<cr><lf>
Please enter your choice:<sp>

A. (H)eader

Responding with an **H** will show a header identifying the data fields with name and units. In addition, the instrument ID is displayed:

HM?:<sp>**H**<cr><lf>
<cr><lf>**Instrument Id**<cr><lf>
<cr><lf><sp><sp>**Time**<sp><sp><sp>**Temp**<sp><sp>**Ibatt**<cr><lf>
HHMMSS<sp><sp><sp><sp><sp>**°C**<sp><sp>**Volts**<cr><lf>

The first line is free-field text up to 20 characters in length. The next line is skipped and the data names are printed. The names are right-justified (with leading spaces inserted) to produce a constant width field. Most fields are 6 characters wide, however fields can also be 5, 7, or 8 characters wide. The name fields are always separated with a space. Any name may appear in any field depending on how the user configured the data display in ANSI mode.

The next line shows the corresponding units for the data fields. The units are right-justified text (with leading spaces inserted) to produce a constant-width field equal to the name field. The name fields are always separated with a space.

B. (M)easure

Responding with an **M** will force the multiprobe to send one line of data without waiting for the next data display interval. This is useful for synchronizing data acquisition software with the multiprobe data output. The data values are right-justified to fill a constant-width field of 5, 6, 7, or 8 characters.

HM?:<sp>**M**<cr><lf>
231302<sp><sp>**24.59**<sp><sp><sp>**12.0**<cr><lf>

Data values may also be appended with a special character (*, ~, @, #, or ?). Data values without an appended character will always have a space separator, however, the appended character may be the only separator between values; a space separator is not guaranteed.

Data values that are too large to fit in the constant-width field, are modified so that the numeric digits are displayed as # (##.## for example). The sign and decimal point are preserved.

C. (Q)uit

Responding with a **Q** or **q** will reset the multiprobe to full terminal mode and can then be connected to Hydras 3 LT.

B.6.2 Data Display

If the TTY menu is not used, a line of data is periodically displayed on the next available line. If the screen is full, the lines are scrolled. All data lines are terminated with <cr><lf> and have the same formatting as the (M)easure command described above.

Appendix C Using HyperTerminal

C.1 HyperTerminal Setup

1. After starting Windows, click on the Start button.
2. Select Programs>Accessories>Communications>Hyperterminal.
3. Enter a name and choose an icon for the application and click **OK**.
4. Select the COM port and click **OK**. Set the communication in a 19200/8/N/Xon-Xoff format.
5. Configure HyperTerminal so the functions, arrows, and **Ctrl** keys act as terminal keys, not window keys. Select the ANSI terminal emulation. File>Properties>Settings.
6. Use Table 9 to communicate with or recover information from the multiprobe.

Table 9 HyperTeminal Commands

Follow the Path:	**To:**
Help: Help Topics: Index: HyperTerminal	Displays the on-line help utility.
File: Properties: Phone number: Configure: Maximum speed	Access and select the modem transfer speed (e.g. 19200 bps)
File: Properties: Phone number: Configure: Connection	Access and select the connection settings (e.g. N, 8, 1)
Transfer: Capture Text	Enable a capture file, to log the data currently received to a disk or to a hard drive. You will be prompted for a file name and a path.
File: Capture to printer	Turn the printer on.
Transfer: Send file	Upload a file and choose the transfer protocol (e.g. Xmodem). You will be prompted for a file name and a path.
Transfer: Receive File	Download a file and choose the transfer protocol (e.g. Xmodem). You will be prompted for a file name and a path.
File: Open	To view a file or find a saved file in the HyperTerminal folder.

Appendix D Glossary & Abbreviations

Ammonia (NH3)—A colorless gaseous alkaline compound which is soluble in water. It has a characteristic pungent odor, and is used as a fertilizer. In water and soil, ammonia is present primarily as NH_4^+ ions and is readily assimilated by plants during nutrition.

Ammonium (NH_4^+)—Ammonium is a form of ammonia by the addition of a hydrogen ion (H^+) to an ammonia molecule (NH_3). Ammonia is converted to ammonium as the pH of a solution drops. Ammonium is less harmful to aquatic life than ammonia. Below a pH of 7.3, more than 99% of the total ammonia is present as ammonium.

Assembly—A unit containing the component parts of a mechanism, machine, or similar device. Probe Assembly: The unit containing the component parts of a sensor (e.g. D.O.:component consisting of the D.O. sensor which is made up of 2 electrodes the cathode and the anode).

Chloride (Cl–)—A common anion, present in both fresh and sea water. It is essentially non-toxic, and is present in all living cells.

Conductivity—Conductivity is inversely related to the resistance of a solution. Conductivity is the ratio of the electric current density to the electric field in a material, also known as electrical conductivity. In limnology, conductivity is a measure of the ability of water to pass an electrical current. Compensation of this measurement to 25 °C constitutes specific conductance. This parameter indicates the amount of dissolved substances (salts). Salts and their concentration dictate osmoregulatory (salt-balancing) functions in plants and animals. The ionic strength of water also regulates the toxicity of many substances. (See: Specific conductance)

Data collection platform (or DCP)—Hardware system and system software used with a computer program to collect data at one or more locations.

Depth—The vertical distance between the water surface and another level (for a multiprobe: 0–10, 0–25, 0–100, or 0–200 meters). (See: Vented depth)

Derating—The reduction of the rating of a device to improve reliability or to predict operation at higher or lower ambient temperatures.

Dissolved oxygen (or D.O.)—A measure of the amount of oxygen present in water and available for respiration. The concentration of D.O. is controlled by many factors including: consumption by aerobic (requiring D.O.) organisms (bacteria, fish, amphibians, and invertebrates); consumption by plants (algae, vascular plants, particularly during dark hours); and water temperature, water flow, and depth

Drift— The long-term lack of repeatability caused by fouling of the sensor, shifts in the calibration of the system, or slowly failing sensors.

Dump—To copy the contents of all or part of a storage, usually from an internal storage device to an external storage device.

Eh—(See: Redox potential)

Electrode—An electric conductor which either measures the potential of a solution (pH, reference, redox, and ammonium electrodes) or forces electric current into or out of a solution (D.O. and conductivity electrodes).

Emery cloth—An abrasive cloth or paper with an adherent layer of emery powder; used to polish and clean metal. (No. 400 or finer is recommended.)

Hysteresis error—The maximum separation due to hysteresis between upscale-and downscale-going indications of a measured value. A difference in parameter readings which occurs due to a variation in the conditions under which the sensor approached the readings. (See: Response time)

Isopotential point—The point at which the ion activity is the same on both sides of a sensor membrane. At the isopotential points, there is a zero potential across the membrane. The observed potential of the sensor may not be zero, due to the differences in reference electrodes.

Milliohm (mΩ)—Unit of resistance (not conductivity or conductance.)

MilliSiemens (mS) = millimho (m)—Units of electrical conductance.

Millimho (m)—(See: MilliSiemens)

Molar concentration—Molar solution: Aqueous solution that contains one mole (unit = gram-molecular weight) of solute in one liter of water. For example: KCl (potassium chloride) molar concentration.

Multiprobe—The combination of several sensors, electrodes, or probe assemblies into a complete, stand-alone piece of equipment which simultaneously measures several parameters for profiling, spot-checking, or logging readings and data. A multiprobe is a multi-parameter instrument.

Nitrate (NO_3^-)—Nitrate is the most oxidized form of nitrogen, and is the primary form of biologically available nitrogen present in aerobic environments. Nitrate is a less toxic form of nitrogen than ammonia, and is readily assimilated by plants and bacteria.

Oxidation reduction potential (or ORP)—(See: Redox potential)

Parameter—A quantity which is constant under a given set of conditions, but may be different under other conditions.

pH—Describes the hydrogen-ion activity of a system: pH 0–7: acid solution, pH 7: neutral, pH 7–14: alkaline (or basic) solution. The "p" in pH stands for power (puissance) of the hydrogen ion (H+) activity. pH is a major factor affecting the availability of nutrients to plants and animals. It controls in part the concentration of many biochemically active substances dissolved in water, and it affects the efficiency of hemoglobin in the blood of vertebrates (e.g. fish) and invertebrates (e.g. shrimp), as well as the toxicity of pollutants.

Probe—A small tube containing the sensing elements of electronic equipment. The probe is an essential part of the water quality monitoring system, since it obtains measurements and data which can be stored, analyzed, and eventually transferred to a computer.

Probe assembly— (See: Assembly)

Profiling—Electrical exploration wherein the transmitter and receiver are moved in unison across a structure to obtain a profile of mutual impedance between transmitter and receiver = lateral search. In water quality, this term is used as the contrary of unattended monitoring. An operator connects the multiprobe to a computer equipped with a communications software. Then, he lowers the multiprobe in the water and receives measurements from the instrument. The data is displayed on the computer screen. The multiprobe can be lowered to different locations along the sample area in order to study the nature of the water based on several points of reference.

Quinhydrone ($C_6H_4O_2$. C_6H_4 $(OH)_2$)—green, water-soluble powder. Quinhydrone is used to calibrate redox sensors. The quinhydrone's redox potential is dependent on the pH of the solution.

Reading—The indication shown by an instrument.

Redox potential = Oxidation-reduction potential (or ORP) = Eh—Voltage measured at an inert electrode immersed in a reversible oxidation-reduction system; measurement of the state of oxidation of the system. The redox potential measures the tendency of electrons to "flow" either toward or away from a noble metal electrode. A substance gains electrons in a reduction reaction and loses electrons in an oxidation reaction. ORP varies from substance to substance, and oxidation-reduction reactions occur simultaneously, hence the determination of the "potential" rather than of a discrete or qualitative value. Oxidation and reduction are in a constant state of flux, continuously seeking equilibrium. Applications for ORP measurement include, but are not limited to, the following: monitoring oxidation of cyanide and chromate wastes (e.g. metal plating), bleaching pulp (e.g. paper manufacturing), manufacture of bleach (e.g. monitoring chlorination), water pollution (e.g. acid mine drainage) and monitoring ozone treatment (e.g. water disinfection). ORP data has been used to understand more about how substances in sediments affect the water quality at the bottom of lakes, reservoirs, and ponds.

Reduction—A reaction that increases the electron content of a substance.

Reference electrode—A nonpolarizable electrode that generates highly reproducible potentials; used for pH, ORP, and ammonium measurements and polarographic analyses (e.g. silver-silver chloride electrode).

Resistivity—Resistivity is the electrical resistance offered by a material to the flow of current, times the cross-sectional area of current flow and per unit length of current path. It is the reciprocal of conductivity and is also known as electrical resistivity and specific resistance. Resistance declines as ion content increases.

Response time— The time required for a system to react, by a prescribed amount, to a step change in some variable. The extent of the response must be stated, as in "to 95% of total change" or "to within 0.1 mg /l of the final reading" (example for D.O.).

Salinity—Salinity is the measure of the total quantity of dissolved salts in water. Salinity refers to the ionic strength of natural waters. Salinity and salt concentration are the only terms that can be used when referring to the relative concentration of certain salts in bays, estuaries, and oceans.

SDI-12—SDI-12 is a standard used to interface data recorders with microprocessor-based sensors. SDI-12 stands for serial-digital interface at 1200 baud. SDI- 12 is intended for applications with the following requirements: battery-powered operation with minimal current drain, low system cost, use of a single data recorder with multiple sensors on one cable, and up to 200 feet of cable between a sensor and a data recorder.

Sensor—The generic name for a device that senses either the absolute value or a change in a physical quantity such as temperature, pressure, flow rate, or pH, and converts that change into a useful input signal for an information-gathering system.

Service loop—A loop in a wire or cable to reduce the load on the wire or cable.

Slope—Slope is the operation applied to the system's response once the zero has been set. Slope is a measure of the sensitivity of a sensor. Slope scales the sensor's output to the correct units. (Also see: Zero)

Specific conductance = conductivity at 25 °C—The ratio of the electric current density to the electric field in a material. The ability of a fluid to conduct electricity. Specific conductance is the inverse of electrical resistivity, corrected at 25 °C, since fluids conduct more at higher temperatures.

Spot-checking—The collection of data using readings at irregular intervals.

Temperature—A measure of heat present in water. Aside from dissolved oxygen, temperature is considered the single most important parameter. Knowledge of water temperature is essential to the measurement of dissolved oxygen, conductivity (salinity), pH, alkalinity, biological/biochemical oxygen (needed to meet the metabolic needs of aerobic - requiring D.O. - organisms) and virtually every other water quality parameter. Temperature controls metabolism (utilization of inorganic and organic matter for life processes) of aquatic animals and plants. Temperature is largely responsible for biochemical reactions and is one of the most important cues for beginning and ending of spawning, migration, and many other phenomena.

Titration—A method of analyzing the composition of a solution by adding known amounts of standardized solution until a given reaction - color change, precipitation, or conductivity change - is produced. Winkler titration (in calibration): A wet chemical method for estimating the D.O. in water.

Tolerance—Refers to the maximum difference between the true value of a parameter and the actual "operator-acceptable" reading. Usually used as a synonym for accuracy.

Total dissolved gas (TDG)—The amount of gaseous compounds dissolved in a liquid.

Total dissolved solids (TDS)—The amount of materials in a body of water that are dissolved or too small to be filtered. These solids include ions, which are important to the internal water balance in aquatic organisms. The amount of substances (calculated in Kg/l) dissolved in one liter of water. A measure primarily of alkaline earth metals and their salts dissolved or in very fine suspension. It provides information regarding the potential buffering capacity of water, water hardness, and the potential lethality of toxins. The concentration of dissolved solids affects osmoregulation (salt balancing) and is often a cue for migration and spawning. TDS concentration affects the buoyancy of fish eggs and other organisms.

Transducer—Any device or element which converts an input signal into an output signal of a different form (ex: doorbell, microphone). The depth or vented level transducer.

Turbidity—The measure of the clarity of a liquid by using colorimetric scales. It is also the expression of the optical property that causes a light to be scattered and absorbed rather than transmitted in straight lines through a sample. Turbidity is the opposite of clarity (ITM) A measure of the opacity or translucence of water. The main objective is to determine the scattering of light by particles of a body of water and report that scattering in some unit of measurement, usually nephelometric turbidity units (or NTU) based on a primary turbidity standard called formazin. Turbidity is caused by plankton (both animal and plant), clay, suspended clay, silt, etc. Although these substances impart color, color resulting from turbidity is referred to as "apparent color" and should not be confused with true color (resulting from dissolved substances). Apparent color can also result from overshading by vegetation or substrate (bottom material) color.

Vented depth—(ITM) The multiprobe transducer measuring depth from 0 to 10 meters. (See: Depth)

Zero—(ITM) A system's "zero" is an anchor point set either temporarily by calibration or permanently by design. This point can easily be established either electronically or by using laboratory standards. (Also see: Slope).

Abbreviations

AgCl	Silver Chloride	**MS**	MiniSonde
Ah	Ampere hour	**mS/cm**	MilliSiemens per centimeter
AWG	American wire gauge	**mV**	Millivolt
BDR	Basic data recorder	**nm**	Nanometer
BP	Battery pack; barometric pressure	**PA**	Probe Assembly
CC	Calibration cable	**PCB**	Printed circuit board
°C	Degrees Celsius (centigrade)	**ppt**	Part per thousand
CSV	Comma-separated value	**psu**	Practical salinity unit
DS	DataSonde	**psiag**	Pound per square inch absolute
EPA	External battery pack	**psig**	Pound per square inch gage
°F	Degrees Fahrenheit	**RBP**	Rechargeable battery pack
GFI	Ground fault interrupt (device)	**RGA**	Returned good authorization
IBP	Internal battery pack	**SDI**	Serial-digital interference
IC	Interface cable	**STDREF**	Standard reference electrode
K	Degrees Kelvin, or kelvin. A unit of absolute temperature.	**CIRCLTR**	FreshFlow™ miniature sample circulator
KCl	Potassium chloride	**SVR**	Surveyor
l or L	Liter	**WSG**	Weighted sensor guard
mmHG	Millimeter of mercury (hectoPascal and millibar also used in Europe)	**4PF**	4-pin female connector
mil	A unit of length, equal to 0.001 inch	**4PM**	4-pin male connector
...M KCl	...molar potassium chloride	**6PF**	6-pin female connector
µm	Micrometer	**6PM**	6-pin male connector
m	Millimho = milliSiemens (mS)	**9PF**	9-pin connector
µS/cm	MicroSiemens per centimeter = micromho per cm	**9PM**	9-pin male connector
m	Meter (1 meter = 3.281 ft)		