WILD RICE SULFATE STANDARD FIELD SURVEYS 2011, 2012, 2013:

FINAL REPORT

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Abbreviations

AVS – acid-volatile sulfide (or acid-volatile sulfur)
DNR – Minnesota Department of Natural Resources
LacCore – National Lacustrine Core Facility of the University of Minnesota
LLO – Large Lakes Observatory of the University of Minnesota-Duluth
LOI – loss-on-ignition
LRC – Limnological Research Center of the University of Minnesota-Twin Cities
MDHL – Minnesota Department of Health Laboratory
MDL – method detection limit
MPCA – Minnesota Pollution Control Agency
PI – principal investigator
QAQC – Quality Assurance/Quality Control
RL – reporting limit
SCWRS – St. Croix Watershed Research Station of the Science Museum of Minnesota
SIL – Stable Isotope Lab of the University of Minnesota
SO_4 – sulfate
TC – total carbon
TIC – total inorganic (carbonate) carbon
TN – total nitrogen
TOC – total organic carbon
TS – total sulfur
UMN – University of Minnesota

Contents

Introduction

Context for This Report

This report is one part of a larger study—the Wild Rice Sulfate Standard Study—coordinated by the Minnesota Pollution Control Agency (MPCA) on the effect of elevated sulfate concentrations on wild rice. Minnesota currently has a water quality standard of "10 mg/L sulfate - applicable to water used for production of wild rice during periods when the rice may be susceptible to damage by high sulfate levels." (Minn. R. 7050.0224, subpart 2). In 2010, the MPCA initiated a multi-year effort to clarify implementation of the state's wild rice sulfate standard, which had recently come under increased questioning and contention. Based on a review of available studies and information, the MPCA determined that additional studies were needed to evaluate the effects of sulfate on wild rice before a revision to the numeric sulfate standard could be considered. In 2011 the Minnesota Legislature provided funding in the Legacy Amendment Bill (Laws of Minnesota, 2011, First Special Session, ch. 6, Art. 2, Sec. 5(j)) to gather this additional information.

Wild rice is an important aquatic plant in parts of Minnesota, particularly northern Minnesota. It provides food for waterfowl, is also a very important cultural resource to many Minnesotans, and is economically important to those who harvest and market wild rice.

The goal of the overall Wild Rice Sulfate Standard Study is to enhance understanding of the effects of sulfate on wild rice and to inform a decision as to whether a revision of the wild rice sulfate standard is warranted. The Study consists of several research efforts that have been conducted by several groups of scientists at the University of Minnesota campuses in Duluth and the Twin Cities, under contract with the MPCA. The data collection phase of the study was completed in December 2013, and is documented in individual reports, along with associated data, from the researchers working on each component of the study.

The primary hypothesis driving the Study has been that if elevated sulfate has a negative effect on the growth of wild rice it is mediated through the formation of hydrogen sulfide in the rooting zone of wild rice, and that elevated iron would mitigate the toxicity of the sulfide by forming insoluble iron sulfide compounds.

The Study components include:

- **Field study of wild rice habitats** to investigate physical and chemical conditions correlated with the presence or absence of wild rice stands, including concentrations of sulfate in surface water and sulfide in the rooting zone.
- **Controlled laboratory hydroponic experiments** to determine the effect of elevated sulfate and sulfide on early stages of wild rice growth and development.
- **Outdoor container experiments utilizing natural sediments** to determine the response of wild rice to a range of sulfate concentrations in the surface water, and associated sulfide in the rooting zone, across the growing season.
- **Collection and analysis of rooting zone depth profiles of dissolved chemicals at wild rice outdoor experiments and field sites** to characterize sulfate, sulfide, and iron in the rooting zone of wild rice.
- **Sediment incubation study** to explore the difference ambient temperature has on the rate that elevated sulfate concentrations in water enter underlying sediment, convert to sulfide, and later release sulfate back into the overlying water.

The MPCA will review the results from individual reports along with existing monitoring data, other relevant scientific studies, pertinent ecological, cultural and historical information, and the original basis for the wild rice sulfate standard to determine if a change to the current wild rice sulfate

standard is warranted, and what that change might be. If change(s) are proposed, they would be adopted into Minnesota Rules via the administrative rulemaking process and subject to U.S. EPA approval before the changes could be implemented.

This report focuses on the field study of wild rice habitats, including site selection rationale, methods, and summary and analysis of data collected.

Overview of the 2011, 2012, and 2013 Field Surveys

Under contract from the Minnesota Pollution Control Agency (MPCA), the University of Minnesota (UMN) conducted a survey of water bodies across Minnesota in the summers of 2011, 2012, and 2013, to assist the evaluation of the State's sulfate water-quality standard to protect wild rice waters (also known as the "wild rice sulfate standard"). This activity, henceforth referred to as the "survey" (or "2011 survey," "2012 survey," and "2013 survey") is intended to provide (1) information to the MPCA about correlations between wild rice presence and environmental parameters and (2) data collected in a comparable way at both field sites and in the mesocosms themselves. The 2011 survey was a preliminary effort to collect initial data on wild rice stands and to develop the methods for larger field surveys in 2012 and 2013.

Team Structure, Management, and Duties

Lead personnel of this project are: Principal Investigator Dr. Amy Myrbo, Research Associate, LacCore and Limnological Research Center (LRC), Department of Earth Sciences, UMN; Dr. Daniel Engstrom, Director, St. Croix Watershed Research Station (SCWRS); and Dr. Nathan Johnson, Assistant Professor, Department of Civil Engineering, UMN-Duluth. This team worked closely with Dr. John Pastor, Professor, Department of Biology, UMN-Duluth. Field technicians for the surveys were drawn from the staff of LRC/LacCore. Laboratory technicians were employees of LRC/LacCore, SCWRS, the Department of Civil Engineering, or the Large Lakes Observatory (LLO), UMN-Duluth, as appropriate for the lab at which analyses were conducted. The Minnesota Department of Health Laboratory (MDHL) conducted analyses of many samples collected in 2012 and 2013, whereas in 2011 these analyses were conducted by SCWRS. The research team developed and conducted the survey in close contact with MPCA personnel.

Content of This Report

This revised final report comprises an overview of the 2011, 2012, and 2013 field survey seasons and a small amount of data analysis.

The 2013 Field Survey

The 2013 survey differed from the 2011 and 2012 surveys in three important ways. First, in 2013, selected sites were visited multiple times during the growing season, while in 2011 and 2012 each site was only sampled on a single day. Second, in 2013, the wild rice experimental growth mesocosms (operated by PI John Pastor) were sampled by field crews, in addition to their field surveys of natural water bodies and cultivated wild rice paddies. Third, in 2013, field crews coordinated with project co-investigator Nathan Johnson to sample at the same time as his team retrieved porewater sampling devices ("peepers") installed in both field sites and mesocosms. The 2011 and 2012 field surveys were described in previous reports to the MPCA (Myrbo 2012 and Myrbo 2013, respectively). Except where noted, methods remained the same for the 2013 survey.

Duration of the 2013 Field Survey

The 2013 Field Survey began with on-site training of the field crew on May 20, 2013. Eight field technicians were trained in boat operation, sampling and sample processing methods, QAQC (quality assurance/quality control) procedures, and chain of custody forms by the field crew leader (S. Rogers) and PI Myrbo. Additional training occurred during field sampling itself. The last day of the field survey was September 20, 2013, and a crew made one additional visit to the Pastor mesocosms on October 8-9.

Site Selection

Site selection was conducted in close collaboration with MPCA personnel. Seventeen sites were selected as "multiple visit" sites that were visited three to five times between May and September 2013, while 19 were selected as sites to be sampled one time. Sites included lakes, rivers, wetlands, and cultivated wild rice paddies, and were selected based on information provided by stakeholders, and on data on the chemistry and distribution of wild rice waters and other shallow water bodies. Figure 1 shows the locations of approximately 1300 wild rice sites identified by the Minnesota Department of Natural Resources (DNR) in its report *Natural Wild Rice in Minnesota* (2008), along with contours of surface water sulfate (SO_4) concentrations based on data in the DNR database.

For statistical purposes of investigating the hypothesized relationship between sulfate and wild rice growth, the team sought sites with a range of values in both parameters (i.e., low-sulfate/low-rice, low-sulfate/high-rice, high-sulfate/low-rice, and high-sulfate/high-rice). The team also strove to sample widely across the state, and to sample sites that had a history of past or present drainage of waters high in sulfate into wild rice waters as well as relatively unimpacted sites. The MPCA gained permission for the field teams to sample some wild rice waters of the Red Lake, White Earth, and Fond du Lac Reservations accompanied by Tribal resource management personnel; additional permissions allowed sampling in wildlife management areas, State parks, cultivated rice paddies, and sites accessed across private land. The sites sampled in the 2011 survey were selected from a large list of waters for which there were historical reports of wild rice growth. In contrast to site selection in the 2011 survey, the 2012 and 2013 surveys included sites without a known history of wild rice presence. Figure 2 presents the sites sampled in the 2011 and 2012 surveys, and Figure 3 presents the sites sampled in the 2013 survey. Appendix 4 provides details of the sites sampled in the 2011, 2012, and 2013 surveys.

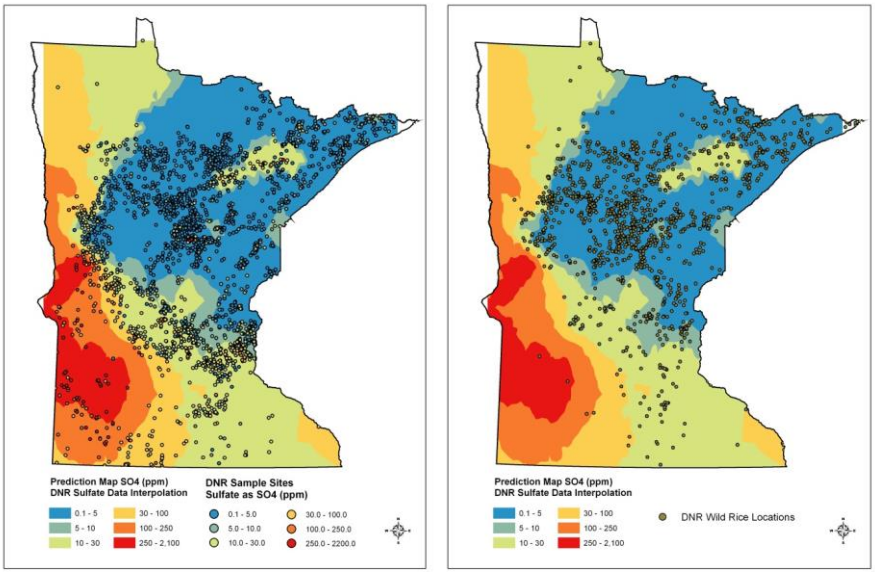


Figure 1. Sulfate (SO_4) concentrations measured in surface water (left) and distribution of identified wild rice water bodies (right), overlain on interpolated contours of surface water sulfate. Datasets from DNR. Figures prepared by Shawn Nelson, MPCA.

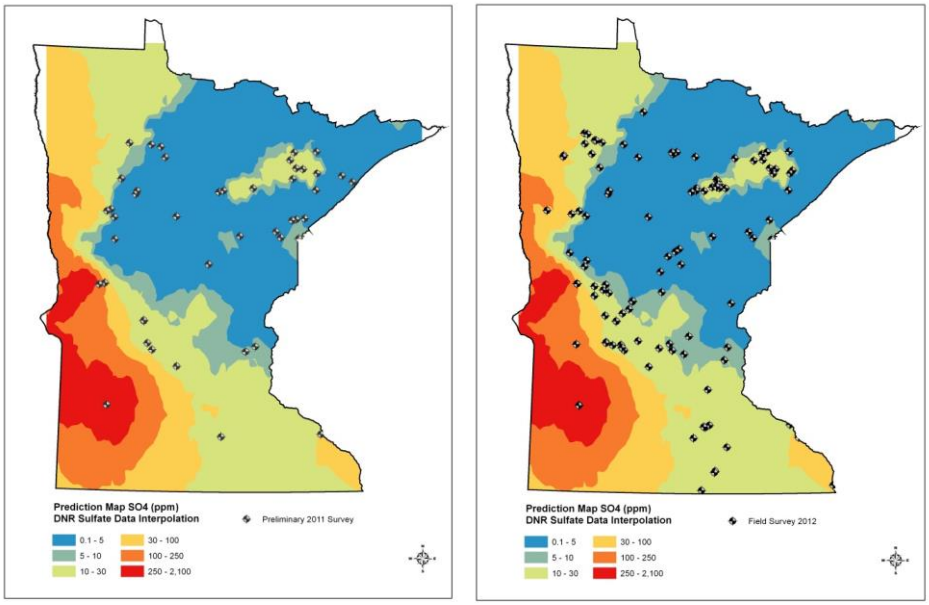


Figure 2. Locations of sites sampled in the field surveys: 2011 survey (left), 2012 survey (right), overlain on surface water sulfate contours as in Figure 1. Figures prepared by Shawn Nelson, MPCA.

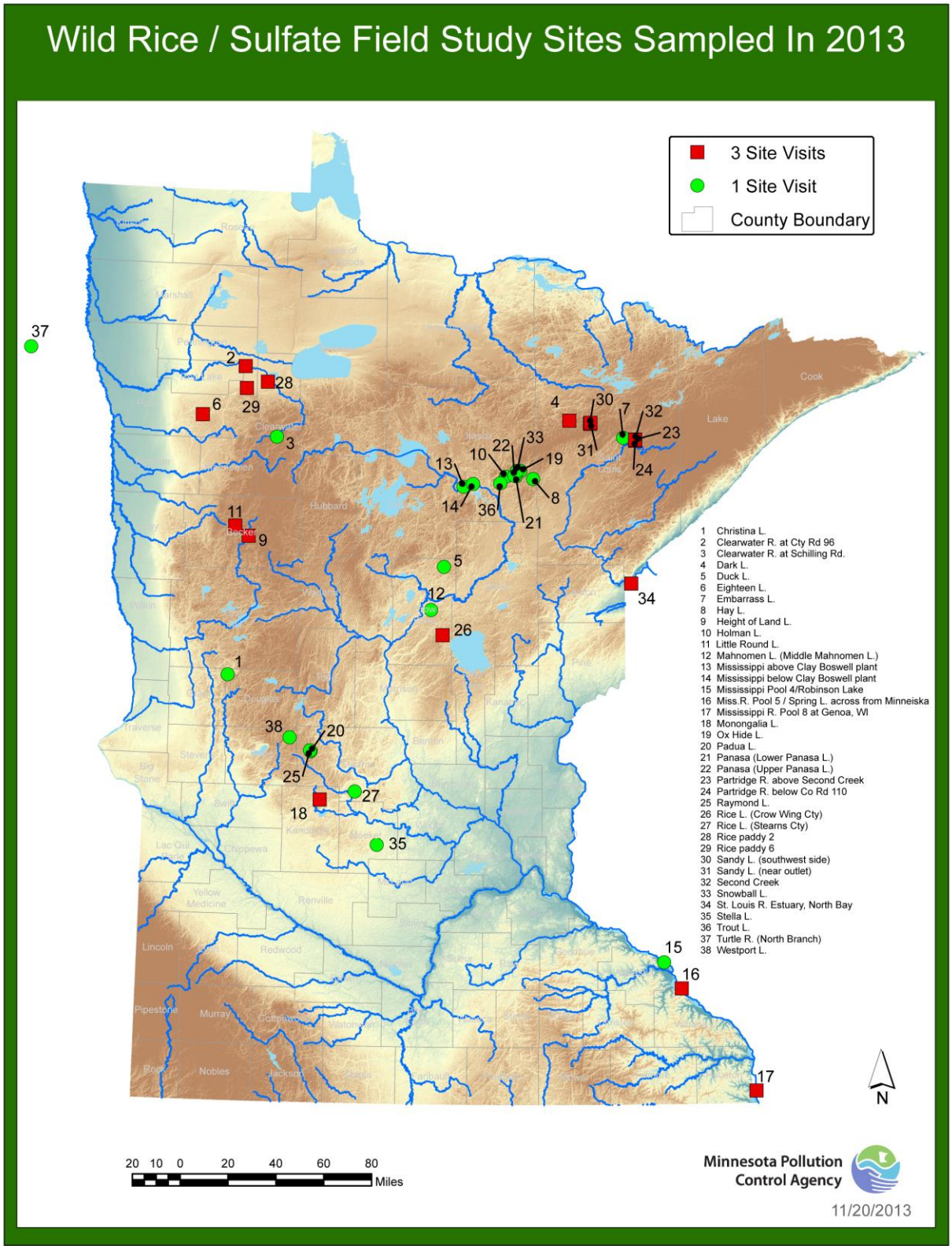


Figure 3. Locations of sites sampled in the 2013 field survey. Figure prepared by Shawn Nelson, MPCA.

Field Campaign

Field protocols were developed in collaboration with MPCA staff. The 2013 field sampling protocol is included in Appendix 1;the 2011 and 2012 protocols are available in previous reports (Myrbo 2012 and Myrbo 2013, respectively). The QAPP document is available from the MPCA. The data sheet completed by field crews for each site is provided as Appendix 2. In the 2013 survey field crews sampled 83 sites (see Fig. 3), some more than once, in addition to making nine visits to sample the mesocosms, including one visit during which 30 mesocosms were sampled in one day (the "mesocosm synoptic"). Location information for the field sites, as well as sites sampled in 2011 and 2012, is included as Appendix 4.

A team of two persons (or three, during training) sampled each site. One or two teams were deployed at any given time. From a canoe or motorboat, depending on access conditions, the team sampled surface water and collected eight short (~50 cm) core samples around the boat. As in the 2012 survey, water and sediment were collected at the same site, unlike in the 2011 survey where water was collected in open water away from submerged plants, but usually within a few hundred feet of the rice bed, and cores were collected in a wild rice bed. Also in contrast to the methods of the 2011 survey, but identically to the methods of the 2012 survey, field crews were instructed to collect cores at all sites, not only those with wild rice present. In the absence of wild rice, field crews were instructed to collect cores at a site selected using a schema developed by the MPCA and included at the end of the field protocol in Appendix 1. At the sampling site the team also conducted a plant survey using 1 m diameter rings placed at four locations around the boat. Plant taxa present were identified, and the percent coverage and number of stems of wild rice were recorded. Plant voucher specimens were collected in some cases. Each 2012 and 2013 sampling site was assigned a unique "LacCore ID" number beginning with "FS-" for "field survey." In 2011 samples were assigned LacCore IDs beginning with "P-" for "preliminary field survey 2011."

After the 2011 preliminary survey it became apparent that choosing lakes that were known to support wild rice populations was not yielding samples from many lakes with elevated surface-water sulfate concentrations (i.e. greater than about 15-20 mg/L). Unless a number of elevated-sulfate systems were sampled, the ability to characterize the biogeochemical consequences of elevated sulfate would be significantly limited. Prior to the field survey in the summer of 2012, an effort was made to identify potential field sites that would simultaneously satisfy two criteria: 1) that the site at least superficially afforded habitat that could support wild rice, and 2) might have elevated sulfate concentrations, based on geographic location. Criterion (1) was satisfied if the site supported rooted macrophytes such as water lilies that sometimes co-occur with wild rice, which also indicated that the surface water was clear enough that light could penetrate to the bottom. Extremely turbid waters do not support rooted macrophytes. Criterion (2) was met by searching for candidate sites on the western and southwestern edges of the wild rice range, where gradients in surficial geology naturally produce surface waters higher in sulfate, and by searching for sites that have taconite waste rock piles in their watershed, which might elevate sulfate concentrations. Candidate sites were also identified from MPCA and MDNR databases (Figs. 1,2). In particular the database maintained by the MDNR Shallow Lakes Program was useful in identifying shallow lakes to sample on the western edge of the known range of wild rice (Fig. 1).

In the spring and early of summer 2012, prior to that year's field survey, remote (phone, databases, maps, and other available resources) and physical reconnaissance were conducted to collect information about potential new sites (physical access, access permissions, wild rice presence, and water chemistry) for possible inclusion in the 2012 survey. At sites visited in person, basic

limnological parameters and a water sample were collected by crew members standing on the shore of the water body. A limited set of analyses was conducted on the water sample. In particular, sulfate was analyzed because much of the goal of the reconnaissance effort was to identify sites for full sampling that had surface-water sulfate concentrations greater than 10 mg/L. These samples were assigned LacCore IDs beginning with "R-" for "reconnaissance." Because of the difference in method of collection and scope of analytical data, R- samples will not be included in the final data analysis; the data were just used to aid in the selection of sites for full sampling. 93 sites were sampled under the reconnaissance effort.

Except for the reconnaissance effort in spring of 2012, during all three field seasons (2011-2013) water and core samples were brought to shore and processed as described in Appendix 1. Individual cores were sampled for porewater using 10 cm long Rhizon™ samplers with 0.2 μm nominal pore size (Shotbolt 2010), inserted vertically into the core tops after overlying water was extruded, drawing into evacuated (and pre-treated where appropriate) serum vials. Three other cores at each site were extruded to remove overlying water, and then further extruded to a depth of 10 cm below the sediment-water interface, placed in a bowl together under a nitrogen atmosphere and composited by vigorous stirring. A subsample of this composite sediment was immediately frozen on dry ice for acid volatile sulfide (AVS) analysis and the remainder was refrigerated for later analysis. A nitrogen atmosphere was not employed in 2011. In 2012 and 2013 the sample for AVS analysis was preserved with zinc acetate prior to freezing. In a few cases the glass AVS sample vial cracked during the freezing process. In 2013 the AVS sample was preserved in a plastic vial, which did not crack. But because plastic might be slightly permeable to oxygen, which could oxidize sulfide, the plastic vial was then placed in a nitrogen-gas-purged glass bottle prior to freezing.

LacCore developed a relational database for this project as part of its existing database system. All data, after being quality checked, are entered into this database. Field data were recorded on paper copies of the field data sheet similar to that provided in Appendix 2. Field technicians entered data into the LacCore database in the evenings as soon as possible given internet connectivity. Data were quality-checked by a different field technician at LacCore. At the end of each day, each field crew sent an email reporting on the day's activities, providing observations on the site(s) sampled and reporting any equipment problems. Site information from these emails is included in the database.

Laboratory Analyses

Sample Handling and Analyses

Water samples were kept refrigerated, and AVS samples kept frozen; these were transferred to MDHL within seven days of collection. The remaining sediment samples were kept refrigerated and returned to LacCore, where they were thoroughly homogenized, subsampled, and distributed as shown in Appendix 3. Some subsamples were transferred to SCWRS, and the remainder were processed at LacCore.

Remaining Tasks and Timeline for Completion

Analysis of samples from the 2013 survey is ongoing. Completion of all analyses, with the exception of acid volatile sulfide (AVS) is anticipated by 12/31/2013. AVS data are anticipated to be available from the MPCA in February, 2014.

Field sampling sites have been established in the EQuIS database by MPCA staff in collaboration with LacCore, and datasets are uploaded to that database after they undergo QAQC.

Results

A limited number of parameters are presented in graphical form below. Data used in these graphs is included in Appendix 4.

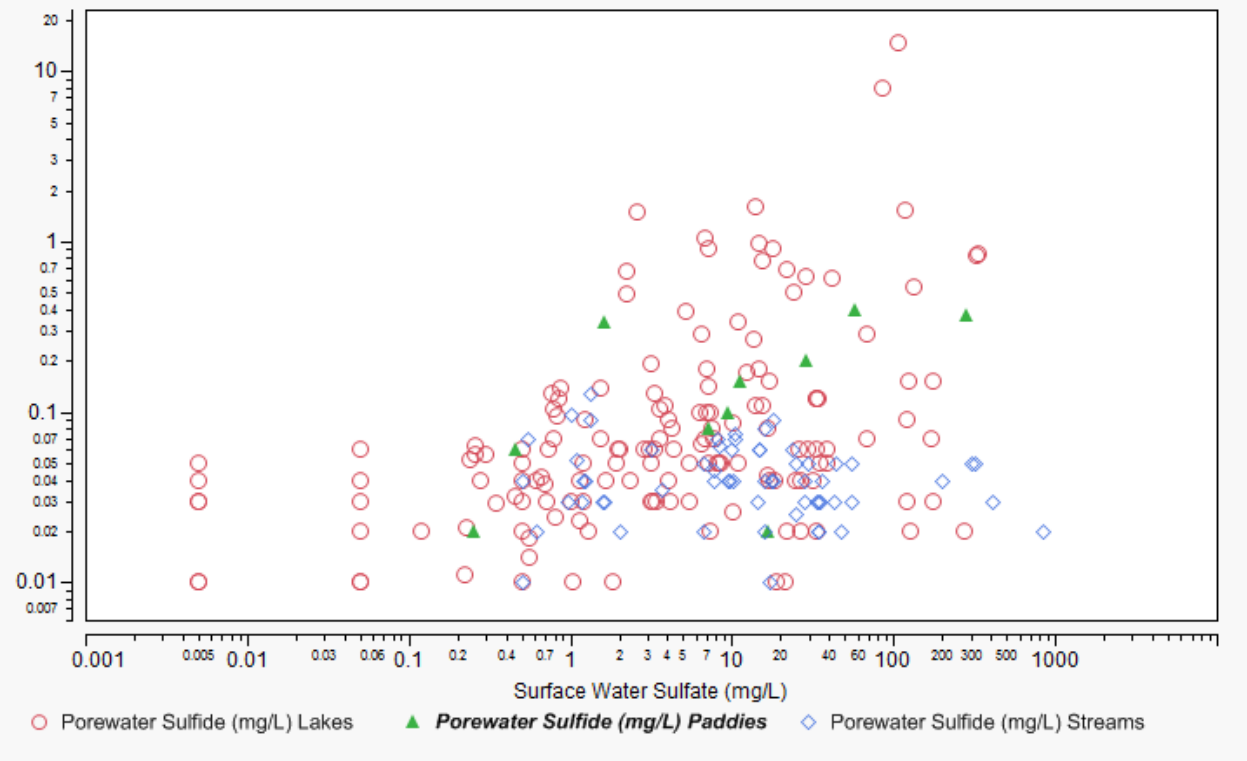


Figure 4. Surface water sulfate and porewater sulfide.

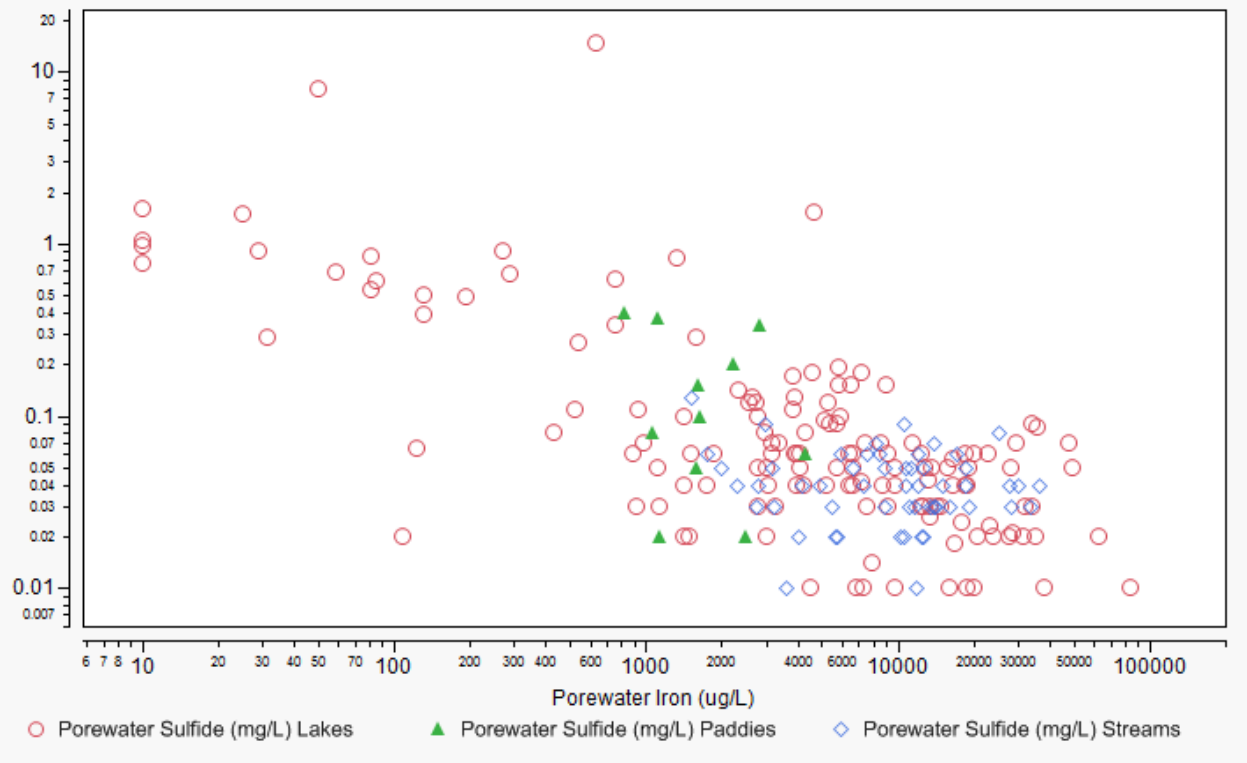


Figure 5. Porewater iron and sulfide.

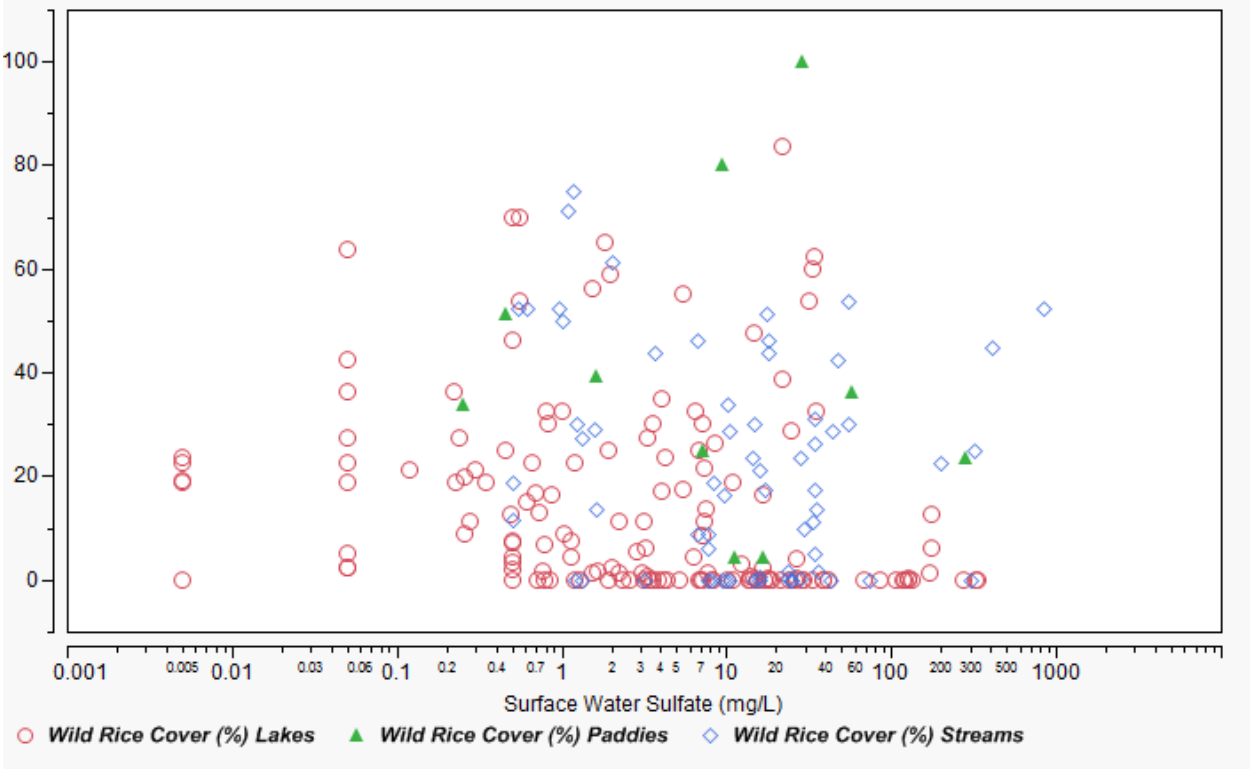


Figure 6. Surface water sulfate and wild rice coverage average in four 1-meter plant rings around the boat at the sampling site.

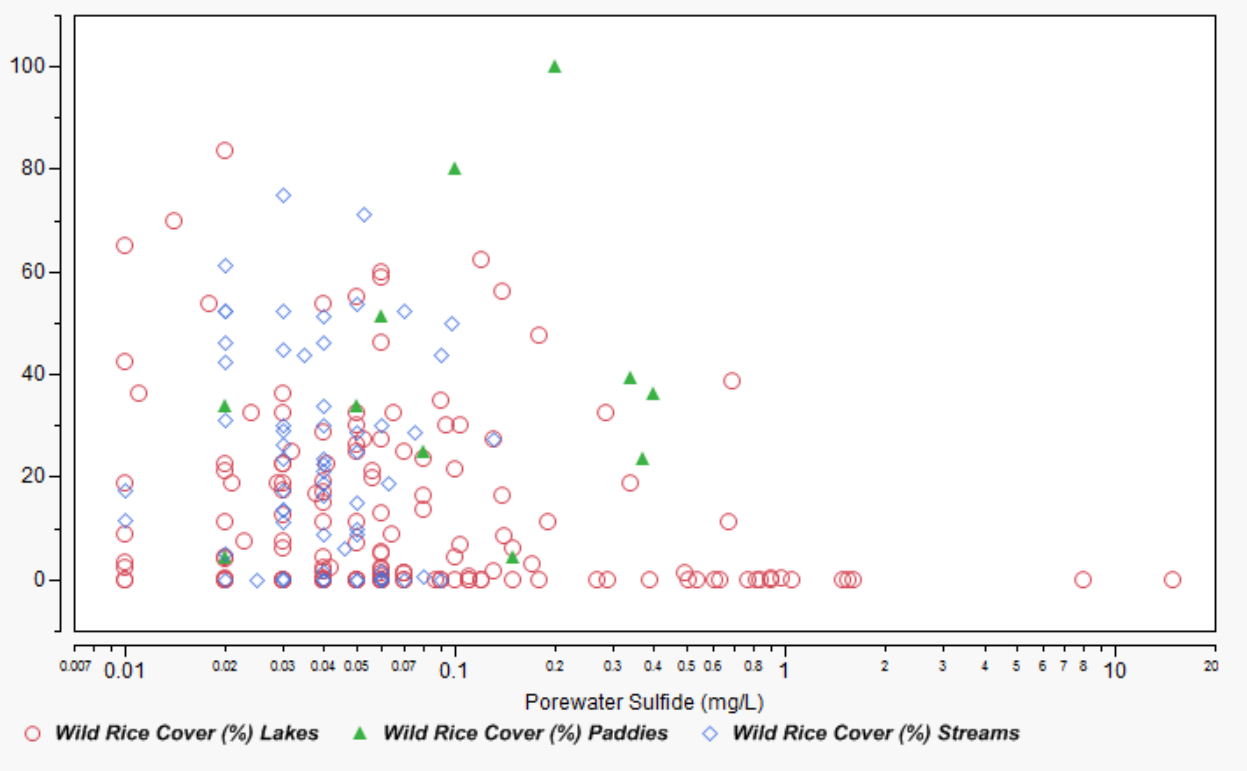


Figure 7. Porewater sulfide and wild rice coverage average in four 1-meter plant rings around the boat at the sampling site.

Appendices

Appendix 1. Sampling protocols and analytical methods

Appendix 2. Field data sheet template

Appendix 3. Sediment subsampling flowchart

Appendix 4. Site information and selected field and analytical data from the sites sampled in 2011, 2012, and 2013

References

Myrbo, 2012. Wild Rice Sulfate Standard Preliminary Field Survey 2011. Final Report. Submitted to the Minnesota Pollution Control Agency. August 27, 2012.

Myrbo, 2013. Wild Rice Sulfate Standard Field Surveys 2011& 2012. Final Report. Submitted to the Minnesota Pollution Control Agency. May 21, 2013.

Minnesota Department of Natural Resources 2008. Natural Wild Rice in Minnesota. A Wild Rice Study document submitted to the Minnesota State Legislature. http://files.dnr.state.mn.us/fish_wildlife/wildlife/shallowlakes/natural-wild-rice-inminnesota. pdf, accessed June 20, 2011.

Shotbolt, L. 2010. Porewater sampling from lake and estuary sediments using Rhizon samplers. *Journal of Paleolimnology.* 44:695-700.

Appendix 1: Sampling protocols and analytical methods

Field protocol

1.0 Amy Myrbo, 8/14/11
rev. 2.0 Amy Myrbo, 8/28/11 (adjusted protocol to reflect changes made during field trials with MPCA)
rev. 3.0 Amy Myrbo, 9/11/11 (added pore water chemistry sample protocol and blanks/duplicates protocol; removed advice to re-pump serum bottle)
rev. 4.0 Cindy Frickle 7/9/12; Ed swain 7/10/2012; Amy Myrbo 7/10/2012 (adjusted protocol to reflect changes made during field training)
rev. 5.0 Cindy Frickle, 7/12/12 (adjusted protocol to reflect changes made during field crew meeting)
rev. 5.1 Amy Myrbo, 7/19/12 (accepted most changes in document and made penultimate recommendations prior to beginning of 2012 field survey)
Rev 5.2 Ed swain 7/19/12 used pages software on an iPad to add a few suggestions framed by double asterisks: **suggestion**
Rev 6.0 Val Stanley 5/16/2013 Changes as per communication with Ed Swain for 2013.
Rev. 6.1 Val Stanley 5/26/2013 Changes as per communication with Amy Myrbo for 2013.
Rev. 6.2 Val Stanley 6/24/2013 COC form protocol updates and clarification.
Rev. 6.3 Amanda Yourd 10/25/2013 Clarification edits.
Rev. 6.4. Ailsa McCulloch 10/29/2013 Clarification edits.
Rev. 6.4. Ailsa McCulloch/Amy Myrbo 11/7/2013 Clarification edits and formatting.

⇓ symbol indicates that data should be recorded at this point, or that a note should be made that a sample was taken.

- Review equipment checklists and restock any required equipment.
- Unload canoe/boat and load equipment (when using Kevlar canoe, load only on water). Lash equipment to boat.
- Check for signage about invasive species and take note of any special decontamination procedures. Check box on data sheet where appropriate. ⇓
- Calibrate Hach pH meter: Complete calibration log that goes with the meter, noting date, time, calibration, and who completed it. ⇓ Insert pH probe into small, color-coded vial with pH 7.0 solution. Take reading and record as “Before.” Calibrate, but do not record the value that appears on the screen. Take another reading and record as “After.” Discard used solution into hazardous waste container and re-fill vial from pH 7.0 bottle. Rinse probe with de-ionized water, then repeat process with pH 4.0 and pH 10.0 solution. Rinse probe again and re-cap in storage solution. Complete process similarly with 1001 µS conductivity solution—using small vial of solution, calibrate to 1001 µS spC. Discard used solution into hazardous waste container. Refill small vial of solution, rinse probe, and re-cap in storage solution. Ensure that calibration log was properly completed, leaving no fields blank. ⇓

- Calibrate Hydrolab Quanta for pH and SpC (every site) ⇓and DO (weekly) ⇓. Note in calibration log.
- Soak Rhizons. ⇓
- Lock vehicle.
- Site selection depends on seasonality; proceed according to the month of the year (below):

    - Single visit to a site in July, August, or September: Proceed to relatively dense wild rice bed (at the same GPS location as a previous crew had sampled the wild rice in this water body, if possible) or, if wild rice cannot be located, choose a site in a water lily bed (either yellow or white). If neither wild rice nor water lilies can be located, use the attached decision tree to decide where to take samples.

    - Repeated visits to a site starting in May or June, and continuing approximately monthly through the summer, for a total of 3 or 4 visits to a given site ("Intensive field sampling"—an effort in the 2013 field season): Repeated visits would ideally be to the same GPS location. Ideally, the first sampling in May or June would be where wild rice plants will be growing in July and August. However, it may be difficult to identify such a site if young wild rice seedlings are not identified growing up in the water column, although floating leaves may be evident, which are characteristic of wild rice. Sample sites would thus ideally be where wild rice is growing; if no wild rice is evident, use a previous year's GPS location for wild rice occurrence. However, if you see wild rice leaves at a different site than the provided lat lon, it would be better to go where the wild rice is growing, rather than using the provided lat lon. If wild rice is not observed and a provided GPS location does not make sense or has been affected by lake-level change (e.g., the GPS location is on dry land) then use the field site decision tree, below. At later site visits, use the GPS location established at the initial sampling in May or June. However, if in July wild rice is not growing at that GPS location, but is at another location, subsequent sampling locations should be shifted to the site that at which wild rice is actually growing.

- Run boat entirely into thickest part of wild rice bed. Avoid uprooting or otherwise destroying wild rice plants whenever possible. *Caution:* when wild rice seeds are ripe, the elongate part of the seed cover is sharp and barbed, and can pierce the eyeball. Use caution when moving through wild rice beds. Sunglasses and long sleeves are recommended.
- Assess sediment matrix characteristics using soil probe, ⇓ then anchor boat by pushing 2 drive rods into sediment and lashing rods to canoe thwarts. Bottom (female) ends of drive rods that do not have a sediment probe attached must be duct taped over so that sediment does not get stuck up in the rod. Avoid resuspending sediment in the water column by pressing drive rods directly and firmly into sediment surface. Extend outriggers to stabilize canoe.
- Deploy thermal stick and record time. ⇓

- Fill in site info. Record weather and flow conditions. Note which equipment you are using. ⇓
- Record orientation of boat relative to compass rose on data sheet using ball compass. ⇓
- Take site photos: First, photograph lake data sheet, making sure that lake name is visible. Then, in order, photograph toward north, east, south, and west, keeping the boat slightly in the frame at the bottom of each photo. Review photos to ensure that each has acceptable exposure and focus. Delete and re-take any that are unacceptable. ⇓
- Turn on GPS, wait until accuracy is optimal (Garmin 76 should be around ±3m), save waypoint, and then record latitude, longitude, elevation, accuracy, and waypoint number. ⇓

Water and sediment sampling:

- Deploy Hydrolab or Hach instrument so that probes are just below water surface on upwind side of boat and collect multiparameter data. Be sure that circulator is on. Record temperature (˚C), specific conductance (spC), and pH. Note spC units. ⇓ If using Hydrolab, return probe into cap filled with tap water. If using Hach, rinse probe with deionized water before replacing caps containing storage solution.
- Find Secchi depth on shady side of boat, without wearing sunglasses. Then find water depth in the same location using Secchi disk. ⇓
- Deploy Hydrolab Quanta for DO profile of water column. Measure from surface (first measurement) to sediment-water interface (last measurement), at 10 cm intervals, which are taped off on the instrument's cord. The last interval may be less than 10 cm in length, and the distance from the previous 10 cm interval should be recorded in addition to the DO reading. ⇓
- Put on nitrile gloves and rinse 2 master water sample bottles 3 times with subsurface water, filling on sunny side and dumping on shady side of boat. Hold bottle underwater and fill, being careful not to disturb sediment or collect submerged macrophytes or scum, etc., floating on surface of water. ⇓ Cap bottles and place in cooler.
- Rinse polycarb tubes. ⇓ Collect 8 cores using tabbed tubes: Retain tension on rope while pushing gravity corer deeper into sediment. Pull corer up and – keeping bottom end of core underwater and holding core upright – insert piston. Cores should be taken 1 m apart from each other and from the thermal stick. Always hold cores upright. Avoid any area where sediment may have been disturbed, including area where Secchi disk was lowered. Place cores in core holder.
- Collect core for repository in an undisturbed location. Using polycarb without tabs, hold corer so that piston is **not** pushed all the way up and wrap duct tape around corer and tube to hold in place. After pulling up core, cut down to 5-10 cm above sediment/water interface and top thoroughly with Zorbitrol, cap, tape, label, and measure core sediment length and total core length. ⇓ On core tube, write lake name, site ID (FS - - -), and draw "up" arrow. Place in shade.

- If a core is lost, spilling sediment that might contaminate the next core, record this, move position one boat length, record new position, and continue, taking remainder of cores as specified above. Take care not to core in any spoiled or previously cored location. ⇓

Vegetation sampling:

- Use 1 m diameter hoop to characterize abundances of plant types. Place hoop even with seats on both sides at front and back of boat.
- Estimate rice coverage in each hoop. ⇓ Count rice stems in each ring. ⇓ Note abundance of other plants within hoop area, including specific species (%sp) whenever possible, using plant guide for identification. ⇓ Look under floating leaves; sum can be over 100%. Take a picture of each survey space. ⇓
- Describe the condition of the wild rice plants and general locations of stands. Estimate relative abundances of flowering rice plants and plants with seeds. Note overall abundance of wild rice (vs. open water) in representative rice stands on the lake, or as far as you can see. ⇓
- Collect voucher specimens: Pull out two wild rice plants (with flowers or seeds if possible), rinse sediment from roots, accordion fold and place into zippered plastic bags, noting site name and water depth. Put in cooler. If rice is not present, collect other species as per decision tree instructions.
- Remove and rinse thermal stick. Record time. ⇓ Remove drive rods, cleaning off any sediment. Secure all equipment and return to shore.

Shore processing:

- Keep all samples either in the shade (repository core) or in the cooler (all other samples, including plants) at all times.
- Pore water sampling: Extrude so that sediment surface is at top of core tube. Set up ring stand with core, in a secure spot in the shade. Place a piece of Saran wrap over the top of the core and poke a small hole in the center. Evacuate serum bottle for 70 mL sample for TP/TN/DOC, first, using a needle and hand pump. Pump to approximately 25 inches Hg internal pressure, then remove needle slowly. Attach needle to Rhizon connection, sealing connection with Teflon tape. Seal any other connections between tubing pieces as well. Insert Rhizon sampler into sediment so that clear connector is just below the sediment surface. Place serum vial upside-down in ring stand clamp. Pierce septum with Rhizon needle, take out most of slack in Rhizon tubing, and tighten clamp rod on vertical rod so that tubing is as straight as possible. Leave to draw until fluid reaches the desired volume of 70mL . Use calibration bottle to measure fluid volume. Insert additional Rhizon(s) if sipping becomes slow due to clogging of the porous part. Keep pushing Rhizon in if core surface lowers. When finished sipping, remove the needle from the bottle before removing the Rhizon from the core. Remove needle slowly to avoid air invasion. If that core will not yield 70 mL, consider achieving the 70 mL goal by switching the serum bottle to the spare short core. Make a note on the field sheet if you do so. ⇓

- Meanwhile, set up second, third, and fourth short cores as described above. Evacuate the serum bottle for nitrate/metals, sipping 50 mL of pore water. Follow same procedure for 30 mL sample used for silica/chloride/sulfate. For sulfide bottle, (preloaded with zinc acetate, with nitrogen headspace), place Rhizon end into sediment then stick needle attachment into an evacuated "sacrificial" bottle to draw air out of the sipper. Once you see water pulling through into the bottle, remove needle and stick into sulfide bottle. (NB: Do not re-use needles from sulfide bottle in other bottles as this bottle contains zinc acetate and may contaminate other bottles.) Record start weight of sulfide bottle. ⇓ Sip 50 mL of pore water into sulfide bottle.

- Take practical samples – as much as you can get until it gets slow and oxygen invasion is a concern. Ensure that top of Rhizon is always below the sediment surface, sipping pore water, and not above it, sipping air. **It is particularly important that air is not introduced into the sulfide sample.** If you observe oxygen invasion in the samples, record it in the comments section of the datasheet. ⇓

- Compositing cores: (This procedure and those below should be done while sulfide sample is sipping.) Turn on high-purity N_2 gas to fill glove bag. Set up extruder. Place first core on extruder and push up piston until all water has been removed (i.e., sediment surface is at top of tube).

- Note whether or not sediment smells like H_2S. ⇓

- Porewater pH measurement: Using one of the cores that will be composited, insert the pH probe below sediment surface to 5 cm depth marked on probe. (If sand or gravel is present in core, use spatula to push aside sediment to make a narrow hole for the pH probe, to avoid scratching the electrode.) Hold there until reading stabilizes. Record reading. ⇓

- Place extruder tray on top of tube. Extrude top 10 cm of core, scraping mud into small, clean bowl bucket as necessary, mixing as little as possible. Discard remainder of core. Repeat with other two compositing cores, adding to the same bucket. Place sample into nitrogen glove bag. Seal bag that has been flushing with high-purity N_2 for at least 10 minutes. Record observations of sediment texture, color, odor, etc. Stir composited sample vigorously to homogenize. Using rubber scraper, scoop enough subsample into labeled, plastic ~60 mL AVS yellow-lidded jar to fill approximately 50 mL. Add zinc acetate preservative from vial (equivalent to 1 mL of 1N zinc acetate/50 mL sediment), then mix well to incorporate preservative. Fill headspace with N_2 and close cap. Place plastic 60 mL jar inside of the black-lidded glass Qorpak or Mason jar filled with N_2 and close that cap. Turn off nitrogen gas. Place AVS jar in cooler with dry ice, but not directly on the dry ice because rapid freezing at the bottom may cause breakage.

- Scoop remaining sediment into large ULINE jar. ⇓ Place large jar in cooler with ice.

- Water sample splitting: Put on nitrile gloves. Shake bottle. If sample contains particulate matter, let settle prior to splitting and do not shake. Split among the 3 subsample bottles; avoid direct contact between bottles while pouring. Add sulfuric acid to the "Nutrients" bottle, close tightly and invert to distribute acid. Put empty acid vials into a Ziploc bag labeled with a hazardous waste label for disposal. DO NOT add acid to the "Metals" bottle—

this will be done in the MDH lab. Tighten all caps *again* to ensure that samples are tightly sealed. Place all samples in cooler. Triple rinse sample bottles with deionized water. ⇓

- Hach water color: Fill outer test tube with clear water, either distilled water or clear drinking water so that the meniscus is even with the upper line. Fill inner test tube to same level with unshaken water sample. Look through tubes towards sky and turn color wheel until the two tubes' colors match. Record value. If water is too colored (>100), remove mirror and multiply value by 5. If still too colored, leave mirror out and dilute sample by half (empty to lower line and add distilled water to reach the second of the three lines), and then multiply the observed value by 10. Rinse lake sample tube with deionized water. ⇓

- Transparency: Shake second water sample bottle. Fill Secchi t-tube. In the shade, and without sunglasses, lower Secchi disk until it is no longer in view from above. Record depth. ⇓ Bring disk back up until just in view again. Record depth. ⇓ Average these two values and record as t-tube Secchi depth. ⇓ If Secchi disk is visible at the bottom of tube, record depth as 101 cm on data sheet. ⇓

- Review data sheet to ensure that all fields have been entered. Do not leave any spaces blank. Make any necessary field notes. ⇓

- Decontamination: IF SITE HAS BEEN IDENTIFIED AS HAVING INVASIVE SPECIES PRESENT, while at the site, decontaminate all equipment using appropriate method (boiling water, alcohol, abrasion, etc.). Make sure to add AIS labels to all samples from infested waters. At all other locations follow DNR protocol of removing aquatic plants from boat and trailer: rinse, drain, and dry. ⇓

- Complete MDH Chain of Custody form for samples: Using attached model, fill out one form for each site. Record names of one sampler, location ID, sample point, date, time when samples were collected (i.e., from lake, not time of shore subsampling), lat/long of the site (under Sampler Comments), and sulfide vial weight (under Sampler Comments). The "Location ID" combined with date and time are the unique identifiers that are used to identify that site on that day, so that all samples collected that day can be merged together in a database. So, make sure all 3 items are identical for all samples that we want to merge. For instance, don't take the "time" literally--just pick an approximate time, like 15:00 and use that for all samples from that site that day. The only reason to use more than one time in a day is if we want 1) a field replicate, or 2) to see how the data for that kind of sample would change over a day. A different time, even by one minute, is regarded as a different sampling effort and the data will not be merged together. Sometimes that is what we want: we do want 10% of our field sites replicated, in which case use a different Location ID, the same date, but a different time (and a different lat-lon). Use the same location ID, date, and time as for the surface water samples, even if they were taken an hour or more apart. When you write by hand on bottles and COC forms, be very neat. Only use sharpies on bottles, not ball point or pencil. Do not use pencil on the COC forms, only pen or ultrafine sharpie. If you make a mistake, draw a line through the mistake and initial the change, right next to the change. Do not write heavily over a mistake in an attempt to make it clear what the correct information is--just cross it out and write the correct information (with initials). Any kind of change or blacking out must be initialed. The tiny computer labels are hard to correct with a sharpie, since there isn't much room--so try to get those right the

first time. If you use the Thermal stick at a field site, please write on the COC form in the comments which stick you used--there are Sticks A, B, and C--which are written on the white PVC. We have a problem with the AVS samples, which are a bottle inside a bottle. The most important bottle to label is the inside one, which has the mud in it. However, only labeling the inside bottle would make it hard to read. If possible, label both bottles. If only one label is available, labeling the one with the mud is preferable. Sign form. All fields pertaining to Matrix code, quantity of containers/preservatives, and analyses should be already completed on the form. Direct any questions about form to Ed Swain. Place forms in Ziploc bag inside of cooler with samples. ⇓

- Do not take completed field data sheets back out onto boat. Enter data daily into database.

- Upload and name photos in a new folder in Transfers. Folder name should contain Location ID (FS-xxx) and Sample Point (Site Name/DOW#). All files should include the Location ID in their names.

- Press plants within two days of sampling (but preferably the same day of sampling), provided the plants have been kept cool and out of the sun. For each individual plant, arrange it accordion-like across the inside of a folded sheet of newspaper. With a waterproof Sharpie, write on the outside of the newspaper the type of plant (WR for wild rice, unknown for an unknown plant, etc.) location, date the plant was sampled, and your last name. You may put more information on paper with the plant, but the purpose of writing on the top of the newspaper is to be able to sort samples without opening the newspaper. Put the folded newspaper between blotting paper sheets, which are between ventilating cardboard pieces, within the plant press. Compress the stack of plants and allow to dry. Change newspaper daily to avoid mold, especially in the first few days after collection, including weekends after returning from the field.

- Re-freeze cold packs as needed and ensure that there is sufficient dry ice to keep AVS samples frozen.

- Bill supplies and services for the day.

Send brief report of day's activities to SO4-project listserv, including lots of information, specifically the site's GPS location, problems and solutions in access, locals you talked to, and generally what the site looked like. Insert relevant text in the "Additional Comments" text field on the field data sheet in FileMaker.

- When relinquishing samples to MPCA or MDH representative, complete "Relinquished by/Affiliation" along with date and time on lower part of Chain-of-Custody form. The accepting representative must complete the "Accepted by/Affiliation" portion of the form.

Field Duplicates and Blanks

Version from 8/11/2012
Updated 10/4/2013 by Amy Myrbo to reflect new locations of Milli-Q systems

Field duplicates (10% of sites, about half from high-sulfate and half from low-sulfate sites):

- After completing shore processing of cores and water from a site, return to the general area you sampled (within 10-20m, and with similar vegetation).
- Collect a complete duplicate set of cores and water samples from this second site. Do not collect a duplicate set of wild rice plants. Treat as above, using a separate COC form, and labeling with a separate Location ID.

Field blanks (five or six total over the course of the study; use a blank to fill out your day if you don't have time to do an additional site):

- Use Milli-Q high purity denioized water (MQDW) from unit in Shepherd or Pillsbury Hall
- Surface water blank: Rinse amber sample bottle with MQDW. Pour MQDW into the amber sample bottle and then fill each surface water sample bottle.
- Porewater blank: Fill four bottles (supplied by MDHL) with MQDW. Set up and sip into serum bottles as for sediment pore water, using one Rhizon per bottle.
- Fill in CoC forms for all bottles, there will be no AVS sample.

Decision tree for determining location to take cores at wild rice study sites
Ed Swain draft July 17, 2012

1	Site has wild rice.....2
	No wild rice evident.....3
2	Is a previously-cored site that has GPS location..... Core as close as possible to earlier site, in a wild rice bed.
	Is not a previously-cored site that has GPS location..... Choose a wild rice bed to sample in.
3	No aquatic macrophytes evident anywhere at the site (ignore cattails)..... Note lack of macrophytes and take reconnaissance samples (UA-1). Don't take sediment samples.
	Aquatic macrophytes evident at the site.....4
4	White or yellow water lily pads present…….. Core in midst of the lily pads, noting lily species.
	White or yellow water lily pads not present……5
5	Other floating-leaved macrophytes (ignore duckweed) present…….. Choose a bed of such plants, sample, and take a voucher plant of the major type for identification.
	Other floating-leaved macrophytes (ignore duckweed) not present……..6
6	Submerged macrophytes present…… Choose a site in about 3 feet of water or less and sample. Take a voucher plant of the major type for identification.
	Submerged macrophytes not present……7
7	Only emergent macrophytes (ignore cattails) present…….. Choose a site in about 3 feet of water or less and sample. Take a voucher plant of the major type for identification.

Mesocosm Sampling Protocols

Contents:

General site sampling

Rev. Val Stanley 05/30/2013. Copied field protocol and made minor changes.
Rev. Amy Myrbo 6/25/2013. Prior to, during, and after day out with field crews, and in consultation with Ed Swain and Nate Johnson, wrote procedure for mesocosm sampling.
Rev. Ed Swain 6/27/2013 to fix typos and change "pore water" to "porewater"
Rev. Amy Myrbo 7/19/2013 to reflect serum bottle sinking protocol

The ⇓ symbol indicates that data should be recorded at this point, or that a note should be made that a sample was taken.

Overall order of activities:

1. Porewater sampling using Rhizons
2. Water profiling using Hydrolab Quanta or equivalent instrument
3. Water sampling
4. Mini-coring next to peeper

(The previous four steps must take place prior to the peeper being retrieved. Step 1 should be started two hours prior to scheduled peeper retrieval. Step 4 should take place immediately before peeper retrieval. Let Nate Johnson's crew know when you are ready to proceed with Step 4.)

5. Porewater pH measurement
6. Hach color wheel analysis *(can occur whenever convenient)*

Wear long nitrile gloves at all times while working in the mesocosms. Change into new gloves right before you put your hands into the water. If you use sunscreen, thoroughly rinse your forearms at the pump before putting your hands into the mesocosm water.

Preparation:

- Review equipment checklists and restock any required equipment.
- Soak Rhizons. ⇓
- Calibrate Hach pH meter: Complete calibration log that goes with the meter, noting date, time, calibration, and who completed it. ⇓ Insert pH probe into small, color-coded vial with pH 7.0 solution. Take reading and record as "Before." Calibrate, but do not record the value that appears on the screen. Take another reading and record as "After." Discard used

- solution into hazardous waste container and re-fill vial from pH 7.0 bottle. Rinse probe with deionized water, then repeat process with pH 4.0 solution. Rinse probe again and re-cap in storage solution. Complete process similarly with 1001 µS conductivity solution—using small vial of solution, calibrate to 1001 µS spC. Discard used solution into hazardous waste container. Refill small vial of solution, rinse probe, and re-cap in storage solution. Ensure that calibration log was properly completed, leaving no fields blank, and record calibrator's initials. ⇓
- Calibrate Hydrolab/Quanta for pH and SpC (every site) ⇓ and DO (weekly) ⇓. Note in calibration log. The Hach pH and SpC meter will not need to be recalibrated between mesocosm readings.
- Make a table with the designations for the four peeper locations to be sampled that day, so that you can refer to it during the day to avoid confusion. Also prepare all labels. An example of a complete mesocosm site and sample designation is S29HA MS-199-1. Labels comprise the following information:
    - MS-site (MS-###);
    - (for stratigraphic sediment samples only:) depth designator for LacCore ("1" for top sample, "2" for second sample, etc.);
    - UMD sample ID
        - Tank code, S# or S##
        - H, M, L, or C for high, medium, low, and control, respectively
        - A for the root zone side or B for the rootless side
- Fill in site information. Record weather conditions. Note which equipment you are using. ⇓
- Take site photos: Take an overview photograph from the gate of the mesocosm enclosure, framing all 30 of the sulfate treatment tanks. Photograph each mesocosm you are working on that day, making sure to include in the frame the code written on the edge of the mesocosm tank. Review photos to ensure that each has acceptable exposure and focus. Delete and re-take any that are unacceptable. ⇓

Porewater sampling:

- Prepare two Rhizons per peeper (usually 8 total, plus 2 spares) with tubing and needles, Teflon-taping all connections to seal. With a needle, puncture the Teflon tape connections so that water can flow through easily.
- Prepare one serum bottle per Rhizon by attaching taping four nails that have been pre-washed (detergent and water) and rinsed to the outside of the serum bottle to weigh it down so that it sits on the sediment surface but does not sink in. Use long (24 inch) Rhizon tubing if available, and secure serum bottles to ring stands next to tank.
- Evacuate serum bottle for metals first, using a needle and hand pump. Pump to approximately 25 inches Hg internal pressure, then remove needle slowly. Put on a new pair of nitrile gloves. Insert the Rhizon vertically into the sediment of the mesocosm about 15 cm away from the peeper so that the clear connector is just below the sediment surface. Submerge the serum bottle near the Rhizon and pierce the septum of the serum bottle with the Rhizon needle. Leave to draw until fluid reaches the desired volume of 70 mL. When finished sipping, remove the needle from the bottle before removing the Rhizon from the core. Remove needle slowly to avoid air invasion.
- Evacuate sulfide bottle, which is preloaded with zinc acetate and has a nitrogen headspace. Put on a new pair of nitrile gloves. Place Rhizon end into sediment as above, and then

insert the needle into an evacuated "sacrificial" bottle to draw air out of the sipper. Once you see water pulling through the tubing into the bottle, remove the needle and insert into

- the sulfide bottle, using the same technique as above. Do not re-use needles from sulfide bottle in other bottles as this bottle contains zinc acetate and may contaminate other bottles. Record start weight of sulfide bottle. ⇓ Sip 50 mL of porewater into sulfide bottle. At end of this sipping, check that weight written on the bottle is not obliterated or caused to bleed.
- *Take practical samples – as much as you can get until it gets slow. Ensure that top of Rhizon is always below the sediment surface, sipping porewater, and not above it, sipping the overlying water.* ***It is particularly important that air is not introduced into the sulfide sample.***

Water profile and sampling:

- Turn on Hydrolab circulator and deploy Hydrolab to collect multiparameter data at 10 cm intervals. Measure from surface (first measurement) to just short of sediment-water interface (last measurement), at 10 cm intervals, which are taped off on the instrument's cord. The last interval may be less than 10 cm in length, so make sure to record the ending depth on the form (1-9 cm below the previous). ⇓ Record temperature (T, in units of ˚C), specific conductance (spC, in units of μS/cm or mS/cm), pH, and dissolved oxygen (DO, in mg/L). Note spC units. ⇓ Rinse probes with deionized water before replacing cap containing storage solution.
- Put on a new pair of nitrile gloves. Being careful not to disturb the sediment or submerged or floating plant material and scum, collect water samples by holding each subsample bottle (not the master amber bottle) a few cm below the water surface to fill. ⇓ Note any disturbance of the sediment that occurred during this activity. ⇓ Tighten all caps to ensure that samples are tightly sealed. Label and place all samples in cooler.

Mini core procedure:

- Set up glove bag and fill/flush with N_2 for at least 15 minutes.
- Cut a section of 1.5" tubing of about 25 cm in length. Insert a top piston and thread the rod onto the threaded top hole of the top piston. Position a board or sawhorse across the mesocosm near the peeper. Lower the mini-corer and rod assembly so that the base of the corer is level with the second peeper slot visible above the sediment surface. Clamp the rod and hold it tightly to keep the piston in place vertically. Wearing gloves, push the tubing down until it hits the sand at the base of the soft sediment, being careful not to overpenetrate and lose the piston out the top of the tube. Unclamp the rod and carefully lift up the rod and tube together. Wearing gloves, place a bottom piston at the bottom of the core tube before lifting the core tube out of the water. Stand the core tube on top of the bottom piston on top of the board. Using a piece of rubber tubing, siphon the water out of the tube above the piston. Using the pipe cutter, cut the tubing just above the black gasket of the top piston. Carefully remove the top piston by gently rocking the rod to one side to break the vacuum. Insert the bottom piston into the bottom of the tube. Move the core to the extruder. Measure the length of the sediment. ⇓
- Note whether or not sediment smells like hydrogen sulfide, H_2S. ⇓
- Using the HTH extruder, extrude the core at intervals of 1.5 cm (three turns) for the top sample, 1 cm (two turns) for the second sample, and 1.5 cm for each sample below that. For

more detail on the stratigraphic AVS technique, see "Mesocosm Stratigraphic AVS Sampling" SOP. Place each sample in a specimen cup, labeled in advance on both the lid and cup with complete stratigraphic sediment label as described above. Sample down to the seventh

sample (equivalent to 10 cm of sediment) or farther; if you reach the sand, stop and do not sample. Immediately place specimen cups in a nitrogen atmosphere *without caps on*.

- Under a nitrogen atmosphere in the glove bag, thoroughly mix each sample with a spatula. Remove half of the sample into a small Nalgene jar. Add the small pre-measured portion of zinc acetate to the small plastic jar, mix well, blow off headspace with N_2, and cap. Place plastic jar inside glass jar (still under the nitrogen atmosphere), blow off headspace of the glass jar, and cap. Label as above. Check caps and place AVS sample on dry ice and metals sample on ice in coolers. Note number of samples extruded on datasheet and write down sample IDs for recordkeeping.

Porewater pH measurement:

- Put on a new pair of nitrile gloves. Insert the pH probe below sediment surface in the mesocosm to 5 cm depth marked on probe. Hold there until reading stabilizes. Record reading. ⇓

Hach water color:

Fill outer test tube with clear water, either distilled water or clear drinking water so that the meniscus is even with the upper line. Collect mesocosm water in the same way as water samples, holding the test tube under water to fill without collecting particles. Inner test tube should be filled to the same level as the reference tube. Look through tubes towards sky and turn color wheel until the two tubes' colors match. Record value. If water is too colored (>100), remove mirror and multiply value by 5. If still too colored, leave mirror out and dilute sample by half (empty to lower line and add distilled water to reach the second of the three lines), and then multiply the observed value by 10. Rinse mesocosm sample tube with deionized water. ⇓

HTH core collection *(First and last sampling dates of the season only):*

- Rinse polycarbonate HTH corer tube. ⇓ Collect one core per peeper. Retain tension on rope while pushing gravity corer deeper into sediment. Pull corer up and – keeping bottom end of core underwater and holding core upright – insert piston. Always hold cores upright. Avoid any area where sediment may have been disturbed.

End of day wrap-up:

- Review data sheet to ensure that all fields have been entered. Do not leave any spaces blank. Make any necessary field notes. ⇓
- Complete MDH Chain of Custody form for samples: Using attached model, fill out one form for each site. Record names of both samplers, location ID, sample point, date, time when

samples were collected (all samples should show the same time, when first samples were pulled), and coordinates (latitude/longitude) of the site (under Sampler Comments). Sign form. All fields pertaining to Matrix code, quantity of containers/preservatives, and analyses should be already completed on the form. Direct any questions about form to Ed Swain. Place forms in Ziploc bag inside of cooler with samples. ⇓

- Enter data daily into database.
- Upload and name photos in a new folder in Transfers. Folder name should contain Location ID (MS-xxx) and Sample Point (Site Name/designation). All files should include the Location ID in their names.
  Re-freeze cold packs as needed and ensure that there is sufficient dry ice to keep AVS samples frozen.
- Bill supplies and services for the day.
- Send a report of the day's activities to the SO4-project listserv, and insert relevant text in the "Additional Comments" text field on the field data sheet in FileMaker.
- When relinquishing samples to MPCA or MDH representative, complete "Relinquished by/Affiliation" along with date and time on lower part of Chain-of-Custody form. The accepting representative must complete the "Accepted by/Affiliation" portion of the form.

Stratigraphic AVS sampling

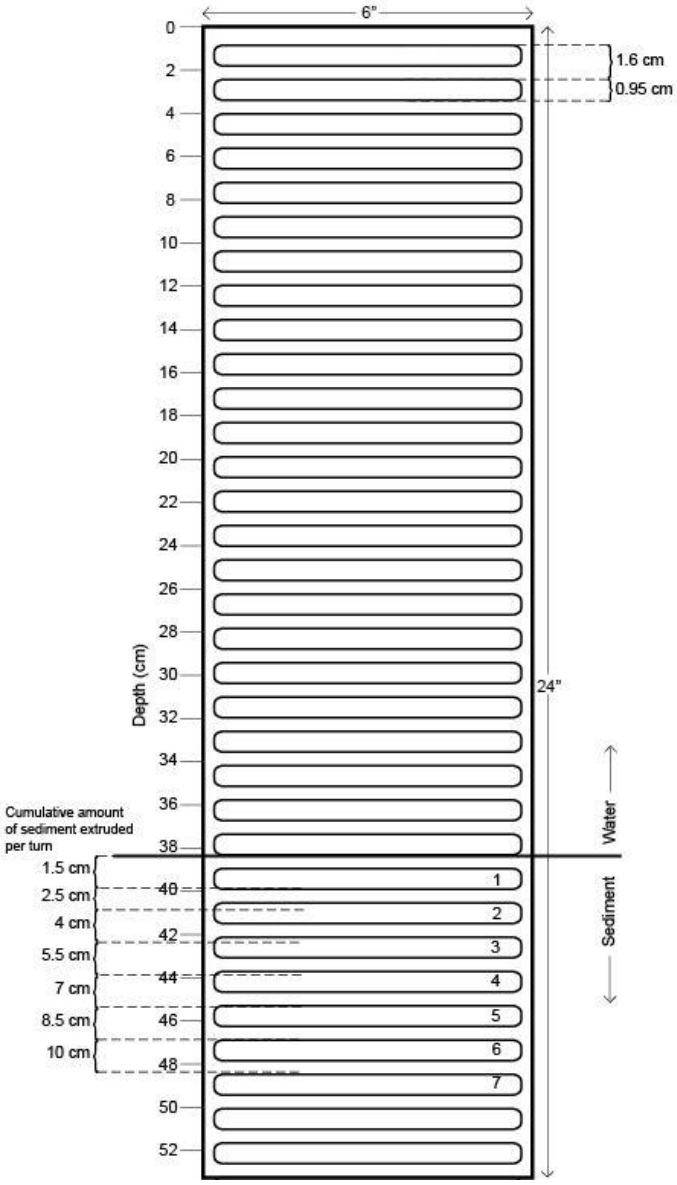
Stratigraphic bulk sediment samples for the analysis of extractable metals (including iron), extractable phosphorus, and acid-volatile sulfide (AVS; also called acid-volatile sulfur) were taken during the 2013 field season from mesocosm tanks at the University of Minnesota Duluth Research and Field Studies Center. Timing of sample retrieval corresponded to the pulling of in situ pore water samplers (peepers) that had been in place in the mesocosm sediment for two to three weeks. See Johnson's report for a detailed discussion of in situ pore water sampling. The aim of stratigraphic bulk sediment sampling in the mesocosm tanks is to relate water chemistry data from the peepers to solid phase metals, especially iron, and acid-volatile sulfide data. Phosphorus was analyzed because the reaction of iron and sulfide can make phosphorus more bioavailable. Water chemistry data is obtained from each peeper cell. The distance between the top of one individual each peeper cell to the top of the next peeper cell is approximately 1.6 cm. Because each extruder turn corresponds to 0.5 cm of sediment extruded, it is easy to match up water chemistry and AVS data at approximate 1.5 cm intervals. See Figure 1 (attached porewater peeper diagram) for more information.

In order to obtain stratigraphic bulk sediment samples adjacent to the peeper in each tank, a simple piston coring technique is used to take a 3.8 cm diameter core with polycarbonate tubing, following the mini-core procedure. See "mini-coring procedure" in "General site sampling" protocol above for information on coring procedure and extrusion.

Correlating solid sediment and water chemistry data:

The correlation between the sediment and porewater peeper assumes that the sediment-water interface is at the top of the first peeper cell. See porewater peeper diagram and table below. A full counter-clockwise turn of the extruder corresponds to 0.5 cm of extruded sediment, so in order to extrude at a corresponding interval with the pore water peeper cells (~1.6 cm), the extruder was turned three times, except for the second turn set, which was only two turns. The first sediment extrusion will match from the top of the non-cell area (white space above first peeper cell) to the bottom of the first peeper cell. The second sediment extrusion will match from the bottom of the first peeper cell to the middle of the second, and of all the extrusions, matches up to the porewater peeper data least well (see Figure 1). The following turns will encompass some non-cell area and most of the peeper cell. The reasoning behind including a two-turn set is that the overall offset (between the total sediment extruded and peeper depth) is significantly less than when doing all three turn sets. There is still some error, with the modified turning set, but the offset is 0.7 cm for 10 cm sediment extruded when substituting a two-turn set instead of 1.1 cm when not.

Figure 1. Diagram of porewater sampler (peeper) with dimensions and corresponding depths in a sampled mesocosm tank.



Turn Set	# Extruder Turns	Total sediment extruded (cm)	Peeper depth (cm)	Cumulative Offset (cm)	Percentage Peeper Analyzed in AVS samples
1	3	1.5	1.6	0.1	100% Peeper 1
2	2	2.5	2.5	0.0	38% Peeper 2
3	3	4.0	4.0	0.0	62% Peeper 2, 24% Peeper 3
4	3	5.5	5.9	0.4	76% Peeper 3, 15% Peeper 4
5	3	7.0	7.5	0.5	85% Peeper 4, 5% Peeper 5
6	3	8.5	9.1	0.6	95% Peeper 5
7	3	10.0	10.7	0.7	100% Peeper 6

Table 1. Correlation between sediment extrusion and pore water peeper depth substituting one two-turn set. Note that the column "percentage peeper analyzed in AVS samples" gives an indication of AVS sample accuracy.

Mesocosm synoptic sampling

The mesocosm synoptic sampling includes selected procedures as described in the "Site sampling" protocol with some amendments, which are noted below. The purpose of the mesocosm synoptic is to constrain temporal variability by sampling porewater and surface water on a single day for all 30 mesocosm tanks.

The ⇓ symbol indicates that data should be recorded at this point, or that a note should be made that a sample was taken.

Synoptic overall order of activities:

1. Porewater sampling using Rhizons and serum bottles
2. Water sampling
3. Porewater pH measurement

Porewater sampling is conducted using four Rhizon and serum bottle set-ups per mesocosm tank. Two serum bottles are used to sample on the rooted side (A) and two on the rootless side (B). On each side, one metals serum bottle and one sulfide serum bottle. Water sampling includes one water sampling kit per mesocosm tank (one nutrient bottle, one metals bottle, and one general chemistry bottle). A porewater pH measurement is also taken on both sides of the tank (A and B).

Wear long nitrile gloves at all times while working in the mesocosms. Change into new gloves right before you put your hands into the water. If you use sunscreen, thoroughly rinse your forearms at the pump before putting your hands into the mesocosm water.

Preparation:

- Review equipment checklists and restock any required equipment.
- Soak rhizons (4 per tank plus 10% extra). ⇓
- Calibrate Hach pH meter: Complete calibration log that goes with the meter, noting date, time, calibration, and who completed it. ⇓ Insert pH probe into small, color-coded vial with pH 7.0 solution. Take reading and record as "Before." Calibrate, but do not record the value that appears on the screen. Take another reading and record as "After." Discard used solution into hazardous waste container and re-fill vial from pH 7.0 bottle. Rinse probe with deionized water, then repeat process with pH 4.0 and 10.0 solution. Rinse probe again and re-cap in storage solution. Complete process similarly with 1001 µS conductivity solution—using small vial of solution, calibrate to 1001 µS spC. Discard used solution into hazardous waste container. Refill small vial of solution, rinse probe, and re-cap in storage solution. Ensure that calibration log was properly completed, leaving no fields blank, and record calibrator's initials. ⇓
- Calibrate Hydrolab Quanta for pH and SpC ⇓ and DO (weekly) ⇓. The Hach pH and SpC meter will not need to be recalibrated between mesocosm readings. Note in calibration log.
- Make a table with the designations for the mesocosm locations to be sampled that day, so that you can refer to it during the day to avoid confusion. Also prepare all labels. An

example of a complete mesocosm site and sample designation is S29HA MS-199. Labels comprise the following information:

- MS-site (MS-###);
- UMD sample ID
  - Tank code, S# or S##
  - H, M, L, or C for high, medium, low, and control, respectively
  - A for the root zone side or B for the rootless side

- Fill in site information. Record weather conditions. Note which equipment you are using. ⇓
- Take site photos: Take an overview photograph from the gate of the mesocosm enclosure, framing all 30 of the sulfate treatment tanks. Photograph each mesocosm you are working on that day, making sure to include in the frame the code written on the edge of the mesocosm tank. Review photos to ensure that each has acceptable exposure and focus. Delete and re-take any that are unacceptable. ⇓

Porewater sampling:

- Prepare four Rhizons per mesocosm tank (plus 10% extra) with tubing and needles, Teflon-taping all connections to seal. With a needle, puncture the Teflon tape connections so that water can flow through easily.
- Prepare one serum bottle per Rhizon by taping 4 nails that have been pre-washed (detergent and water) and rinsed to the outside of the serum bottle to weigh it down so that it sits on the sediment surface but does not sink in. Use long (24 inch) Rhizon tubing if available, and secure serum bottles to ring stands next to tank.
- Using a needle and the MPCA's automatic vacuum pump, evacuate the serum bottles. This can be done the night before the synoptic will be conducted to save time. Evacuate serum bottle for metals first. Pump to approximately 25 inches Hg internal pressure, then remove needle slowly. Put on a new pair of nitrile gloves. Insert the Rhizon vertically into the sediment of the mesocosm so that the clear connector is just below the sediment surface. Make sure the Rhizon is inserted away from any rice plants that are growing. Submerge the serum bottle near the Rhizon and pierce the septum of the serum bottle with the Rhizon needle. Leave to draw until fluid reaches the desired volume of 70 mL. When finished sipping, remove the needle from the bottle before removing the Rhizon from the core. Remove needle slowly to avoid air invasion.
- Evacuate sulfide bottle, which is preloaded with zinc acetate and has a nitrogen headspace. Put on a new pair of nitrile gloves. Place Rhizon end into sediment as above, and then insert the needle into an evacuated "sacrificial" bottle to draw air out of the sipper. Once you see water pulling through the tubing into the bottle, remove the needle and insert into the sulfide bottle, using the same technique as above. Do not re-use needles from sulfide bottle in other bottles as this bottle contains zinc acetate and may contaminate other bottles. Record start weight of sulfide bottle. ⇓ Sip 50 mL of porewater into sulfide bottle. At end of this sipping, check that weight written on the bottle is not obliterated or caused to bleed.
- *Take practical samples – as much as you can get until it gets slow. Ensure that top of Rhizon is always below the sediment surface, sipping porewater, and not above it, sipping the*

overlying water. It is particularly important that air is not introduced into the sulfide sample.

Water Sampling:

- Put on a new pair of nitrile gloves. Being careful not to disturb the sediment or submerged or floating plant material and scum, collect water samples by holding each subsample bottle (not the master amber bottle) a few cm below the water surface to fill. ⇓ Note any disturbance of the sediment that occurred during this activity. ⇓ Tighten all caps to ensure that samples are tightly sealed. Label and place all samples in cooler.

Porewater pH measurement:

- Put on a new pair of nitrile gloves. Insert the pH probe below sediment surface in the mesocosm to 5 cm depth marked on probe. Hold there until reading stabilizes. Record reading. ⇓

End of day wrap-up:

- Review data sheet to ensure that all fields have been entered. Do not leave any spaces blank. Make any necessary field notes. ⇓
- Complete MDH Chain of Custody form for samples: Using attached model, fill out one form for each site. Record names of both samplers, location ID, sample point, date, time when samples were collected (all samples should show the same time, when first samples were pulled), and coordinates (latitude/longitude) of the site (under Sampler Comments). Sign form. All fields pertaining to Matrix code, quantity of containers/preservatives, and analyses should be already completed on the form. Direct any questions about form to Ed Swain. Place forms in Ziploc bag inside of cooler with samples. ⇓
- Enter data daily into database.
- Upload and name photos in a new folder in Transfers. Folder name should contain Location ID (MS-xxx) and Sample Point (Site Name/designation). All files should include the Location ID in their names.
- Re-freeze cold packs as needed.
- Bill supplies and services for the day.
- Send a report of the day's activities to the SO4-project listserv, and insert relevant text in the "Additional Comments" text field on the field data sheet in FileMaker.
- When relinquishing samples to MPCA or MDH representative, complete "Relinquished by/Affiliation" along with date and time on lower part of Chain-of-Custody form. The accepting representative must complete the "Accepted by/Affiliation" portion of the form.

Final sampling

First draft by Amy Myrbo; revised by Ed Swain 10/3/2013 and Amy Myrbo 10/4/2013

Please continue to be careful of the plants when you sample. They are senescent, but Brad and John are still collecting data on them. The plants are delicate; try not to bend or break them, as they will be harvested and weighed. Take a photo of each mesocosm to document the state of the plants prior to beginning work in the mesocosms.

One point of this sampling event is to compare porewater methods: *in situ* Rhizon sampling like we've done over the summer in the mesocosms vs. Rhizon sampling from short cores as we've done at the field sites. By using both methods side by side, we'll determine how comparable the porewater chemical datasets are.

Overview:
In each of **eight** mesocosms (the eight remaining tanks of interest that have not yet been sampled), on the **rooted side only**:

- Surface water sampling;
- *In situ* Rhizon sampling (two serum bottles);
- *In situ* porewater pH measurement;
- Three HTH-size 10-cm long cores: two for pore water sipping, and a third for solid-phase analysis after porewater pH measurement in that core.
- One smaller diameter core, cut stratigraphically for preservation of AVS, SCWRS metals, and CNS samples.

Supplies:

- Mason jars are fine as the outer glass jar for AVS sampling.
- MDH lab just gave Ed 125 more serum bottles that could be used for this sampling, plus zinc AVS preservative vials. The field crew should obtain these from Ed's garage.
- Small-diameter coring equipment (core tubes, pistons of both sizes, extruders, and supporting equipment)
- Dry ice for AVS
- Sample bottles for about 60 stratigraphic AVS samples and 8 AVS samples from the large homogenized cores.
- Sample bottles for 60 stratigraphic samples for Science Museum analysis.
- Specimen cups or large poly-cons for SCWRS samples.
- Poly-cons for immediate subsampling for CNS (½ of strat sample for AVS, then split remaining ½ into SCWRS and CNS)

For the coring of the mesocosms, please cut ten pieces of LacCore standard “HTH size” polycarb tubing, 20-25 cm long. These do not need HTH wings on them.

Find six appropriate endcaps (marked as 2 3/4") and punch or cut a ~1 cm diameter circular hole in the center of the cap. Make sure the cut is clean, i.e., that there are no cuts extending from the hole.

Choice of mesocosms:
control: 4, 13
50 ppm SO4: 6, 16
150 ppm SO4: 11, 27
300 ppm SO4: 1, 14

All samples should be taken towards the middle of the mesocosm, **at least 6 inches away from the sides of the mesocosm, and away from peeper site**. Work around the wild rice plants. Do not knock them over.

What to sample:
***In situ* - in each mesocosm:**
First, before disturbing the sediment, perform a regular sampling of the surface water (3 MDH bottles);

- Collect surface was DO, pH, and spC using Hach meter
- Conduct DO profile using Hach meter
- Insert probe for pH of porewater;
- Insert Rhizon directly into the sediment: 1 for metals; 1 for sulfide (with preservative and stir bar).
- Hach color wheel analysis

***"In polycarbo"* - on cores collected from each mesocosm after sipping is complete:**
Two cores for porewater sipping: 1 for metals; 1 for sulfide (with preservative and stir bar);
One core for pH of porewater and solid phase analyses (CNS; Organic grain size; LOI; TIC; AVS);
One small diameter core for stratigraphic sampling.

How to sample:
The technique for coring the mesocosms will be a bit different from the field site method, but is based on the same principles of the function of a corer with a flap or cap at the top that is closed after the core tube has been inserted into the sediment.

- Insert three short (~20 cm) tubes near each other, and at least six inches away from the tank walls, into the rooted zone of a mesocosm. Push down until you feel resistance and the sand at the bottom of the organic sediment. The top of the tube may be under the water, which is good.
- Place an endcap (with hole) securely on each tube. Place a piece of duct tape (3M duct tape sticks pretty well to wet surfaces) over the hole in the cap and press to seal as well as possible.

- Leaving the 3 cores in place, and using the sawhorse method used throughout this field season, take the small diameter stratigraphic core and cut into the same 1.5 cm intervals and process as was done monthly this past summer.
- After the small diameter stratigraphic samples are stored, remove the three larger diameter cores: Twisting the core tube a little at the beginning, and holding a finger over the hole covered with duct tape, remove the cores one at a time. Get a bottom piston started in the base of the tube before the base of the core comes out of the water. Holding the bottom piston in, stand the core up on the ground or the extruder stand, and vent the cap (remove the tape) to relieve pressure and allow the bottom piston to enter the tube. Sip porewater or measure pH from this core as per the normal field protocol. Repeat with the remaining two cores. Note the length of sediment above sand in each core, if less than 10 cm.

The core used to measure pH is the one that will be extruded and homogenized for solid phase analyses as per the usual field protocol. The sediment from the two cores used for porewater sipping should be **returned** to that area of the appropriate mesocosm.

Sample transfer:
Gery will pick the samples up at Ed's garage and deliver them to MDH. Gery's cell phone number is 612-850-8071—please call that when samples are dropped off at Ed's garage.

Homogenizing and subsampling bulk sediment samples

Draft v.1.0 (10/30/2013) by Sean Rogers
Revised by A. Myrbo v.2.0 (11/7/2013)

Equipment:
Drill press
Workforce 1-gallon helix paint mixer with 0.25 inch stainless steel hex shaft.
1 oz. red polycon for TP/P-frac/metals sample
1oz. red polycon for CNS sample
1oz. red polycon for TIC sample
Specimen cup for organic grain size sample
Crucibles (pre-weighed) for LOI samples
16 quart plastic sterilite bin
1000 ml beakers (2) for cleaning
400 mL Kimax beaker labeled with its sample ID
Spatulas
10 or 12 cc sampling syringe with tip cut off
1 cc syringe with tip cut off
KimWipes
DI water
Label tape or label

Safety:
The primary hazard in drill press homogenizing is injury to eyes from material that is splashed out of the container while the sediment is being mixed. Proper eye protection should be worn while operating machinery.

Procedure:
1. Insert helix paint mixer into the chuck of the drill press and tighten with chuck key.
2. Adjust the spindle speed to 375/500/750 rpm setting (the drill needs to be running when you do this).
3. Place the sterilite bin on the table of the drill press. This will contain any spills and splashes as the material is being mixed and allow for easy cleanup between samples.
4. Shake the bulk sediment sample thoroughly by hand to mix some of the overlaying water with the sediment. This will prevent a sudden splash-out, and loss of water content when the drill is activated.
5. Place the bulk sediment sample jar in the sterilite bin.
6. Lower the helix paint mixer into the sediment by turning the handle counterclockwise.
7. Holding the bulk container firmly, activate the spindle by flipping the switch to the low-speed setting.
8. Thoroughly homogenize the sample by lowering the paint mixer all the way to the bottom of the jar. Move the jar around so the paint mixer comes in contact with the sides of the jar. The paint mixer/spindle can be raised and lowered by slight turns of the wheel.
9. With the drill running and mixing the sample, begin to pull your subsamples with syringes/spatulas.

10. When all necessary subsamples have been collected, turn drill off, remove helix paint mixer with chuck key. Rinse clean with tap water followed by another rinse with low-purity DI water. Wipe dry with KimWipes.

Volumetric subsampling (phytoliths):

1. Weigh labeled beaker and record weight.
2. Pull sample of sediment with spatula and pack into 1 cc volume space of syringe.
3. Discharge sediment from syringe into the beaker.
4. Weigh beaker plus sediment and record weight.
5. Clean syringe and spatula in between sampling with DI water and KimWipes.

Volumetric subsampling (LOI):

1. Weigh crucibles and record weight.
2. Pull ~2 cc of sediment with spatula.
3. Empty sediment into crucible.
4. Cover crucibles in-between samples to avoid evaporation of water content.
5. Weigh crucible plus sediment and record weight.

Known-mass sampling (organic grain size):

Weigh approximately 20 g of sediment into a tared specimen cup.

All other subsampling:

Use water content data (from LOI) to calculate a minimum mass (2 g dry equivalent) and subsample this much (but not a lot more)

Loss-on-Ignition

Purpose:
Water, organic matter, carbonate mineral, and siliciclastic+diatom content are estimated by sequentially measuring weight loss in sediment core subsamples after heating at selected temperatures.

A compositional profile can be generated rapidly and for very low cost. This profile is sufficient to develop a general sense of core stratigraphy and often is sufficient for correlation between cores.

The results are accurate to 1-2% for organic matter and carbonate in sediment with over 10% organic matter. In clay- or diatom-rich sediment, water of hydration is lost during the carbonate burn, resulting in errors of up to 5% for carbonate analyses (and "false positive" carbonate content in carbonate-free sediments). If high precision (0.1%) is needed, or if sediment is in short supply, coulometric analysis is recommended.

A nonprogrammable Lab Line L-C oven is used for the 100ºC drying step. Other drying ovens are also available in the lab. A drying oven rather than a furnace is used for the 100°C step because the furnaces ramp up to temperature quickly and may overshoot 100°C by an amount that could affect the analysis.

A Fisher Scientific Isotemp programmable muffle furnace is used for the 550°C and 1000°C steps. This is a multiple mode instrument capable of reaching 1125°C and controlling temperatures to better than ± 15ºC with a ± 10ºC temperature uniformity. The programmable circuit will provide the necessary corrections to maintain temperatures at established set point. This furnace holds 200 samples at a time. Another furnace, the Lindberg Blue M, is available for use as a backup, but only holds 50 samples. This is a 2 mode instrument capable of controlling temperatures to better than ± 10ºC. The automatic reset circuit will provide the necessary corrections to maintain temperatures at established set point.

Procedure Summary:
Subsamples are placed in weighed crucibles and weighed. Weight loss is measured after heating at 100ºC overnight to remove water, at 550ºC for four hours to remove organic matter, and at 1000ºC for two hours to remove carbonates. After each heating step, the firebrick holding crucibles is allowed to cool completely in the oven or furnace before weighing, or placed in a desiccator if crucibles cannot be weighed immediately. Samples must be cool so that convection currents do not affect the balance, and kept in the oven, furnace, or desiccator so that they do not absorb atmospheric water. Samples must not be placed in a non-venting desiccator when warm.

Ash left at the end of the procedure can be saved for analysis of remaining elements as oxides.

Only one heating step can be accomplished each day, because the 100°C drying time, and the ramp-up and cool-down times of the furnaces are all >8-10 hours. The user should thus plan five days, ideally consecutively:

Day 1 Weigh crucibles (if necessary), subsample, and weigh (initial or wet weight); place samples in drying oven at *100°C* (allow several hours for these steps, depending on subsampling complexity).
Day 2 Turn off oven, let samples cool, weigh (100°C, dry weight, or water loss); place samples in furnace at *550°C for 4 hrs*
Day 3 Weigh (550°C or organic matter loss); place samples in furnace at *1000°C for 2 hr*s
Day 4 Weigh (1000°C or carbonate loss), discard or save sample residues, wash crucibles, place crucibles back in trays, place trays of crucibles in furnace at 1000°C for 2 hours to completely clean the crucibles.
Day 5 Make sure crucibles are cool, remove trays from drying oven, cover with foil, and place in desiccator for reuse. Label these trays as clean and ready to be reused.

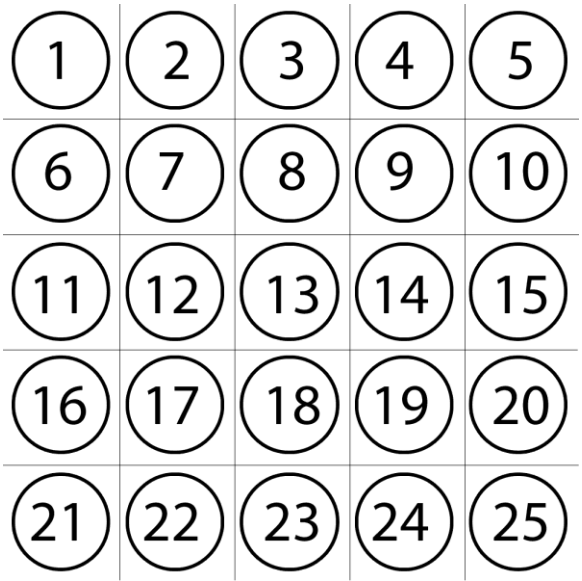
Equipment:
Ceramic crucibles
Firebricks drilled to accept crucibles
Sampling device (spatula, syringe)
Desiccator(s)
Drying oven
Muffle furnace capable of reaching 1000°C
Balance weighing in grams to 4 decimal places

Safety:
The most obvious hazard in LOI is being burned by hot samples fresh out of the furnace. Be patient. The high-temp gloves and mitts are only good to about 350°C and can be awkward to use.

The muffle furnaces each have a thermocouple (looks like a white stick with metal protruding from the end) which penetrates in through the back of the chamber. It is easily damaged, so be careful not to bump it when adding or removing samples.

Procedure Detail:
1. Select and weigh 25 crucibles per fire brick tray. You can prepare up to 200 samples (eight trays) for analysis at a time.
    a. Never touch crucibles with your hands. Skin oils will add weight and introduce error to your results. Always use a pair of tweezers when handling crucibles.

   b. The crucibles are numbered with permanent glaze. Check to see that they are in order in the brick (see below) and that you are starting with the lowest numbered crucible in your tray. Keep good notes! As soon as you've mixed things up the data are useless.
   c. When not burning or being weighed, the crucibles and the samples they contain must be stored in a desiccator. Make sure there is enough desiccator space for the number of samples you hope to analyze. Wait until the samples are cool (<30°C) before putting them in the desiccator: the decrease in pressure upon cooling of the air in the desiccator will vacuum-seal the desiccator shut and it'll be very difficult to open.
   d. Always remove aluminum foil before placing trays in drying oven or furnace
2. Record your crucible weights in the LOI template spreadsheet [*saved in transfers/SOPs/LOI/ as* **LOI.Template.xls**]. This new template will highlight (in red) any weights entered that are clearly incorrect (i.e., less than or equal to the empty crucible weight or greater than or equal to the weight of the preceding burn). Care must still be taken to ensure correct values are entered into the spreadsheet.
3. Place some sample (~1-5 cc) in each crucible and weigh. Weighing should be done as soon as each tray is filled, do not wait to fill multiple trays before weighing. This is your *wet weight*. Record in LOI spreadsheet. *Note:* if you use the **LOI macro** (more about which later), the samples do not have to be volumetric.
4. Heat these samples at **100-105°C overnight or for at least 12 hours in** the drying oven. This will evaporate water and the resulting weight will be your *100°C weight*. Turn off the oven and let samples cool (until <30°C), before removing and weighing. If you cannot weigh the samples immediately after they cool, place in a desiccator with aluminum foil between each tray until you can weigh them.
5. After weighing and recording your *100°C weight*, return the samples to the furnace for a **4-hour burn at 550°C**. This will burn off organic matter. The following day, after samples have cooled in the furnace, samples can be weighed. If you cannot weigh the samples immediately after they cool, place in a desiccator with aluminum foil between each tray until you can weigh them. This will prevent samples from taking in air moisture and throwing off your weights. See furnace directions below.
6. Record your *550°C weight* burn weights in the spreadsheet and return the samples to the oven for a **2-hour 1000°C** burn. This will burn off a combination of carbonate material and some of the water stored in the lattice of clay minerals and diatom silica. See furnace directions below.
7. After cooling, record this *1000°C weight* as your final measurement. You may discard the sample remaining in the crucible, or save it for another analysis.
8. Run the LOI Macro. See directions below.
9. Clean the crucibles for the next user.
   a. Two buckets are needed and should be in or near the sink in room 680A. Fill one bucket



with warm tap water and add soap from the bottle labeled "Lab Soap" above the sink. Fill the other bucket with low-purity deionized (DI) water from carboy above the adjacent sink.

b. Remove any remaining residue and place the crucibles in the warm tap-water bath.
c. Using a brush found above the sink, scrub the crucibles until all baked on residue is gone. Some discoloration will remain.
d. Rinse the crucibles in the DI water bath, shake dry and place in the firebrick tray following the numbering system designated in the figure on the right.
e. Place all washed trays of crucibles in the furnace and burn at 1000°C for two hours (in the same manner as the carbonate burn).
f. The following day, remove all trays from the furnace, cover with aluminum foil and place in the appropriate desiccators. Use the "Cleaned 1000°C" crucible icons or label the trays "Cleaned and burned at 1000°C" between each tray so that the next person knows they are ready to be used.

How to use the Lab Line L-C oven:

1. Open the oven and place your trays on the shelving in the oven. Load in the top trays first to prevent contamination of the samples (and, similarly, remove the bottom trays first when emptying the oven). Close the oven door
2. Turn on the oven using the switch on the front panel.
3. Set the temperature knob about ¾ of the way between a setting of 4 and 5 to heat the oven to 100°C.
4. The oven does not have a temperature feedback control system, it is a good idea to periodically check the temperature on the oven to make sure it reaches 100°C but does not greatly exceed 100°C.

How to use the Isotemp muffle furnace in Ramp and Soak mode:

1. Open the flue on top of the furnace.
2. Turn on the Furnace using the switch on the front control panel.
3. Open the furnace and place your trays on the shelving in the furnace. Load in the top trays first to prevent contamination of the samples. The muffle furnace has a thermocouple (looks like a white stick with metal protruding from the end) which sticks in through the back of the chamber. It is easily damaged, so be careful when adding or removing samples not to bump it. Close the furnace door.
4. Verify that the Run LED is not on. If it is on, press Run until the light goes out.
5. Press the following sequence of buttons in the left most column.

Button	Top Display	Lower Display	Description
Menu	No	program	Furnace is not in program mode.
UP	Yes	program	Select yes to set program parameters
Menu	1	step	The first step in the program

Menu	SP (Set Point)	styp (step type)	is a set point.
Menu	(550 or 1000)°C	sp	This is the temp from the last time the program ran.
Up/Down	(550 or 1000)°C	sp	Use the up or down keys to set to 550 or 1000°C.
Menu	20° C	rate	The ramp up rate should always be 20°C for LOI.
Menu	No	retn (return)	No return for this step,
Menu	2	step	move on to step two in the program,
Menu	Soak	styp	a soak step.
Menu	(4 or 2)	hour	This is the length from the last time the program ran
Up/Down	(4 or 2)	hour	Use the up or down keys to set to 2 or 4 hours,
Menu	0	min	0 minutes,
Menu	0	sec	0 seconds,
Menu	No	retn	No return for this step.
Menu	3	step	Move on to step three in the program,
Menu	end	styp	to end the program,
Menu	off	end	by letting the furnace cool to room temp.
Menu	yes	save	Save the program.
Menu	actual temp	set temp	You have exited the program parameters.

6. Press run twice to run the program, the run light should be solid (not flashing) and the program will automatically start to ramp up to temperature. You can see the set temperature and the actual temperature on the display.
7. Once the furnace has cooled down, you can close the flue and turn off the furnace. If the alarm LED is lit up on the control panel please notify staff, this means that the actual furnace temperature exceeded the set temperature by more than 25°C
8. Remove the bottom shelf of samples first, to prevent contamination.

How to use the LOI Macro:
To use the macro [*saved in transfers/SOPs/LOI as* **LOI.Macro.xls**], the spreadsheet that contains your LOI data must be in the following format (column titles):

Depth, Crucible Weight, Wet Weight, Weight (100°C), Weight (550°C), Weight (1000°C)
or
Depth (Top), Depth (Bottom), Crucible Weight, Wet Weight, Weight (100°C), Weight (550°C), Weight (1000°C).

These columns and the data within them are the ONLY cells that may be filled in on the page, or the macro will malfunction. If you have supplementary data such as crucible number, core names, notes to self, etc., cut them from this spreadsheet and put them in another sheet in the same workbook.

Save the data spreadsheet as a **new file** (the macro overwrites the opened file) and close it. Open up the **LOI.Macro.xls** file. (Click "enable macros" in the warning window that pops up.) Go to Tools--> Macro--> Macros (or press Alt-F8). The "macro name" line should read "A1" and be highlighted. The first line of the section below A1 should read "LOI." Click "Run."

The next box that pops up asks for the file name (as saved on the spreadsheet), some info about the coring site (nonessential), whether your depth intervals are single or you've used two columns for top and base depths (determined by which format for column titles you used above), and whether you did a $CaCO_3$ (1000°C) burn. Once you've filled in and selected the appropriate options, click "OK" and the macro will perform its magic. It will probably not find your file at first, but you have the option of browsing for it.

The resulting data will be placed in columns to the right of your original data.

*For a comprehensive review of best practices and comparative LOI methods, please see Heiri et al, Journal of Paleolimnology 25, p. 101-110.

Organic grain size procedure

Amy Myrbo
V1. 12/2011
V2 10/2013 additions by Sean Rogers

Safety:
Similar to LOI. Use caution when handling materials that have been in the oven or furnace. Discard chipped or cracked crucibles.

Setup:
Sieve stack: 1mm, 500 um, 250 um; 400mL beaker underneath. Label each beaker and sieve stack. Keep same sieve stack on the same beaker.
Always handle crucibles with tweezers, never your fingers.
Weigh 1000° -burned crucibles and enter masses along with crucible numbers in spreadsheet.
Record sample ID and fraction designation (1mm, 500um, 250um, fine) for each crucible in the spreadsheet.

Sieving:
Spray through until each sieve holds only material coarser than the mesh
For each completed sieve, spray material to edge of sieve to collect material; transfer as much as possible to crucible using spatula, tweezers, etc. Then spray to edge again, and spray into crucible from the back of the sieve. Place inverted sieve onto a clean glass petri dish and blow any remaining material stuck onto the mesh with compressed air. This is a quantitative procedure, so be sure to transfer all of solid material. The amount of water does *not* matter, so don't worry about water on the outside of the crucible, etc. Centrifuge down samples that no longer fit in one crucible. Large plant pieces that do not fit easily in the crucible may be cut in pieces. Handle with tweezers and use a pre-cleaned pair of scissors. Do this in a clean glass petri dish set over a piece of white paper so that you can easily collect all the material back into the crucible
Allow the material in the beaker to settle, then sip off or decant water without removing any sediment.
After decanting, swirl material in beaker and immediately (but carefully) pour into a labeled test tube.
Centrifuge down, decant, vortex, quickly pour into crucible. Rinse remainder from centrifuge tube into crucible.

Loss on Ignition:
Follow general LOI SOP, with the following changes:
When a tray is full, place it carefully in the Isotemp muffle furnace and dry at 100°C (you do not need to weigh before drying).
Weigh cooled samples after drying.
Burn at 550°C.
Weigh cooled samples after burning.
Clean crucibles. When done with all samples, burn crucibles at 1000°C.

EAS: CHN/CNS analysis

Sampling protocol:

1. Measure out 15mg of woody samples, 55mg of mixed woody/sandy samples and 75mg of sandy samples into large tin containers using a three decimal place balance (weighing to 0.001 mg).
2. Use tweezers to fold each tin into a sphere and remove any protrusions.
3. Place each sample into the sample tray according to locations listed on Excel spreadsheet and make sure that each sample moves freely within the tray.
4. Measure out accompanying standards (BBOT, Methanonine and Acetanilide) into small tin containers and place in the sample tray according to masses and tray locations listed on Excel spreadsheet.
5. Repeat steps 2 and 3 with standards.
6. Keep trays taped closed and place in desiccator until samples are run.

EAS Computer Program:

1. Double click on EAS 32 desktop icon (Either on Desktop or Start Menu)
2. Click where it says "login" under the channel 1. Channel 2 has no function.
3. Type in your name under the analyst name. Hit enter
4. Once the Channel 1 Window opens, go to the Setting menu along the top and click on the Export option.
5. Once you get to the export window, click on the ellipses in the bottom-right of the screen to browse for the folder to which the file will export (Fig 2).

EAS Sample Table Setup:

1. Click on the Sample table Icon from the Channel 1 Window.
2. On the sample table, click File
    a. Click New or use existing file to make a copy (rename).
3. Type in a sample table name of your choosing, then the number of lines that you want your sample table to be (will generally be 49)
4. Copy and Paste your 'Sample Identifier' from Excel input file into 'Sample'
5. Change "Sample Type" for each sample to reflect either Bypass, Unknown, Standard, or Blank
6. Paste the masses into sample table.
7. The Filename for each sample should start with '%4n' followed by the 'Sample Identifier', eg. %4n Blank or %4n Acetanilide
    a. Should be able to copy and paste from Excel input File
8. Identify the level for each standard. Number them but the order in which they will be run.
9. Under 'Standard Name' select the correct standard from the pull down menu with in the cell (the known weight% for each element should appear after selection)
10. Check Mark those for analysis under the 'Run' column
11. Save Sample Table.

Running a Sample Table:

1. If running in EAS STANDALONE
    a. Press 'F3' on EA machine control panel so 'off' shows in upper right corner of the machine control panel

b. Open Purge valve on the autosampler
c. Check the alignment of the sample holes so that they line up with oven
d. Bypass the first sample hole and add samples in counter clockwise direction
e. Close lid and tighten top three bolts evenly
f. Press 'F3' and vent purge valve for 5-8 mins
g. Close purge valve
h. Wait 5-7 mins
i. Click 'Detector signal' within the flow chart of the EAS-Channel 1
    i. You should see a blue line that indicates the current voltage within the system. It will increase while the autosampler and purge valve are open.
    ii. Once the purge valve is closed, if the line remain constantly flat for >5 mins then proceed with the run.
    iii. If the blue line wavers, then there is a leak or atmosphere within the system from improper venting of purge valve.
        1. If so, repeat steps e-g (Purge valve venting and closing)
j. Assure that silver-cylindrical toggle switch (to the left of computer keyboard) is switched to 'EAS'
k. Press 'Remote' then "Enter' on the EA machine control panel
l. After saving sample table, click 'Run' within EAS Sample Table
    i. The first row should highlight in red, indicating the current sample for analysis
    ii. Sample table should queue up but a 'State: Running' should appear at the bottom of the Sample Table.
    iii. 'Running' should appear on the LCD screen of EA machine
m. Watch the first sample drop and check all is going well

2. After the sample table has completed
    a. Press 'Abort' then 'Enter' on the ECS Control Panel
    b. Press 'F3', standby gas saving mode

For all EAS Runs – Calibration Files:
This can be done during or after the run. However if you do it right after the first standard has run the remaining standards will be added automatically.

1. Open the Calibration Window by clicking the icon with a linear red line within an XY plot.
2. **Creating Calibration File**
    a. From the Calibration Window, go to File →Open, then open the calibration file that corresponds with your sample table name.
    b. You may need to open the chromatogram for the first standard within the Calibration Window.
    c. Enter retention time for Nitrogen as the number above the first peak in the graph.
    d. Enter retention time for Carbon as the number above the second peak in the graph.
    e. Enter retention time for Sulfur as the number above the third peak in the graph.

f. If you set the peaks for the first standard after is has completed and before the second standard run, the peaks for all remaining standards will be recognized and added to the calibration file. The peak area and mass will be added automatically.

g. If creating calibration curve after run has completed

i. Under the 'Weight' column enter your offline calculation of the weight percent

1. The weight percent is equal to the known %C, %N or %S (You can find this value from your sample table) multiplied by the weighed sample mass

a. This should also be calculated within your Excel input file so you can cut and paste the values into the calibration file

h. After all standards have been run,

i. Click on the Nitrogen tab at the bottom of the page (Fig. 7)

1. All of the points should lie on or very close to the line. The correlation factor should be near 1. (0.99 is acceptable, 0.999 is great, and 0.9999 is impressive)

ii. Click on the Carbon tab at the bottom of the page.

iii. Repeat Step 3.

1. All of the points should lie on or very close to the line. The correlation factor should be near 1. (0.99 is acceptable, 0.999 is great, and 0.9999 is impressive)

iv. Click on the Sulfur tab at the bottom of the page.

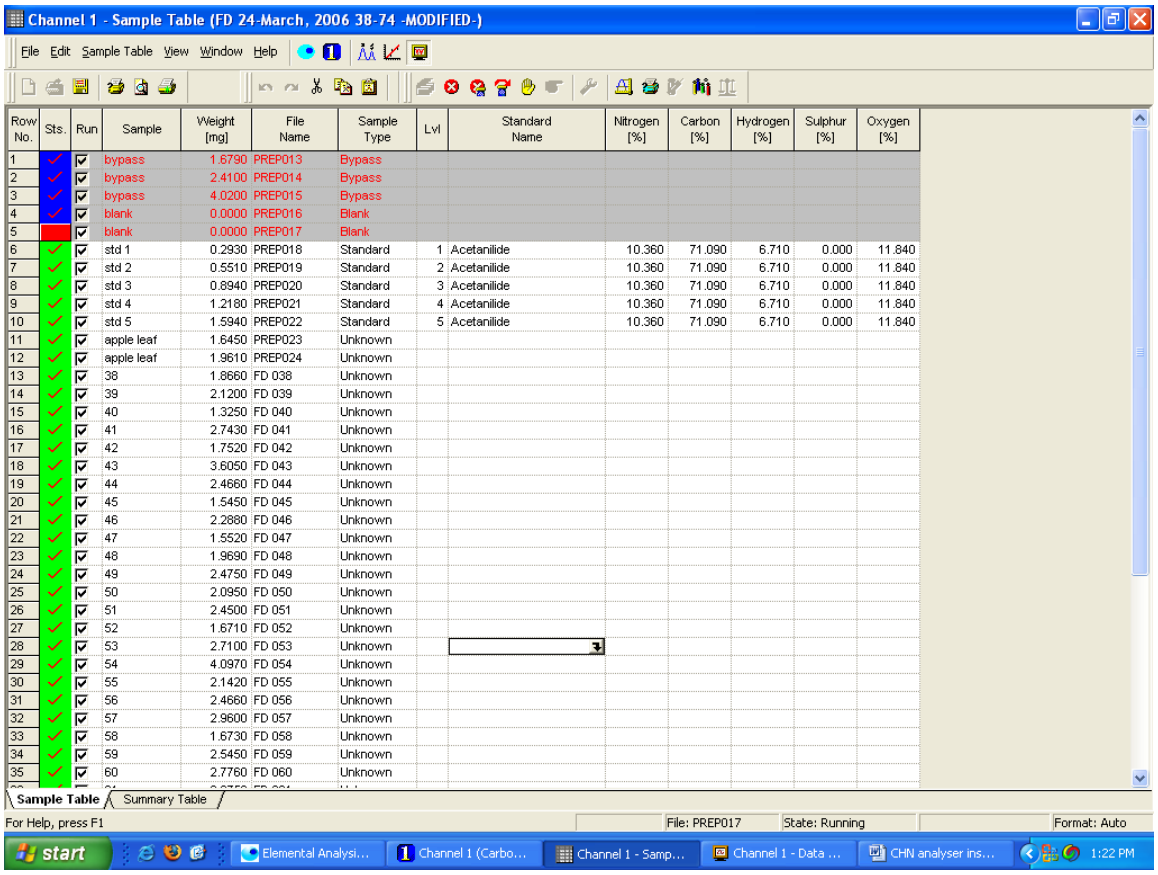
v. Repeat Step 3.

1. All of the points should lie on or very close to the line. The correlation factor should be near 1. (0.99 is acceptable, 0.999 is great, and 0.9999 is impressive)

vi. If you do at least 5 standard points, and one is way off the line, you can delete it from the table on the left hand side of the calibration window.

Data Export:

3. After the run has completed, click 'Summary Table' at the bottom of the finished Sample Table.
   a. Click save.
4. Highlight the entire table, copy (ctrl+c) and paste (ctrl+v) into an Excel spreadsheet.
   a. You will need to type in the column headings on your own
   b. Copy and paste the Mass values from your input file



Row No.	Sts.	Run	Sample	Weight [mg]	File Name	Sample Type	Lvl	Standard Name	Nitrogen [%]	Carbon [%]	Hydrogen [%]	Sulphur [%]	Oxygen [%]
1	✓	☑	bypass	1.6790	PREP013	Bypass							
2	✓	☑	bypass	2.4100	PREP014	Bypass							
3	✓	☑	bypass	4.0200	PREP015	Bypass							
4	✓	☑	blank	0.0000	PREP016	Blank							
5		☑	blank	0.0000	PREP017	Blank							
6	✓	☑	std 1	0.2930	PREP018	Standard	1	Acetanilide	10.360	71.090	6.710	0.000	11.840
7	✓	☑	std 2	0.5510	PREP019	Standard	2	Acetanilide	10.360	71.090	6.710	0.000	11.840
8	✓	☑	std 3	0.8940	PREP020	Standard	3	Acetanilide	10.360	71.090	6.710	0.000	11.840
9	✓	☑	std 4	1.2180	PREP021	Standard	4	Acetanilide	10.360	71.090	6.710	0.000	11.840
10	✓	☑	std 5	1.5940	PREP022	Standard	5	Acetanilide	10.360	71.090	6.710	0.000	11.840
11	✓	☑	apple leaf	1.6450	PREP023	Unknown							
12	✓	☑	apple leaf	1.9610	PREP024	Unknown							
13	✓	☑	38	1.8660	FD 038	Unknown							
14	✓	☑	39	2.1200	FD 039	Unknown							
15	✓	☑	40	1.3250	FD 040	Unknown							
16	✓	☑	41	2.7430	FD 041	Unknown							
17	✓	☑	42	1.7520	FD 042	Unknown							
18	✓	☑	43	3.6050	FD 043	Unknown							
19	✓	☑	44	2.4660	FD 044	Unknown							
20	✓	☑	45	1.5450	FD 045	Unknown							
21	✓	☑	46	2.2880	FD 046	Unknown							
22	✓	☑	47	1.5520	FD 047	Unknown							
23	✓	☑	48	1.9690	FD 048	Unknown							
24	✓	☑	49	2.4750	FD 049	Unknown							
25	✓	☑	50	2.0950	FD 050	Unknown							
26	✓	☑	51	2.4500	FD 051	Unknown							
27	✓	☑	52	1.6710	FD 052	Unknown							
28	✓	☑	53	2.7100	FD 053	Unknown							
29	✓	☑	54	4.0970	FD 054	Unknown							
30	✓	☑	55	2.1420	FD 055	Unknown							
31	✓	☑	56	2.4660	FD 056	Unknown							
32	✓	☑	57	2.9600	FD 057	Unknown							
33	✓	☑	58	1.6730	FD 058	Unknown							
34	✓	☑	59	2.5450	FD 059	Unknown							
35	✓	☑	60	2.7760	FD 060	Unknown							

Total inorganic carbon (TIC, carbonate) coulometry

Draft v.4.0
Written by A. Myrbo (with C. Schodt), 11/6/2013

Total inorganic carbon coulometry (TIC) measures the amount of inorganic carbon contained in lake sediments or water samples. The TIC content of sediments can be related to a number of factors such as rates precipitation of carbonate minerals or introduction of detrital carbonates into the system. The UIC CO_2 coulometer can be used to rapidly determine TIC from either water or sediment samples.

Principles:
Carbon dioxide gas evolved by dissolution in acid from carbonates in the sample is swept by a gas stream into a coulometer cell. The coulometer cell is filled with a partially aqueous medium containing ethanolamine and a colorimetric indicator. Carbon dioxide is quantitatively absorbed by the solution and reacts with the ethanolamine to form a strong, titratable acid which causes the indicator color to fade. The titration current automatically turns on and electrically generates base to return the solution to its original color (blue).

Equipment and Procedure:
To measure TIC, carbon contained within carbonate minerals such as calcite, dolomite, siderite, etc., we use a UIC model 5030 carbonate carbon apparatus [NOTE: in August 2013, we replaced this instrument with an AutoMateFX carbonate carbon apparatus with the CO_2 coulometer. For analyses conducted after this date, please also refer to the AutoMate SOP]. 10-15 mL of acid (we prefer phosphoric) serves to evolve CO_2 from the sample which is swept into the carbon coulometer where it is detected and displayed on a digital screen in terms of micrograms (or any other operator-selectable units) of carbon.

Cell Preparation:
The coulometer cell should be cleaned and refilled at minimum every 48 hours, more if results begin regularly taking 15 min+, or if the cell will not return to 29.6% transparency and 0 current.

Note: you must wear appropriate gloves when handling coulometry solutions. A pair of butyl rubber gloves hangs in the cabinet with the columetry solutions. For the rest of this procedure, you must wear regular lab gloves to protect your hands from the acid. Cell filling and cleaning must be done in a fume hood.

1. Clean the cell by filling the cathode half (the larger chamber) half full of DI water and using vacuum pressure to pull the water through the frit. Repeat until the frit rinses clear.
2. Refill the cathode chamber with acetone or methanol, and once again use vacuum pressure to pull it through the frit. This removes water residue. Additional acetone/methanol on a kimwipe can be used to remove fingerprints or smudges.
3. Once the cell has dried completely, fill the main chamber of the coulometer cell with 50-75 mL of cathode solution (to the marked line) (large plastic bottle).

4. Place the magnetic stir bar in the bottom of the cell body and insert the cell top with the coiled platinum electrode into the cell. The electrode should be opposite the fritted arm.

5. Add 0.25 cm (enough to cover the bottom) potassium iodide (KI) to the bottom of the side arm (anode compartment) of the cell.

6. Fill the side arm with 10 or more mL of anode solution so as to cover the filter and submerge at least 0.5 cm of the silver anode in solution; the level of the anode solution should at least match that of the cathode solution, but the amount of liquid will vary as the anode is consumed (over a period of months) in the analysis.

7. Place the solid silver electrode into the side arm with the silver submerged in the solution.

8. Make sure the glass of the cell is clean and free of grease, fingerprints, water spots, etc., which affect transparency (and thus %T). Wipe/polish with a paper towel or Kim-Wipe, or wash if necessary.

9. Place the assembled cell into the coulometer cell holder. The side arm should extend out the front and against the right wall of the holder, with the platinum electrode and gas inlet tube toward the back of the holder, *out of the light path*.

Operation:

10. Make sure coulometer cell current is OFF.

11. Turn ON the main power switch.

12. Set air flow for internal and adjust to 75-125 cc/min.

13. Connect the cell to the cell to the Carbonate Carbon Apparatus using a one-way (check) valve. Only inset the gas tube in the cell when air is flowing, to avoid coulometer cell solution being siphoned back into the KI scrubber.

14. Attach the electrodes to the cell outlet terminals - red to red, black to black.

15. Select “Cell Set Up”. The value displayed should be between 2700-4000 units. If so, select back. If not, something is obscuring the light beam. NOTE: if you are using the same cell chemicals two days in a row (i.e. cell chemicals are blue, not clear), skip this step.

16. Select “Begin Analysis”. Allow cell current to titrate the cell solution to its endpoint (solution color becomes blue with %T at 29.6 If it is lower than 29.6%, check that the light path is unobstructed and adjust the arrangement of the tube and electrode to correct). For best results, allow the cell to titrate for at least 30 minutes.

17. Set the heater at #5

You are now ready to begin the analysis.

Analysis Procedure:

1. Run a blank sample using an empty sample container. The blank is normally less than 1 μg C per minute. If the blank is higher than this, it may not be an issue. Re-run the blank twice. If the values are consistent, higher values may be acceptable; the machine will correct for the blank value. If the blanks are above 1 μg per minute and inconsistent, then either the system has a leak, the gas is not flowing, something is obstructing the light beam, or the cell was set up incorrectly. A blank of 0 is also a sign that the machine is not reading correctly.

2. Follow the blank by two or more standards (standard $CaCO_3$ is found in a desiccator near the balance). For best precision, material for each analysis should contain 1-3 mg of C. For our standard calcite, this means that you should use about 10-25 mg of standard. Using larger samples sizes (e.g., 30-35 mg) may improve accuracy, but will deplete your cell chemicals more quickly, and may slow the sample's run-time.

3. Weigh a sample or standard into a, dry, tared size 00 gelatin capsule. Close the capsule loosely and place it into a clean test tube. Make sure the gelatin capsule is at the bottom of the test tube and attach to apparatus. Sample should contain 1-3 mg of C; adjust the quantity as you begin to see how much carbon tends to be in your samples. Record sample weight in spreadsheet.

4. Enter the sample number and weight into the machine and press enter. Wait for 60-90 seconds for the test tube to heat, and for the CO_2 in the test tube to be flushed.

5. Select "Begin Analysis" then pump 5-10 mL of acid into the reaction tube.

6. If you add too much acid to the reaction tube, or if the sample material especially reactive, the acid may foam and carry sample material onto the walls of the sample column, Unless the material can be rinsed down with more acid, the sample is lost. Discard the sample rise down the reaction column with DI water. To prevent foaming, allow the test tube to heat for an additional minute, used a smaller initial volume of acid, periodically adding more as the reaction progresses and, if possible, use a smaller sample volume.

7. The machine will calculate results and end the analysis when the result has less than 1% change from one minute to the next. For best results, it is advisable to add an additional pump of acid to the test tube at 3-4, minutes, especially if the reaction has foamed any of the sample onto the test-tube walls.

8. Remove the sample tube, pour residue into a waste container, begin next analysis. Wash tubes and rinse in DI water and place in drying rack. If the

samples are being loaded into gelatin capsules, it is not necessary to completely dry the test tubes.

9. Neutralize the waste acid with soda ash as you go.

10. Run one standard and one duplicate analysis every ten samples (or more if desired).

Calculations:

There is a spreadsheet to calculate %TIC (ccoul.xls on the desktop). The calculation is as follows:

%TIC = {(µg C[display value] - per minute µg C[blank value]) / µg sample weight} x 100

Note: the formula subtracts the per minute blank value (blank value/minutes of counting).

For pure calcium carbonate the value should be 12.00%. (Ideal results are 11.85-12.15 but we accept values from 11.75%-12.25% However, if values are consistently outside of the +/- .15% range, there may be an issue.) Other carbonates will have different carbon percentages according to the table below.

Mineral	Cation(s)	C	O_3	mw	%C
$CaCO_3$	40.08	12.01	48.00	100.09	12.00%
$MgCO_3$	24.31	12.01	48.00	84.32	14.24%
$(Ca,Mg)CO_3$	64.39	12.01	48.00	184.41	13.03%
$FeCO_3$	55.85	12.01	48.00	115.86	10.37%
$ZnCO_3$	65.38	12.01	48.00	125.39	9.58%
$MnCO_3$	54.94	12.01	48.00	114.95	10.45%

Shut-down (Carbonate Carbon Apparatus):

The Carbonate Carbon Apparatus should be shut down during periods of non-use.
Remove the tube from the cell (disconnect the gas line).
Turn off main power switch.
Note: To prevent residual acid from marring the exterior of the apparatus, keep a test tube connected to the apparatus when the system is not in use.

Shut-down (Coulometer):

Short periods (during the day)
Before turning off air flow, disconnect inlet gas flow line into the coulometer cell. This prevents coulometer solution from being siphoned out of the cell.

Overnight or longer
The coulometer's main unit can take two hours or more to warm up, and during this time may give artificially low results. For best results, if you will be working on the coulometer for consecutive days, turn off the cell current but leave the main power on.

Cell Changing and Clean-up:
Note: Solutions should also be replaced when over 100 mg of carbon have been titrated (for 100 mL of cathode solution). At this point the coulometer may not be able to return the cell to 29.6% transparency between samples, leading to long sample run times and possibly inaccurate results.

Turn OFF cell current and main power supply.

Unplug electrodes and remove cell from holder.

Dispose of the main chamber solution (cathode solution) into the cathode waste solution bottle. Be sure to remove the stir bar first or be prepared to retrieve it from the waste bottle with a magnetic stir bar retriever. Start a new waste bottle (and label correctly) if necessary.

Dispose of the side arm solution (anode solution) and residual KI into the anode waste solution bottle. Start a new waste bottle (and label correctly) if necessary.

Rinse both cell body and the electrodes thoroughly with DI water. Pull DI water through the frit with a vacuum until it runs clear.

Clean the glass frit and remove excess water in the anode compartment by pulling methanol or acetone through the frit with a vacuum.

If any build up is visible on the platinum electrode, rinse the electrode with concentrated nitric acid to remove the precipitate. Then rinse the electrode thoroughly with DI and dry - nitric acid residue will ruin your cell chemicals.

Rinse and dry all components.

Store cleaned cell in cell holder and return other components to the drawer.

Periodic Maintenance and Scrubber Changing:

If the cell current routinely fails to reach 200 during standards and initial titration, the frit may be clogged. This is rare, but if it does occur, fill both side of the cell 1/3 full of nitric acid and allow it to sit overnight. Pull some of the nitric acid through the frit with a vacuum,

and then clean the cell thoroughly with DI water. Residual nitric acid anywhere (especially in the frit) will ruin the cell chemicals.

Changing Scrubbers:

Air Scrubber (40% KOH)

The KOH solution removes CO_2 from the carrier gas. Running the machine on ultra-high-purity gas, the KOH scrubber will rarely need to be changed (once every 1-2 months at most). If the KOH solution is foamy, it should be diluted with DI water.

Preparation

Weigh out 40-45 g of KOH and dilute to 100 mL with DI water. Note: Use caution when adding water to KOH as the reaction is exothermic.

Filling

Remove the dispersion tube, bushing and O-ring from the air scrubber assembly.
Place 15-20 mL of KOH solution in the body of the air scrubber.
Replace the dispersion tube, O-ring and bushing. Slide the dispersion tube through the bushing and O-ring so the fritted end is near the bottom of the scrubber.
Hand-tighten the bushing/O-ring seal and place the filled scrubber in its clamp.

Sample Scrubbers:

The gas coming from the sample should first be run through a scrubber containing magnesium perchlorate or another desiccant (as with the Total Carbon procedure). This scrubber should be changed as needed, likely daily or more. Place a piece of glass wool in one end of the scrubber tube, carefully fill with magnesium perchlorate granules, and cap the other end with another plug of glass wool.

The second scrubber (used to remove sulfur compounds) should contain ~10g of reduced silver granules, also contained between glass wool. Note: As the granules collect sulfur, they blacken and increase in size. This may cause them to grow together and block the gas flow, or even crack the scrubber tube. To prevent this, you may wish to mix the silver with another non-carbon bearing granulated solid such as silica or alumina. You should also periodically stir the scrubber material to distribute the silver sulfate granules.). The scrubber should be changed when 75% of the silver is black. If you are feeling adventurous, the black silver sulfide potentially may be regenerated electrochemically with soda ash and aluminum foil.

One can use a variety of acids to react with the carbonates. Originally we used a 2N HClO4 solution and then switched to using 2N HCl. Currently; best results have been achieved with phosphoric acid. Unless there are exceptional circumstances, phosphoric acid will yield the best results. The procedure for mixing these solutions is given below.

2N H_3PO_4	Dilute 48ml of 85% H_3PO_4 in 452ml of DI water.
2N $HClO_4$	Dilute 109 mL of 9.2 N $HClO_4$ in 391 mL of DI water.
2N HCl	Dilute 172 mL of 37% HCl in 328 mL of DI water

Note: Always add the acid to the water, not the water to the acid.

Automate instructions

Written by Jason Curtis v.1.9
August 2013
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Initial set up of AutoMate:
The AutoMate is composed of 4 parts:
One box with the electronics (called the Controller or Electronics box)
One box with the pumps, valves, flow controls (called the Wet box)
The carousel
The computer (supplied by customer) with the control software

Positioning:
(See AutoMate positioning image)
Place the carousel on a lab counter. Then place the Wet box (the one with the flow meter on the front panel) to the right of the carousel. Then place the Controller box on top of the Wet box. (Don't put the Wet box on top of the Controller box as liquid might get on the electronics).

Tubing:
All tubes are labeled – just match them to the label on the part they go to.

Compressed tank gas (UHP N_2) runs through 1/8" nylon tubing from tank regulator to the back of the carousel. Inside the carousel is a tee that splits the tank gas into 2 streams. One runs the pneumatics that move the needle assembly. The other stream goes into a solid CO_2 scrubber (Ascarite, NaOH on a media). Following removal of CO2, this is now considered the carrier gas. The carrier gas runs to the 1/8" quick fitting labeled "Carrier Gas In" on the Wet box.

Use of UHP N_2 avoids the need to scrub CO2 from the supply gas. Then the Ascarite trap can be bypassed and gives one fewer consumable to worry about.

The other 2 inputs to the Wet box are "Acid In" and "DI Water In". The fittings are such that you cannot reverse the bottles. Once you get the whole thing set up and acid and water in the bottles you will need to prime the liquid lines (instructions below).

The output from the Wet box is through a 1/8" Teflon tube that runs to the longer needle. This just slips over the needle. We have never had leaks at this slip junction. If you need to cut off the tubing remove as little as possible. Then use something like a ballpoint pen to slightly flare the tubing. Then slip it onto the needle. (1/8" tubing have varying ID and some work to slip over the needle and form a leak tight seal while most batches do not -contact AutoMate FX, Inc. for replacement 1/8" Teflon tubing). Do not attach the tube from the Wet box to the shorter needle or the liquid in the sample vial will end up in the scrubber and eventually in the coulometer cell.

The shorter needle is attached to a shorter piece of 1/8" Teflon tubing also by a slip fit. The other end of the tubing goes into the side arm of the post sample scrubber. The Teflon tubing is inserted into a piece of 1/4" OD, 1/8" ID Tygon tubing that acts as a spacer in the screw fitting that attaches to the scrubber. The output tubing from the needle attaches to the side arm of the post sample scrubber. Do not attach the output from the shorter needle to the body of the scrubber or all the liquid in the scrubber will end up in the coulometer cell.

The output from the scrubber travels through a 1/8" Teflon tubing. Again, this tubing is also inserted into a piece of 1/4" OD, 1/8" ID Tygon tubing that acts as a spacer in the screw fitting that attaches to the scrubber. The downstream end of this tube goes into a one-way valve and then into the cathode side of the coulometer cell.

Scrubbers:
Pre-scrubber is mounted horizontally on the right of the main instrument. This pre-scrubber holds Ascarite (NaOH on a media) or another CO2 absorbent. Put a wad of glass wool or quartz wool one end of the glass trap and then fill trap with absorbent and then put a wad of glass wool or quartz wool at the other end. The glass wool or quartz wool acts to trap the absorbent so that it does not get blown out of the trap.

Post-sample scrubber is necessary for two reasons. First, to collect any acid vapors or acid drops and keep them from getting to the coulometer cell -this requires simply water or a silver nitrate solution (the coulometer cell is basically titrating CO2 acidity so acid from reaction cell messes things up). Second, to collect any SO_2 that might be released from samples --this requires a silver nitrate solution (3% silver nitrate in DI – see coulometer manual).

Cables:
There are 4 cables, 1 USB to RS232 adapter cable, and one electrical cord for the AutoMate. Each connection is labeled and they cannot be incorrectly attached. (1) A 25 pin cable connects the Controller and Carousel. (2) A 15 pin cable connects the Controller and the Wet box. (3) A 9 pin cable connects the controller to the USB to RS232 adapter and then to computer and (4) another 9 pin cable connects the controller and the coulometer. The connections for the UIC 5012 and the 5011 coulometers are different. The 5012, 5014, and 5015 connect simply with a 9 pin cable into the "Serial" port on the back. The 5011 connects with a 25 pin cable. The 5011 top must be removed by taking out the hex head screws. The 25 pin connection is at the back of the topmost board. The 5011 needs the 9 to 25 pin optional adapter cable. Attach it to the 9 pin cable and attach the appropriate 25 pin connector to the coulometer.

Coulometer communication set up:
(see communication switches images on page 12)
For 5014 and 5015, install "5011 Emulator" from UIC following their instructions.
The software communicates with the coulometer at 9600 baud. This must be set using dip switches inside the coulometer. This is very easy on the 5011. Remove top and the uppermost board has the dip switches. Find the red dip switches with 4 switches. Set 1 and 4 to 0 (zero) and 2 and 3 to 1 (one). (see 5011 communication switches image). Replace top. For the 5012 it is a little more in

depth. First you must remove the right side cover (right hand cover as you face the front of the instrument). There are 6 Phillips head screws, 3 on the back and 3 on the side. Once the cover is off you are looking at several boards. The one that you want is the uppermost one (see 5011 communication switches image). You cannot see it very well due to the mounting design. The board is the same one as in the 5011. On the edge of the board facing you is the same red 4 dip switch fitting. The settings are the same as for the 5011. Set 1 and 4 to 0 (zero) and 2 and 3 to 1 (one). (see 5011 communication switches image) Once the dip switches are set correctly I suggest trying communications with the AutoMate system prior to putting the top or right side cover back on. (see software sections for checking coulometer communications)

Also on the coulometer set the time to 50 minutes and the roller switch to 1 or ug C.
UIC 5014 and 5015 CO_2 coulometers need a new firmware chip to communicate with the AutoMate system. These are available directly from UIC, Inc. Contact AutoMate FX for part numbers.

Compressed Gas:
Carrier gas can be a variety of gases. The best is ultra-high purity N_2 (UHP N2). Industrial N_2 and compressed air are not recommended as the Ascarite scrubber will clog quickly. We don't recommend a liquid NaOH scrubber like used on manual UIC TIC systems because they require daily maintenance.

Software installation:
Software for the AutoMate Prep Device is pre-installed for use with UIC coulometers.
Updated versions of AutoMate software will run with no issue. Just place them in theAutoMate folder on the Desktop and double click on them. You can place a shortcut on the desktop. An installer is included in case a new computer is needed (Win 8, Win7 and WinXP).

AutoMate control software:
(see Screenshot image below)The graphical interface for the AutoMate is all on one screen for simplicity. The various functions are roughly divided into groupings.
Across the top are some general buttons."Close AutoMate Application" = quit the program "STOP After Current Sample Complete" = Stop Autorun when current sample finished Just below this across the top are a series of dots with text above. These dots light up to show the sequential happening in an auto run. They are mostly self-evident. "Controller" is the step where the software checks communication with the controller. Likewise, "Coulometer Test" check communication with the coulometer.

On the left side of the screen are a series of buttons with pull down menus. The top one is "System Control" which controls many of the functions of the automate. See below for specifics of "System Control." Use "System Control" with caution as you can do bad things like start the acid pump running and then leave it on, consequently pumping acid all over the place. The next button down is "Run Control" which starts autoruns. The rest of the buttons (7 in total) control parameters during autoruns. See below for specifics of these autorun parameter buttons.

System control pull down buttons. Most are self-evident. Here are a few that might require

explanation: "Get system status" = check the status of all the functions of the automate and update the current status lights (see below)"Test Controller Communications" is somewhat repetitive as this is also done in "GetSystem Status" above but it is also useful other times. "Test Coulometer Communications" talks with the coulometer and gets the current value from the readout from the front of the coulometer. This is displayed in the box at the bottom center of the screen "Data From Coulometer"

Autorun Parameters Autorun parameter buttons have pre-set value that should work for most situations. They can be modified as needed. If you find that you need different values AutoMate FX can easily re-compile the software with your specific values so that whenever the software starts you specific values come up."% Difference" = This is part of the stability equation that determines if a sample analysis is complete. This equation is based on the rate of increase in the counts of micrograms of C versus the blank. It is basically the same formula that UIC has in their manual for the coulometer "Sample Run Time" = Minimum length of time for each sample. Will run longer based on the stability equation. (Dirty cell or poorly operating cell will lead to long runtimes)"Sample Purge Time" = Time to purge atmosphere from vial prior to coulometer reset and acid injection. "Minimum Purge Counts" = Micrograms of C that must be recorded during the above purge. If this minimum is not met then the run will stop with an error. This is a safety to ensure that a vial is in place, the needles pierced the septa, the cap and septa on the vial are tight, that the connections to the scrubber and the coulometer are tight, etc. If any of these problems exist the run stops and does not waste samples or pump acid and water all over the place. "Acid Inject Time" = Time to inject acid. The main requirement here is that the level of the acid in the vial, combined with the water manually added to the sample during loading, is high enough so that the longer needle is in the liquid. If the liquid level is lower than the longer needle run time will be much greater. "DI Inject Time" = Time to inject water following sample analysis. This is mainly to clean the insides of the tubing and valves. The outside of the needle is squeegeed off when the needles withdraw. "DI Purge Time" = Time to allow the water in the valves and lines to be blown out by thecarrier gas "Set Sample Rate" = Frequency of data collection. 1 minute works well In the middle of the screen are controls for the data table. "Table Functions" is a button with a pull down menu. Here you can create a new table, save a table, save as, or open an existing table. Easiest way to get data into the file is to copy the cells with sample ID’s and masses from Excel and paste it into the correct cells. Don’t paste into the Blank row – It will not allow it. Also you have to use the paste function in the "Table Functions" pull down – right clicking is not enabled. The long box to the right shows the location of where the data will be stored on the hard drive. Default is a folder called AutoMate data in My Documents. I suggest leaving it there and creating a shortcut to the folder on the desktop. Sample name and weight can be entered directly into the table. Data is exported as .dat files and can be directly imported into spreadsheet programs like Microsoft Excel. Below the Table are a second series of dots that show the current status of the functions of the AutoMate. Most are self-explanatory. Grey is off and green is on. Needle down = needle assembly is in the full down position (carousel will not turn)Needle up = needle assembly is in the full up position. Supply pressure = carrier gas pressure is above 5 psi Vial in position = carousel is lined up directly under the needle assembly. Carousel zero = carousel position 0 is lined up directly under the needle assembly Carrier gas = carrier gas is flowing Controller = Automate software is communicating with the Controller Coulometer = Automate software is communicating with the coulometer.

At the bottom center of the screen are a series of read outs that show timing during runs, the position the carousel is in, and reading from the coulometer.

At the bottom right of the screen is a pull down to select the vial position, a button to go to the above select position, and a button to go directly to zero.

During an auto run the software looks at the table to determine which samples to run. Basically it looks at the weight column from the top and finds the first row with a weight in the cell. Then it looks to see if the total counts cell has any data. If there is no data it runs that sample. If there is data in the total counts cell it assumes that the sample has been run and looks for a row with a weight but no data in the total count cell. It will then run that cell. Once all the rows with weights have associated data in the total count cell the auto run ends. During an auto run data is stored into the table and the export file at each sample reading (normally 1 minute intervals).

Priming liquid lines:
The AutoMate system is shipped with no liquid in the lines for safety. You must remove the air in the lines and replace it with water and acid. Here are the priming instructions: Fill and lightly cap the water and acid bottles (caps must not be tightened -air needs to be able to get in otherwise a vacuum forms). Put Exetainers without caps in positions 1,2,3, Advance carousel to position 1. Put needle down. Turn on Carrier gas. Click on "acid on for 2 seconds" button, then select ok. Repeatedly select "Acid on for 2 seconds" for about 6-10 times until acid flows to the vial. Make sure that you do not overfill the Exetainer. If needed turn carrier gas off, move needle up and move to next vial, put needle down, carrier gas on. Then repeat for water using "Water on for 2 seconds" button. Repeatedly select "water on for 2 seconds" for about 6-10 times until water flows to the vial. This only has to be done when first filling the bottles, unless you let the bottle go dry.

If you have problems with priming a pump see the troubleshooting section at the end of this document. Also contact jason@automatefx.com

Solid sample loading:
Samples are loaded into Exetainer vials. These are 12 ml screw top vials with septa tops. They are made by Labco in the UK. 12ml Borosilicate Vials -Round Bottomed. 938W or 538W.
http://www.labco.co.uk/usaandcanada/

Note: Fewer than 1 in 100 of the Exetainers are slightly too large in diameter for the AutoMate carousel. AutoMate FX suggests discarding any abnormally large vials. Caps can be used 3-5 times each. Vials can be washed and dried and reused forever. New caps and septa can be purchased from Labco.

Standards are usually 6-10 mg. Samples are usually 10-15mg unless the %C is really low in which case more material can be used. Sample weighing is one of the most important steps to getting good results. A microbalance (0.000mg)is recommended. Samples can be weighed out in a couple of ways:

1. Use a macrobalance (reads to 0.0 mg) and place the vials upright on the balance and then tare the balance. Powder is the placed in the bottom of the vial until the correct weight is reached. Record weight.
2. Use a microbalance (0.0mg) or a microbalance (0.000mg). Cut strips of weighing paper about 1cm wide by 5 cm long. Fold the strips lengthwise. Place strips on balance and tare. Add material onto weighing paper until correct weight is reached. Record weight. Then pick up strip with sample using a forceps and slide into a vial until the strip is near the bottom. Tap powder off so that it all gets to the bottom of the vial.

Then cap each vial. The tops seal surprisingly well and do not need to be tightened very much. If the cap puckers down then it is tightened too much. Examine a septa. You will notice a slightly raise ring in the rubber at about the same diameter out from the center as the opening in the cap of the vial. Watch closely when you tighten a vial. When this ring start to shrink toward the center slightly the vial is tightened enough.

Samples can be placed in standard test tube racks. Sample vials can be written on with marker pen. Tape is not recommended unless it is kept right near the top of the vial (this is because the holes in the carousel are just slightly bigger than the vials).

Solid samples can be weighed out prior to analysis and stored for a long time as needed.

DIC of water samples:
The AutoMate Prep Device can also be used to measure total DIC in waters. 5ml is the recommended volume. Contact Automate FX for more information. Different default timing is required. The best way to do this is to ask AutoMate for a different version of the software with the correct defaults.

Total CO2 in headspace:
The AutoMate Prep Device can also be used to measure total CO2 in headspace for soil incubations. Contact Automate FX for more information.

Supplies:
The AutoMate requires three main consumables that must be replenished on a regular basis. These are DI water, acid (nitric, hydrochloric, or phosphoric are all compatible with the materials in the AutoMate -do not use perchloric acid), and a CO_2 free gas (ultra-high purity N_2 or CO_2 free air).
AutoMate FX highly recommends 10% phosphoric acid. 1N nitric and 1N hydrochloric acid also work but see the needle will not last as long (see consumable section below). Special coated needles help with 2N sulfuric acid – contact AutoMate for more info.

Recipe for 10% Phosphoric acid. Wear gloves and goggles60 ml 85% ortho-phosphoric acid (reagent grade) 440 ml DI water Add acid to water, mix, and wait a minute or so. Container will be slightly warm.

Water and Acid are stored in 1 liter bottles with the tubing out through the top shoulder. Water

goes in the bottle labeled Water and acid goes in the bottle labeled Acid. One filling of bottles should last about 6 to 8 sample runs (45 total vials per run). There is no safety to stop the run if the liquid bottles are allowed to go empty so check at the start of each run. Two cautions. First, the acid and water quick connects on the back of the Wet box are not valved. If they are unplugged the contents of the bottles will flow out. Fill the acid and water bottles with a beaker and a funnel. Second, do not tightly cap the water and acid bottles when running the AutoMate. This will cause a vacuum to form and eventually no liquid will flow.

Other more long lasting / durable consumables are Ascarite for the CO_2 scrubber, 3%silver nitrate solution for the post sample scrubber, needles, and septa top Exetainer vials. The Ascarite in the trap will last a long time if the input gas is low in CO_2. The silver nitrate scrubber will last several runs depending on the amount of sulfur in the samples. The needles will last for differing times depending on the acid that is used. The needles are made of 316 stainless and are most readily attacked by nitric acid, then less so by hydrochloric acid, and least by phosphoric acid. Septa that are shoved into the vials are a pretty good indication that one of the needles is dull or broken (the run will automatically stop). This will almost always be the longer needle because it actually sits in the acid during sample reaction. A simple tool is included to help install the longer needle to the correct depth in the needle holder assembly (an extra-long and short needle are included).

Running samples on the AutoMate:
The carousel holds 45 vials total. Vial zero is always reserved for a blank. I suggest 100% calcium carbonate standards in 1,2,3,22,44.

Place samples in carousel. You will have to turn carousel to get samples into the positions right around the needle guide. Use the vial button and Go to vial button.

Enter sample names and weights into the table. Or better yet, cut and paste from excel spreadsheet. Use the "Table Functions" pull down and save the table (or allow the automatic save prompt after run is started).

Ensure that water and acid bottles have enough liquid.

Check gas supply pressure (basically the amount of gas left in the cylinder) and delivery pressure on the regulator (set to about 30 psi).

Check that the carrier gas pressure on the front of the wet box is about 8 or 9 psi. If it is not, you must adjust the pressure regulator. This is under the carousel. Lift the front of the carousel until the carousel is at about a 30° angle. Then reach under the front right side and pull the knob downward to unlock the regulator adjuster. Then turn knob while watching the gauge on the front of the Wet box. When adjusted to 8-9 psi push knob upward to lock.

Prepare post sample scrubber (3% silver nitrate scrubber) and connect into flow path.

Prepare coulometer cell and titrate to 29% transmittance. Connect AutoMate post sample scrubber

to coulometer cell with Teflon tubing.

Check system status lights (the following should be lit -needle up, supply pressure, vial in position, controller)

Check coulometer communications in system control. Light should go on indicating communication with the coulometer.

Go to "Run Control" and select "Run samples"

Once the needle goes into vial 0 and starts the purge check that the carrier gas flow rate is about 100 ml / min on the front of the wet box. Check that the Blank is in the range of 2 to 6 micrograms of C and the standards are within your acceptable range. Very short suggestions for setting up the coulometer. Additional info can be found in the manuals for the Coulometer.

Setting up 5011 and 5012 coulometer:

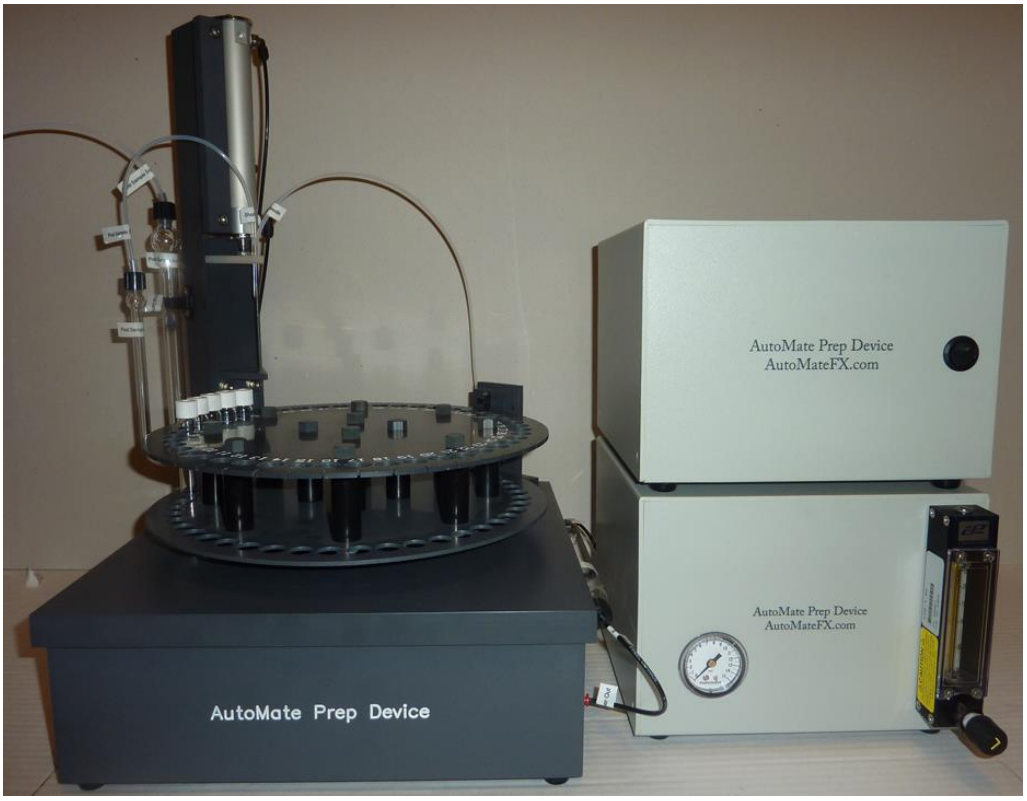
1. Turn main power on to Coulometer (upper switch, not cell current switch)
2. Get clean coulometer cell and stir bar and platinum electrode and silver anode
3. In hood, with gloves and eye protection, fill larger portion of cell (cathode) with Carbon Cathode solution (larger gallon bottle) to between 125 and 150ml.
4. Using a funnel place about 1/4" of Potassium Iodide in smaller portion of the cell(anode)
5. Fill smaller portion of the cell with Carbon Anode Solution to same level as larger side
6. Place platinum electrode in cathode side with the electrode and tube away from the anode and perpendicular to the long axis of the cell
7. Place silver anode in cell (avoid silver touching KI or glass)
8. Gently place cell in coulometer (confirm stir bar)
9. Plug in wires to same colors and attach inlet tubing
10. Rotate cell to get maximum transmittance (%T), then adjust transmittance to 100%Make sure Pt electrode and tube are as far back from light path as possible. Do not tilt rubber stopper backward as this will put the Pt electrode and tube forward.
11. Reset coulometer and turn cell current on
12. After a few minutes transmittance will drop to 29%, color will change and total µg C should be about 1200-5500µgC (depends on the age of the Cathode Solution).

Post run:

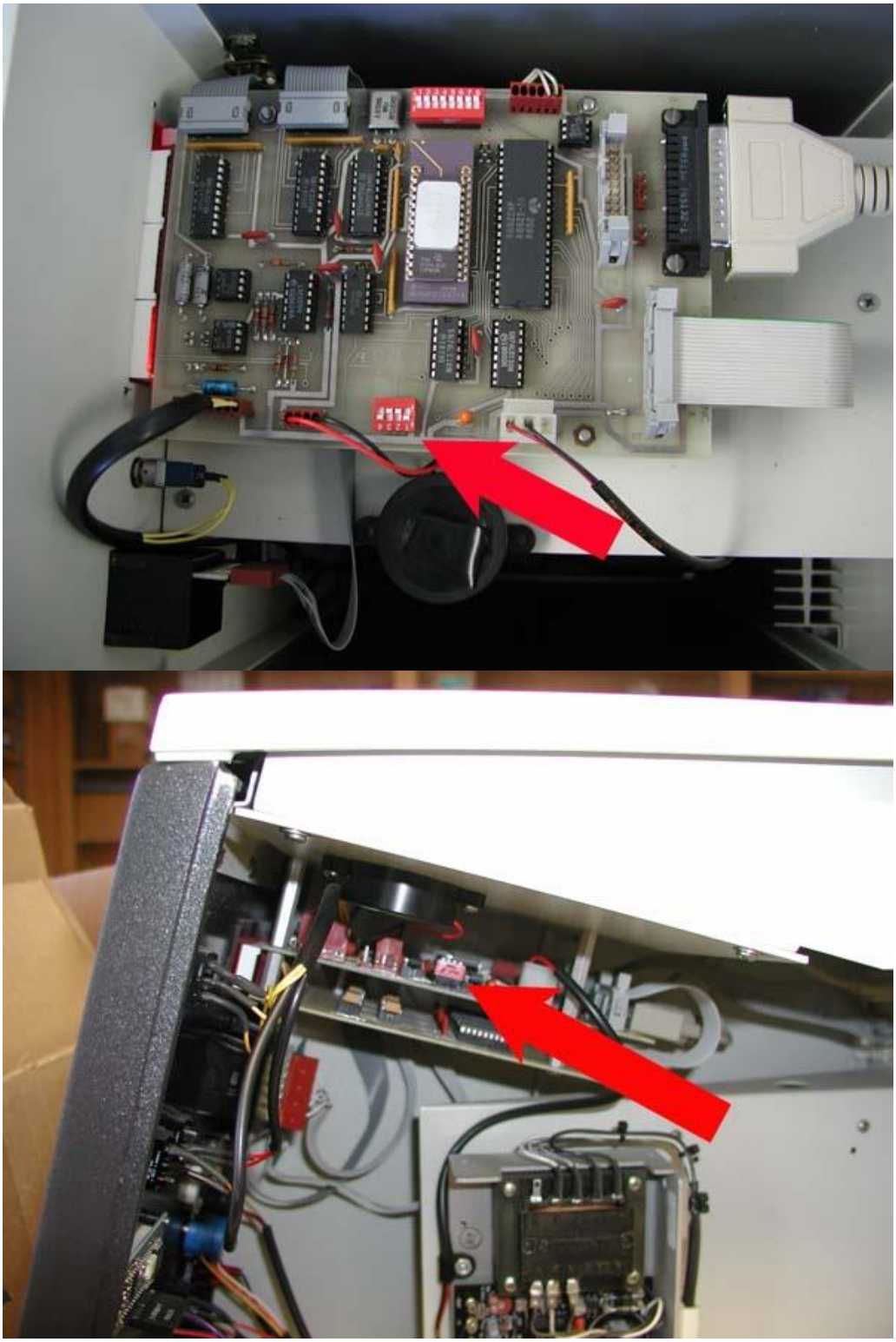
You should be able to do at least 2 complete runs on one set of coulometer chemicals

1. Turn off cell current
2. Unplug wires and tubing from cell
3. Remove cell from coulometer
4. At sink, remove cathode electrode and anode electrode and rinse each with DI water, use metal bottle brush handle to get stir bar out
5. Take #4 stopper and plug anode side and dump cathode solution in to cathode solution waste bottle in hazardous waste collection site. Rinse cathode side of the cell with DI water. Then place stopper in sink.
6. Dump anode solution and KI in to anode solution waste bottle in hazardous waste collection site

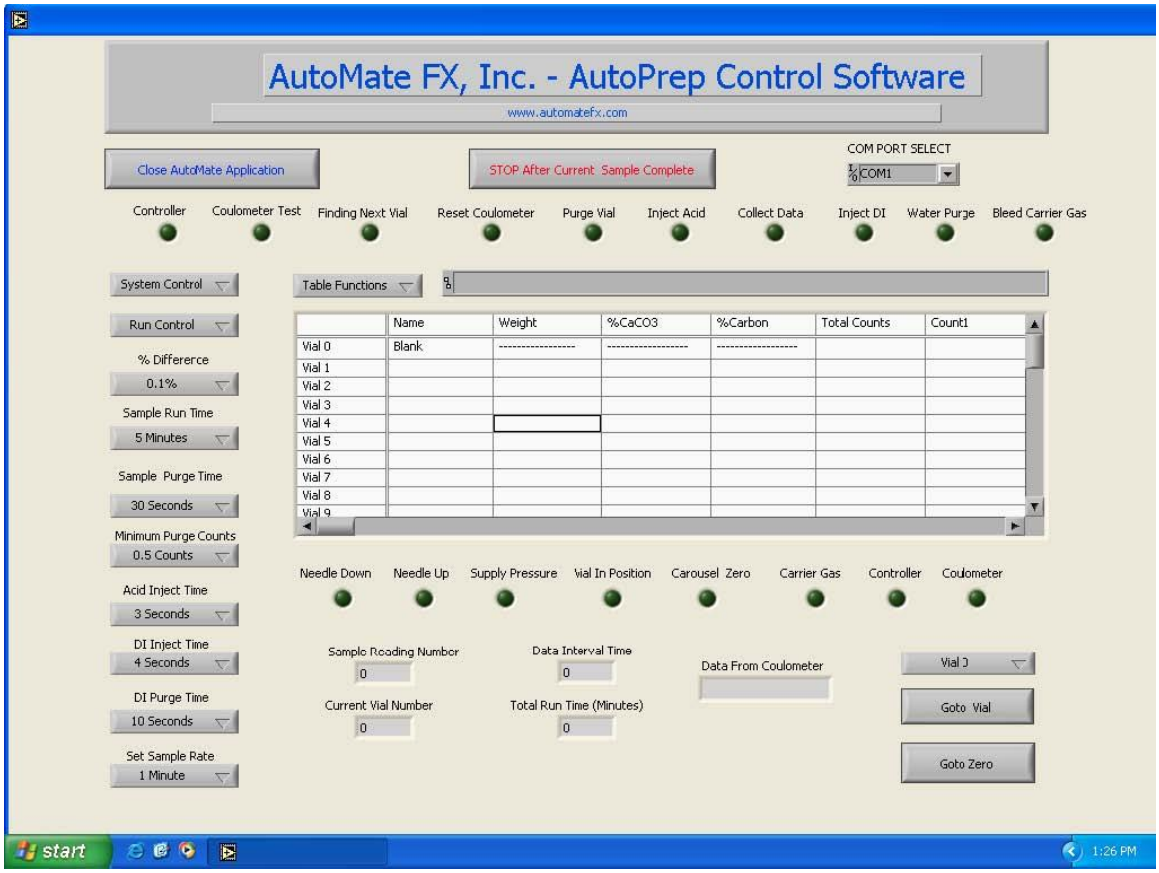
7. Rinse stopper and cell in sink with DI water. Use brush and soap to clean cell and stir bar. Rinse again and place stir bar in cell and place both in oven to dry.
8. If cathode electrode is not shiny, place in 12N nitric acid for a few minute. Then rinse with DI.



AutoMate Prep Device positioning 5011 communication switches



5012 communication switches AutoMate Software Screenshot



Instructions flushing acid and water prior to shipping:
If you need to ship the system you must flush the acid and water out of the system as a safety. To do this empty acid bottle. Then remove 1/8" tube from "Air/liquid to long needle" and replace with disposable 1/8" tubing. Do not lose the 2 ferrules in this fitting. Aim the replacement 1/8" tubing into a beaker. Turn on acid pump (System control, acid pump on). The acid pump will run for a maximum of 7 seconds. Run until acid stops flowing out. Add some DI water to acid bottle and run pump again until water comes out of replacement tube and flows into beaker (again, in 7 second increments). Dump rest of water out of acid bottle. Dump water out of water bottle. Turn on water pump and run until water stops flowing (System control, water pump on) -again in 7 second increments.

Needle replacement instructions:
To remove the needle from the needle holder you need to do the following:

1. Turn off compressed gas
2. Turn the carousel to a position where there is no vial
3. Manually push the needle assemble down
4. Remove the tubing from the needles
5. By hand unscrew the steel pneumatic rod from the needle holder. If you can not move it by hand use a wrench on the lowest portion of the rod where there are 2 flats for grabbing. Do

not use pliers on the steel pneumatic rod.
6. Push the steel pneumatic rod up. Then lift the needle holder assembly up and gently rotate it so that the needle comes out of the needle holder.
7. Once the needle holder with the needles is free measure the length of needle sticking up and the length of needle sticking down. Record this.
8. After recording the stickup and stick down you can just take a pair of pliers and crush down on the longer portion of the needle you want to remove and then twist the needle out. You sort of wind the needle around the jaws of the pliers as you twist the handles of the pliers.

To install new needle:
1. In the supplies that I sent to you were 2 "tools" for replacing the needles. The 2 tools are a piece of stainless steel tubing 4" long and a brass rod (together these will give a stick up that works for your system).
2. It is best to find a counter top (or some flat surface) with a small hole in it so that you can place the needle holder flat on the counter with the remaining needle through the hole. You can also do it over the edge of a counter but this is a little more tricky.
3. Use a hammer to gently tap the needle into the holder -only about 1/16". Then slip the stainless steel tube over the needle, hold the stainless steel tube up a little, insert the brass rod into the stainless steel tube, and hammer gently on the brass rod. This will hammer in the needle without bending the needle. Before the rod gets all the way flush to the stainless steel tube stop and measure the stickup and stick down. The stainless steel tube and the brass rod should be the correct length so that when you have hammered the brass rod flush with the stainless steel tube the needle should be in the correct position. But it is better to stop and check a couple of times.
4. To reinstall the needle assembly you should hold the assembly off to the side and aim the long needle into the smaller hole in the needle guide. When it is started you can then sort of rotate the assembly so that the needles will slide down into position.
5. Then rethread the steel pneumatic rod into the needle holder until the screw on the steel pneumatic rod just is flush with the bottom of the needle holder. Run the nut down to the holder.
6. Reattach the tubing
7. Visually check that the long needle is in the needle guide but not sticking down below the needle guide.
8. Put a vial in the next position, turn to it, plunge needle in, turn on carrier gas, confirm that gas flows to post sample scrubber. If no flow either the tubing is not on tight or, if you changed the shorter needle, maybe the shorter needle is not in far enough.

Troubleshooting:
No liquid delivery (either acid or water)

Some users have had problems with getting the pumps to prime (fill with liquid at first usage). There are two possible causes of this problem. (1) a stuck valve (or diaphragm)in the KNF pump or (2) an air block in the tubing between the water bottle and the water pump (most likely between the bulkhead fitting and the pump).

The good thing is that the same procedure should solve either problem. Solution below is outlined

for the water system but the acid system is fixed the same way.

To solve it you will need a disposable plastic syringe of about 25 to 50 ml (no needle).
One with a locking Luer connection is best but not important.

1. Pull the tube out of the water bottle.
2. Fill syringe with DI water
3. Insert tip of syringe into water tube
4. Turn carousel to a position with an empty Exetainer vial with no cap
5. Move needle down under System
6. Turn on carrier gas under System
7. Turn on water pump under System (you should hear the pump hum and a click)
8. Push on the syringe and gently force water into the tube (you will have to hold the tube onto the syringe)
9. You should see water leave the syringe and go into the tube. Pump should change sound as it starts to pump water (it will slow down a little).
10. Turn off water pump under System or after 7 seconds it will turn off automatically.
11. Replace tubing into water bottle
12. Run the water pump a couple of time to check
13. Turn off carrier gas under System
14. Move needle up

If this does not work try taping (gently hitting) on the tubing inside the wet box while the pump is running. The next sentences tell where to tap. The water bulkhead fitting is attached to a larger diameter tube which goes to a reducing fitting then to a smaller diameter tube and then to the pump. Tap on the reducing fitting and also anyplace the tubing loops up and allows a place an air block could occur. You could also bend the tubing down so that any loops are forced downward to allow air to move.

Lake sediment phytolith extraction

Written by Chad L. Yost (chadyost@email.arizona.edu)
Modified for use at LacCore by Jessica Heck (jheck@umn.edu, LacCore.org)

Safety:
All personnel are required to follow strict safety requirements. The chemicals, glassware, and equipment used in this procedure can be hazardous if mishandled. Lab staff must be specifically trained by experienced personnel.

Required protective gear: lab coat, splash goggles and gloves must be worn at all times. Staff must also wear closed toe shoes and long pants. Hair must be tied back.

All chemical reactions must be performed in a fume hood. Any sample with acid in it must be decanted in a waste bucket for neutralization before disposal. Once neutralized, these chemicals (along with the non-acids) may all be washed down the drain with tap water.

Record Keeping:
Notebook: Each preparer should have a lab notebook to log the process as it is done. The book should be a permanent bound volume, not just loose pieces of paper. We also keep a computer log. Make hardcopies of the printout, one going to each researcher that had samples processed in the batch.

All labeling is important. A perfectly prepared sample that is mislabeled is useless. Sample vial labels are permanent and part of the research archives. The label should include site name, location, core #, sample depth, sample volume, preparation date, and spike quantity. Make sure to double check that the label matches what you have written in your lab notebook. Additionally it is critically important to label all tubes and beakers correctly to minimize errors related to the mixing up of samples.

There is no substitute for careful personal instruction in the subtleties of this procedure. Samples vary more than you might expect, and it is often necessary to give some of them individualized treatments. While learning, we encourage you to ask if you are unsure what to do. We want you to know that mistakes happen, even to those who have years of laboratory experience. The critical requirement is the *mistakes be noted and discussed* when they happen so corrections or adjustments can be made. It is generally best to start over.

Quantitative procedure notes:
This is a quantitative procedure**.** This means that in theory, there is no loss of phytoliths during the course of the treatment. Be aware of the causes of lost sample material as discussed below. Concentrate on what you are doing.

Phytolith data is only as good as the preparation. An analyst must be confident that the differences between samples are due to natural differences in the samples and not due to differences in the phytolith preparation procedures. The same lab technician should process all the samples in a project.

Contamination problems often come from poor housekeeping. Keep your area clean.

When transferring samples, make sure the entire sample is transferred. Be very careful in decanting samples as this is the greatest potential source of loss of materials in the sample short of dropping the sample.

Reagents:
Reagents are dispensed from repipettes, squeeze bottles, or special anti-drip bottles if dangerous. To prevent contamination, do not touch the tip of the squeeze bottle to the side of a test tube. These chemicals are dangerous and should be treated as such. Please be familiar with the SDS (Safety Data Sheets) for each chemical before beginning.

Hydrochloric acid (HCl), 36%, use as supplied.

Nitric acid (HNO_3), 67%, use as supplied.

Potassium hydroxide (KOH), 10%, 100 g potassium hydroxide pellets plus 900 mL DI water. Make sure the accuracy is to the nearest gram and measure DI water in a graduated cylinder. Put weighed pellets in container and SLOWLY add DI water.

Sodium hexametaphosphate (SHMP), 5% = 950 mL DI water plus 50 g sodium hexametaphosphate. Make sure the accuracy is to the nearest gram and measure water in a graduated cylinder.

Soda ash-for neutralization

Equipment:
- We currently use an Eppendorf 5810 centrifuge. Please see the Centrifuge SOP for proper use, care, and maintenance. You are responsible for following the information in the Centrifuge SOP. It is essential that the centrifuge buckets be balanced by weight.
- You need centrifuge vapor caps capable of capping both 15 mL and 50 mL centrifuge tubes. Since at some stages hazardous chemicals will be centrifuged, it is necessary to have a system that protects room air quality. The buckets are loaded and capped inside the fume hood to seal in fumes before being transferred to the centrifuge outside of the fume hood. We use the vapor caps supplied by Eppendorf for the 5810 centrifuge.
- Specific centrifuge tube adaptors are needed for each style of tube used in the centrifuge. We use adaptors supplied by Eppendorf with minor modifications.
    - 15 mL plastic conical tubes – 15 mL falcon adaptors, blue and the bottom layer of the adaptor is conically formed. Holds 9 tubes per bucket; maximum of 36 tubes in the centrifuge. See Fig. 2 on p. 5.
    - 50 mL plastic conical tubes – 50 mL falcon adaptors, orange with blue conical inserts that must be placed in the bottom of the adaptors before loading the tubes. The blue conical inserts must be used or the bottom of the tube may deform or split open. Holds 4 tubes per bucket; maximum of 16 tubes per centrifuge. See Fig. 3 on p. 5.
    - 1 dram vials – 3-15 mL adaptors, orange, includes rubber mat at base. Assemble adaptors but only use two orange layers on top of the base (with the rubber mat). Holds 12 vials per bucket; maximum of 48 tubes per centrifuge. See Fig. 4 on p. 5.
- Electric hot plate and a flat-bottomed sauce pan for maintaining a boiling water bath.
- A Griddle may be used as large hotplate for heating beakers
- Vortex mixer.

- Fume hood: all reactions must be done in a fume hood
- Test tube racks. Need several for each test tube type.
- Test-tube rack that fits into the sauce pan. We take standard test tube racks and cut them in half so that they fit in our sauce pan.
- Conical centrifuge tubes:15 mL polypropylene (Nalgene #3103-0015/Fisher #05-502-10A) and 50 mL polypropylene (Falcon/BD# 2098/Fisher #14-959-49A). *Do not use polycarbonate*. Carefully inspect all tubes (plastic and glass) before each use for any signs of crazing, cracking, or splitting. The multiple chemical assaults weaken them over time.
- Adjustable pipette and magnetic stir plate (if using LacCore Phytolith Spike suspension). We use an Eppendorf Research pipette that adjusts from 100-1,000 µL.
- Sieves system: We use "custom" designed 70 micron sieves. They are constructed out of screen, 1" plastic (PVC, ABS, …) tubing, and plastic epoxy such as J-B Weld or Loctite epoxy plastic binder. Our current screen supplier is SEFAR 1-800-995-0531, their site is: www.labpak@sefaramerica.com. They sell by the yard, but the better deal is their LabPaks, which hold several 12" squares and are available in a wide range of nylon or polyester mesh sizes.
- Glassware: 400ml tall form beakers, 100 mL graduated cylinders, microscope slides and cover slips for checking samples.
- Cleaning supplies: washbasin, sponges, paper towels, detergent, glassware brushes, gloves, etc…
- Funnels for holding sieves.
- Vacuum sipper set up. We use an Erlenmeyer flask with a side port connected to the in-house vacuum lines. A stopper and tube for sipping is attached to the top of the flask for sipping.
- Dispensing bottles (500 mL): Squeeze bottles and repipettes are appropriate for most reagents. For the screen steps, spray bottles are helpful in moving material through the screens.
- Benchkote for protecting the fume hood surface and countertops. This should be changed frequently to keep the area clean.

Procedure
Follow the steps in sequence**.** The procedure is designed so that the steps do not interfere with each other. Examples: Potassium hydroxide breaks up the sample so it screens well. **Ask questions if you are not sure what to do next.**

This procedure takes roughly 3 days to prepare 12 sediment samples for phytolith analysis. Except where noted, it can be stopped at the end of each step. At the end of a work period, cover both the samples and the test tubes for holding the stir sticks. Label the test tube rack with the bill to/project code/user information, date, your name, and the current preparation stage. Leave the samples covered in the fume hood until you are able to continue the procedure.

There are 9 basic steps to the phytolith extraction procedure.
Step 1: Set-up
Step 2: Hydrochloric and nitric acid treatments
Step 3: Potassium hydroxide treatment
Step 4: Screening
Step 5: Gravity settling
Step 6: Freeze drying

Step 7: Heavy liquid separation
Step 8: Polystyrene spike
Step 9: Final storage medium/slide preparation
Step 10: Record completion
Step 11: Clean up

Steps can be conveniently divided up into 3 days. Day one usually consists of steps 1-3. Day two usually consists of steps 4-6. The final day is steps 7-10. Depending on initial sample volume and composition this may vary significantly.

Step 1: Set up and spike

1. Before you begin, label the test tubes and racks you plan to use. You will need three test tubes for each sample: one 50 and one 15 mL. Our current setup allows for 12 samples to be run simultaneously.
2. Transfer 1 cc of sediment into 400 ml tall form beakers.
3. Add exotic palm phytolith spike-details to be determined. Polystyrene spike will be added at the end of the procedure if it is used instead of exotic palm spike.

Step 2: Hydrochloric and nitric acid treatments

1. Add 10mL 36% HCl, swirl sample and place on hotplate. Record the reactivity of the HCl with the carbonates (none, weak, moderate, strong).
    a. Add slowly, especially if carbonates are present.
    b. HCl will digest carbonates and oxidize some organics.
2. Add 50 mL 68% HNO_3 to beakers, swirl sample and place back on hotplate for 1 hour, or until oxidation has visibly stopped. Bring solution temperature up to 115C Cover beakers with a watch glass to prevent sediment from baking to the sides and swirl beakers every 15 minutes to prevent sediment from baking on.
    a. For sediments low in organics and/or charcoal, the acid solution will turn yellow when oxidation is complete. Otherwise, the solution may appear reddish-orange. It's possible that the boiling time may be > 1 hour.
    b. 10 mL HCl + 50 mL HNO_3 should be enough to completely immerse 1 cc of sediment. If more acid is required add it preserving a 1:5 HCl:HNO_3 ratio. The addition of HCl to HNO_3 lowers the boiling point of the solution and reduces the "knocking" of beakers on the hotplate.
    c. Nitric acid strongly oxidizes organics, and will work on carbonates as well. It will also remove iron (Fe) and aluminum (Al) oxide clays.
3. Once the reaction is complete, based on the color of the liquid and the gas trapped under the watch glass, remove the watch glass and boil-off enough liquid so that ~20 mL remains. Once 20 mL of sample remains remove the sample from the hotplate and cool until the beaker can easily be handled (5-10 minutes).
4. Transfer the sample with high purity dH_2O into 50 mL centrifuge tubes.
    a. Swirl the beaker, pour, and then rinse the beaker with a fine stream of high purity dH_2O into a 50 mL centrifuge tube.
5. Rinse to neutral with high purity dH_2O.
    a. Rinses 1-2: centrifuge 3,000 rpm for 1min. Rinses 3-4: centrifuge 3,000 rpm for 3 minutes. Add 2-3 mL high purity dH_2O, vortex for 30 seconds in-between rinses, then top off to 40 mL with high purity dH_2O using a squeeze bottle with enough force to mix the sample.

6. Transfer the sample with high purity dH_2O into 15 mL centrifuge tubes. Vortex, and centrifuge 3,000 rpm for 3 minutes. Decant.

Step 3: Potassium hydroxide treatment

1. Add 1 mL of room temp 10% KOH to each tube, vortex and add another 9 mL 10% KOH. Let stand for about 5 min. Vortex sample after 2.5 minutes if sample size is large.
    a. <u>Caution:</u> *Warm to hot 10% KOH can result in some phytolith dissolution after as little as 20 to 30 minutes exposure*.
    b. Potassium hydroxide is used to remove humates from the sample. KOH also acts to deflocculate the sample for sieving.
2. Top off with high purity dH_2O and conduct another 5 reps of rinsing to neutral pH as described in step 8.
    a. This is a good time to stop in order to save screening and gravity settling for the next day. However, if sample size, clay-load, and diatom abundance require more than one day of gravity settling, Day 2 steps could be started at the end of Day 1.

Step 4: Screening

1. Set up sieves. Use a small diameter sieve, placed in a funnel, which is then placed on top of a 100 ml graduated cylinder.
    a. 70 micron sieve will remove some of the diatom and sponge spicule fraction. Although gravity settling is a clay-separation technique, it also works to remove a portion of the diatom and sponge spicule fraction.
    b. Sieves are constructed of two pieces of 1 1/8" diameter PVS or ABS. A screen is glued into place between two cylinders with J-B Weld.
    c. Phytoliths typically range in size from 10 to 200 microns. Depending on the context and goals of the phytolith study, a larger sieve mesh (100, 150, 200, etc.) may be selected for use. Sieve size will be determined before the start of the extraction procedure. This procedure is optimized for the recovery of phytoliths diagnostic of wild rice (Zizania palustris), so the 70 micron sieve size was selected.
2. Using a squeeze or spray bottle filled with a 5% sodium hexametaphosphate (SHMP) solution, rinse the sample through the sieve (Figure 1).
    a. Use a spray bottle where you can adjust the water stream so that it is a wide forceful shower rather than a single stream.
    b. Sieves can be placed upside-down in a sonic bath with 1% SHMP solution.

Step 5: Gravity Settling

1. Once the sample is through the sieve, use a forceful stream of 5% SHMP solution from the squeeze bottle to thoroughly mix the sample as you fill the graduated cylinder to the 100 ml mark. Make sure the sample is well mixed and then allow it to settle for 1 hour.
    a. The sample can be more thoroughly mixed by covering the graduated cylinder and inverting it. Be sure to rinse the entire sample off of the cover and back into the graduated cylinder.
2. After 1 hour, sip the top 10 cm of supernatant in each graduated cylinder using a vacuum sipper and discard (Figure 2).
3. Next, use a squeeze bottle with 5% SHMP to deliver a forceful stream of solution to mix the sediment and to fill the cylinder back to the 100 ml mark (+/- 3 mL). Allow the sample to settle for 1 hour and then aspirate the top 10 cm as previously described.

4. Repeat steps 5.2-5.3 until the sample has reached a state where after 1 hour, the top 10 centimeters are completely clear (all clay-sized particles have been removed).
    a. When staring 1 cc of lake sediment, it typically takes 4 to 5 rounds of gravity settling. Starting more than 1 cc of sediment may require additional settling reps. It may be possible to conduct 1 or 2 rounds of settling after the acid/base steps on the 1st day of extraction.
    b. When it appears that only one more round of gravity settling will be necessary, only fill the graduated cylinders to the 75 ml level, and after 1 hour, aspirate 10 cm down from there. This should leave 20 ml of solution at the bottom of each cylinder.
    c. This method is based on Stokes law of settling, $= \frac{g(\rho_s - \rho_l)d^2}{18\eta}$, solved for time, $t = \frac{18\eta h}{v} = \frac{18\eta h}{g(\rho_s - \rho_l)d^2}$. Where v =velocity of fall, g=acceleration due to gravity, ρ_s=particle density, ρ_l=liquid density, d= particle diameter, η =liquid viscosity, t = time to sip, h = depth to sip down to.
5. Transfer the samples from the graduated cylinder to 50 mL centrifuge tubes. Pick up the graduated cylinder with the first sample, swirl by hand to fully suspend the sediment and quickly pour into a 50 ml centrifuge tube. Keep cylinder inverted while using the RODI water squeeze bottle to thoroughly rinse all sediments out of the cylinder and into the centrifuge tube.
6. Vortex, centrifuge (10 minutes at 3000 rpm), and carefully decant.
    a. Pellets at this stage can be fairly loose and easily disintegrate. Stop decanting when the meniscus reaches the pellet; however, enough water needs to be decanted to allow subsequent transfer into a 15 ml tube.
    b. If more than 4 ml of sediment remain after gravity settling, heavy liquid separation should be conducted in a 50 ml tube, otherwise continue by transferring the samples into 15 ml centrifuge tubes, spin for 10 min at 3000 rpm, decant all remaining water, and vortex to loosen pellet. Samples are now ready to dry for heavy liquid separation.

Step 6: Freeze dry

1. (Optional) Freeze the samples, place in a vacuum chamber and dry overnight. Samples are typically dry when atmosphere in the chamber is less than 200 millitorr (mTorr). When dry, remove samples from the vacuum chamber, cap, and vortex for 30 to 45 seconds to loosen the sediment.
    a. Freeze drying helps to release clays and to separate particles that may otherwise adhere to each other. Drying samples will also ensure that the heavy liquid density (next step) is not reduced due to the presence of rinse water retained in the sample. This step may not be necessary and its inclusion or omission in the extraction procedure will be determined on a project-by-project basis.

Step 7: Heavy liquid separation

1. Prepare a heavy liquid solution (LST – solution of lithium heteropolytungstates in water) with a density of 2.3 g/ml and add a 3 ml to each sample.
    a. The exact amount of heavy liquid that you add to each sample may vary. For 15 ml tubes, I like to have around 2 or 3 ml of separation between the sample pellet and the heavy liquid surface. For 50 ml tubes, I like to have at least 5 ml of separation.
    b. Caution – *sediments dried in a vacuum chamber are so dry that the addition of heavy liquid can result in a wisp of material out of the top of the tube. Slowly add the heavy liquid and use a fume hood to minimize cross-contamination between other samples and the heavy liquid container.*

2. Place caps on the centrifuge tubes and vortex thoroughly for at least 30 seconds (starting and stopping several times for through mixing), inverting once to check for complete mixing of each sample at the bottom of the tube.
3. Centrifuge samples for 10 minutes at around 1500 rpm (spinning at 3000 rpm can cause some phytoliths to sink that otherwise would not).
4. Decant the thin film of particles (phytoliths, diatoms, charcoal, etc.) and retain in either a 15 or 50 ml centrifuge tube.
5. Add another 2 to 3 ml of heavy liquid to each sample, mix, and spin as outlined above. Add this even thinner and possibly imperceptible film of residue to its respective tube from Step 24.
    a. The LST steps are repeated for thoroughness, to make sure that all phytoliths and exotic markers have been extracted from the sediment.
    b. The material that pelletized during the heavy liquid spins can be discarded.
    c. Please note – *the total amount of heavy liquid containing the phytolith extract should not exceed 6 ml in a 15 ml tube, and 20 ml in a 50 ml tube. This is because a H_2O to heavy liquid ratio of 2.5 to 1 must be maintained in order to ensure that phytoliths sink and pelletize during the rinse steps outlined below*.
6. The tubes containing the retained heavy liquid and floated phytolith fraction need to be filled with RODI water and very thoroughly mixed, often requiring inverted shaking to make sure that the less dense water fraction combines with the more dense heavy liquid fraction.
7. Centrifuge the samples for 10 minutes at 3000 rpm and decant, making sure that the meniscus does not disturb the phytolith-laden pellet while decanting.
    a. If recycling the LST, the decanted heavy liquid should be retained for recovery and reuse.
8. If the phytoliths that floated in heavy liquid were retained and rinsed in 50 ml tubes, they should now be transferred to 15 ml tubes for the remainder of the rinses.
9. The phytolith extract samples should be rinsed (mix with H_2O, centrifuge, decant) at least 4 more times to make sure that all heavy liquid is removed.
    a. If it is not, the addition of alcohol (next step) with result in phytoliths sticking together during microscope slide prep. Rinse centrifuge times can probably be reduced to 5 min at 3000 rpm unless the phytolith extract pellets are too loose.

Step 8: Polystyrene spike

1. After the last water rinse, add 1.0 mL of polystyrene spike, vortex, centrifuge, decant.
2. Rinse 3 times.

Step 9: Final storage medium/slide preparation

(Add polystyrene spike and rinse 3x if used in place of exotic phytolith spike.)

3. After the last water rinse, add about 3 ml of 95 to 99 % alcohol to each sample, centrifuge (5 min 3000 rpm), and decant. Transfer using alcohol to 1.5 ml screw-cap vials with o-rings for storage. Fill the vials with alcohol to prevent any drying out during shipment.
4. If diagnostic slides need to be made place 1 drop of sample onto a microscope slide, add 1 drop of immersion oil, mix, and let the alcohol evaporate (heating is fine). Place a cover slip over the sample and seal the edges of the cover slip to the microslide with nail polish. The nail polish may need to be added 2-3 times, with drying in between.

Step 10: Record Completion

Make sure that everything is properly complete in your own lab book. Your book is sometimes checked for details on a procedure done to a specific sample, sometimes years later.

Transcribe your log into the FileMaker Pro log. Record any errors or discarded samples. Be certain the sample owner is aware of any problems; e-mail them to make them aware of any problems and the reasons for such problems. **Do not wait for the sample owner to get a copy of the log and ask about the problems, contact them first and notify a staff member.** Print a copy of the FileMaker Pro log entry and give it to the sample owner with their samples. Do not begin another batch until this is done. It is easier to find samples in this log if it is in chronological order.

Update the billing database with the number of samples processed for each project code.

Step 11: Clean Up

Laboratory glassware: Wash every item after use. Wash with lab detergent and bleach, rinse 3 times with tap water followed by 2 DI water rinses. Gloves and plastic apron are recommended when washing glassware. The glassware should have no spots or rings when dry. Put the dishes away when they are dry.

Countertops, shelves, hood surface: Clean regularly. Hood surface and countertops after each use and shelves at least once a month. Place benchkote on the surface of the fume hood and change it often.

Since the acids and bases used are inorganic, they may be disposed of down the drain after they have been neutralized (pH > 7) in the waste bucket. . All decanting involving hazardous chemicals was done into the waste bucket. Neutralize it with a scoop or two of soda ash as needed throughout the day. Foaming can be controlled by squirting the reaction with 95% EtOH (ethanol) or TBA (tertiary butyl alcohol). ALWAYS completely react wastes in the waste bucket by adding more soda ash until they test neutral or slightly basic using pH tape. DO NOT leave un-reacted wastes in the bucket overnight. Clean it for the next person.

References:

C.L. Yost, M.S. Blinnikov and M.L. Julius, 2013. Detecting ancient wild rice (*Zizania* spp. L.) using phytoliths: a taphonomic study of modern wild rice in Minnesota (USA) lake sediments. *Journal of Paleolimnology* 49(2), 221-236.

Aleman, J.C., et al., Estimating phytolith influx in lake sediments, Quaternary Research (2013), http://dx.doi.org/10.1016/j.yqres.2013.05.008

Figure 1. Small diameter sieve on graduated cylinder set up

This is the suggested set up for Day 2, Step 5 sieving of sample into a graduated cylinder for subsequent gravity settling repetitions. (photo credit: C. Yost)

Figure 2. Suggested set up for gravity settling steps

Yellow slide markers are placed at the 10 cm level. Vacuum pump and Erlenmeyer flask with pipette on right used to aspirate the supernatant to the 10 cm level. (photo credit: C. Yost)

Total P and total N (TPTN) and/or dissolved P and dissolved N (DPDN) digestion

1.0 Kelly Thommes, 6/13/2000

DIGESTION:

1. Samples will be analyzed on the Lachat autoanalyzer for both Total Phosphorus and Total Nitrogen (TPTN, unfiltered) and/or Dissolved Phosphorus and Dissolved Nitrogen (DPDN, filtered through a 0.45 µm filter). Forty-eight samples can be processed per batch (this includes QA/QC samples).

2. Print out sample names using the plastic labels and place on acid-washed 60-mL HDPE bottles. Include project initials, site #, type of water sample (SW or GW), TPTN or DPDN, site name, date, and time. Include calibration standards, check standards, blanks, digestion efficiency standards, duplicates, spikes, lab-fortified blanks, and samples. Ten percent blanks and duplicates should be included. If enough sample exists, use the same sample for the duplicate as for the spiked sample. Include one spiked-sample and one lab-fortified blank for phosphorus and one spiked-sample and one lab-fortified blank for nitrogen. Use Deionized (DI) water for the zero calibration standards, blanks, and lab-fortified blanks.

3. Using the spreadsheet generated for labels, record the weight of the labeled bottles (with cap) using the analytical balance connected to the laptop computer.

4. Remove cap, and tare the 60-mL HDPE bottle on the balance. Pour 20 g (+/- 0.5 g) calibration standard, check standard, efficiency standard, duplicate, blank, or sample into the 60-mL HDPE bottle. Remove the bottle and replace cap. Tare the balance and record weight of the bottle+sample with cap.

5. When pouring out the spiked-sample or lab-fortified blank, record the sample weight (20 g +/- 0.5 g). Using a calibrated auto pipette, add 3 mL of the 100 µg P/L calibration standard for the phosphorus spiked-sample and phosphorus lab-fortified blank. Add 3 mL of the 8.00 mg N/L calibration standard for the nitrogen spiked-sample and nitrogen lab-fortified blank. Record weights of spike added.

6. Using the calibrated 5-mL auto pipette, add 5 mL of digestion solution (made from the ND-SOP) to each bottle. Cap tightly and shake to mix. Place loosely capped sample bottles in autoclave and digest for 15 min at 121 ^{o}C and 16 psi. Remove samples from autoclave and cool in freezer for 20-30 min (keep caps loosened). When cool enough to handle, add 0.5 mL of 11 *N* H_2SO_4 to each bottle, cap tightly, and shake to mix. Place loosely capped bottles back into autoclave for an additional 30 min at 121 ^{o}C and 16 psi. Again, cool samples in freezer. When cool enough to handle, tightly cap and shake bottles. Dry bottles if wet and record bottle+sample weight.

7. Samples can now be run using the Lachat autoanalyzer. Samples should be run preferably the same day or no more than a couple of days after the digestion.

DIGESTION REAGENTS AND STANDARDS:

Digestion Solution

To a 1-L volumetric, dissolve 10.48 g of granular sodium hydroxide (NaOH) and 42 g of potassium persulfate ($K_2S_2O_8$) in approximately 900 mL of DI reagent grade water. When dissolved, bring to volume.

11 *N* Sulfuric Acid (H_2SO_4)

To a 1-L volumetric and in a fumehood, add 305 mL of concentrated sulfuric acid to about 600 mL of DI reagent grade water. The volumetric should be surrounded by an ice bath while at the same time swirled to reduce the heat. When cool, bring to volume.

Phosphorus Stock Standard 25 mg P/L

To a 1-L volumetric, dissolve 0.1099 g primary standard grade anhydrous potassium phosphate monobasic (KH_2PO_4) that has been dried for one hour or overnight at 105 ^oC in about 800 mL DI reagent grade water. Bring to volume and invert to mix.

Phosphorus Working Stock Standard 250 µg P/L

To a 1-L volumetric, dilute 10 mL Phosphorus Stock Standard to the mark with DI reagent grade water. Invert to mix.

Nitrogen Stock Standard 200.0 mg N/L as NO_3^-

To a 1-L volumetric, dissolve 1.444 g potassium nitrate (KNO_3) in about 600 mL DI reagent grade water. Dilute to mark and invert to mix.

Phosphorus Working Standards 0, 5, 10, 25, 50, 100, 200 µg P/L

5 µg P/L	5 mL of P Working Stock Standard (250 µg P/L) in a 250-mL volumetric
10 µg P/L	10 mL of P Working Stock Standard (250 µg P/L) in a 250-mL volumetric
25 µg P/L	0.25 mL of P Stock Standard (25 mg P/L) in a 250-mL volumetric
50 µg P/L	0.50 mL of P Stock Standard (25 mg P/L) in a 250-mL volumetric
100 µg P/L	1.00 mL of P Stock Standard (25 mg P/L) in a 250-mL volumetric
200 µg P/L	2.00 mL of P Stock Standard (25 mg P/L) in a 250-mL volumetric

Nitrogen Working Standards 0.00, 0.20, 0.40, 1.00, 4.0, 8.0, 20.0 mg N/L

0.20 mg N/L	0.25 mL of N Stock Standard (200.0 mg N/L) in a 250-mL volumetric
0.40 mg N/L	0.50 mL of N Stock Standard (200.0 mg N/L) in a 250-mL volumetric
1.00 mg N/L	1.25 mL of N Stock Standard (200.0 mg N/L) in a 250-mL volumetric
4.0 mg N/L	5.00 mL of N Stock Standard (200.0 mg N/L) in a 250-mL volumetric
8.0 mg N/L	10.0 mL of N Stock Standard (200.0 mg N/L) in a 250-mL volumetric
20.0 mg N/L	25.0 mL of N Stock Standard (200.0 mg N/L) in a 250-mL volumetric

Check Standards Amp 2 for TN and TP (Record Lot # on volumetric and benchsheet)
5 µg P/L, 25 µg P/L, 100 µg P/L with 0.30 mg N/L, 10 mg N/L

Stock Adenosine 5'-triphosphate disodium salt hydrate (Aldrich A26209) 99% pure, 50 mg P/L

To a 1-L volumetric, dissolve 0.2996 g Adenosine 5'-triphosphate disodium salt hydrate that has been dried for one hour or overnight at 105 ^{o}C in about 800 mL DI reagent grade water. Bring to volume and invert to mix.

Phosphorus Efficiency Standard 100 µg P/L
To a 250-mL volumetric, add 0.50 mL Stock Adenosine (50 mg P/L) and bring to volume.

Phosphorus Efficiency Standard 25 µg P/L
To a 250-mL volumetric, add 0.125 mL Stock Adenosine (50 mg P/L) and bring to volume.

Stock Glutamic Acid 100 mg N/L
To a 1-L volumetric, dissolve 1.3366 g glutamic acid that has been dried for one hour or overnight at 105 ^{o}C in about 800 mL DI reagent grade water. Bring to volume and invert to mix.

Nitrogen Efficiency Standard 8.00 mg N/L
To a 250-mL volumetric, add 20.0 mL Stock Glutamic Acid (100 mg N/L) and bring to volume.

Nitrogen Efficiency Standard 1.00 mg N/L
To a 250-mL volumetric, add 2.50 mL Stock Glutamic Acid (100 mg N/L) and bring to volume.

AUTOMATED COLORIMETRIC PROCEDURE ON THE LACHAT QUICHEM 8000 AUTOANALYZER

	Phosphorus	Nitrogen
Method	SCWRS Method	10-107-04-1-A
Sample Loop	133 cm	Microloop
Interference Filter	880 nm	520 nm
Chemistry	Bipolar	Direct
Inject to Peak Start		
Peak Base Width		
% Width Tolerance		
Threshold		
Method Cycle Period		
Probe in Sample		
Sample reaches 1st Valve		
Load Period		

LACHAT REAGENTS

PHOSPHORUS MANIFOLD
Stock Ammonium Molybdate Solution
To a 1-L volumetric, dissolve 40.0 g ammonium molybdate tetrahydrate $[(NH_4)_6Mo_7O_{24}\bullet 4H_2O)$ in approximately 800 mL of DI reagent grade water. Dilute to mark and mix with a magnetic stirrer for at least four hours. Store in plastic and refrigerate.

Stock Antimony Potassium Tartrate Solution
To a 1-L volumetric, dissolve 3.0 g antimony potassium tartrate (potassium antimony tartrate hemihydrate $K(SbO)C_4H_4O_6 \bullet 1/2H_2O$) in approximately 800 mL of DI reagent grade water. Dilute to mark and mix with a magnetic stirrer until dissolved. Store in a dark bottle and refrigerate.

Working Molybdate Color Reagent
To a 1-L volumetric, add approximately 500 mL DI reagent grade water and 20 mL concentrated H_2SO_4. Swirl until cool and add 213 mL of Stock Ammonium Molybdate Solution, then add 72 mL of Stock Antimony Potassium Tartrate Solution. Dilute to mark and invert to mix. Degas with helium.

Working Ascorbic Acid
To a 1-L volumetric, dissolve 60.0 g ascorbic acid in approximately 900 mL of DI reagent grade water. When dissolved, dilute to mark. Degas with helium. Add 1.0 g sodium dodecyl sulfate ($CH_3(CH_2)_{11}OSO_3Na$). Invert to mix. Prepare fresh weekly.

Phosphate Carrier 0.231 N H_2SO_4
Dilute 21 mL of 11 N Sulfuric Acid to 1-L volumetric with DI reagent grade water. Degas with helium.

Sodium Hydroxide-EDTA Rinse
To a 500-mL volumetric, dissolve 32.5 g sodium hydroxide (NaOH) and 3 g tetrasodium ethylenediamine tetraacetic acid (Na_4EDTA). Dilute to mark and invert to mix. Store at room temperature. Use this to clean phosphorus manifold lines. Pump reagent through for about five minutes followed by DI water for five minutes.

NITROGEN MANIFOLD
15 *N* Sodium Hydroxide (NaOH)
To a 500-mL volumetric, add 75 g NaOH very slowly to approximately 250 mL of DI reagent grade water. Caution: the solution will get very hot. Swirl until dissolved. Cool and store in a plastic bottle at room temperature.

Ammonium Chloride Buffer, pH 8.5
To a 1-L volumetric, dissolve 85.0 g ammonium chloride (NH_4Cl) and 1.0 g disodium ethylenediamine tetraacetic acid dihydrate ($Na_2EDTA \bullet 2H_2O$) in approximately 800 mL DI reagent grade water. Dilute to mark and invert to mix. Adjust pH to 8.5 with 15 N sodium hydroxide.

Sulfanilimide Color Reagent
To a 1-L volumetric, add approximately 800 mL DI reagent grade water. Add 100 mL 85% phosphoric acid (H_3PO_4), 40.0 g sulfanilimide, and 1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride (NED). Shake until wetted and stir to dissolve for 30 min. Dilute to mark and invert to mix. Store in a dark bottle. This solution is stable for one month.

REFERENCES

Standard Operating Procedure For the Analysis of Total Phosphorus and Total Nitrogen in Water From an Alkaline Persulfate Digest, North Dakota Dept. of Health, Chemistry Div.

EPA (March 1983) Method 353.2 (colorimetric automated, cadmium reduction)

Lachat (Aug 1994) QuikChem Method 10-107-04-1-A (Nitrate/Nitrite)

Lachat (Feb 1996) QuickChem Method 10-115-01-1-B (Determination of Orthophosphate by FIA Colorimetry)

AVS and Aq-sulfides

1.0 Jill Coleman Wasik, 11/2009
2.0 E. Mittag, 11/26/2009, edited to include a procedure for extracting sulfides from pore waters.

This SOP is developed from Standard Methods 4500-S2- A., B., C., D., G., and I, as well as lab SOPs provided by P.E. Drevnick and C.R. Hammerschmidt, and articles by Hsih and Shieh (1997), Hsieh et al. (2002), Hammerschmidt and Burton (2010), and Brumbaugh and Hammerschmidt (2011).

Sulfide is a reactive constituent in natural environments, readily binding with soft metals and complexing with natural organic matter. In anaerobic sediments sulfide plays an important role in sequestering iron and certain heavy metals. The acid-volatile sulfide (AVS) fraction in sediments is thought to represent dissolved sulfides, amorphous FeS species, and sulfide-bound, acid-extractable, cationic metals. This SOP describes the method used to extract AVS from sediments and prepare a sample for simultaneously extracted metals (SEM) analysis by ICP-MS.

Sample collection: Samples should be collected and processed as much as possible in a manner that preserves the sulfur speciation within the sediments. The primary concern in sample processing and preservation is exposure to oxygen because the sulfide can rapidly oxidize in the presence of oxygen. Thus subsampling, stirring, and exposing samples to air can all compromise AVS concentration. Following collection samples should be quickly homogenized, if necessary, placed in sample containers with little to no headspace, and frozen under nitrogen, argon, or CO_2. In the field, a cooler of dry ice works well for fast preservation following processing. In the lab, a glove bag or anaerobic chamber is useful for processing samples. If SEM analyses are not anticipated, sediments may be preserved with ~1 mL of 1 N zinc acetate solution/50 mL sediment. The zinc binds dissolved sulfides reducing the effects of oxygen exposure. However, it is still recommended that the chemically preserved samples be frozen under an anoxic atmosphere as soon as possible.

Equipment:
4-6 120mL Teflon jars (Savillex #100-0120-01)
4-6 70mm 1/8th inch MNPT stopcock (Savillex #751-PVT2-2-2); 1/8th inch tubex 1/8th inch MNPT stopcock (Savillex #751-PVT
8-12 60mL round bottom Teflon vials (Savillex #200-060-2)
8-12 33mm transfer closures w/2 1/8th inch push-in tubing side ports (Savillex #600-033-23)
3-5 PFA Tees (Savillex #751-UT2N)
25ft FEP 18th inch ID tubing (Cole Parmer #S-06406-64)
25ft Nalgene FEP 1/8th inch OD tubing (VWR #63014-710)
4-6 position stirring plate
4-6 sediment stir bars (VWR #58949-232)
4-6 CP 65-mm flowmeter w/ SS fittings, valve, max air-flow 50mL/min (Cole-Palmer #S-

32015-01)
1mL pipet and tips
10mL pipet and tips
Balance
Ultra hi-purity nitrogen (5.0)
2-stage nitrogen gas regulator
Agilent BOT 1/8th inch high capacity oxygen trap (VWR #HPBOT-2)
Restek indicating O2 trap (Fisher Scientific #06-711-815)
Silver/Sulfur ISE probe (Fisher Scientific #13-620-545) and meter
Lachat FIA autoanalyzer
Lachat manifold for sulfide QuikChem Method #10-116-29-1-X
Glove bag (optional)
Assorted labware for making up solutions:
2: 2L Erlenmeyer flasks fitted with rubber stoppers and diffusion tubes for deoxygenation
1: 100mL amber volumetric flasks
1: 2L volumetric flask
1: 100mLgraduated cylinder
1: 200mL glass bottle
1: 20mL glass vial

Saturate Stock Preparation:

Prepare a saturated stock solution of sulfide in a glove bag for use in preparation of working Lachat standards and daily check standards. Pipet 5 mL of DDIW into an amber vial. Remove a crystal of Na_2S*9H_2O from bottle and wash the crystal surface with DDIW to remove oxidation products. Dry crystal and place in vial with DDIW. Add enough crystals to the vial until the solution is saturated and no more will dissolve (~10mL of Na_2S*9H_2O in 5mL DDIW will suffice). Aid dissolution by slowly inverting or turning vial. Allow solution to sit overnight. Add more crystals if necessary and allow to sit for another 4 hours. Once solution is saturated (no more crystals will dissolve) determine concentration using an ISE probe.

- Make 50 mL of sulfide anti-oxidant buffer (SAOB) by dissolving 4 g NaOH, 3.75 g Na_2EDTA, and 1.8 g ascorbic acid (in that order) in 50 mL DDIW. Make sure each chemical dissolves completely before adding the next chemical. This solution should be remade daily or if the color has turned a deep purple (indicating oxidation).
- Dilute 0.1 mL of saturated sulfide stock in 9.9 mL of SAOB in centrifuge/Nunc tube.
- Place ISE probe in standard-SAOB solution. Stir solution with a micro stir bar. Record initial probe reading.
- Titrate sulfide-SAOB with 0.1M $Pb(ClO_4)_2$ recording probe response after each addition. The sulfide-Pb titration has a very sharp endpoint between -865 and -730 mV. If the sulfide solution was saturated and unoxidized then the endpoint will typically occur between 2 and 2.5mL 0.1 M $Pb(ClO_4)_2$ has been added. So larger volumes can be added at the beginning of the titration (0.5mL Pb(ClO4)2) and smaller volumes as the endpoint is approached (0.1mL $Pb(ClO_4)_2$). Add $Pb(ClO_4)_2$ until the probe response is <-700mV.

- Calculate the concentration of the saturated stock solution using the equation $C_1V_1=C_2V_2$ where C_1V_1 are the concentration and volume of $Pb(ClO_4)_2$respectively and C_2V_2 are the concentration and volume of the original sulfide solution added to the SAOB.
- Dispose of waste from titration in special containers meant for hazardous waste disposal

AVS extraction:
A measured amount of sample is acidified with HCl in an extraction vessel. Theoretically, all acid-extractable sulfides in the sample should be protonated to form H_2S. The H_2S is flushed from the extraction jar by oxygen-free N_2 gas into a trapping vial containing 0.25M NaOH trapping solution.

Note 1- there are several methods available for AVS extractions and countless variations for each. The lab should choose one method and prove through rigorous QAQC testing that the chosen method is capable of consistently and adequately recovering sulfide from the sample matrix.
Note 2- it is assumed that wet samples are analyzed with this method. Samples initially preserved with Zn-acetate may be freeze-dried first, however rigorous QAQC testing should have been performed to assess the effect of freeze-drying on AVS loss prior to processing samples in this manner. If dried sediments are used, then an equivalent dry weight to the 1-3 grams wet weight specified in the method should be used.

Reagents:
*Any mixing should be done by slowly turning and inverting the reagent. Do not shake or vigorously stir as this could introduce oxygen to the solution.

- Dexoygenated, deionized water (DDIW) – all reagents should be prepared using DDIW! Stir 2 L of deionized water in a large Erlenmeyer flask on a hot plate until water boils vigorously. Carefully remove flask from hot plate, cap with a flow-through top, and bubble with nitrogen for at least 45 minutes.
- 0.25 M NaOH diluent – In a 2 L volumetric flask dissolve 20g of NaOH in 2 L of DDIW. Be sure to actively stir/agitate this solution until all the NaOH is dissolved because concentrated NaOH can dissolve glass and weaken the flask. Therefore, this should be stored in plastic containers.
- Alkaline DDIW – dilute 1.6 mL of NaOH diluent to 200mL with DDIW
- 6N HCl – dilute 250mL of concentrated HCl to 500mL with DDIW under a fume hood. Cap flask with a flow-through top attached to an HCl vapor trap and deoxygenate solution with N_2 for approximately 30 minutes.
- 1M Ascorbic Acid – Dilute 17.6 g of ascorbic acid to 100 mL in an amber volumetric flask with DDIW.

Procedure:

Procedures for extracting AVS from sediment and from pore waters differ.
For AVS in sediment:

- Record tare weight of each trapping vial.
- Pour 50 mL of 0.25 M NaOH into first trap vials and record weight (vials connected to extraction jars). If expect sample to be low in sulfides, add 40-45 mL of 0.25 M NaOH to the first trap vials to pre-concentrate the sulfide.
- Pour 25 mL of 0.25 M NaOH into second trap vials and record weight (vials connected to 1st trap vials).
- Pour ~50 mL of alkaline DDIW into extraction jars. *The final volume of sediment slurry in the extraction jar should be 50mL. The water content and volume of sediment added to the jar will affect the amount of DDIW poured out.* Record weight of alkaline DDIW added.
- Assemble extraction manifold (jars and traps) and degas under N_2 (flow meter = 45) while stirring vigorously (stir plate = 5).
- While extraction and trapping solutions are degassing, weigh 1-3 grams of wet sediment onto a 2x2 inch piece of Parafilm. Record sediment weight.
- Fold Parafilm into thirds over sediment sample and pinch one end so Parafilm holds this "burrito" shape being careful not to spill any sediment.
- Turn stir plate off (leave N_2 on), open an extraction jar, and insert Parafilm with sample. Close extraction jar and repeat until all samples are in their jars. Check trap vials for vigorous N2 bubbling to ensure that gas is flowing through manifold. If Parafilm gets caught in jar threads the jar will not seal correctly, flow to the traps will be reduced to non-existent, and sulfide will be lost following acidification.
- Turn stir plate back on the highest speed at which the stir bars will stir consistently with the Parafilm in the jar (usually ~4).
- Degas the extraction manifold for 15 minutes.

For AVS in pore waters:

- Record tare weight of each trapping vial.
- Pour 50 mL of 0.25 M NaOH into first trap vials and record weight (vials connected to extraction jars). If expect sample to be low in sulfides, add 40-45 mL of 0.25 M NaOH to the first trap vials to pre-concentrate the sulfide.
- Pour 25 mL of 0.25 M NaOH into second trap vials and record weight (vials connected to 1st trap vials).
- Pour entire contents of the sample bottle into the extraction jar. Record weight of sample added. Close jars tightly.
- Add 5 mL of SAOB solution to sample bottle. This is the same SAOB solution used to prepare the saturated stock. Stopper sample bottle and slowly swirl contents until all white precipitate is dissolved or has been removed from the sides of the sample jar. Pour SAOB into extraction jar. Close extraction jar tightly. This step helps to solubilize any AVS that is associated with a ZnAc preservative.
    - Note: If sufficient headspace exists in the sample jar, 5 mL of SAOB can be

injected through the septum. A spare needle can be used to vent the bottle. The entire pore water sample with the SAOB will then be emptied into the extraction jar. This allows solubilizing of AVS while minimizing the sample's exposure to oxygen.

- Add 5 mL of DDIW to sample jar, swirl, and pour into extraction jar. Close extraction jar tightly.
- Assemble extraction manifold (jars and traps) and degas under N_2 (flow meter = 45) while stirring vigorously (stir plate = 5). Check trap vials for vigorous N2 bubbling to ensure that gas is flowing through manifold and extraction jars are completely closed.
- Degas the extraction manifold for 15 minutes.

From this point, the method for extracting AVS from sediment and from pore waters is the same:

- Turn off stir plate and N_2. Monitor the gas inlet line on the extraction jars for solution backing up and adjust N_2 pressure accordingly.
- Add 1 mL of 1 M ascorbic acid to each extraction jar through the stopcock fixing and then stir briefly. The ascorbic acid protects sulfides from the oxidizing effects of iron.
- Add 10 mL of 6 N HCl and then 1 mL of DDIW to each jar through the stopcock fixing.
- Turn stir plate on to lowest setting and allow samples to react with acid for 45 minutes. N2 should not be bubbling through solution at a rate more than 1-2 bubbles every 10 seconds.
- Prepare Lachat for sulfide analysis during this and the subsequent break.
- Turn stir plate up to highest speed at which the stir bars will stir consistently and turn N_2 up to ~30 on the flowmeter. Flush extraction jars for 45 minutes.
- Turn off N_2 and slowly open the first extraction jar. Test for H2S vapors. If present close jar, turn on stir plate and N_2, and continue flushing in 15-minute intervals until there are no vapors detectable.
- Disconnect trap vials from extraction jars before acid backs up jar inlet lines and pour out aliquots of trap vial solutions into glass vials for analysis on Lachat.
- Analyze samples on Lachat as soon as possible in order to avoid losing sulfides to oxidation.
- If also analyzing for SEM, then either pour extraction jar solution into containers for storage or filter solution immediately to 0.45 µM and dilute 2:1 with deionized water to create a final 0.5 acid solution.
- Clean extraction jars, trapping vials, and caps with deionized water. If processing samples for SEM, then soak extraction jars and caps in a 20% HCl bath for 15-30 minutes. Thoroughly rinse jars and caps and place in drying oven for at least 15 minutes.

Lachat Analysis

The colorimetric method for sulfide analysis relies on the formation of methylene blue through the reaction of H_2S and dimethyl-p-phenylenediamine in the presence of ferric iron in an acidic matrix. Solution absorbance is measured at 660 nM. Standards and samples must be analyzed within minutes of creation because sulfide is quickly oxidized in the presence of oxygen.

Reagents:

- 0.2M HCl – dilute 8.5 mL concentrated HCl to 500 mL with DDIW under a fume hood. Cap flask with a flow-through top attached to an HCl vapor trap and deoxygenate solution with N_2 for approximately 30 minutes.
- 3 M HCl – dilute 125 mL concentrated HCl to 500mL with DDIW under a fume hood. Cap flask with a flow-through top attached to an HCl vapor trap and deoxygenate solution with N_2 for approximately 30 minutes.
- FeCl3*6H2O – dissolve 6.65 g of $FeCl_3*H_2O$ in 500 mL of 0.2 M HCl.
- Dimethyl-p-phenylenediamine color reagent – dissolve 0.5g of dimethyl-p-phenylenediamine in 500 mL of 3 M HCl.
- 0.25 M NaOH carrier – dissolve 20 g NaOH in 2 L of DDIW (this is the same solution as the diluent used in the AVS extraction).

Standards:

- Saturated S2- stock solution – place several DDIW washed crystals of Na_2S in an amber vial and add DDIW to ~half the volume of the crystals.
- 500 ppm S2- working stock – after calculating concentration of saturated stock solution from the $Pb(ClO_4)_2$ titration, calculate volume needed to create 500 ppm solution by the following equation:

  Saturated stock (mL) = saturated stock (ppm)/50,000

  Dilute calculated volume of saturated stock to 100 mL with 0.25 M NaOH in an amber volumetric flask.
- 2 ppm S^{2-} working standard – dilute 0.4 mL of 500 ppm working stock to 100 mL with 0.25 M NaOH
- Using graduated, 50 mL centrifuge tubes make the working standards for the calibration curve by weight according to the following:

Standard:	A	B	C	D	E	F	G	H
Concentration (ppm):	2	1	0.5	0.25	0.1	0.05	0.02	0
2ppm stnd wt. (g):	50	25	12.5	6.25	2.5	1.25	0.5	0
Division Factor	1	0.5	0.25	0.125	0.05	0.025	0.01	-

- Weigh the appropriate amount of 2 ppm standard into a centrifuge tube for a given standard.
- Divide the actual weight of 2 ppm standard by the Division Factor listed to calculate the final, total weight of the standard once diluted with 0.25 M NaOH (it should be around 50 if you were close to the standard weight).
- Slowly add the 0.25 M NaOH diluent to weight, cap, invert gently so as not to oxidize the standard.
- Run standard curve immediately. Standards are only good for a short period of time after creation.
- Analyze calibration standards and samples according to Lachat QuickChem Method #10-116-29-1-X
- A check standard should be made separately from the calibration standards and run with each set of samples. Typically a 20 ppm standard can be made and dilutions of this standard made new with each set of samples to check that the instrument is calibrated.
- If samples are expected to run over 2 ppm then the Lachat should be calibrated for the higher 1-100 ppm range. Make working standards according to the following using a 100 ppm working standard from the saturated stock solution.

Standard:	A	B	C	D	E	F	G	H
Concentration (ppm):	100	50	25	10	5	2	1	0
2ppm stnd wt. (g):	50	25	12.5	5	2.5	1	0.5	0
Division Factor	1	0.5	0.25	0.1	0.05	0.02	0.01	-

DIC, DOC (Dohrmann Procedure)

1.0 Erin Mortenson, 11/2009

1. Prepare **Reagents** (once per month): DI H2O, Phosphoric acid, and Sodium Persulfate AND **DIC/DOC Standards** (once per month) [see end of SOP for directions]

- auto sampler is connected by white lines, not the boat sampler
- Dohrmann needle is attached, tighten thumb screw
- take keypad out (top gray GS10C), put in RS232, change dip switch (SW2) with small screw driver
  - UV Vis=0, DOC/DIC=S3
- turn on switch in back
- check waste containers
- clean sparging tube and UV reactor with DI water and "no soap" brush
- change liquid in gas/liquid separator every other time you make reagents
  - DI H2O and 3 drops of Phosphoric Acid
- check Maxiumum Integration Time…should be set at four (4) minute default

2. Turn on computer and program: Programs/Phoenix 8000/TOC Talk 3.0 ***

- turn on Nitrogen gas-both large gray valve (all the way) and small black (all the way and 1/2 turn back)
- put instrument in ready mode and verify gas flow rates=200cc/min±10%
  - Setup/Instrument/Ready mode

3. Check septum, gas flow level-gas liquid separator should be at line – check for moisture in perm drier

4. Prime lines: Set up/Diagnostics/Syringe:
   - valve position to reagent, move to 5000 μl, move to waste, move syringe volume to 0, move to.
   - same procedure for acid (5000 μl) and DI H2O (10000 μl)
   - Click "Home Position" at end

5. Wait until baseline is stable before moving on to #6
   - Tekmar techs suggest waiting for 2 hours for baseline to stabilize

6. To Clean: Run/Sample setup/Open/CLEAN.SET
   - choose 5 reps and ready/save changes/start

7. Run a reagent blank (5 reps): open:
   - TOCBLANK.SET = TC Blank Range 2 – TOC Range 0.1-20 ppm
   - OCRGTBLK.SET = TC Blank Range 3,4,5 – TOC Range 20-10000 ppm
   - ICRGTBLK.SET = IC Blank Range 2 – IC Range 0.1-20 ppm
   - ICBLANK.SET = IC Blank Range 3,4,5 – IC Range 20-10000 ppm

Dohrmann Procedure 11/09 (page 2)

8. Run Calibration Curve or Cal Verification:
    - Calibration Curve
        - sample setup/open:
            - TOCCURVE.SET for **low DOC**
            - HIGHCAL.SET for **high DOC**
            - LOWICCAL.SET for **low DIC**
            - ICCAL.SET for **high DIC**
        - check positions, sample type, method, 3 reps, ready
            - must go through each standard to save it
    - Cal Verification
        - sample setup, open either CALVERIC or CALVEROC, use one of the standards in the middle of range from verification
        - choose correct Sample and Method ID

    ❖ make sure cal curve R=0.999 or greater to proceed

9. Run CLEAN.SET again with 5 reps

10. Run Samples – Start & End with Cal Verification: create a new folder and save as the date with the letter indicating the run you are on.
Example: 070515A (year, month, day)

    ❖ make sure to add a cal verification at end of run

11. Run CLEAN.SET again with at least 3 reps

12. To get results: put instrument in standby mode and turn gas off
    - go under Results/Multiple analysis/Save file as a report
    - print summary report and detailed report if one rep must be thrown out
    - write report file name on print-out + DOC/DIC
    - staple and file in DOC/DIC output files binder
    - Edit/View in Excel – calculate average ppm C on spreadsheet for each sample
    - Save above as Excel workbook in C/Project Results in its respective folder

TURN OFF NITROGEN GAS WHEN FINISHED!

Dohrmann Procedure 11/09 (page 3)

Reagent Preparation

Phosphoric Acid (H_3PO_4) 85%

- Measure 37 mL 85% phosphoric acid into rinsed bottle (first filled with partial amount of DI water in directions below)
- Total of 188 mL DI water added to bottle

Sodium Persulfate ($Na_2S_2O_8$) 98 + % (used in TOC analysis only)

- Measure 25 g 98+% sodium persulfate into rinsed bottle
- Add 9 mL 85% phosphoric acid
- Add 213 mL DI water

DOC/DIC Stock Standard Preparation

TC (DOC) Stock Solution (1000 ppm – 100 mL)

- Dry KHP ($C_8H_5KO_4$) at 100°C for 1 hour
- Measure 0.2126 g KHP into 100 mL volumetric flask
- Add ~ 75 mL DI water
- Add about 0.1 mL 85% phosphoric acid to adjust pH below 3
- Fill to mark

IC Stock Solution (1000 ppm – 100 mL)

- Dry Na_2CO_3 (Anhydrous) at 100°C for 1 hour
- Measure 0.883 g Na_2CO_3 into 100 mL volumetric flask
- Fill to mark with DI water

Dohrmann Procedure 11/09 (page 4)

DOC/DIC Timing & Volume Required

- based on 2 reps, start to finish

Low DOC	~ 19 min / sample	(medium slugs)	28 mL
High DOC	~ 15 min / sample	(1 big slug)	14 mL
Low DIC	~ 7 min / sample	(small slugs)	20 mL
High DIC	~ 7 min / sample	(small slugs)	

*** if Dohrmann hasn't been run in a month or more, warm up time is much longer and the baseline acts "strange"….it's best to warm up the Dorhmann the day before the run for several hours and run a clean run when a stable baseline is reached

Simultaneous determination of ammonia-N and nitrate/nitrite-N in surface waters

Using the Lachat QuikChem 8000

1.0 K. Thommes and J. Coleman, 7/2003

(This SOP is modified from Lachat QuikChem methods 10-I07-06-1-B, 10-107-04-1-A, and 10-107-04-1-B)

Notes:

- This SOP is used in the analysis of surface waters for NH3 (range = 0.01-2.0 mg N/L) and NO3/NO2 (range = 0.001-0.1 and 0.2-20.0 mg N/L).
- All samples may be run using the NH3 and low NO3/NO2 methods. Any samples falling in the high NO3/NO2 range may be rerun later with the 2nd NO3/NO2 method.
- Place any suspected high NO3/NO2 samples at the end of the run to prevent poor integration of subsequent normal samples.
- If high DOC colors the samples that fall into the low NO3/NO2 range, then a background color analysis should be performed after the initial analysis.
- The color reagent line should be moved to a solution of 10% H3P04. Skipping this step can result in background values that are higher than the original NO3/NO2 readings. The H3P04 concentration of the background solution is the same as the H3P04 concentration of the color reagent. This is critical because the acid removes a certain amount of that background color.

5-10% blanks and method duplicates, 1 spike and Lab Fortified Blank for each analyte, and 1 check standard should be run in each tray. The spike solutions for the LFB and spiked samples are 1 mL of 0.10 mg N/L as NH3 cal standard, and 1 mL of 0.025 mg N/L as NO3 cal standard. The check standard should be made from the AMP 1 stock standard and its concentration should be such that the two analytes of interest fall within the analysis range of those analytes (0.01-2.0 mg N/L as NH3, and 0.001-0.1 mg N/L as NO3).

Lachat analysis of aqueous sediment phosphorus extractions (0.01-2.0 mg P/L)

1.0 K. Thommes (SCWRS) with A. Craft-Reardan (Metropolitan Council, Environmental Services), modified from LaChat's Quickchem Method 10-115-01-1-A.

(This SOP was developed by Kelly Thommes, SCWRS, in conjunction with Angela Craft-Reardan, Metropolitan Council, Environmental Services. It is modified from Lachat's Quikchem Method 10-115-01-1-A)

1. There are 3 phosphorus fractions to be analyzed for this set of extractions (plus a background color analysis for the NaOH-P fraction):
   1.1. Total P (TP) =Total phosphorus
   1.2. Apatite P (HCI-P) =Inorganic phosphorus (Ca-bound P)
   1.3. NAIP/Inorganic P (NaOH-P) =Non-apatite inorganic phosphorus (Al-P, Fe-P)

2. The analyst performing the sediment extractions should include 5-10% method duplicates, blanks, lab-fortified blanks, and spikes. Also, when selecting the HCl and NaOH samples for the method duplicates and spikes, it is helpful if the same sample is used for each fraction (i.e. if selecting sample A in the HCl fraction for a spike, select its counterpart in the NaOH fraction for that fraction's spike). This keeps confusion to a minimum when extracting the different fractions, pouring out samples prior to the Lachat analysis, and setting up trays in the Lachat software.

3. TP fraction (seems to be the most problematic for the Lachat)
   3.1. All TP fraction samples may possibly contain residual H202 from the extraction. Adding a small amount of sodium metabisulfite (Na2S2O5) to each sample neutralizes the H2O2. To correct for this dilution, one can either recalculate the Lachat results, or dilute the calibration standards by the same factor as the samples so the Lachat results are correct as they are output. We have successfully used the latter.
   3.2. The TP samples can display double peaks at low analyte concentrations. This may be a matrix effect due to changes in pH resulting from the addition of Na2S2O5.

   In order to make QA/QC calculations it is imperative to record weights for calibration standards, samples, spikes, and bisulfite additions for each QA/QC sample in each fraction.

Lachat Analysis

1. Prior to analysis, pour out 15 mL (by weight) of each calibration standard.
2. Pipette 0.3 mL of Na2S2O5 into each cal standard vial. Invert to mix.
3. The actual concentration of the calibration standards should be calculated accounting for the dilution. These values MUST be entered into the analyte table prior to running the calibration curve.
4. Run the calibration curve.
5. Pour out 6 mL, by weight, of each sample in test tubes.
6. Pipette 0.12 mL of Na2S2O5 into each test tube. Invert to mix.
7. Make up a 0.5mg PIL check standard using Ampule 1 stock nutrient solution.
   7.1. The check standard can have Na2S2O5 added to it in the same ratio as the samples and calibration standards (Measure 6 mL of check standard into tube and add 0.12 mL of Na2S2O5. Invert to mix.). However, this may not be necessary.
8. Make up instrument spikes by pouring out 6 mL of the intended spike sample, adding 0.12 mL of 1 mg P/L phosphorus calibration standard to the spike, and then adding 0.12 mL of Na2S2O5. Invert several times to mix well.
9. Make up instrument Lab Fortified Blanks by following the procedure in step 8 using an extraction blank instead of sample.
10. Other QA/QC samples are as follows:
    10.1. Run a check standard at the beginning, middle, and end of a tray.
    10.2. Run a DI water blank before each check standard.
    10.3. Run at least 5-10% instrument duplicates at the end of each tray.

After the TP run, a new calibration curve must be run using fresh calibration standards. The HCl fractions and NaOH fractions do not have the extra bisulfite additions. After the second calibration curve is finished, run the HCl tray first, followed by the NaOH fraction. NO BISULFITE should be added to any of the HCl or NaOH samples, spikes, check standards, or lab-fortified blanks. However, the same general QA/QC procedures and guidelines should be followed (steps 8-10).

Once the HCl fraction is finished, run the NaOH fraction. If the QA/QC samples are the same for the two fractions (as recommended above), the Lachat tray file used for the HCl fraction may be used for the NaOH fraction.

The NaOH fraction samples have a yellow/brownish color to them, and it should be determined if this color being read by the Lachat detector. After the NaOH fraction is finished, pull the Lachat line out of the color reagent bottle and place it in the DI (carrier) bottle. Next rerun the NaOH tray to obtain background color/interference results. This set can be difficult for the software to integrate properly because the peaks are so small.

Look for suspicious values such as numbers in the ten-thousandths range (e.g. 0.0006). Also, double check that each peak is integrated with a start and an end tick mark located properly about the peak. If a peak is not integrated properly, or has a suspicious result, then the analyst may need to rerun those samples (either by tacking them onto the end of a tray, or by rerunning them in a new run). If simply rerunning them does not fix the integration problems, then the Peak Base Width may need to be changed, the calibration curve reanalyzed with the new PBW changes, and the background tray reanalyzed under the new parameters.

Standard preparation

Dry ACS grade anhydrous potassium phosphate monobasic (KH2PO4) at 105 degrees C for at least one hour.

Stock Standard (250ppm P)
In a 1-L volumetric dissolve l.099 g KH2PO4 in approximately 800 mL of DI water.
Dilute to mark and invert to mix. Good for 6 months.

Working Stock Standard (20ppm P)
In a 1-L volumetric dilute 80 mL of stock standard to mark and invert to mix. Good for up to 6 months.

Set of 7 Working Standards
2.0, 1.0, 0.5, 0.2, 0.05, 0.01, 0.00 mg P/L

By Volume: To 6, 250-mL volumetric flasks add:
25, 12.5, 6.25, 2.5, 0.625, 0.125 mL
of 20 ppm working stock standard respectively (the 0.00 pm standard is fresh DI water). Dilute each working standard to the mark and invert to mix.

By weight: To 6, 250-mL volumetric flasks add:
25, 12.5, 6.25, 2.5, 0.625, 0.125 g
of 20 ppm working stock respectively (the 0.00 ppm standard is fresh DI water). Record the exact weight of standard in each flask. To get the final weight of the solution after dilution, divide the exact weight of added standard by:
0.1, 0.05, 0.025, 0.01, 0.0025, 0.00005,
respectively. Dilute each working standard up to its respective calculated weight with DI water.

Metal analysis by ICMP-MS

1.0 IDENTIFICATION AND PURPOSE

This standard operating procedure (SOP) provides the procedures for analysis of elements using an Agilent 7700x Inductively Coupled Plasma -Mass Spectrometer (ICP-MS) in the Gustavus Adolphus Environmental Chemistry Lab. This document encompasses only the dilution and analysis of sediment extracts and filtered water samples for trace metals and elements.

2.0 MATRIX OR MATRICES

This procedure applies to the analysis of trace metals and elements in filtered water samples and sediment extracts.

3.0 SCOPE AND APPLICATION

This procedure describes the dilution and analysis of filtered water samples and sediment extracts samples using an Agilent 7700x (ICP-MS). The method is appropriate for standard analysis of trace and major elements.

4.0 METHOD SUMMARY

This SOP covers the preparation and analysis of metals in water and sediment extracts that have been submitted to the Gustavus Environmental Chemistry Lab. The sediment extracts have been previously preserved with hydrochloric acid (HCl) as have the filtered water samples. Water samples are preserved to 0.5% HCl and sediment extracts are typically 0.83% HCl when they arrive. The extracts are diluted with 2% nitric acid (HNO_3) and internal standards are added prior to analysis by ICP-MS. Typically, 1-5 mL of each water sample is diluted to a final volume of ~10 mL after addition of internal standard. For sediment extracts, typically 100 uL is diluted to 10 mL after addition of internal standard. Analysis is completed using MassHunter software on the Agilent 7700x ICP-MS following proper tuning of the instrument.

5.0 CLEAN PROTOCOLS AND INTERFERENCES

Minimizing laboratory contamination and blank levels is key to providing accurate results. The Gustavus Adolphus Environmental Chemistry Lab is not a Class 100 clean lab, but certain steps have been taken to minimize mercury and metal background levels in the lab. Incoming air is filtered, a HEPA laminar flow bench is available, and other steps are taken to minimize mercury and metal background levels. This section outlines how contamination is minimized during sample processing at the Gustavus Adolphus Environmental Chemistry Lab.

5.1 Measures taken to minimize laboratory contamination

- 5.1.1 Wear nitrile gloves at all times while processing samples
- 5.1.2 Cover all laboratory work surfaces with metal-free mats
- 5.1.3 Laboratory personnel are advised against using certain personal cosmetics
- 5.1.4 Pipets used are only designated for use with trace metal samples
- 5.1.5 All pipet tips used are disposable trace-metal free tips
- 5.1.6 All samples are diluted in trace metal free plastic centrifuge tubes

5.2 Measure taken to minimize trace metals in solvents

- 5.2.1 All sample containers used for solvents are either acid-washed Teflon or PETG and are used consistently for the same solution

5.2.2 Only Milli-Q water with a resistivity greater than 18.2 Ωohms is to be used in preparing solutions
5.2.3 Acid used for solutions used for sample dilutions is J.T. Baker ULTREX® II Ultrapure Reagent grade acid.

6.0 SAFETY

6.1 Personal protection should be used for all work used in the inorganic laboratory, (e.g., gloves, safety glasses, laboratory coats, etc.).
6.2 The liquid argon tank is stored and handled according to relevant safety codes
6.3 Sample vials are kept in racks to prevent spills
6.4 All personnel are trained in the exaction and analysis of acid samples for inorganic analysis.
6.5 Strong acids are only used in the hood and stored sealed
6.6 Personnel follow applicable laboratory safety procedures

7.0 EQUIPMENT

The Agilent 770x Inductively Coupled Plasma-Mass Spectrometer - consists of an ASX-500 Autosampler, nebulizer pump, nebulizer, spray chamber, 27 MHz inductively coupled plasma source, stacked ion optics, octapole reaction system, quadrupole mass spectrometer, dual mode electron multiplier detector, a computer running MassHunter G7201B B.01.02 software that controls the 7700x, data acquisition, and data processing.

7.1 Typical Operating Conditions:
Plasma forward power: 1550 W
Plasma Ar flow rate: 16 Lpm
Nebulizer flow rate: 0.79 Lpm
Makeup gas flow rate: 0.29 Lpm
Spray chamber temperature: 2°C
Modes: No Gas and Helium modes
Integration time: 0.1 sec to 0.3 sec
Replicate integrations: 3
Rinse 1: 2% HNO_3/0.05% HCl for 90 seconds
Rinse 2: 5% HNO_3/0.05% HCl for 30 seconds
Rinse 3: 2% HNO_3/0.05% HCl for 60 seconds
Total acquisition time: 6 min/sample

8.0 MATERIALS

8.1 15 mL trace metal free Polypropylene centrifuge vials with screw caps.
8.2 Micro-pipettes with disposable tips, 5 □l to 1 mL.
8.3 Repeating pipeters
8.4 Teflon and PETG solution bottles, 125 mL to 2 L
8.5 Miscellaneous: nitrile gloves; disposable laboratory wipes and bench covers

9.0 CHEMICALS, REAGENTS, AND STANDARDS

9.1 Nitric Acid, Hydrocholric Acid ultrapure and concentrated (J.T. Baker Ultrex II UltraPure)

i. To prepare the 2% Nitric or Hydrochloric Acid Solution, dilute 40 mL of acid in 2 L of MilliQ water.

9.2 MilliQ water: RO water, filtered and dispensed by a MilliPore system. MilliQ water is checked to ensure a resistivity of greater than 18.2 Ωohms.

9.3 Argon gas supplied by a pure liquid Argon dewar.

9.4 Internal Standard: Inorganic Ventures: IV-ICPMS-71D Internal Standard contains ^{6}Li, Sc, Y, In, Tb, and Bi at 10 □g/mL in 3% HNO_3.

9.5 Calibration Standard: Inorganic Ventures: IV-ICPMS-71A Calibration Standard contains 71 elements at a concentration of ~10 □g/mL in 3% HNO_3.

9.6 Major elements added to the Calibration Standard are Na, Mg, Al, K, Ca, and Fe. All of these standards are purchased from Inorganic Ventures.

9.7 Check Standard: Inorganic Ventures IV-Stock1643 certified reference material contains 30 elements at various certified concentrations

9.8 Water Reference Material SLRS5: River Water Reference Material for Trace Element Analysis purchased from the National Research Council Canada.

9.9 Tuning Solution: Agilent Stock Tuning solution containing: Li, Y, Ce, TI and Coat 10 mg/L in 2% HNO_3 which is diluted to 100 □g/L with 2% HNO_3.

9.10 Pulse to Analog (P/A) Solution is prepared from Agilent Tuning Solution 1 and Agilent Tuning Solution 2 which diluted to known concentrations for each element.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Warm-up and tuning: The ICP-MS is allowed to warm up for 30 minutes prior to running a performance report. The Agilent 7700x is set to automatically optimize performance following the warm up period. If all performance measures are met, a performance report is generated which is used to determine whether the instrument needs to be tuned further.

10.2 Performance Report: The following parameters/procedures are optimized while tuning solution is running through the ICP-MS:

Torch Axis: is adjusted using masses 7, 89, and 205

Electron multiplier: proper sensitivity is checked using mass 80

Standard Lenses Tune: Lens voltages are optimized using masses 7, 89, and 205

Resolution/Axis: proper assignment of masses and mass width are checked and adjusted

Plasma Performance: oxides and doubly-charged ions are ensured to be below the proper range

P/A Factor: The electron multiplier has a pulse and an analog mode and this procedure sets the proper correction if the detector switches modes during analysis.

The performance report must be adequate prior to the proceeding with analysis.

10.3 Additional tuning can be performed using the MassHunter Software. Acceptable tune values are as follows:

No Gas Mode: (integration time = 0.1 sec, 3 replicates and concentrations = 100 ppb)

Mass	**Count**	**RSD**	**W-50%**
7	>1000	<3%	0.6 to 0.8
89	>3000	<3%	0.6 to 0.8
205	>3000	<3%	0.6 to 0.8

Oxide (156/140)	<2%
Doubly Charged	<2%

He Mode: (integration time = 0.1 sec, 3 replicates, concentrations = 100 ppb, He flow = 4.3 mL/min)

Mass	**Count**	**RSD**	**W-50%**
59	>500	<3%	0.6 to 0.8
89	>1500	<3%	0.6 to 0.8
205	>1500	<3%	0.6 to 0.8

Oxide (156/140)	<2%
Doubly Charged	<2%

Calibration of target analytes. Target analytes reported and mode of operation:

Analysis Mode	Mass	Name	Analyte or Internal Standard
2: He	23	Na	Analyte
2: He	24	Mg	Analyte
2: He	27	Al	Analyte
2: He	39	K	Analyte
2: He	44	Ca	Analyte
1: No Gas	45	Sc	ISTD
2: He	45	Sc	ISTD
2: He	51	V	Analyte
2: He	52	Cr	Analyte
2: He	55	Mn	Analyte
2: He	56	Fe	Analyte
2: He	59	Co	Analyte
2: He	60	Ni	Analyte
2: He	63	Cu	Analyte
2: He	66	Zn	Analyte
2: He	69	Ga	Analyte
2: He	75	As	Analyte
2: He	85	Rb	Analyte
2: He	88	Sr	Analyte
1: No Gas	89	Y	ISTD
2: He	89	Y	ISTD
1: No Gas	107	Ag	Analyte
1: No Gas	111	Cd	Analyte
1: No Gas	115	In	ISTD
2: He	115	In	ISTD
1: No Gas	133	Cs	Analyte
1: No Gas	137	Ba	Analyte
1: No Gas	159	Tb	ISTD
1: No Gas	208	Pb	Analyte
1: No Gas	209	Bi	ISTD
1: No Gas	232	Th	Analyte
1: No Gas	238	U	Analyte

10.4 Calibration standard concentrations: Six concentrations levels are generally used for trace element analysis: 0 ppb, 0.1 ppb, 0.5 ppb, 1.0 ppb, 5 ppb, and 100 ppb. Major element concentrations (Na, Mg, Al, K, Ca, and Fe) are known exactly, but are slightly different for each element due to differences in stock standard concentrations. Approximate concentrations used are 0 ppb, 5 ppb, 25 ppb, 50 ppb, 250 ppb, and 500 ppb. Additional major element standards are prepared if the concentration range of the some samples exceeds the highest standard.

10.5 Internal Standards: Internal standards are added directly to each calibration standard at a concentration of 100 ppb.

11.0 QUALITY CONTROL

11.1 Blanks: There are three types of blanks that are quantified: calibration blank, instrument or rinse blank, and method blank. The calibration blank is the 0 ppb calibration standard and is solvent matched with the sample matrices, which is typically 2% HNO_3 and 0.08% HCl. The rinse blank is also solvent matched and is run before and after each set of calibrations to ensure there is no carry over between samples. The method blanks are sent to our lab and processed exactly like other samples.

11.2 Calibration check standard: This is the 1643 standard (see 9.7) and is used to verify the accuracy of the calibration. It is typically diluted 20:1 and internal standards at a concentration of 100 ppb are added. Acceptable values are 80 to 120% of the certified value.

11.3 River water certified reference material: The SLRS-5 (see 9.8) is typically used undiluted, but solvent matched by addition of nitric and hydrochloric acid. Acceptable values are 80 to 120% of the certified value for each element.

11.4 Spike samples: These are samples spiked with the calibration standard and quantified like other samples. Acceptable values for recovery of the spiked elements are between 80 and 120%.

11.5 Instrument performance: Instrument performance is checked by monitoring internal standard counts and by constructing calibration curves every 25 samples or less.

a. Internal standard responses are monitored for changes in response over time and for precision. If Internal standard response falls below 80%, a change in instrument response or a matrix effect is being observed. If possible, sample can be diluted further to remove matrix effects or the cause of the drop in response must be determined. Precision is monitored by tracking relative standard deviations (RSD) of replicate measurements (3 replicates of each sample is run in both modes of operation). If the RSD is greater than 5% the operator must determine the cause before continuing.

b. Calibration standards calibration check standard, and certified reference material is run after a maximum of 25 samples. If the counts for the calibration standards change greater than 10%, the samples in between the calibration standards are rerun with new calibration curves. Calibration curves for each element are considered satisfactory if $R^2>0.99$. If values are less than 0.99, calibration curves are reconstructed after rerunning standards that are causing variance.

11.6 Lab duplicates are run every fifteen samples and values are flagged if there is greater than a 20% relative percent difference between duplicates.

11.7 Calibration curves are constructed by the MassHunter software

12.0 OPERATIONAL PROCEDURE

12.1 An appropriate amount of sample is transferred with a pipet to a 15 mL centrifuge tube. Typically 100 □L of a sediment extract and 1 mL of a filtered water sample are used. These volumes are adjusted if a matrix effect is observed or if many of the elements are too concentrated or dilute.

12.2 100 □L of internal standard is added with a repeating pipet.

12.3 Samples are diluted to 10 mL with 2% nitric acid.

12.4 Samples are shaken to ensure homogeneity and capped until analysis
12.5 ICP-MS calibration and standardization is performed as outlined in Section 10
12.6 An initial set of calibration standards, check standards, certified reference material, and blanks are run.
12.7 If standard curves, check standards, certified reference materials, and blanks meet all criteria, samples are run. Samples are run in batches of 10-25.
12.8 MassHunter is used to calculate the concentration of each element in the centrifuge tube. Dilution factors are applied to calculate concentration on a per gram dry weight for sediments and per L of water for filtered water samples.
12.9 Results are exported to spreadsheets.

Phosphorus extraction procedure (high sample throughput)

All Fractions

1. Freeze-dry and powder sediments.

Total-P

1. Weigh out 0.1 ± 0.01 g dry sediment into pre-weighed 50mL NUNC centrifuge tube on analytical balance; record exact weight of sediment.

2. In a fume hood: add 10 ml of 30% H_2O_2, and place in hot water bath (85-90°C); allow reaction to proceed one hour taking care that contents do not boil out during the early stages of the reaction (use stream of MeOH to control bubbles).

3. Remove samples from heat and bring weight to 30 g with d-H_2O on top-loading balance; add 10 ml of 2.0 N HCl and return to hot water bath for 30 min.

4. Cool samples in cold water bath and record final weight on top-loading balance; centrifuge for 10 minutes at 3500 rpm.

5. Pipette 5.0 ml of extract with pipet into pre-weighed 60-ml pp. bottle; record extract weight and dilute to 50 g with d-H_2O on top-loading balance; label with sample number and code **TP**.

6. Add 6.25 mL of 1 M $Na_2S_2O_5$ (sodium meta bisulfite) to each bottle. Record weight added.

Ex-P

1. Weigh out second aliquot of 0.1± 0.02 g dry sediment into labeled, pre-weighed 50-ml centrifuge tube on analytical balance; record exact weight of sediment.

2. Label two 60 mL PP bottles for each sample, one set with code **Ex-P 1st** and the other with **Ex-P 2nd**.

3. Add 40 mL of 1 M NH_4Cl to centrifuge tube and record total weight. Cap tubes tightly.

4. Shake on shaker tray for 2 hours at 120 RPM. Position tubes so that sediment is disturbed during shaking.

5. Centrifuge for 10 minutes at 3500 RPM.

6. Pipette 5 mL of supernatant into a pre-labeled, Ex-P 1st 60 mL PP bottle. Record weight of extract.

7. Bring weight extract weight to 50 g with d-H_2O and record final solution weight.

8. Sip off remaining supernatant in centrifuge tube with sipper and vacuum pump taking care that no sediment is lost.

9. Repeat steps 2-7 for a second extraction this time using the Ex-P 2nd bottles.

10. Add ca. 25 ml d-H_2O, swirl contents and centrifuge for 10 min; remove supernatant with vacuum "sipper" and discard.

NaOH-P

1. Using sediment already extracted with NH_4Cl add 25 ml of 0.1 M NaOH and record total weight; cap tubes tightly and shake for 16 hr at room temp on rotary shaker 120 RPM.

2. Centrifuge for 10 minutes at 3500 rpm.

3. Pipette 5.0 ml of extract with pipet into pre-weighed 60-ml pp. bottle; record extract weight and dilute to 50 g with d-H_2O on top-loading balance; label with sample number and code **NaOH-P**.

4. Drain as much remaining supernatant from centrifuge tube with a vacuum "sipper" into waste container, taking care that no sediment is lost.

5. Add ca. 25 ml d-H_2O, swirl contents and centrifuge for 10 min; again remove supernatant with vacuum "sipper".

HCl-P

1. Bring contents of centrifuge tube containing residual sediment to 20 g with d-H_2O water on top-loading balance.

2. Add 6.25 ml 2.0 N HCl; cap tubes tightly and shake on rotary shaker for 20 hr at room temp. (Keep shaker RPMs at highest level that does not cause sample to slosh at top of centrifuge tube—only need to keep sediment lightly agitated.)

3. Centrifuge for 10 minutes at 3500 rpm.

4. Pipette 5.0 ml of solution with pipet into pre-weighed 60-ml pp. bottle; record solution weight and dilute to 50 g with d-H_2O on top-loading balance; label with sample number and code **HCl-P**.

Reagents

1. 30% (low-P) hydrogen peroxide H_2O_2

2. 1 M NH_4Cl
   53.5 g NH_4C dissolved in 1 L DI

3. 0.1 M sodium hydroxide NaOH
   4 g solid NaOH to 1 L

4. 2.0 N hydrochloric acid HCl
   165 mL conc. HCl to 1 L

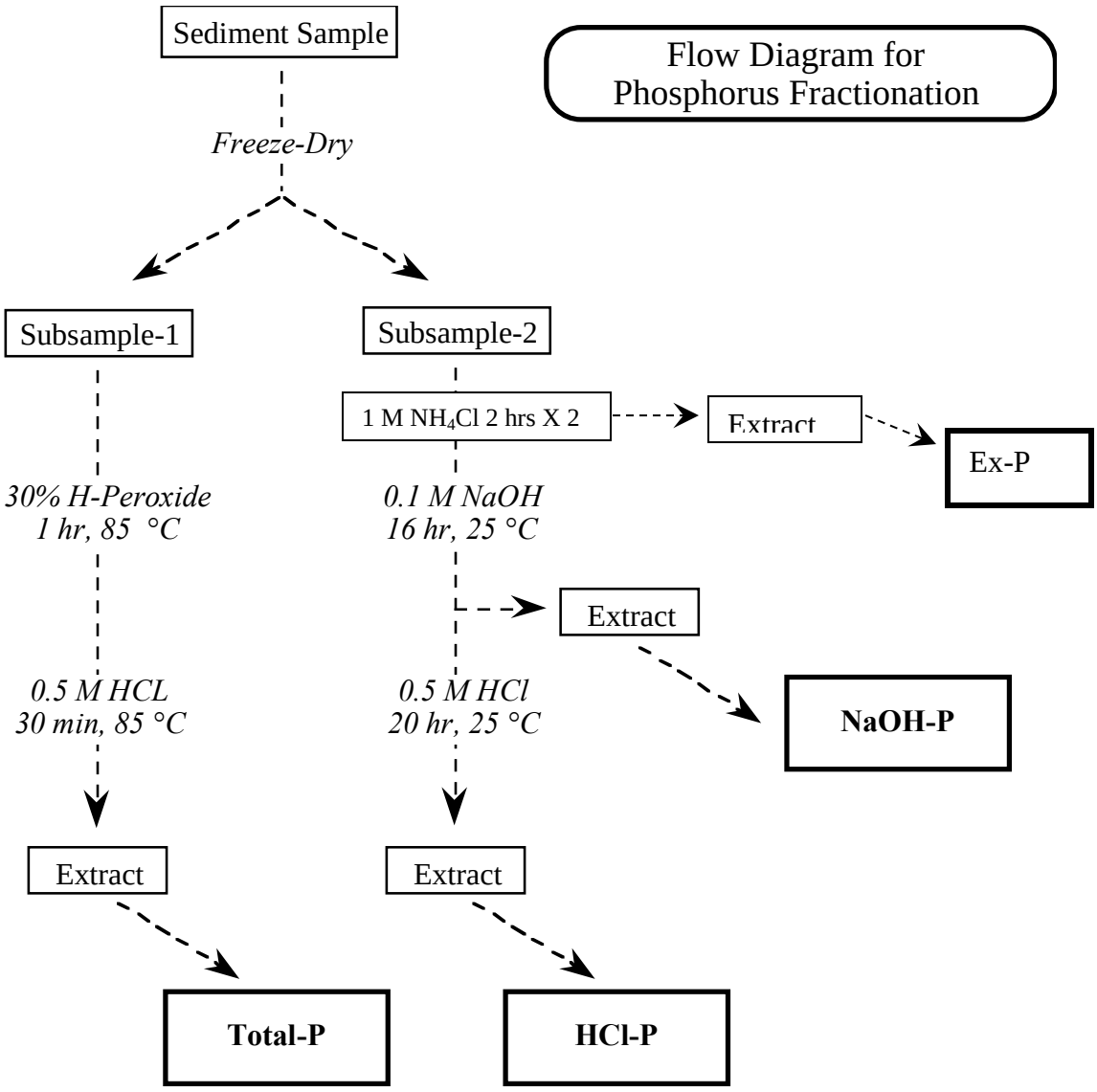
5. 1.0 M sodium metabisulfite $Na_2S_2O_5$
   19.01 g solid $Na_2S_2O_5$ to 100 g DI

Analytical

Analysis	Method	DF	Expected Conc.	Matrix
Total-P	Ascorbic Acid	2500	400 ppb	0.05 M HCl
NaOH-P	Ascorbic Acid	2500	200 ppb	0.01 M NaOH
HCl-P	Ascorbic Acid	2500	80 ppb	0.05 M HCl

Phosphorus Analysis

- use 5-cm flow-through cell (200 ppb = ca. 0.4 Abs)
- standards for all fractions (0, 10, 50, 200, 500, 1000, 2000 ppb)
- spike Total-P samples (50 ml) and standards with 6.25 ml of 1 M $Na_2S_2O_5$ prior to addition of color reagent to remove any remaining peroxide
- split NaOH-P fraction into two 50-ml aliquots; add complete color reagent to one aliquot and color reagent w/o ascorbic acid to the other; read the second aliquot as background correction for absorbance from extracted DOC.



Ex-P represents exchangeable-P

NaOH-P (NAIP) represents Exchangeable-P + Fe/Al-bound P

HCl Extractable-P (Apatite-P) represents Ca-bound P

Residual-P (Organic-P) = Total-P – (NaOH-P + HCl-P)

Color analysis for Marcell samples (440nm and 254nm)

1. Make sure autosampler is hooked up to the uv-vis spectrophotometer by plugging the pump and keypad into the back outlet of the autoanalyzer. Disconnect the Dohrmann by removing the RS232 cable. Connect the pump into the GSIOC outlet and the keypad into the appropriate outlet. The SW1 and SW2 on back of autoanalyzer should be moved using a small Phillips screwdriver to "0" and "0" (when switching to the Dohrmann SW1 and SW2 should read "0" and "3"). Turn on autosampler (on back left side), this will also turn on keypad. Turn on pump (on back above power cord). Use the sample probe (needle) dedicated for uv-vis by placing into autosampler probe holder (Dohrmann probe may need to be removed). Tighten screw to keep sample probe in place.

2. Fill DI bottle with fresh DI water and place line in bottle. Verify waste containers are empty and waste lines are placed in container. Clamp tension holder around tubing on pump making sure tubing passes through guides in front (pump turns clockwise, so make sure tubing is facing in the right direction).

3. Marcell color samples will be run using a 1-cm flow cell. If flow cell needs to be changed, the flow cell holder should be removed by unscrewing the one thumbscrew that holds it in place. The flow cell holder for the 1-cm flow cell should be put into its place and tightened using the thumbscrew. Verify placement of holder by turning off lab lights and placing a piece of paper on the side where the light begins to pass through the flow cell (this must be done while the instrument is on. Turn instrument on by selecting the "align" program within the Cary software. Select "connect" to turn lamp on. To turn off, exit out of program). Next, place paper on other side of flow cell where the light is to be passing through. Verify a small square beam of light that has passed through flow cell. If this is confirmed, flow cell holder is in place. Also, remember that the arrow on flow cell indicates the direction of flow "into" the flow cell.

4. Align samples into rack including space for duplicate. Add blank of DI water at end.

5. Turn on computer and select "Advanced Reads" from Cary program. Connect to spectrophotometer if needed. In "setup" menu, select correct wavelength (either 440nm or 254nm). "Average time" should read "0.100". "Replicates" should read "3". Select the "samples" tab and enter in sample ID's starting first with project acronym (MAR). ID's may need to be modified to fit into field. When complete select "OK".

6. Place rack with samples onto autosampler. Remove caps, but keep caps in order to replace onto bottle if needed.

7. On the keypad for the autosampler, ignore time, date, month, year (hit "enter" through them). Chose program "Cary.exe" (1), then "custom rack" (6), and then "TD-40" (8). "First sample" should be "1". "Last sample" should be the last place number on tray that

has a sample. "Sample analysis time" should be set to "1.5" minutes and "rinse time" to "3" minutes. "Sample read time" should be kept at "0.05". "Pump speed" default should remain at "24.0". Press "enter" after "+/-". You should know have the option of turning pump on by selecting the "pump on" button on the keypad. This will pump water to the rinsing well. Do this until the rinsing well is overfilling with water.

8. Select "pump off" on keypad and proceed to "load analyzer" (make sure caps are off vials!). When probe enters into first sample, immediately select "stop sipping". This will send probe to rinsing well. Once probe is in rinsing well, you may select "pump on" to pump DI water through flow cell. Once flow cell has been filled with DI water, verify that flow cell has no air bubbles by removing flow cell from holder. Be careful not to touch outside of flow cell where light goes though. If there are air bubbles, try to move flow cell upside down to remove them while water is pumping.

9. Upon verifying no air bubbles in flow cell, place flow cell back in holder and on keypad select "stop pump". You may now blank the system by selecting "zero" on the "Advanced Reads" program screen. Try a few times to get as close to 0.0000 as you can.

10. From "Advanced Reads" program, select "start". Verify selected sample tray. Sample batch file (.BAB file) should be saved with the run date in "Marcell Color" folder. Select ok. When the 'present sample" screen appears, do not select anything. Leave this screen up.

11. Select "load analyzer" from keypad. Sample probe will go into first sample. You may want to verify that there are no air bubbles in flow cell while sample is sucking. Just make sure the flow cell gets put back into the holder prior to the instrument taking it's reading (note time left on keypad). It appears that as the DI water warms up, bubbles form more frequently in the bottle and tubing. This can become a problem as air bubbles enter the flow cell when a reading is being taken.

12. When running duplicates, make sure sample vial is moved into correct position. Do this when sample probe is rinsing by slightly rocking tray outward and repositioning the vial into the correct location on tray.

13. When all samples have been analyzed at the first wavelength (ex. 440nm), change wavelength to 254nm in "setup". Sample ID's will already be entered (from 440 run), just remember to move the duplicate sample back to the original place on the tray. Rezero the instrument with DI at the new wavelength.

14. After printing out a hardcopy of the .BAB file, .BAB files should then be imported into excel as a .csv file. Once converted to a .csv file, make any changes on the sample ID's that could not fit into the field. Save the .csv file by selecting "save as" and creating a Microsoft Excel Workbook file (.xls). Save the file with the date it was run, a letter indicating what run it was during that day, and also include the wavelength (ex.

120501a440.xls). Batch files (.BAB) should be saved in the "Varian output" folder. Files saved as .csv should be deleted when the .xls file has been created.

15. When sample runs are completed for the day, remove DI water line and pump flow cell and rinsing well dry. Release tension on pump cartridge. Disengage pump tubes from guides, and turn off auto sampler and pump. Exit out of Cary program and turn off computer. Waste in waste containers from Marcell samples can go down drain since this is only water.

Trace-metal extractions for non-point source sediments

1.0 D.R. Engstrom, 8/6/98
2.0 J.C., Revised 02/27/2002

1. Dry sediments in freeze-drier; grind to a fine powder.

2. Make labels for 60ml acid-washed, PP bottles; record weight of bottle+ label.

3. In a dispenser bottle, make up 1L of 0.5N HCl from concentrated, high purity, Seastar HCl. Include lot # of acid on benchsheet. When making up acid, anything coming into contact with the acid must be *extremely* clean. Volumetric should be acid washed, triple rinsed with DI water, and rinsed with a small amount of high purity acid before using.

4. Weigh out 0.25 +/- 0.02 g dry sediment into a 50-ml, acid washed centrifuge tube on analytical balance; record exact weight.

4. Add 25ml high purity 0.5 N HCl using a dispenser bottle and record exact weight of acid used. Loosely cap vials and allow carbonates to react for a few minutes before proceeding. Include in set 10% duplicates and blanks of just 25ml HCl.

6. Heat in a hot-water bath at 80-85 degrees C for 30 minutes, plus time for samples to arrive at temperature (determine this using a thermometer in a water blank); place samples in refrigerator to stop reaction. Allow to cool 5 minutes.

7. Dry outside of tubes and centrifuge for 10 minutes at 1000 rpms (until solution is clear).

8. With an auto-pipettor, pipette 10.0 ml of sample supernatant into pre-weighed, 60ml, acid-washed, PP bottles; record solution weight.

9. Dilute sample solution with 40 +/- 0.5g DI H_2O on top-loading balance; record exact weight of water added.

Reagents

0.5 N high purity HCl
42g conc. HCl to 1.0 kg

Analytical

Analysis	Method	DF	Matrix	2nd Dilution
Trace Metals	ICP-MS	500	0.1 N HCl	none
Majors	ICP-MS	5000	0.01 N HCl	1:10

Table of Results

Sample No.	Vial No.	Sed. Wt.	HCl Wt.	Bottle Wt.	Extract Wt.	DI H2O Wt.

Notes for sediment fingerprinting

Use approximately 1g of sediment

Use high purity Seastar Baseline HCl
Include 250ml 0.5 N HCl for Rick to make up standards
Sample matrix will be 0.1 N HCl
Include 5-10% duplicates and blanks

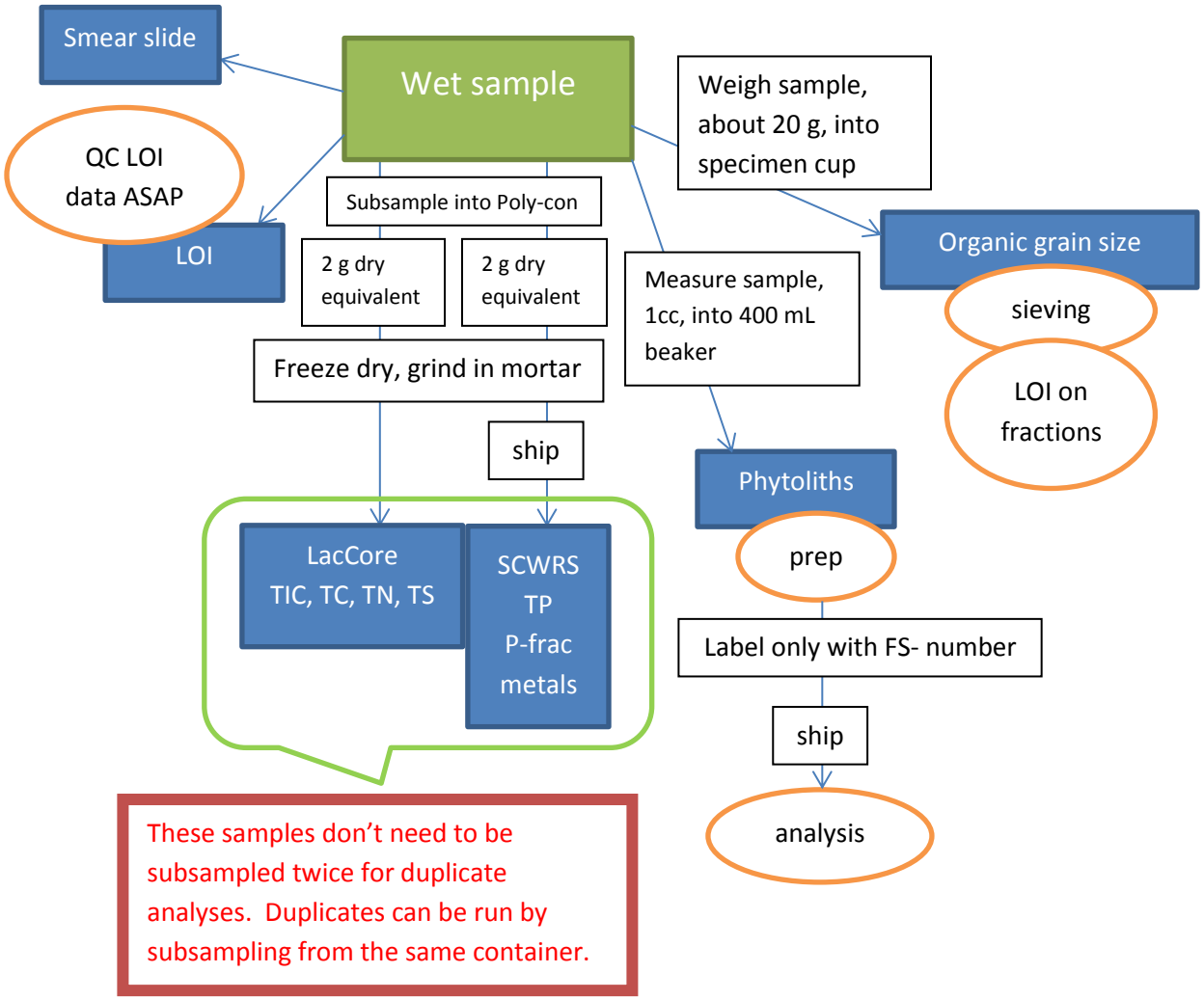
Appendix 3. Sediment subsampling flowchart.

Overview protocol for SO4 sediment sample processing

11/7/11 (original)
10/18/12 **Draft:** revised by Amy to reflect 2012 protocol
10/19/2012 revised by Amy after discussion w/lab staff
11/3/12 revised by Amy to change protocol for freeze died samples
5/19/13 revised by Amy to reflect change in lab that analyzed TS, and size of organic grain size and phytolith samples
7/19/2013 revised by Amy to increase size of SCWRS sample (many samples were too small)
9/19/2013 revised by Amy to reflect new plan to run CNS at ESci Stable Isotope Lab and blinding of phytolith sample labels

IMPORTANT:

Use FS- or MS- number AND site name on all labels
Tare balance for sample container
HOMOGENIZE FOR WATER CONTENT AND SEDIMENT TEXTURE BEFORE ALL SUBSAMPLING
Record all information, including sample weights, into database



Subsample XX sites for duplication of all analyses (same spls for all) (but see comment directly above to left) – Amy/Ed will select

Field sampling data sheet **MCPA/LacCore SO4 project** **Revision 2.9, 5/26/2013** **VS**

State Location ID _____ LacCore ID _____

Site ID _____ AIS status ○ Yes ○ No

Water quality sondes ☐ Hach 13 ☐ Hach 2 ☐ Quanta Hydrolab + Hach calibrated ○ Yes ○ No DO calib? ○ Yes ○ No

Date (mm/dd/yyyy) _____ Time (24 hr) _____ GPS device ○ 1 ○ 2 ○ 3 GPS map datum ○ NAD83 ○ WGS 84

Thermal stick deployed ○ Yes ○ No which stick? ○ A ○ B ○ C Time in: _____ Time out: _____

Crew member _____ Weather _____

Crew member _____ Air temp _____ ○ °C ○ °F Wind _____

Others present _____ Flow conditions _____

Boat ○ Canoe ○ Motorboat ○ Ice Sediment probe observations < surface _____ depth _____

Location

Boat orientation ○ N ○ NE ○ E ○ SE ○ S ○ SW ○ W ○ NW Site photos ☐ Map/Site ☐ N ☐ E ☐ S ☐ W

Latitude _____ Longitude _____ lat/long type ○ Exact ○ Approximate ○ General

Elevation _____ ○ m ○ ft Accuracy _____ ○ m ○ ft Waypoint _____ Water sample collected ○ Yes ○ No

Temperature _____ ○ °C ○ °F spC _____ ○ mS/cm ○ µS/cm pH _____ Rinse core tubes ○ Yes ○ No

Secchi depth _____ ○ m ○ ft actual water depth _____ ○ m ○ ft Cores collected ○ Yes ○ No

Repository core total length _____ ○ cm ○ in Repository core sediment length _____ ○ cm ○ in

Comments on location

Water DO profile

measure from top to bottom

Select unit for all values: ○ mg/L

0 cm		40 cm		80 cm		140 cm	
10 cm		50 cm		90 cm		150 cm	
20 cm		60 cm		100 cm		160 cm	
30 cm		70 cm		110 cm		170 cm	
				120 cm		180 cm	
				130 cm		190 cm	

200 cm _____

DO value at sed-water interface: first choose depth to last interval, then enter its value. _____ cm _____ *(0-9)*

(If water depth exceeds 209 cm, enter all other data in Additional Comments field, write in field notebook, and email Val Stanley.)

Plant rings

Taxa present

Rice Notes

Plant comments

% Open water in sight _____ 2 wild rice plants collected for pressing (with seeds if possible) ○ Yes ○ No

DO_all_surface_mg/L

Email information

Site type (L/S/P)

Mining impacted?

% Floating Leaf Avg

Lily pads? (1/0)

Lily pads? (y/n)

Wild Rice? (y/n)

color wheel no mirror_both

color wheel_ALL

Wild Rice_pct_Avg_plant_ring (1/0)

Appendix 3. Sediment subsampling flowchart.

Overview protocol for SO4 sediment sample processing

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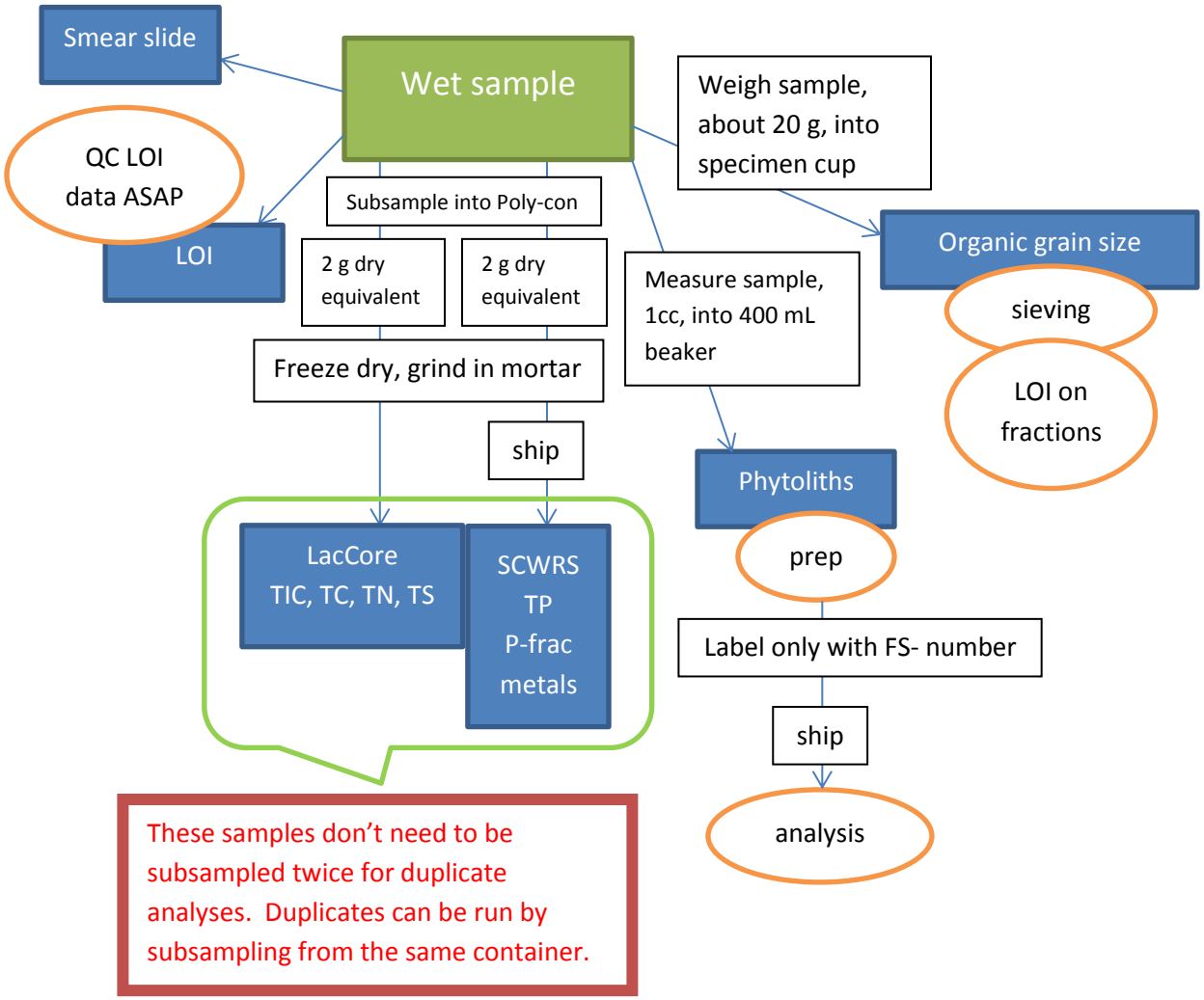
IMPORTANT:

Use FS- or MS- number AND site name on all labels
Tare balance for sample container
HOMOGENIZE FOR WATER CONTENT AND SEDIMENT TEXTURE BEFORE ALL SUBSAMPLING
Record all information, including sample weights, into database



Subsample XX sites for duplication of all analyses (same spls for all) (but see comment directly above to left) – Amy/Ed will select

Appendix 4. Site information and selected data.

LacCore ID	Site name	State Location ID	Date of Visit	County	Latitude (core)	Latitude (surface water)	Longitude (core)	Longitude (surface water)	Site type (lake, stream, paddy)	Surface water SO4_mg SO4/L	Pore water sulfide_mg S/L	Pore water Fe_µg/L	Wild Rice % coverage Avg in plant ring	Wild Rice present (y/n)
P-1	Height of Land	03-0195-00-209	08/22/2011	Itasca	46.91287	46.90075	-95.60952	-95.5985	L	0.24	0.053		27.5	yes
P-2	Mud	S004-735	08/23/2011	Otter Tail		46.626597		-95.575145	S					no
P-3	Little Round	03-0302-00-202	08/24/2011	Itasca	46.97592	46.97392	-95.74036	-95.73608	L	0.46	0.032		25	yes
P-4	Little Flat	03-0217-00-201	08/24/2011	Itasca	46.99813	46.99815	-95.66412	-95.66429	L	0.22	0.011		36.25	yes
P-5	Itasca	15-0016-00-208	08/25/2011	Clearwater	47.23806	47.23325	-95.20648	-95.20274	L	0.26	0.056		20	yes
P-6	Elk	15-0010-00-203	08/25/2011	Clearwater	47.19464	47.19471	-95.22538	-95.22292	L	0.28	0.04		11.25	yes
P-7	Itasca	15-0016-00-207	08/25/2011	Clearwater	47.23323	47.23325	-95.19852	-95.20274	L	0.26	0.064		8.75	yes
P-8	Pelican	26-0002-00-219	08/26/2011	Grant		46.06158		-95.82964	L					no
P-9	Embarrass	69-0496-00-202	08/29/2011	St. Louis		47.53402		-92.31641	L	6.35				no
P-10	Pike	S006-927	08/30/2011	St. Louis	47.73249	47.73088	-92.34676	-92.34602	S	8.31	0.063		18.75	yes
P-11	Sand	S003-249	08/30/2011	St. Louis	47.63478	47.63493	-92.42349	-92.42336	S	7.69	0.046		6.25	yes
P-12	Birch	69-0003-00-205	08/30/2011	St. Louis	47.73573	47.73594	-91.94279	-91.94066	L	3.58	0.104		30	yes
P-13	Partridge	S007-443	08/31/2011	St. Louis	47.52119	47.52072	-92.18994	-92.19102	S	10.39	0.075		28.75	yes
P-14	Mississippi River above Clay Boswell	S007-163	09/01/2011	Itasca	47.23788	47.23836	-93.71957	-93.71935	S	1.09	0.053		71.25	yes
P-15	Mississippi River below Clay Boswell	S006-923	09/01/2011	Itasca	47.25469	47.25493	-93.63435	-93.63383	S	3.65	0.035		43.75	yes
P-16	St. Louis	S006-929	09/01/2011	St. Louis	47.40147	47.40128	-92.37725	-92.37654	S	24.5	0.025		0	no
P-17	St. Louis	S007-208	09/01/2011	St. Louis	47.46679	47.46642	-91.93551	-91.93532	S	1.23	0.04		30	yes
P-18	Lax	38-0406-00-203	09/02/2011	Lake		47.35081		-91.29211	L	1.43				no
P-19	Wolf	69-0143-00-202	09/02/2011	St. Louis	47.25856	47.25806	-91.96183	-91.95989	L	1.54	0.139		56.25	yes
P-20	Gull	04-0120-00-203	09/06/2011	Beltrami	47.65592	47.66061	-94.69439	-94.70406	L	0.78	0.103		6.75	yes
P-22	Ham	02-0053-00-201	09/06/2011	Anoka		45.25721		-93.22642	L	0.95				no
P-23	Gourd	04-0253-00-201	09/07/2011	Beltrami	47.81198	47.81139	-94.96541	-94.96584	L	0.69	0.038		16.75	yes
P-24	Second	15-0091-00-201	09/07/2011	Clearwater	47.82545	47.82674	-95.36347	-95.3653	L	0.87	0.139		16.25	yes
P-25	Lower Rice	S006-985	09/08/2011	Clearwater	47.37925	47.38293	-95.48338	-95.49054	S	1.02	0.097		50	yes
P-26	Lower Rice	S006-985	09/08/2011	Clearwater	47.38171	47.3817	-95.49261	-95.49323	S	0.55	0.07		52.5	yes
P-27	Pleasant	11-0383-00-206	09/09/2011	Cass	46.928	46.92616	-94.47565	-94.48247	L	0.49			12.5	yes
P-28	Raymond	73-0285-00-203	09/12/2011	Stearns	45.62898	45.63026	-95.0234	-95.02198	L	0.82	0.094	5108.98	30	yes
P-29	Padua	73-0277-00-203	09/13/2011	Stearns	45.62015	45.62101	-95.01923	-95.02202	L	0.76	0.13	2633.29	1.5	yes
P-30	Stella	47-0068-00-203	09/14/2011	Meeker	45.06591	45.0675	-94.43388	-94.429	L	7.59	0.08	2971.46	13.75	yes
P-31	Cloquet	38-0539-00-201	09/14/2011	Lake	47.43134	47.4315	-91.48443	-91.48416	L	0.81	0.024	17831.31	32.5	yes
P-32	Caribou	69-0489-00-205	09/15/2011	St. Louis		46.89907		-92.32165	L	0.63				no
P-33	Pelican	26-0002-00-219	09/15/2011	Grant		46.06158		-95.82964	L	5.79				no
P-34	Anka	21-0353-00-201	09/16/2011	Douglas	46.07692	46.07955	-95.7292	-95.73271	L	2.23	0.671	289.2	11.25	yes
P-35	Anka	21-0353-00-201	09/16/2011	Douglas	46.07689	46.07955	-95.73765	-95.73271	L	2.23	0.493	193.49	1.25	yes
P-36	Wild Rice Reservoir	69-0371-00-204	09/16/2011	St. Louis	46.90982	46.90947	-92.16364	-92.16947	L	1.13	0.023	23166.7	7.5	yes
P-37	Ina	21-0355-00-201	09/16/2011	Douglas		46.08222		-95.72596	L	2.17				no
P-39	Grand	69-0511-00-203	09/17/2011	St. Louis		46.88717		-92.39877	L	0.83				no
P-40	St. Louis Estuary	S007-444	09/19/2011	St. Louis		46.6588		-92.28186	S	4.9				no
P-41	St. Louis Estuary Pokegama Bay	S006-928	09/19/2011	Vernon, WI		46.68548		-92.16187	S	2.33				no
P-42	Monongalia	34-0158-01-201	09/20/2011	Kandiyohi	45.34807	45.34706	-94.95092	-94.94984	L	16.51	0.042	13294.92	2.5	yes
P-43	Wild Rice	09-0023-00-201	09/20/2011	Carlton		46.67353		-92.60233	L	0.37				no
P-44	Dead Fish	09-0051-00-202	09/20/2011	Carlton	46.74512	46.74591	-92.68634	-92.68664	L	0.3	0.056	16584.55	21.25	yes
P-45	Hay	31-0037-00-201	09/21/2011	Itasca	47.28743	47.28664	-93.10167	-93.09866	L	10.24	0.087	35586.37	0	no
P-46	Hay	31-0037-00-201	09/21/2011	Itasca	47.28691	47.28664	-93.10181	-93.09866	L	10.24	0.026	13402.25		no
P-47	Little Birch	77-0089-00-101	09/21/2011	Todd	45.77473	45.79012	-94.79955	-94.79218	L	3.2	0.05	9818.34	11.25	yes
P-47	Little Birch	77-0089-00-101	09/21/2011	Todd	45.77473	45.79012	-94.79955	-94.79218	L	3.2	0.191	5814.11	11.25	yes
P-47	Little Birch	77-0089-00-101	09/21/2011	Todd	45.77473	45.79012	-94.79955	-94.79218	L	3.2	0.191	5814.11	11.25	yes
P-47	Little Birch	77-0089-00-101	09/21/2011	Todd	45.77473	45.79012	-94.79955	-94.79218	L	3.2	0.191	5814.11	11.25	yes
P-51	Flowage	01-0061-00-205	09/22/2011	Aitkin	46.68956	46.69217	93.33801	-93.33731	L	0.56	0.014	7906.47	70	yes
P-52	Flowage	01-0061-00-206	09/22/2011	Aitkin	46.6895	46.69217	93.33799	-93.33731	L	0.56	0.018	16837.26	53.75	yes
P-52	Flowage	01-0061-00-206	09/22/2011	Aitkin	46.6895	46.69217	93.33799	-93.33731	L	0.56	0.018	16837.26	53.75	yes
P-52	Flowage	01-0061-00-206	09/22/2011	Aitkin	46.6895	46.69217	93.33799	-93.33731	L	0.56	0.018	16837.26	53.75	yes
P-53	Carlos Avery Pool 9	02-0504-00-201	08/19/2011	Anoka	45.31792	45.31905	-93.0587	-93.05851	L	0.35	0.029		18.75	yes
P-55	Lady Slipper	42-0020-00-204	09/22/2011	Lyon	44.57015	44.56899	-95.62737	-95.62617	L	107.71	14.84	638.02	0	no
P-56	Rice	18-0053-00-203	09/23/2011	Crow Wing	46.33956	46.33956	-93.89005	-93.89005	L	0.38				no
P-57	Unnamed	34-0611-00-201	09/23/2011	Kandiyohi	45.2675	45.26739	-94.86501	-94.8653	L	6.42	0.286	1581.48	32.5	yes
P-57	Unnamed	34-0611-00-201	09/23/2011	Kandiyohi	45.2675	45.26739	-94.86501	-94.8653	L	6.42	0.065	123.43	32.5	yes

P-57	Unnamed	34-0611-00-201	09/23/2011	Kandiyohi	45.2675	45.26739	-94.86501	-94.8653	L	6.42	0.065	123.43	32.5	yes
P-57	Unnamed	34-0611-00-201	09/23/2011	Kandiyohi	45.2675	45.26739	-94.86501	-94.8653	L	6.42	0.065	123.43	32.5	yes
P-61	Lily	81-0067-00-202	09/28/2011	Waseca	44.19402	44.19544	-93.64686	-93.64626	L	0.66	0.041	7176.35	22.5	yes
P-62	Lily	81-0067-00-202	09/28/2011	Waseca	44.19402	44.19544	-93.64686	-93.64626	L	0.64				no
P-63	Maloney	79-0001-00-201	09/29/2011	Wabasha	44.22431	44.22404	-91.9328	-91.93253	L	1.83	0.01	20112.02	65	yes
P-64	Maloney	79-0001-00-201	09/29/2011	Wabasha	44.22431	44.22404	-91.9328	-91.93253	L	1.83				yes
P-69	Rice	18-0053-00-203	09/27/2011	Crow Wing	46.33942	46.3397	-93.89134	-93.89018	L	0.23	0.021	28823.03	18.75	yes
R-1	Trout	31-0216-00-213	05/21/2012	Itasca	47.244		-93.384			38.8				no
R-2	Holman	31-0227-00-203	05/21/2012	Itasca	47.31005		-93.3555			21.2				no
R-3	Upper Panasa	31-0111-00-203	05/21/2012	Itasca	47.30542		-93.26135			60				no
R-4	Lower Panasa	31-0112-00-204	05/21/2012	Itasca	47.30247		-93.25487			43.7				no
R-5	Ox Hide		05/21/2012	Itasca	47.33505		-93.21362			28.3				no
R-6	Swan	31-0067-00	05/21/2012	Itasca	47.32226		-93.17913			16.5				no
R-7	Snowball	31-0108-00-203	05/21/2012	Itasca	47.33279		-93.24113			9.38				no
R-8	Hay	69-0150-00-201	05/22/2012	St. Louis	47.71126		-91.97612			21.3				no
R-9	Sandy	69-0730-00-202	05/22/2012	St. Louis	47.62406		-92.59793			169				no
R-10	Little Sucker	31-0126-00-202	05/22/2012	Itasca	47.37749		-93.24564			17.8				no
R-11	Big Sucker	31-0124-00-204	05/22/2012	Itasca	47.37772		-93.26698			9.23				no
R-12	Prairie	S007-209	05/23/2012	Itasca	47.25136		-93.48936			4.5				no
R-13	Pokegama	31-0532-00-218	05/23/2012	Itasca	47.18601		-93.55713			3.63				no
R-14	Jay Gould	31-0565-00-203	05/23/2012	Itasca	47.23718		-93.63357			2.92				no
R-15	Goose	11-0096-00-201	05/23/2012	Cass	47.21346		-93.96119			0.72				no
R-16	Laura	11-0104-00-203	05/23/2012	Cass	46.97673		-94.01865			0.45				no
R-17	Round	01-0137-00-203	05/23/2012	Aitkin	46.72015		-93.65897			1.96				no
R-18	Wilkins	01-0102-00-204	05/23/2012	Aitkin	46.63786		-93.48876			1.21				no
R-19	Mississippi Crow Wing	S007-205	05/24/2012	Crow Wing	46.43977		-94.11918			3.54				yes
R-20	Mahnomen	18-0126-00-201	05/24/2012	Crow Wing	46.49621		-94.00834							no
R-21	Rabbit	18-0093-00-204	05/24/2012	Crow Wing	46.53059		-93.92867			16.9				no
R-22	Knife	33-0028-00-209	05/24/2012	Kanabec	45.98625		-93.27904			2.08				no
R-23	Knife	33-0028-00-209	05/24/2012	Kanabec	45.98625		-93.27904			2.04				
R-24	Fish	33-0036-00-103	05/24/2012	Kanabec	45.82044		-93.32621			1.6				no
R-25	Fish	33-0036-00-103	05/24/2012	Kanabec	45.82044		-93.32621			1.6				
R-26	Rice	73-0196-00-215	05/24/2012	Stearns	45.38302		-94.63342			27.9				no
R-27	Welby family farm	86-0237-00-202	05/25/2012	Wright	45.3596		-94.07668			2.72				no
R-28	Mink	86-0229-00-206	08/08/2012	Wright	45.26847		-94.03331			4.22			0	no
R-29	Hunt	66-0047-00-209	05/25/2012	Rice	44.33699		-93.44116			22				no
R-30	Hunt	66-0047-00-209	05/25/2012	Rice	44.33699		-93.44116			21.9				
R-32	Rice	66-0048-00-204	05/25/2012	Rice	44.33181		-93.49765			22.4				no
R-35	Rice	74-0001-00-202	06/04/2012	Steele	44.08848		-93.06828			6.8				no
R-36	North Geneva	24-0015-00-210	06/04/2012	Freeborn	43.78794		-93.28062			6.73				no
R-37	South Geneva	24-0015-02-211	06/04/2012	Freeborn	43.77282		-93.27283			16.1				no
R-38	Bear	24-0028-00-207	06/04/2012	Freeborn	43.5603		-93.49417			3.09				no
R-39	Minnesota	22-0033-00-204	06/04/2012	Faribault	43.84762		-93.86974			44.9				no
R-40	Pelkey	49-0030-00-202	06/05/2012	Morrison	45.99719		-94.22702			0.31				no
R-41	Mortenson	34-0150-02-201	06/05/2012	Kandiyohi	45.29934		-94.90542			0.47				no
R-42	Holstad	34-0150-01-202	06/05/2012	Kandiyohi	45.29878		-94.90265			0.05				no
R-43	Field	34-0151-00-202	06/05/2012	Kandiyohi	45.29825		-94.90475			0.34				no
R-44	Eighteen	60-0199-00-202	06/06/2012	Polk	47.64008		-96.06025			8.69				yes
R-45	Rice paddy	WT00029	06/06/2012	Clearwater	47.85161		-95.47261			23.8				yes
R-46	Rice paddy	WT00029	06/06/2012	Clearwater	47.85159		-95.47272							yes
R-47	Rice paddy	WT00025	06/06/2012	Clearwater	47.86191		-95.49449			22.5				yes
R-48	Big Rice	11-0073-00	06/06/2012	Cass	46.99193		-93.95384			1.41				no
R-49	Cromwell	14-0103-00-202	06/07/2012	Clay	46.96142		-96.31579			37.3				no
R-50	Bray	56-0472-00-203	06/07/2012	Otter Tail	46.45319		-95.87476			3.18				no
R-51	West battle	56-0239-00-205	06/07/2012	Otter Tail	46.29664		-95.71265			4.55				no
R-53	Tamarac	56-0192-00-203	06/07/2012	Otter Tail	46.36407		-95.56976			1.01				no
R-55	Blaamyhre	34-0345-00-202	06/08/2012	Kandiyohi	45.37221		-95.17748			0.27				yes
R-56	Blaamyhre	34-0345-00-202	06/08/2012	Kandiyohi	45.37221		-95.17748							yes
R-57	Glesne Slough	34-0353-00-201	06/08/2012	Kandiyohi	45.3512		-95.18901			0.18				yes
R-58	Louisa	86-0282-00-206	06/08/2012	Wright	45.30735		-94.24302			11.2				no
R-59	Gilchrist	86-0064-00-202	06/08/2012	Wright	45.24473		-93.83532			25.6				no

R-60	Rice	02-0008-00-207	06/08/2012	Anoka	45.15895		-93.12277			6.72				no
R-70	Strom Slough	34-0429-00-201	07/31/2012	Kandiyohi	45.36		-95.16			0.5				
R-102	Mill Pond	21-0034-00-202	05/21/2012	Douglas	46.07068		-95.22173			6.28				no
R-103	Unnamed	21-0075-00	05/21/2012	Douglas	46.0869		-95.33871			1.04				no
R-104	Miltona	21-0083-00-207	05/21/2012	Douglas	46.04917		-95.43276			5.3				no
R-105	Christina	21-0375-00-314	05/21/2012	Douglas	46.07177		-95.77089			9.66				
R-106	Unnamed	56-1083-00-202	05/21/2012	Otter Tail	46.1148		-95.70688			1.02				no
R-107	Gray	56-0353-00-203	05/22/2012	Otter Tail	46.71029		-95.67663			0.05				no
R-108	Bean	03-0411-00	05/22/2012	Itasca	46.93521		-95.87353			77.2				no
R-109	Bee	60-0192-00-203	05/22/2012	Polk	47.65104		-96.04806			10.2				no
R-110	Golden	60-0249-00-202	05/22/2012	Polk	47.65866		-96.09902			3.27				no
R-111	Sunset	60-0248-00-202	05/22/2012	Polk	47.65919		-96.10202			4.24				no
R-112	Tamarac	60-0247-00-201	05/22/2012	Polk	47.66448		-96.11203			32.6				no
R-113	Snow	56-0110-00-201	05/23/2012	Otter Tail	46.20123		-95.45002			0.05				no
R-114	Unnamed	56-0094-00-202	05/23/2012	Otter Tail	46.19843		-95.45203			0.25				no
R-115	Gourd	56-0139-00-202	05/23/2012	Otter Tail	46.39515		-95.53843			1.36				no
R-116	Davies	56-0311-00-202	05/23/2012	Otter Tail	46.44246		-95.69922			3.82				no
R-117	Dead	56-0383-00-215	05/23/2012	Otter Tail	46.44317		-95.80669			2.39				no
R-119	Lizzie	56-0760-00-202	05/23/2012	Otter Tail	46.62974		-96.0327			7.83				no
R-120	Rice	56-0363-00-204	05/23/2012	Otter Tail	46.67111		-95.6911			1.32				no
R-121	Mud	56-0222-00-203	05/23/2012	Otter Tail	46.62681		-95.57515			5.37				no
R-122	Clearwater	S002-121	05/24/2012	Clearwater	47.93697		-95.68995			21.5				no
R-123	Pine	15-0149-00-206	05/24/2012	Clearwater	47.70389		-95.51252			13.4				no
R-124	Sixth Crow Wing	29-0093-00	05/24/2012	Hubbard	46.92246		-94.87059			2.09				no
R-125	Sixth Crow Wing	29-0093-00-203	05/24/2012	Hubbard	46.92246		-94.87059							no
R-126	Fifth Crow Wing	29-0092-00-203	05/24/2012	Hubbard	46.91923		-94.87821			1.46				no
R-128	Big Swan	77-0023-00-209	05/25/2012	Todd	45.86619		-94.74696			7.86				no
R-129	Big Swan	77-0023-00-209	05/25/2012	Todd	45.86619		-94.74696							no
R-130	Little Birch	77-0089-00-206	05/25/2012	Todd	45.7931		-94.79664			8.36				no
R-131	Westport	61-0029-00-203	05/25/2012	Pope	45.70864		-95.19607			15.5				no
R-132	Johanna	61-0006-00-205	05/25/2012	Pope	45.48433		-95.23197			1.88				no
R-133	Danvers WMA	S005-944	05/25/2012	Swift	45.33264		-95.73311			181				no
R-134	Swan Lake Outlet	34-0223-00	05/25/2012	Kandiyohi	45.33157		-95.0843			14.2				no
R-135	Henchien	34-0207-00-201	05/25/2012	Kandiyohi	45.3213		-95.05106			10.9				no
R-150	Lady Slipper	42-0020-00-202	07/23/2012	Lyon	44.56988		-95.62762			325				no
R-160	Laura	11-0104-00-203	08/20/2012	Cass	46.97678		-94.01846			0.5				yes
R-206	Cedar	70-0091-00-206	08/13/2012	Scott	44.58428		-93.53098			7.77				
R-300	Polk County Pond	WT00047	06/12/2013		47.69215		-95.72807							
FS-50	Swan	34-0223-00-201	07/30/2012	Kandiyohi	45.32598	45.32598	-95.067	-95.067	L	11.7				no
FS-51	Glesne Slough	34-0353-00-201	07/31/2012	Kandiyohi	45.35142		-95.18872		L	0.005	0.03	31800	22.5	yes
FS-52	Blaamyhre	34-0345-00-203	08/01/2012	Kandiyohi	45.36402		-95.18596		L	0.62	0.04	4220	15	yes
FS-53	Raymond	73-0285-00-203	08/02/2012	Stearns	45.62855		-95.02245		L	0.005	0.04	1420	19	yes
FS-54	Little Birch	77-0089-00-207	08/03/2012	Todd	45.7779		-94.79778		L	7.4	0.02	1420	11.25	yes
FS-55	Pelkey	49-0030-00-202	08/26/2012	Morrison	45.99617		-94.22732		L	3.42	0.03	34300	0	no
FS-56	Rice	18-0053-00-203	08/27/2012	Crow Wing	46.33887		-93.89151		L	0.5	0.01	84600	3.5	yes
FS-57	Mississippi River below Clay Boswell	S006-923	08/28/2012	Itasca	47.25507		-93.63415		S	10.3	0.07	8160	0	no
FS-58	Mississippi River above Clay Boswell	S007-163	08/28/2012	Itasca	47.23861		-93.7197		S	1.19	0.04	10700	0	no
FS-59	Upper Panasa	31-0111-00-202	08/29/2012	Itasca	47.30604		-93.26517		L	29.6	0.06	6470	0	no
FS-60	Lower Panasa	31-0112-00-205	08/29/2012	Itasca	47.30175		-93.25209		L	33.6	0.12	5300	0	no
FS-61	Swan	31-0067-02-206	08/30/2012	Itasca	47.28875		-93.21268		L	12.5	0.17	3840	3	yes
FS-62	Swan	31-0067-02-206	08/30/2012	Itasca	47.28903		-93.21235		L	14	0.11	3860	0.75	yes
FS-63	Caribou	69-0489-00-206	09/03/2012	St. Louis	46.89134		-92.31347		L	1.21	0.05	28100	0	no
FS-64	Dead Fish	09-0051-00-202	09/04/2012	Carlton	46.74541		-92.68645		L	0.71	0.03	12400	0	no
FS-65	Wild Rice	09-0023-00-202	09/04/2012	Carlton	46.67121		-92.60551		L	0.5	0.04	18500	0	no
FS-66	St. Louis Estuary	S007-206	09/05/2012	St. Louis	46.65449		-92.27386		S	16	0.02	12500	0	no
FS-67	St. Louis Estuary Pokegama Bay	S006-928	09/05/2012	Vernon, WI	46.68589		-92.16064		S	9.97	0.06	5920	0	yes
FS-68	Wolf	69-0143-00-101	09/06/2012	St. Louis	47.25644		-91.963		L	2.01	0.06	6520	2.25	yes
FS-69	St. Louis	S007-208	09/07/2012	St. Louis	47.46712		-91.92786		S	1.33	0.09	10500	0	no
FS-70	St. Louis	S006-929	09/07/2012	St. Louis	47.4015		-92.37719		S	73.8			0	no
FS-75	Mortenson	34-0150-02-201	07/24/2012	Kandiyohi	45.29996		-94.90617		L	0.005	0.05	13600	0	no
FS-76	Field	34-0151-00-201	07/25/2012	Kandiyohi	45.29644		-94.90575		L	0.005	0.03	12700	0	no

FS-77	Monongalia	34-0158-02-204	07/26/2012	Kandiyohi	45.33308		-94.92678		L	21.7	0.69	59.2	38.75	yes
FS-78	Lady Slipper	42-0020-00-202	07/27/2012	Lyon	44.5699		-95.62753		L	335	0.84	80.5	0	no
FS-79	Lady Slipper	42-0020-00-203	07/27/2012	Lyon	44.57233		-95.62164		L	330	0.82	1320	0	no
FS-80	Mission	S001-646	08/06/2012	Pine	45.86229		-93.00109		S	0.62	0.02	10200	52.25	yes
FS-81	Flowage	01-0061-00-204	08/07/2012	Aitkin	46.68799		-93.33704		L	0.78	0.07	8630	0	no
FS-82	Rabbit	18-0093-02-204	08/08/2012	Crow Wing	46.5313		-93.9285		L	15.3	0.11	937	0	no
FS-83	Mississippi Crow Wing	S007-205	08/08/2012	Crow Wing	46.43864		-94.12505		S	3.13	0.06	7550	0	no
FS-84	Pleasant	11-0383-00-207	08/10/2012	Cass	46.92276		-94.48735		L	0.005	0.01	6830	0	yes
FS-85	Bean	03-0411-00-201	08/21/2012	Itasca	46.93371		-95.87056		L	85	8	50	0	no
FS-86	Eighteen	60-0199-00-203	08/22/2012	Polk	47.63968		-96.06066		L	4.29	0.08	4270	23.75	yes
FS-87	Bee	60-0192-00-202	08/23/2012	Polk	47.65274		-96.05038		L	11	0.34	759	18.75	yes
FS-88	Clearwater	S004-204	08/24/2012	Clearwater	47.51741		-95.39043		S	2.04	0.02	5770	61.25	yes
FS-89	Birch	69-0003-00-205	09/10/2012	St. Louis	47.73577		-91.943		L	8.61	0.05	3010	26.25	yes
FS-90	Sand	S003-249	09/11/2012	St. Louis	47.63505		-92.42335		S	15.9	0.08	25000	0.75	yes
FS-91	Pike	S006-927	09/11/2012	St. Louis	47.7327		-92.34726		S	14.2	0.03	13500	23.75	yes
FS-92	Partridge	S007-443	09/12/2012	St. Louis	47.52065		-92.19088		S	36.3	0.04	29800	1.5	yes
FS-93	Turpela	69-0427-00-201	09/12/2012	St. Louis	47.46127		-92.23709		L	3.3	0.06	3870	0.75	yes
FS-94	Sturgeon	S004-870	09/13/2012	St. Louis	47.65602		-92.93154		S	1.62	0.03	33700	13.75	yes
FS-95	Embarrass	69-0496-00-203	09/14/2012	St. Louis	47.53339		-92.2979		L	18.8	0.01	9790	0	no
FS-101	Rice paddy	WT00026	06/25/2012	Beltrami	48.2161		-94.61883		P	11.3	0.15	1600	4.25	yes
FS-102	Rice paddy	WT00027	06/26/2012	Polk	47.92646		-95.63134		P	1.61	0.34	2820	39.25	yes
FS-103	Rice paddy	WT00028	06/26/2012	Polk	47.80534		-95.67319		P	279	0.37	1120	23.75	yes
FS-104	Gourd	04-0253-00-201	06/27/2012	Beltrami	47.8121		-94.96502		L	0.27				no
FS-105	Second	15-0091-00-202	06/27/2012	Clearwater	47.82582		-95.36372		L	0.74	0.06	887	13	yes
FS-106	Rice paddy	WT00029	06/28/2012	Clearwater	47.85229		-95.47315		P	7.14	0.08	1060	25	yes
FS-107	Rice paddy	WT00030	06/28/2012	Clearwater	47.85207		-95.49525		P	9.46	0.1	1650	80	yes
FS-108	Rice paddy	WT00031	06/29/2012	Crow Wing	46.24597		-94.25481		P	0.25	0.02	2500	33.75	yes
FS-109	Carlos Avery Pool 9	02-0504-00-202	07/03/2012	Anoka	45.31919		-93.0611		L	0.005	0	28500	23.75	yes
FS-125	Tamarac	56-0192-00-203	08/19/2012	Otter Tail	46.36373		-95.57136		L	2.33	0.04	18900	0	no
FS-126	Bray	56-0472-00-202	08/20/2012	Otter Tail	46.4518		-95.87825		L	1.65	0.04	1740	1.75	yes
FS-127	Height of Land	03-0195-00-210	08/21/2012	Itasca	46.91326		-95.60953		L	0.5	0	6750	70	yes
FS-128	Cromwell	14-0103-00-201	08/22/2012	Clay	46.96507		-96.31714		L	41.2	0.61	85.5	0	no
FS-129	Mink	86-0229-00-207	08/23/2012	Wright	45.27674		-94.02988		L	1.22	0.09	5700	0	no
FS-130	Hay	31-0037-00-202	09/06/2012	Itasca	47.28737		-93.10201		L	31.7	0.04	16600	53.75	yes
FS-131	Hinken	S007-207	09/05/2012	Itasca	47.72708		-93.99228		S	0.5	0.04	7260	18.75	yes
FS-132	Ox Hide	31-0106-00-203	09/07/2012	Itasca	47.33502		-93.21339		L	26.4	0.02	20900	4	yes
FS-133	Mahnomen	18-0126-02-201	09/17/2012	Crow Wing	46.49849		-93.99583		L	16.9	0.15	5870	0	no
FS-134	Bass	31-0576-00-207	09/18/2012	Itasca	47.28437		-93.62759		L	1.01	0.03	2800	32.5	yes
FS-135	Blank, Field		09/18/2012											
FS-136	Itasca	15-0016-00-208	09/19/2012	Clearwater	47.23425		-95.20494		L	0.5	0.03	7520	7.5	yes
FS-137	Elk	15-0010-00-204	09/19/2012	Clearwater	47.19524		-95.22493		L	0.5	0.05	15900	7.25	yes
FS-138	Little Round	03-0302-00-203	09/20/2012	Itasca	46.97259		-95.73496		L	0.5	0.06	3970	46.25	yes
FS-139	Welby family farm	86-0231-00-202	09/21/2012	Wright	45.35915		-94.07824		L	0.5	0.06	9100	2	yes
FS-140	Lower Panasa	31-0112-00-204	08/29/2012	Itasca	47.30334		-93.2569		L	33.6	0.12	5300		
FS-175	Maloney	79-0001-00-201	07/23/2012	Wabasha	44.22507		-91.93212		L	3.15	0.03		0	no
FS-176	North Geneva	24-0015-00-209	07/24/2012	Freeborn	43.78757		-93.27095		L	15.6	0.77	10	0	no
FS-177	South Geneva	24-0015-02-208	07/24/2012	Freeborn	43.77086		-93.28506		L	14.1	1.6	10	0	no
FS-178	Bear	24-0028-00-206	07/25/2012	Freeborn	43.54652		-93.50282		L	18.3				no
FS-179	Rice	74-0001-00-201	07/25/2012	Steele	44.08417		-93.07372		L	3.84	0.11	523	0	no
FS-180	Lily	81-0067-00-202	07/26/2012	Waseca	44.19471		-93.64699		L	0.005	0.01	7250	18.75	yes
FS-181	Rice	66-0048-00-203	07/27/2012	Rice	44.33322		-93.47337		L	5.22	0.39	131	0	no
FS-182	Hunt	66-0047-00-208	07/27/2012	Rice	44.32748		-93.44428		L	17.1	0.04	6410	0	no
FS-183	Unnamed	34-0611-00-201	07/30/2012	Kandiyohi	45.26748		-94.86498		L	16.8	0.08	428	16.25	yes
Fs-184	Rice	73-0196-00-216	07/30/2012	Stearns	45.3864		-94.63087		L	2.58	1.49	25	0	no
FS-185	Hoffs Slough	76-0103-00-201	08/01/2012	Swift	45.32545		-95.70587		L	273	0.02	109	0	no
FS-186	Westport	61-0029-00-204	08/01/2012	Pope	45.68974		-95.21696		L	7.11	0.9	29	0	no
FS-187	McCormic	73-0273-00-203	08/02/2012	Stearns	45.72204		-94.91207		L	1.54	0.07	3360	1.25	yes
FS-188	Stella	47-0068-00-204	08/27/2012	Meeker	45.06828		-94.43335		L	18.1	0.9	270	0.25	yes
FS-189	Clearwater	S002-121	08/28/2012	Clearwater	47.93721		-95.69064		S	23.8	0.06	1760	1.75	yes
FS-190	Pine	15-0149-00-205	08/28/2012	Clearwater	47.68412		-95.54141		L	14.7	0.18	4560	47.5	yes
FS-191	Ina	21-0355-00-202	08/29/2012	Douglas	46.07153		-95.7281		L	7.08	0.14	2330	8.5	yes

FS-193	Big Mud	71-0085-00-201	08/30/2012	Sherburne	45.45292		-93.74184		L	0.5	0.02	27800	4.25	yes
FS-194	Gilchrist	86-0064-00-201	08/31/2012	Wright	45.2309		-93.82404		L	6.98	0.18	7230	0	no
FS-195	Fisher	70-0087-00-201	08/31/2012	Scott	44.79424		-93.40609		L	6.85	0.07	47600	25	yes
FS-196	Prairie	S007-209	09/03/2012	Itasca	47.2519		-93.4884		S	9.63	0.04	18500	16.25	yes
FS-197	Snowball	31-0108-00-202	09/04/2012	Itasca	47.33545		-93.24397		L	8.4	0.05	19100	0	no
FS-198	Ox Hide	31-0106-00-203	09/07/2012	Itasca	47.33503		-93.21338		L	26.4	0.04	9730	0.25	yes
FS-199	Rice	S006-208	09/05/2012	Itasca	47.67416		-93.65468		S	1.57	0.03	2730	29	yes
FS-200	Louisa	86-0282-00-205	08/08/2012	Wright	45.29978		-94.25797		L	7.04	0.1	5930	0	no
FS-201	Mink	86-0229-00-206	08/08/2012	Wright	45.27398		-94.02687		L	1.31	0.02	3000	0	no
FS-202	Long Prairie	S007-204	08/09/2012	Douglas	46.00722		-95.26339		S	7.71	0.04	2290	8.75	yes
FS-203	Long Prairie	S007-203	08/09/2012	Douglas	45.9729		-95.16029		S	6.66	0.02	5640	46.25	yes
FS-204	Big Swan	77-0023-00-208	08/10/2012	Todd	45.87954		-94.74201		L	5.49	0.05	1110	55	yes
FS-205	Big Swan	77-0023-00-207	08/10/2012	Todd	45.87952		-94.74184		L	5.47	0.03	916	17.5	yes
FS-207	Kelly Lake	66-0015-00-204	08/13/2012	Rice	44.35416		-93.37434		L	1.92	0.05	2790	0	yes
FS-208	Mississippi Pool 8 at Genoa	S007-222	08/14/2012	Buffalo, WI	43.57581		-91.2334		S	18	0.09	2950	43.75	yes
FS-209	Mississippi Pool 8 at Reno Bottoms	S007-556	08/15/2012	Houston	43.60246		-91.26858		S	18.1	0.04	4870	46.25	yes
FS-210	Mississippi Pool 4/Robinson Lake	79-0005-02-202	08/16/2012	Wabasha	44.35926		-91.98808		S	15.7	0.04	27800	21.25	yes
FS-211	Mississippi Pool 4/Robinson Lake	79-0005-02-201	08/16/2012	Wabasha	44.36106		-91.98969		S	17.7	0.04	12000	51.25	yes
FS-212	Mississippi Pool 5 / Spring	S007-690	08/17/2012	Douglas, WI	44.19931		-91.84608		S	17.2	0.01	11800	17.5	yes
FS-213	Gull	04-0120-00-204	09/10/2012	Beltrami	47.65583		-94.69449		L	1.14	0.04	3070	4.5	yes
FS-214	Bowstring	S007-219	09/11/2012	Itasca	47.70237		-94.06079		S	1.34	0.13	1510	27.5	yes
FS-215	Popple	S006-188	09/11/2012	Itasca	47.7254		-94.0817		S	0.5	0.01	3610	11.75	yes
FS-216	Big Sucker	31-0124-00-203	09/12/2012	Itasca	47.39194		-93.26584		L	7.78	0.07	3140	1.25	yes
FS-217	Blank, Field		09/12/2012											
FS-218	Holman	31-0227-00-202	09/13/2012	Itasca	47.30052		-93.34449		L	24.2	0.51	132	0	no
FS-219	Trout	31-0216-00-212	09/13/2012	Itasca	47.25915		-93.39419		L	38.6	0.06	18400	0	no
FS-220	Padua	73-0277-00-202	08/07/2012	Stearns	45.623		-95.01862		L	0.86	0.12	2580	0	yes
FS-221	Hay Creek Flowage	58-0005-00-202	09/17/2012	Pine	46.08938		-92.41035		L	1.95	0.06	20100	58.75	yes
FS-222	Blank, Field		09/18/2012											
FS-223	Little Sucker	31-0126-00-202	09/14/2012	Itasca	47.37651		-93.24597		L	13.7	0.27	542	0	no
FS-224	Stone Lake	69-0046-00-201	09/19/2012	St. Louis	47.50393		-91.88572		L	3.26	0.03	1140	6.25	yes
FS-225	Miltona	21-0083-00-205	08/13/2012	Douglas	46.04963		-95.42171		L	4.11	0.03	14800	0	no
FS-226	Louise	21-0094-00-202	08/14/2012	Douglas	45.93307		-95.41476		L	4.09	0.04	8760	17	yes
FS-228	West battle	56-0239-00-204	08/15/2012	Otter Tail	46.29057		-95.60486		L	4.03	0.09	5380	35	yes
FS-229	Mill Pond	21-0034-00-202	08/16/2012	Douglas	46.07157		-95.22179		L	7.16	0.05	4080	30	yes
FS-230	Mill Pond	21-0034-00-202	08/16/2012	Douglas	46.07148		-95.22176		L	7.36	0.1	2780	21.5	yes
FS-231	Rice	02-0008-00-206	08/17/2012	Anoka	45.1604		-93.12103		L	3.6	0.07	986	0	no
FS-250	Little Rice	69-0612-00-201	09/20/2012	St. Louis	47.70857		-92.4389		L	1.03	0.01	16100	8.75	yes
FS-251	Sandy	69-0730-00-203	09/21/2012	St. Louis	47.62539		-92.58856		L	3.05	0.06	12500	1.25	yes
FS-300	St. Louis Estuary	S007-444	05/27/2013	St. Louis	46.65147		-92.23759		S	9.4	0.04	15000	0	no
FS-301	Partridge	S007-443	05/28/2013	St. Louis	47.52132		-92.19026		S	14.8	0.06	17100	0	no
FS-302	Partridge	S007-513	05/30/2013	St. Louis	47.51526		-92.18935		S	43.1	0.03	19200	0	no
FS-303	Second	S007-220	05/30/2013	St. Louis	47.52042		-92.1925		S	303	0.05	10800	0	no
FS-304	Rice	18-0053-00-203	06/10/2013	Crow Wing	46.3387		-93.89059		L	0.05	0.01	38400	2.5	yes
FS-305	Sandy	69-0730-00-203	06/11/2013	St. Louis	47.61868		-92.59373		L	135	0.54	81.5	0	no
FS-306	Sandy	69-0730-00-203	06/11/2013	St. Louis	47.62546		-92.58836		L	11	0.05	49300	0	no
FS-307	Rice paddy	WT00046	06/12/2013	Clearwater	47.84819		-95.4865		P	16.6	0.02	1140	4.25	yes
FS-308	Rice paddy	WT00028	06/12/2013	Polk	47.80559		-95.67399		P	57.1	0.4	825	36.25	yes
FS-309	Eighteen	60-0199-00-203	06/13/2013	Polk	47.63686		-96.05987		L	4.36	0.06	4060	0	no
FS-310	Second	S007-220	06/14/2013	St. Louis	47.52052		-92.19245		S	316	0.05	6640	25	yes
FS-311	Mississippi Pool 8 at Genoa	S007-222	06/20/2013	Buffalo, WI	43.57655		-91.23409		S	29.3	0.05	2000	10	yes
FS-312	Mississippi Pool 5 / Spring	S007-660	06/21/2013	Douglas, WI	44.20181		-91.84441		S	28.3	0.04	4180	23.75	yes
FS-313	Monongalia	34-0158-01-203	06/23/2013	Kandiyohi	45.33339		-94.92927		L	34.7	0.05	5760	32.5	yes
FS-314	Clearwater	S002-121	06/24/2013	Clearwater	47.93723		-95.69072		S	28	0.03	11600	0.25	yes
FS-315	St. Louis Estuary	S007-444	06/24/2013	St. Louis	46.65159		-92.2373		S	8.1	0.07	13900	0	no
FS-316	Partridge	S007-513	06/28/2013	St. Louis	47.51371		-92.18993		S	24.9	0.05	11300	0	no
FS-317	Partridge	S007-443	06/26/2013	St. Louis	47.52146		-92.1903		S	7.65			0	no
FS-318	Height of Land	03-0195-00-210	06/26/2013	Itasca	46.91346		-95.61235		L	1.21	0.03	9160	22.5	yes
FS-319	Little Round	03-0302-00-203	06/27/2013	Itasca	46.97241		-95.73503		L	0.05	0.06	1520	5	yes
FS-320	Sandy	69-0730-00-204	07/09/2013	St. Louis	47.61879		-92.59364		L	118	1.54	4670	0	no
FS-321	Sandy	69-0730-00-203	07/09/2013	St. Louis	47.62554		-92.58846		L	122	0.09	34200	0	no

FS-322	Dark	69-0790-00-202	07/10/2013	St. Louis	47.63888		-92.77806		L	175	0.07	11500	1.25	yes
FS-323	Second	S007-220	07/11/2013	St. Louis	47.52039		-92.1925		S	405	0.03	8900	45	yes
FS-324	Rice	18-0053-00-203	07/15/2013	Crow Wing	46.33915		-93.8918		L	0.05	0.06	22900	27.5	yes
FS-325	Rice paddy	WT00046	07/16/2013	Clearwater	47.84806		-95.48647		P	0.46	0.06	4300	51.25	yes
FS-326	Rice paddy	WT00028	07/17/2013	Polk	47.80551		-95.67321		P	28.8	0.2	2220	100	yes
FS-327	Clearwater	S002-121	07/17/2013	Clearwater	47.93712		-95.69057		S	23.7	0.06	8450	0.25	yes
FS-328	Eighteen	60-0199-00-203	07/18/2013	Polk	47.63686		-96.05989		L	3.34	0.13	3900	27.5	yes
FS-330	St. Louis Estuary	S007-444	07/22/2013	St. Louis	46.65184		-92.23721		S	6.71	0.05	8860	8.75	yes
FS-331	Partridge	S007-443	07/24/2013	St. Louis	47.52118		-92.19044		S	14.6	0.06	12000	30	yes
FS-332	Partridge	S007-513	07/24/2013	St. Louis	47.51371		-92.18938		S	54.4	0.05	18600	53.75	yes
FS-333	Embarrass	69-0496-00-203	07/26/2013	St. Louis	47.53326		-92.29764		L	18.2	0.04	3970	0	no
FS-334	Mississippi Pool 8 at Genoa	S007-222	07/29/2013	Buffalo, WI	43.5758		-91.23439		S	44.2	0.05	3140	28.75	yes
FS-335	Mississippi Pool 5 / Spring	S007-660	07/30/2013	Douglas, WI	44.19532		-91.84101		S	47.7	0.02	10500	42.5	yes
FS-336	Mississippi Pool 4/Robinson Lake	79-0005-02-201	07/30/2013	Wabasha	44.36129		-91.99007		S	55.3	0.03	16100	30	yes
FS-337	Clearwater	S004-204	07/29/2013	Clearwater	47.51749		-95.39059		S	0.95	0.03	11100	52.5	yes
FS-338	Height of Land	03-0195-00-210	07/30/2013	Itasca	46.91295		-95.61158		L	0.05	0.03	13400	36.25	yes
FS-339	Christina	21-0375-00-315	08/31/2013	Douglas	46.07337		-95.75669		L	14.6	0.97	10	0.25	yes
FS-340	Monongalia	34-0158-02-203	08/31/2013	Kandiyohi	45.33311		-94.92924		L	33.6	0.06	6660	60	yes
FS-341	Stella	47-0068-00-205	08/01/2013	Meeker	45.06603		-94.43389		L	24.7	0.04	5240	28.75	yes
FS-342	Little Round	03-0307-00-203	08/05/2013	Itasca	46.97213		-95.73579		L	0.05	0.03	3280	18.75	yes
FS-343	Raymond	73-0285-00-203	08/06/2013	Stearns	45.62904		-95.02333		L	1.92	0.05	5740	25	yes
FS-344	Padua	73-0277-00-202	08/06/2013	Stearns	45.62305		-95.01865		L	0.05	0.04	6610	2.5	yes
FS-345	Rice	73-0196-00-216	08/07/2013	Stearns	45.38651		-94.6313		L	6.85	1.04	10	0	yes
FS-346	Westport	61-0029-00-205	08/08/2013	Pope	45.70415		-95.20296		L	6.3	0.1	1420	4.5	yes
FS-347	Snowball	31-0108-00-202	08/12/2013	Itasca	47.33555		-93.24387		L	8.2	0.05	6650	0	no
FS-348	Sandy	69-0730-00-204	08/13/2013	St. Louis	47.61855		-92.59339		L	123	0.15	6570	0	yes
FS-349	Sandy	69-0730-00-205	08/13/2013	St. Louis	47.61906		-92.58983		L	122	0.03	12600	0	no
FS-350	Ox Hide	31-0106-00-203	08/14/2013	Itasca	47.33512		-93.21317		L	25.9	0.06	1870	0	no
FS-351	Second	S007-220	08/15/2013	St. Louis	47.52053		-92.19251		S	838	0.02	12600	52.5	yes
FS-352	Dark	69-0790-00-202	08/15/2013	St. Louis	47.63884		-92.7782		L	173	0.07	7470	1.25	yes
FS-353	Holman	31-0227-00-202	08/12/2013	Itasca	47.3009		-93.34437		L	68	0.29	31.3	0	no
FS-354	Mississippi River above Clay Boswell	S007-163	08/13/2013	Itasca	47.23756		-93.71865		S	1.18	0.03	28200	75	yes
FS-355	Mississippi River below Clay Boswell	S006-923	08/13/2013	Itasca	47.25528		-93.63402		S	10.2	0.04	36600	33.75	yes
FS-356	Trout	31-0216-00-212	08/14/2013	Itasca	47.25913		-93.3942		L	39.1	0.05	12800	0	no
FS-357	Lower Panasa	31-0112-00-204	08/15/2013	Itasca	47.30259		-93.25609		L	28.5	0.63	763	0	no
FS-358	Turtle River, North Branch	S007-662	08/19/2013	Grand Forks, ND	47.99524		-97.62759		S	198	0.04	2790	22.5	yes
FS-359	Eighteen	60-0199-00-203	08/20/2013	Polk	47.63673		-96.05997		L	2.83	0.06	3140	5.5	yes
FS-360	Rice paddy	WT00046	08/21/2013	Clearwater	47.84791		-95.48661		P		0.05	1590	33.75	yes
FS-361	Rice paddy	WT00028	08/21/2013	Polk	47.80541		-95.67443		P				68.75	yes
FS-363	St. Louis Estuary	S007-444	08/26/2013	St. Louis	46.65177		-92.23721		S				18.75	yes
FS-364	Partridge	S007-513	08/30/2013	St. Louis	47.51376		-92.18942		S				57.5	yes
FS-365	Partridge	S007-443	09/03/2013	St. Louis	47.52123		-92.19007		S	34.1	0.02	12700	31.25	yes
FS-366	Partridge	S007-443	09/03/2013	St. Louis	47.52129		-92.18997		S	34.2	0.03	5430	17.5	yes
FS-367	Hay	31-0037-00-202	09/04/2013	Itasca	47.28699		-93.10085		L	22.1	0.02	31400	83.75	yes
FS-368	Dark	69-0790-00-202	09/05/2013	St. Louis	47.63871		-92.77822		L	175	0.15	9050	6.25	yes
FS-369	Dark	69-0790-00-202	09/05/2013	St. Louis	47.63885		-92.77806		L	176	0.03	14400	12.75	yes
FS-370	Mississippi Pool 8 at Genoa	S007-222	09/09/2013	Buffalo, WI	43.5765		-91.23374		S	33.3	0.03	3240	11.25	yes
FS-371	Mississippi Pool 5 / Spring	S007-660	09/10/2013	Douglas, WI	44.20163		-91.84428		S	34.4	0.03	13800	26.25	yes
FS-372	Mississippi Pool 5 / Spring	S007-660	09/10/2013	Douglas, WI	44.20156		-91.84433		S	34.8	0.03	14200	13.75	yes
FS-373	Clearwater	S002-121	09/09/2013	Clearwater	47.93721		-95.69094		S	34.4	0.02	4020	5	yes
FS-374	Little Round	03-0302-00-202	09/10/2013	Itasca	46.97445		-95.738		L	0.12	0.02	1480	21.25	yes
FS-375	Height of Land	03-0195-00-210	09/10/2013	Itasca	46.91301		-95.61111		L	0.05	0	8920	63.75	yes
FS-376	Rice	18-0053-00-203	09/11/2013	Crow Wing	46.33936		-93.89184		L	0.05	0.02	35500	22.5	yes
FS-377	Mahnomen	18-0126-02-201	09/11/2013	Crow Wing	46.49858		-93.99555		L	21.1	0.01	4540	0	no
FS-378	Duck Lake WMA	18-0178-00-202	09/12/2013	Crow Wing	46.75206		-93.88509		L	0.05	0.01	18700	42.5	yes
FS-379	Monongalia	34-0158-02-203	09/13/2013	Kandiyohi	45.3332		-94.92924		L	34.6	0.12	2740	62.5	yes
FS-380	Sandy	69-0730-00-204	09/17/2013	St. Louis	47.61869		-92.59385		L	126	0.02	23900	0.25	yes
FS-381	Sandy	69-0730-00-204	09/17/2013	St. Louis	47.61872		-92.59314		L	126	0.02	23900	0	yes
FS-382	Sandy	69-0730-00-203	09/17/2013	St. Louis	47.62554		-92.58848		L	67.9	0.07	29500	0	no
FS-383	Upper Panasa	31-0111-00204	09/18/2013	Itasca	47.30593		-93.26762		L	33.6	0.02	62900	0	no
FS-384	Second	S007-220	09/19/2013	St. Louis	47.52044		-92.19251		S		0.05	12600	15	yes