MACROINVERTEBRATE COMMUNITY SAMPLING PROTOCOL FOR DEPRESSIONAL WETLAND MONITORING SITES

I. PURPOSE

To describe the methods used by Minnesota Pollution Control Agency's (MPCA) Biological Monitoring Program to collect macroinvertebrate community information at wetland monitoring sites for the purpose of assessing water quality and developing biological criteria.

II. SCOPE/LIMITATIONS

This procedure applies to all monitoring sites for which an integrated assessment of water quality is to be conducted. An integrated assessment involves the collection of biological (macroinvertebrate and plant) and chemical data to assess wetland condition.

III. GENERAL INFORMATION

Sites may be selected for assessment for a number of reasons including: 1) sites randomly selected for condition monitoring as part of the Environmental Monitoring and Assessment Program (EMAP), 2) sites selected for the development and calibration of biological criteria (e.g., Index of Biological Integrity), and 3) sites selected to evaluate a suspected source of pollution.

IV. ACTION STEPS

A. Field Sampling

For sampling wetland macroinvertebrate assemblages a seasonal index period of June - early July is preferred, this can be earlier if spring temperatures are unusually high that year. In previous wetland work, Minnesota Pollution Control Agency (MPCA) researchers found that some of the invertebrates were too immature to identify when sampled in May, especially the dragonfly nymphs. The sampling window was therefore moved forward to June. In stream invertebrate work, the sampling is done in September to ensure base flow conditions, and to obtain a relatively high percentage of mature larval invertebrates. This approach does not work for wetlands because: a) the wetlands may be dry or unsampleable later in the field season, and b) the wetlands will be heavily colonized by invertebrates which have immigrated into them from other waterbodies. In the latter situation, the invertebrate community in September may be less reflective of the water quality of the wetland itself than the invertebrate community in early summer.

Currently the MPCA has emphasized depressional wetlands in their development of invertebrate indices of biotic integrity (IBI). Depressional wetlands can be stratified into nearshore emergent (shore to 1 m water depth), deep emergent (> 1m water depth), and open water submergent vegetation zones. The MPCA has focused on the nearshore emergent vegetation zone for developing the invertebrate index of biological integrity. In this zone there is a high richness and abundance of invertebrates, including the large predatory insects, due in part to the decomposing vegetation and diverse vegetative microhabitats which occur in this zone. Sampling is conducted in areas that are representative of the wetland emergent zone. However, field partitioning of the wetland for invertebrate sampling as above may need to be modified as the MPCA expands assessment to other wetland types (e.g., riparian, forested).

Sampling of invertebrates by the MPCA Biological Unit is restricted to macroinvertebrates, excluding ostracods and the smaller microinvertebrates which are not retained by a U.S. Standard No. 30 sieve (28 meshes per inch, 0.595 mm openings). Macroinvertebrates are collected in the field using two sampling

techniques: dip nets and activity traps. Previous MPCA projects (e.g., Helgen et al. 1993) demonstrated that dip net sampling captures the greatest richness of invertebrates, but the actively swimming or night-active predators may be under-collected by this method. Therefore, activity traps are placed in the wetland for two days to collect the active swimmers (see details below). Previous work by MPCA (Helgen et al. 1993) has shown reduced taxa richness in benthic, or bottom samples taken with core tubes and subsequently this method of sampling is not currently in use.

Dip Net Sampling

Two samples are collected from each wetland using a heavy-handled D-frame aquatic dip net with a 600 micron mesh size (Wildlife Supply Company). The two samples are taken in different areas within the same general location of the nearshore emergent vegetation zone and are not intended to be replicates, but rather are done to sample the wetland more widely. Ultimately, the data from the two samples are combined for purposes of calculating IBI metric scores. Each dip net sample consists of two dipnetting efforts composited into one sample. Each effort consists of sweeping the dip net strongly a few times (3 -5 depending on the density of the vegetation), reaching outward and pulling towards the body in a rapid motion. Each sweep should be through the water column and vegetation downwards to near the bottom. If mud is scraped into the net, the sample should be discarded and the sampling effort must be repeated in an area away from the previous netting, after the net has been cleaned out.

A method utilized by MPCA reduces the amount of time associated with separating invertebrates from the vegetation that invariably gets swept into the dip net. This method involves the placement of the entire dip net contents on top of a framed ½ inch hardware cloth screen set over two small pans (Coleman cooler style) containing sieved water (Figure 1). The frame is placed so no open screen area projects beyond the pans of water below. This frame and pan setup is placed into a larger plastic pan (tote tray) which can be floated on the water. Over a period of ten minutes the vegetation is spread apart on the hardware cloth to allow the invertebrates to drop or crawl out into the pans below. After ten minutes a second dipnetting effort is done in a nearby area, the vegetation from the first dip net effort is removed, and the second net's contents are placed on the cleared screen. The spreading process is repeated for about 10 minutes, after which the vegetation is again discarded.

After both sweeping efforts are completed, the contents in the two small pans are poured through a 200 micron nytex nylon net sieve to drain out the water. The sieve is made with 15 cm length of 4" diameter PVC pipe with the net glued on one end with a ring of the PVC. The 200 micron sieve is used to retain the chironomids dislodged from the vegetation. The contents of the sieve is back-flushed with 100% alcohol with a strong squirt-bottle into a sample jar, thus combining the two dip net efforts into one dip net sample. The goal is to end up with 80% alcohol final concentration. Care must be taken to represerve samples containing a large catch of invertebrates, or to divide the sample between two jars (sample #, jar 1 of 2, jar 2 of 2). The jar should have not more than 1/3 volume of invertebrates to alcohol. Sixteen-ounce plastic jars with foam or polypropylene seals are useful for preservation in the field. Labels made with India ink or pencil on 100% cotton paper or other material known to survive the preservatives are placed within the jar. Any label placed on the outside of the jar is only for convenience in managing samples.

Activity Trap Sampling

The activity traps work as passive funnel traps to collect organisms that swim into the funnel and pass through the neck into the bottle. Made from clear 2-liter plastic beverage bottles obtained from the manufacturer free of labels or opaque parts, the traps are nearly invisible underwater. The top of the bottle is cut cleanly with a hot wire at the shoulder and inverted into the bottle. The bottle traps used by the MPCA are designed with four two inch grooves cut in to the funnel edge by a hot wire to allow the funnel to snap into the bottle opening without the use of clips or visible straps (Figure 2). The traps are supported on a 4 ft ½" dowel, or a 4 ft fiberglass electric fence post, and attached with a flexible half section of 3" thin wall PVC pipe which allows raising or lowering the activity trap on the dowel (Figures 2 & 3).

Ten activity traps are placed in each wetland for two consecutive nights within the nearshore emergent vegetation zone. The ten activity traps are set out in pairs with each trap in a pair located approximately 3 - 4 meters apart. In the shallowest water (15 cm) the traps are placed just under the surface of the water, but

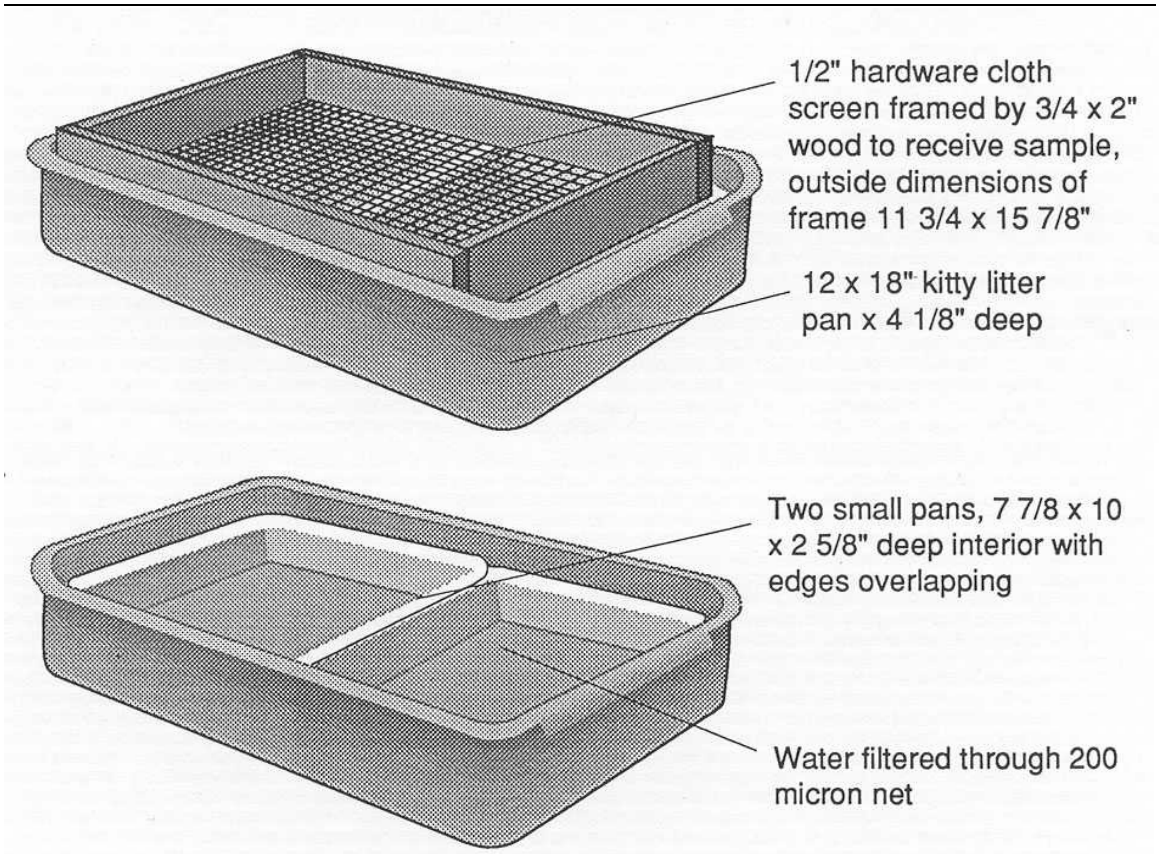

Figure 1. Diagram of hardware cloth and tray apparatus for separating invertebrate specimens from vegetation collected in dip net samples.

should not be resting on the bottom to avoid filling the bottletrap with sediment. In deeper water (> 50 cm) traps are placed horizontally about 15 - 20 cm under the surface. Traps are not placed at the deeper edge of the vegetation in the open water area because capture efficiency goes down as the water gets deeper. The traps are backfilled with water leaving no air bubbles inside in order to reduce predation within the trap. The wingnut should be tightened enough so the trap remains horizontal (see Figure 3a).

After the required two-night period the traps can be collected by slightly loosening the wingnut in order to rotate the trap to a vertical position and slide it up the dowel by slightly compressing the dowel clamp. Then the funnel is removed and the contents of the trap are poured through the 200 micron sieve. The trap is squirted with tap water and the inside is rubbed to dislodge leeches and other invertebrates. Specimens attached to both faces of the funnel opening are also considered part of the sample. These dislodged specimens are then added to the contents of the sieve. The second trap of the pair is collected and its contents are poured into the same sieve. The sieve is back-flushed into a sample jar with 100% alcohol to a final concentration of 80%. Care must be taken to represerve samples having a large catch of invertebrates, or divide the sample between multiple jars (sample #, jar 1 of 2, jar 2 of 2, etc.). The jar should have not more than 1/3 volume of invertebrates to alcohol. Sixteen-ounce plastic jars with foam or polypropylene seals are useful for preservation in the field. Labels with India ink or pencil on 100% cotton paper or other material known to survive the preservatives are placed within the jar. Any label placed on the outside of the jar is only for convenience in managing samples.

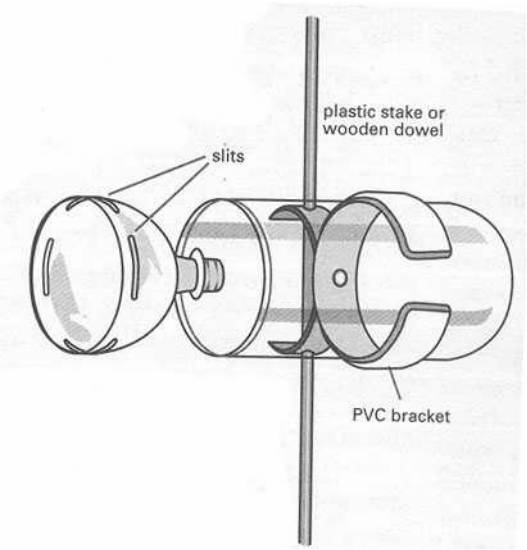

Figure 2. Activity trap design illustrating adjustable PVC bracket and funnel grooves.

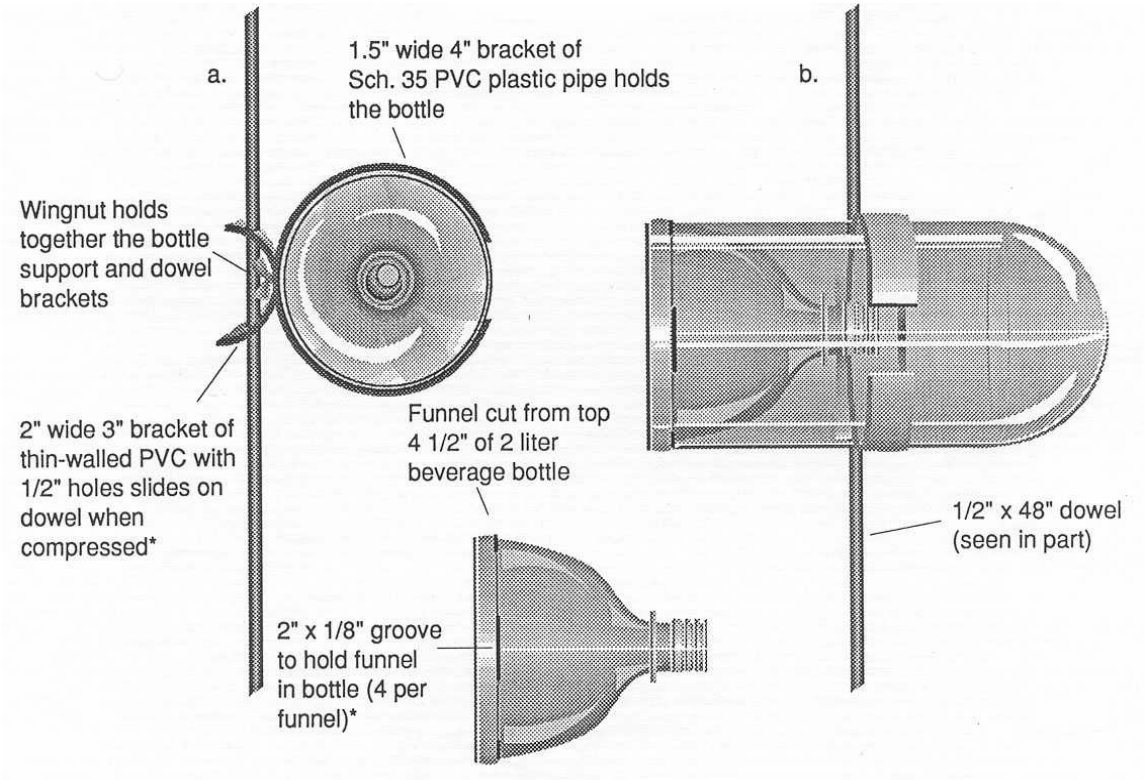

Figure 3. Activity trap design illustrating a) view into funnel and b) lateral view.

B. Sample Storage and Maintenance

All preserved samples are kept in a hazardous materials room. They are checked within a week of field sampling and then periodically for adequate preservative volume, and represerved with 80% alcohol as necessary. For samples that require additional alcohol the lids are tightened or replaced in order to prevent further evaporation.

C. Sample Processing

A combination of dissecting microscopes and compound microscopes are used for sorting and identifying macroinvertebrates in the laboratory. At MPCA there is one Olympus SZX microscope, one Olympus SZH, two Olympus SZ40 microscopes, and one Olympus BX40 compound microscope. The procedures for sorting invertebrates from dip net and activity trap samples are outlined in Table1.

Sample Identifications
Organisms are identified to the lowest possible taxonomic level. Typically this is to the genus level though often it is to the species level, at a minimum they will be identified to the taxonomic level as designated for each group in Table 2. Once specimens are sorted according to the guidelines listed in Table 2, identifications are then made for each specimen within the sorted groups. Identifying all the specimens in a

Table 1. Sorting protocol for dip net and activity trap macroinvertebrate samples.

Procedure	Comments
1) Note start time and site information on to data sheets.	Check/retain inner label.
2) Pour the sample into sieve and rinse with tap water.	Collect, cover, and save alcohol for re-preservation
3) Backflush sample with water to glass picking tray. Tray should be placed over grid transparency upon light box.	Generally, sample is large, and must be separated into two or more efforts to accommodate picking and accuracy.
4) Fill glass tray slightly (1 cm) with water. This helps to separate organisms from debris.	Gentle stirring/probing sample also helps dislodge critters from debris.
5) Using forceps, pick entire sample to sorting trays, jars, and petri dishes according to taxa list (Table 2). Be sure to keep record of separate taxa on mechanical counter. Properly fill in lab sheets.	Pick, count, and visually ID according to taxa List (Table 2). A magnifying lamp may be beneficial for small organisms and juveniles.
6) Specimens may then be combined in general groups (dragons/damsels, snails/sphaeriidae, etc.) and placed into vials/jars with proper labeling for later identification. Preserve in 80% ethanol.	Grouping conserves resources. Label should contain site, date, collector, and sample type written in pencil on cotton stock.
7) Replace original label and sample remnant to sample jar. Backflush using 80% alcohol and fill using previously saved alcohol.	Be sure to check prior alcohol for strength.
8) Note end time on datasheet. Calculate total time.	Calculate in hourly increments.

group (e.g., dragonflies) at the same time facilitates proper designations (e.g., species or genus) by allowing comparisons of closely related taxa. References containing the taxonomic keys for identifications are provided in Appendix A. Where ambiguity exists, specimens will be set aside for identification by an independent invertebrate taxonomist (also see *Reference Collection* section).

Table 2. Invertebrate taxa list indicating which groups are counted and identified for each sample type (dip net or activity trap) and the taxonomic resolution for each group.

	Activity Trap		Dip Net		Identify to
Group	**Total**	**Picked**	**Total**	**Picked**	
[3] Amphipoda (Ad)	x	x	x	x	Genus
[3, 4] Amphipoda (Juv, < 3mm)	x		x		Lowest Level
Anisoptera (Larvae)	x	x	x	x	Genus
Anostraca	x	x	x	x	Genus
[2]Chironomidae (Larvae)			x	x	*
[2] Chironomidae (Larvae, < 3mm)			x		*
Coleoptera (Ad)	x	x	x	x	Genus
Coleoptera (Larvae)	x	x	x	x	Genus
[3] Conchostraca	x		x	x	Genus
[1]Corixidae (Ad)	x	x	x	x	Genus
[1, 3, 4] Corixidae (Juv.)	x		x		Family
Diptera (Larvae)	x	x	x	x	Genus
Ephemeroptera (Larvae)	x	x	x	x	Genus
Gastropoda (Ad)	x	x	x	x	Species
[3]Gastropoda (Juv.)	x		x		Genus
Hemiptera (Ad)	x	x	x	x	Genus
Hemiptera (Juv.)	x	x	x	x	Lowest level
Hirudinea (Ad)	x	x	x	x	Species
Hirudinea (Juv.)	x	x	x	x	Lowest level
Isopoda (Ad)	x	x	x	x	Genus
Isopoda (Juv.)	x	x	x		Lowest level
Lepidoptera (Larvae)	x	x	x	x	Genus
Malacostraca	x	x	x		Family
Megaloptera (Larvae)	x	x	x	x	Genus
[1, 3] Neoplea (Ad & Juv.)	x		x		Genus
[3] Sphaeriidae (Ad & Juv.)	x		x	x	Family
Trichoptera (Larvae)	x	x	x	x	Genus
Zygoptera (Larvae)	x	x	x	x	Genus

[1] Represents Corixidae & Neoplea which were counted/recorded separately from other Hemiptera.
[2] Chironomidae Ids are done on dip net samples only. Estimate abundance of chironomids in activity traps.
[3] Represents groups that may be counted within the glass tray.
[4] Represents group that may be sub-sampled in high numbers.
* Identifications made by Dr. Len Ferrington (University of Minnesota).

Reference Collection
A macroinvertebrate reference collection is maintained for each project at the MPCA Biomonitoring Laboratory (St Paul Office). This collection consists of specimens of each type of macroinvertebrate that has been collected for individual projects conducted by MPCA staff. A few specimens of each taxon are placed in vials or small jars which are labeled inside for the taxon, date, and collection site. This collection will be reviewed by other biologists to confirm the identifications for each project. Specimens for which the identification is uncertain will be reviewed by other biologists with expertise in the particular group.

D. Quality Assurance

At least ten percent of the sites for each project are sampled twice, either on the same date or within a week in an area that is equally representative of the wetland as was first selected for sampling. At least ten percent of the samples are repicked. If organisms were missed, the entire set of samples is repicked. At least ten percent of the picked samples will be reviewed by a qualified invertebrate biologist to verify identifications. In addition, the reference collection from the project will be reviewed by a qualified invertebrate biologist to verify identifications. Chironomidae will be identified by a specialist in the taxonomy of the group (Dr. Len Ferrington, University of Minnesota).

Data is recorded on standard hard copy lab and field data sheets (see Appendix B for examples). These data sheets and field notebooks will be copied and stored in a separate place. In addition, data from each project will be stored and maintained within a Microsoft® Access database that resides on the MPCA network drives and is normally backed up each night.

Following data input all entries are completely proofed before data analysis begins.

E. Literature Cited

Helgen, J.H., K. Thompson, J.P. Gathman, M. Gernes, L.C. Ferrington, and C. Wright. 1993. Developing an Index of Biological Integrity for 33 Depressional Wetlands in Minnesota. Minnesota Pollution Control Agency

Appendix A: Taxonomic References for Identifying Invertebrates

Burch, J.B. 1982. North American Freshwater Snails. Museum of Zoology. University of Michigan. Ann Arbor.

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Klemm, Donald J. 1982. Leeches (Annelida: Hirudinea) of North America. US EPA Cincinnati, OH. EPA-600/3-82-025.

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Needham, James G. and Minter J. Westfall. 1954. The Dragonflies of North America. University of California Press. Berkeley.

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Westfall, Minter J. Jr and Michael L. May. Damselflies of North America. 1996. Scientific Publications. Gainesville, FL.

Wiggins, Glenn B. 1996. Larvae of the North American Caddisflies (Trichoptera), 2nd edition. University of Toronto Press. Toronto.

Appendix B: Field and Lab Data Sheets

MPCA Wetland Monitoring Program

WETLAND INVERTEBRATE VISIT FORM

Wetland Name:		**Date:**	
Field Number:	**County:**	**Crew:**	
COORDINATES	**LATITUDE**	**LONGITUTDE**	**TYPE OF GPS**
Field GPS:	____°____’ ____.____“	____°____’ ____.____“	☐ **2D** ☐ **3D**
GPS TIME	**PDOP**	**ROV. FILE #**	

BOTTLE TRAP PLACEMENT

☐ **Bottle Traps Placed** **TIME:**
Traps place by: **DATE:**____/____/____
TEMP:

Number of Bottle Traps Placed: _____

BOTTLE TRAP RETRIEVAL

Bottle Trap Sample #	**# Jars per Sample**
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

BT’s Had	**None**	**Few**	**Some**	**Many**
Tadpoles				
Minnows				

Salamander larvae--_____
Salamander adults--_____
Frog adults --------_____

Fish Species	**Number**

DIPNET SAMPLE

☐**D-net Sample Taken** **TIME:**
D-net taken by: **DATE:**____/____/____
TEMP:

Number of D-nets Taken: _____

D-net Sample #	**# Jars per Sample**
_____	_____
_____	_____

SAMPLE SITE INFORMATION

Wetland Bottom: **Firm** **Soft** **Mucky** **Help!!**

Comments:

Aquatic Veg:	**None**	**Sparse**	**Moderate**	**Dense**
Submerged				
Emergent				

Shoreline Veg: Grassy Shrubs Wooded Other

Comments:

Weather: Sunny Partly-Cloudy Overcast Windy Calm Rainy

PHOTOGRAPHIC DOCUMENTATION

Looking left from BT’s: Frame Seg. # /

Looking opposite shore from BT’s: Frame Seg. # /

Looking right from BT’s: Frame Seg. # /

DIRECTIONS TO WETLAND / COMMENTS

WATER CHEMISTRY	
DEPARTMENT OF HEALTH SAMPLES	**FIELD MEASURMENTS**
☐ **Turbidity, 1 125ml, ** DO THIS FIRST ****	☐ **PH ----------**
☐ **TSS and Chloride, 1 liter general**	☐ **Conductivity ------**
☐ **Calcium and Magnesium, 1 250 ml metals** ☐ **Preserved with HNO_3**	☐ **Field Turbidity ----**
☐ **Nitrogen and Phosphorus, 1 250 ml nutrient** ☐ **Preserved with H_2SO_4**	☐ **Water Temp--------**
CHLOROPHYLL WATER/FILTER ----to be chilled in cooler, filtered and sent to MDH	
☐**1 Liter amber glass bottle (or 1 liter general)** *****Volume filtered----------**	

SKETCH OF WETLAND

Include roads used to access site, most convenient vehicle parking, location of bottle traps, location of dipnetting, any other relevant info.

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Dipnet ID Sheet Pg 1			Analyzed By:		Date analyzed:	
Site Name and #:				ID Time DN1:	ID Time DN2:	
Sample Collection Date:			Count		Count	
Odonata	Genus		DN-1	Notes/jar/vial	DN-2	Notes/jar/vial
Dragonflies						
Damselflies						
Trichoptera (Caddisflies)						
	Genus		**DN-1**	**Notes/jar/vial**	**DN-2**	**Notes/jar/vial**
Ephemeroptera (Mayflies)						
	Genus	species	**DN-1**	**Notes/jar/vial**	**DN-2**	**Notes/jar/vial**
Hirudinea (Leeches) For Glossiphonidae try to identify species.						
	Genus	species	**DN-1**	**Notes/jar/vial**	**DN-2**	**Notes/jar/vial**
Gastropoda (Snails) Try to identify to species. Counts are given for mature specimens (3-4 whorls)						
	Genus	species	**DN-1**	**Notes/jar/vial**	**DN-2**	**Notes/jar/vial**