Comments received concerning the draft document

The Sulfate Standard to Protect Wild Rice: Study Protocol
(MPCA, June 6, 2011 version)

Comments compiled August 22, 2011

Comments are compiled in reverse chronological order, with most recent first.
To discourage automated harvesting of email addresses when this is posted on the MPCA web site, all “@” symbols in the email addresses have been replaced with “#$”.

Leech Lake Band Of Ojibwe

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***Arthur "Archie" LaRose,* Chairman**
***Vacant,* Secretary/Treasurer**

District I Representative *Robbie M. Howe* **District II Representative** *Steve White* **District III Representative** *Eugene Whitebird*

Date: 7/1/2011

David Thornton
Assistant Commissioner
Minnesota Pollution Control Agency

Dear Mr. Thorton,

It is my responsibility and duty to my people as Leech Lake Division of Resource Management Director to inform you that we do not support the Minnesota Pollution Control Agencies (MPCA) Sulfate Standard Study Protocol. Our reasons for not supporting this study are both technical and cultural.

At previous meetings between the Minnesota Tribes and MPCA the Leech Lake environmental director stated several times that the wild rice experts are the Anishinaabe people. Also that any Mahnomin study should be long term and only with the consent of the tribes. How many of the scientific professionals have been ricing or understand what Mahnomin means to the Anishinaabe people? Common sense says that this study would only be a snapshot in time and not produce any conclusive information. It is this basic flaw that makes it impossible for LLDRM to support or even recognize any such study. Also no Mahnomin will be taken from the water of or border water of the Leech Lake Indian Reservation. Furthermore we must also talk about the Mahnomin within the 1855 treaty area because the LLBO still retains gather rights in that region.

As Anishinaabe people we have many traditions and cultural traits that we hold sacred, none more than Mahnomin (wild rice). Our people have maintained the Mahnomin for generations and more recently Leech Lake Band of Ojibwe Division of Resource Management has been tasked with ensuring the Leech Lake Indian Reservation waters remain capable of producing wild rice. We have successfully maintained the single greatest region of wild rice within the waters of Leech Lake Indian reservation. This success came not because we scientifically study Mahnomin but because we have respected and held true to some of core teachings about Mahnomin.

I want to leave you with one final analogy that may give a better idea of how significant Mahnomin is to the Anishinaabe culture: Would you ask Christians to cut open Jesus Christ's body and study to see if the sulfate would have any impacts on that being?

Bruce M Johnson

Bruce Johnson, Director
Division of Resource Management
Leech Lake Band of Ojibwe

Cc: Leech Lake Tribal Council

MINNESOTA
CHAMBER of
COMMERCE

June 30, 2011

Ms. Shannon Lotthammer
Section Manager
Water Assessment and Environmental Information Section
Environmental Analysis & Outcomes Division
Minnesota Pollution Control Agency
520 Lafayette Road North
St. Paul, Minnesota 55155-4194

Re: **Wild Rice Research Protocol**

Dear Shannon:

On behalf of the Minnesota Chamber of Commerce (Chamber), enclosed are general and technical comments related to the preliminary field study (Exhibit A "Wild Rice-Sulfate 2011 Preliminary Field Study") and proposed laboratory studies ("Draft Sulfate and Wild Rice Protocol" published by Pat Engelking in an email dated June 7, 2011) for derivation of a surface water sulfate standard for the protection of wild rice. Specific technical comments related to the proposed laboratory and field studies are attached.

The Chamber has several overarching concerns related to the laboratory and preliminary field protocols as currently drafted:

- It would be useful if MPCA could present the process that will be used to derive a water column-based sulfate wild rice standard. For other Clean Water Act water quality standards, whether for aquatic life, human health, or wildlife, a process is presented and that process defines the data needed to successfully execute the process resulting in a standard. Without knowing the process, it is difficult to discern what the objectives are for generating the standard.
- It would be useful if MPCA could acknowledge, so that all stakeholders understand the limits of time and investment, where assumptions or boundaries to the research will have to be made in deriving a water column- based sulfate wild rice standard. Numerous instances, spanning decades, can be cited where EPA did not understand all toxicological variables in order to develop water quality criteria (recent examples being selenium and copper).
- MPCA might consider other assumptions to simplify and focus research efforts to generate the data to derive a water column-based sulfate wild rice standard. For example:

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- Critical toxicity endpoint for laboratory studies will be seed germination
- Only critical variables (e.g., porewater ORP(oxidation reduction potential), porewater Fe, pH) should be evaluated in laboratory studies to define their role in toxicity (e.g., inhibiting seed germination) of sulfate to wild rice
- Critical toxicity endpoints must be specified (e.g., seed germination, others listed in the attached comments)

- Because there is no process to derive a surface water sulfate standard to protect wild rice, much of the protocol seems to present experiments that, while interesting, are focused on generating data of little use in deriving a sulfate standard. Understanding the mechanistic chemistry between surface water sulfate and sediment conditions potentially impacting wild rice is unnecessary to the issue at hand.
- There is no timeline proposed to derive the standard before the next triennial review of Water quality standards.
- A toxicity-based approach as outlined by USEPA could much more efficiently determine the need for a surface water sulfate standard to protect wild rice. Such an approach should focus on sensitive early life stages of wild rice and the relationship between surface water sulfate and impacts to wild rice.
- Regardless of the approach taken, EPA Region 5 should continue to be afforded an opportunity to provide input so that any subsequently proposed standard is more likely to be accepted by EPA.

To assure that appropriate investment of resources in the given time-frame, we propose that the draft study protocols be focused on two major components, sediment characteristics impacting wild rice toxicity and translation of those sediment characteristics to a corresponding sulfate water column level.

It is important to keep in mind that the purpose of the sulfate and wild rice laboratory protocol should be to determine whether or not a water column criterion for sulfate is necessary to protect wild rice, and if so, what level would be protective of that use. The timeline for making that determination should be the current triennial standards review. It seemed to be the consensus of the wild rice experts at the gathering on May 9, 2011, that this question could be answered in short order with the protocol for Task A. Therefore, laboratory protocol Task A should be undertaken immediately in order to determine the direct toxic effects of sulfate on wild rice, and completed in time to be used in the current triennial standards review.

Tasks A and B will provide for more definitive assessments of sulfate concentrations detrimental to wild rice in the presence of representative sediments to be completed before the next triennial standards review. While there may be additional compounds and conditions which impact the growth and productivity of wild rice, determining which compounds and conditions and the levels that are protective will likely take considerable time and are not necessary to understanding at what concentrations surface water sulfate is detrimental to wild rice. Many of the other tasks expend resources evaluating sediment chemistry interactions that are not necessary to understand in order to develop a surface water sulfate standard. Such research should be carefully considered with respect to developing a surface water sulfate standard prior to the next triennial review.

As you are aware, Chamber members are facing compliance schedules in their NPDES permits to meet the current water quality criterion of 10 mg/L sulfate in waters supporting wild rice. In about the time it will take to complete the current triennial standards review, Chamber members will need to begin investing millions to tens of millions of dollars to meet the compliance schedules. If removal of sulfate to a 10 mg/L level is not required, such expenditures would do little to protect wild rice, and will result in Minnesota industry being non-competitive with other states and in global competition.

It is also important to keep in mind that because the water quality criterion in question is a water column criterion, the protocols need to reflect US EPA guidance on development of water quality criteria (i.e., USEPA's 1985 Guidance for derivation of water quality critieria for protection of aquatic life, Stephan et al., PB85-227049). It is also important to keep in mind the agency's own regulations for development of water quality criteria found at MN Rules 7050.0218, and the associated guidance and federal citations referenced there. As the research undertaken will become part of rulemaking, it is important that the detailed protocol conform to these standards, in order to withstand challenges from others. It is also important to recall that the Class 4A standard for sulfate applicable to wild rice is a chronic standard, and ultimately expression of a standard will have to incorporate the components of frequency, duration, and magnitude inherent to EPA's expression of water quality criteria.

With specific regard to the other tasks outlined in the laboratory protocols and hypotheses to be tested, three general comments are provided:

- There should be no methyl mercury research conducted as part of the research on wild rice. There is no evidence that mercury or methyl mercury impacts wild rice. The draft protocol itself states that the Priority C hypotheses (methylation of mercury) "...do not address the wild rice issue".
- It seemed the consensus of the wild rice experts at the gathering on May 9 that in-situ, large scale mesocosm studies should be undertaken only after all other studies at all other scales have been conducted. Given the large number of variables which may affect wild rice, such experiments should be undertaken only if a clear hypothesis can be expressed which would be directly answered by such a study, and only if the concerns of the Native American bands are addressed. If such studies are undertaken, it is not necessary to understand all of the intricate interactions between surface water sulfate and sediment chemistry as it relates to potential impacts on wild rice. An excellent surrogate to understand the net effects of these interactions is sediment oxidation reduction potential (ORP). Sediment ORP and pH should be considered as key parameters in mesocosm and all other studies.
- While sediment sulfide may be an important factor in the growth and production of wild rice, there are several hurdles which need to be overcome before research can provide meaningful results with respect to establishing a surface water sulfate standard. A key consideration is the ability to accurately measure sulfide in sediment pore water. When undertaking the sulfide research, the MPCA needs to keep clearly in mind how sediment sulfide conditions (and any other parameter studied) would be translated to a water quality standard.

Thank you for the opportunity to comment on the proposed draft protocol. We look forward to a continued joint effort to better understand potential surface water sulfate impacts on of wild rice.

Sincerely,

Mike Robertson
Minnesota Chamber of Commerce

Cc:
Mark Tomasek Pat
Engelking Rebecca
Flood
MN Chamber Wild Rice Sulfate Task Force Members

Minnesota Chamber Technical Comments — Wild Rice Sulfate Protocols
June 30, 2011

General

- Please include bios, resumes, and publications of the researchers on the website where information from this project will be stored.

- It would be helpful if others were notified of specific study sites as you determine them.

- A "weight of evidence approach" should be utilized in establishing a surface water sulfate standard to protect wild rice if a toxicological threshold cannot be specifically defined from the research ultimately conducted. MPCA should identify a process to determine the weighting criteria and how it will be used well in advance of its application.

There seems to be a general consensus that sediment sulfide is much more toxic than water column sulfate to wild rice. Therefore, given the regulatory time constraints, the primary research objectives should be:

- Generation of data focused on understanding the relationship of a few variables to the generation of sulfide in sediments, and on sediment sulfide toxicity to wild rice. The few variables evaluated should be for conditions most characteristic of Minnesota sediments in areas supporting wild rice. For example, generating data to relate sediment sulfide toxicity to key sediment and porewater variables: oxidation reduction potential (ORP), sediment pH, and sediment porewater iron. This approach would mimic the EPA process of relating metals toxicity to water hardness and total vs. soluble metal. The outcome of this additional data could be a wild rice sediment sulfide protection standard whereby key characteristics of the sediment influence sulfide toxicity to wild rice.

- Generation of data to define the key parameters and water column sulfate concentrations contributing to sulfide levels in sediment detrimental to wild rice. This information could be used to develop a simple model to translate a sulfide sediment standard to a water column sulfate protection level.

Key EPA concepts inherent to developing aquatic life water quality criteria must be considered in developing a study design that must ultimately result in a specific means by which to derive a surface water sulfate standard to protect wild rice:

- Testing of sensitive wild rice life stages.

- Use of well-defined, ecologically-relevant test endpoints, for example: seed germination, and selected others as listed in the technical comments on the laboratory protocols (below).

- Considering appropriate criteria implementation components such as frequency, duration, and magnitude.

- Potential use of a surrogate parameter that integrates the complex sediment physical, chemical, and biological interactions that result in sulfide generation in sediments. For example, under certain sediment ORR conditions, increased water column sulfate concentrations may not be detrimental to wild rice. ORP could be used as a means to indicate relative sediment sulfide

toxicity in the same way pH and water hardness are used to indicate ammonia and heavy metals toxicity, respectively, in current EPA criteria.

Specific technical comments related to the proposed preliminary field study, and laboratory protocols, are presented below.

Preliminary Field Study Protocols

- Additional information should be provided on the porewater protocols to be utilized. As proposed in this study, it is critical to obtain baseline measurements in low and high sulfate sites (including sediment pore water composition, metals, nitrate, sulfate, sulfide to name some) over time. Such baseline parameters will help set the range in treatment levels Imposed in Tasks A and B of the laboratory study.

- The proposed field study is a preliminary assessment of water column characteristics, sediment chemistry, and wild rice productivity to be conducted in the summer of 2011. It is assumed some associations will be extrapolated between water, sediment, and wild rice field data. It is understood that the field data will be used to design additional field studies and a more detailed laboratory protocol. We believe this is a logical progression of activities.

- A limited number of *in situ* plant growth parameters should be measured in the "intensive field study" to establish a baseline for comparison in subsequent laboratory studies.

- In the draft field study protocol, specific metrics to define productivity and water column sulfate concentrations for the five proposed site classes has to be presented so that discrimination amongst these classes is consistent.

- Upon completion of defining the specific metrics for the five site classes, it may be beneficial in later data analysis to reduce the number of site classes (e.g. to three).

- In the draft field study protocol, one of the objectives is to test and refine methods of sediment porewater sampling. We believe that sufficient acceptable porewater sampling methods are already available and detailed evaluation of such methods is not necessary given the limited resources and time available before the next triennial water quality standards review.

- While addressing key sediment chemistry parameters related to factors potentially impacting wild rice, the field study does not address documenting the frequency, duration, and magnitude of water column sulfate concentrations associated with the various wild rice Site Classes. These data characteristics must be understood because they are critical components of water quality standards. This concept is discussed in the proposed laboratory study, but nothing is proposed in the preliminary field study to address this. Repeat sampling of selected field sites could provide useful data in this regard.

- Utilizing oxidation reduction potential (ORP) could prove quite useful to reflect the complex chemical interactions in and between surface water and sediment. Some research has indicated that that wild rice stands generally progress naturally so long as sediment ORP is above approximately -150 mV (Painchaud and Archibold 1990, limura et al. 2002).

- *In situ* sediment ORP measurements should be included in all research conducted.

- A key component of selecting the site classes for assessment should be assessing seasonal water level fluctuations. Water level stability is known to be a key aspect to successful wild rice propagation.

- Natural factors such as invasive species, grazing by herbivorous fish, water level fluctuations, and wild rice population cycles should be considered (and measured where feasible) when selecting study sites.

- Realizing the practical limitations, it is suggested that a spring (ice out) field component be added to the preliminary study to assure that critical life stages and potential sediment conditions are captured in the data. Conditions important to wild rice propagation may be overlooked in subsequently-designed studies based only on summer field data.

- In addition to those proposed, the following sediment parameters should be measured *in situ:* ORP, pH, and dissolved oxygen. Additional sediment and/or porewater chemical parameters that should be analyzed are hydrogen sulfide (H_2S), oxidized and reduced iron and sulfate. Although other parameters such as nitrate, oxidized manganese, and carbon dioxide could be analyzed, the focus of this project should be the impact of the sulfate/sulfide ORP couple on wild rice. As such, analyses and subsequent geochemical speciation modeling should focus specifically on this ORP couple.

- With respect to sediment sample handling prior to chemical analyses, mixing and compositing samples is inappropriate for parameters such as pH, ORP, and H_2S. If compositing is conducted for such parameters, it should be conducted in a glove box under nitrogen or argon.

- Water column TOC should be measured in lieu of or in addition to DOC.

- Water hardness should be calculated from the surface water calcium and magnesium data obtained. Hardness (and chloride) has been shown to alter sulfate toxicity to a variety of (non- plant) aquatic taxa.

- Please clarify that all sediment chemical parameters will be assessed on a porewater basis

- Many porewater parameters are sensitive to the sample handling artifacts. It would be helpful to have a list of methods and references for the pore water sampling methods that will be evaluated.

- Does the water chemistry parameter "nitrate/ammonia" indicate that both nitrate and ammonia will be measured? If the analyte of concern is ammonia, the typical reference is ammonia as N or ammonia-N.

<u>Pore water sampling protocols</u>

- Numerous studies have been published relating to the importance of proper pore water sampling method with respect to insuring data integrity. In general, the following factors have been shown to most commonly impact data quality for sulfide and provide data representative of field conditions:
    - — Proper equipment set-up - e.g., allowing proper equilibration, deoxygenating dialysis samplers.

- Proper sampling — e.g., deploying probes and sensors for sufficiently long time periods
- Proper sample handling — e.g., centrifuging samples in a glove bag, and use of sulfide antioxidant buffers or zinc acetate to stabilize sulfide for analysis.
- Proper equipment cleanup
- Two sources that should be consulted as part of developing these methods include:

  USEPA: Sampling for Contaminants in Sediment and Sediment Porewater (http://www.clu-in.org/programs/21m2/sediment/) and an example SOP developed for the Everglades (http://www.evergladesplan.org).

- Given how challenging it can be to accurately measure sulfide, it is important to assure the protocol is consistently and reliably carried out.

Draft Laboratory Study Protocols

- Specific timelines should be proposed for conducting Task A and Task B, and both should be conducted simultaneously given the regulatory time constraints. Focus should be placed on the most important parameters impacting wild rice, and assessment of other parameters discontinued once critical parameters are identified. Flexibility is key to proper allocation of resources and expediting study results.
- An emphasis should also be placed on focusing on key parameters while providing appropriate numbers of replicates in laboratory and mesocosm studies to insure statistical validity.
- Dose-response studies are appropriate if well-designed. Standard best-fit data evaluation techniques from studies with sufficient replication should be utilized, and extrapolation of values below the no observed effects concentration (NOEC) value should be avoided.
- A key finding from review of existing literature should be defining the "most sensitive life stage" that can be monitored in the lab. For example, seed germination is easy to measure in the lab and might prove to be a critical monitoring tool of wild rice productivity and sustainability. Task A should be amended to have defining a sensitive life stage applicable for lab studies as a key data objective. In addition to seed germination, seedling growth (e.g., leaf and stem length, and number of leaves) and biomass (e.g., dry weight), seed productivity (e.g., number of seed heads per plant and number of seeds per seed head, ratio of biomass of seeds to plant vegetative growth), vegetative growth (e.g., leaf, root, or shoot length), and plant part biomass (again, leaf, root, or shoot) should be considered as key response parameters
- As discussed for the field studies, ORP should be included as a key parameter to integrate the interactions between the numerous variables dictating production of sulfide in sediments. ORP conditions could guide subsequent standards derivation.
- *In situ* sediment ORP measurements should be included in all research conducted.
- There is a presumption in the laboratory study protocol that sulfate is toxic to wild rice, and that its toxicity is a function of influencing sediment sulfide concentrations. While it is important to determine the level at which water column sulfate is detrimental to wild rice, sulfate is a relatively innocuous anion, and it is much more likely that if either sulfur-based compound is especially

toxic to wild rice it would be sulfide. The study should more appropriately focus on whether there is a demonstrable relationship between water column sulfate concentrations and sediment sulfide (or other) conditions harmful to wild rice.

- Although there is brief mention of examples of how toxicity could be defined for wild rice, specific endpoints that will be measured for purposes of developing a standard are not defined. Test durations and effects to be measured to define toxicity should also be specified.

- The laboratory protocol presents numerous hypotheses on very specific chemical interactions in sediment and surface water potentially important to wild rice growth and propagation, and describes in general terms some approaches to assess specific hypotheses related to sulfate and sulfide toxicity. Given that specific study designs will be contingent on the results of the preliminary field work, it is understood that little detail can be provided at this time. In the interim, the following should be considered with respect to the laboratory study's general approach:

  – In order to better expend resources, hypotheses related to mercury methylation (Priority C), and many of the Priority B hypotheses, should not be tested.

  – Hypothesis B topics warranting additional consideration include assessing the role of hydrology and water movement in removing organic matter (5D, 7A), and assessing the role of increased nutrient availability increasing competition from other plants (60, 7C).

  – The approach of determining the direct toxic effects of sulfate, cations, and sulfide on wild rice in laboratory tests is a reasonable starting point, and the use of hydroponic methods is a reasonable initial screening approach. However, simple confirmatory studies in test systems containing sediment should be conducted to account for the various complex chemical interactions potentially overlooked in a hydroponic study.

  – The accumulation and removal of wild rice straw as a parameter dictating development of wild rice stands should be considered in study design.

- Tasks B1 and B2, though interesting, are not necessary to define or develop a water quality criterion. Detailed understanding of "cause and effect" is not critical to the goal of re-developing a sulfate water quality standard. The response of wild rice to sulfate is key, not why or how the response occurs.

- "Intensive field studies" as described in Task D should only be carried out after data review following completion of Tasks A and B. Task D should also be carried with some amendment, for example, choose three different site types (e.g. where wild rice grows well, where it does not grow well, where it historically grew and no longer grows) with a few replicate locations.

- Task E should be conducted only after completion of Tasks A and B.

From: Christine Wagener [mailto:Wagener.Christine#$epamail.epa.gov]
Sent: Thursday, June 30, 2011 4:38 PM
To: Engelking, Pat (MPCA)
Cc: Blaha, Gerald (MPCA); David Pfeifer
Subject: Wild Rice Research Protocol

Dear Pat,

We have reviewed the MPCA Wild Rice/Sulfate Protocol. It is clear MPCA has engaged an expert staff in compiling the document.

We have no further comments. Best of luck to the MPCA staff in moving forward on this effort.

Sincerely,

Christine
Christine M. Wagener, PhD
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From: Russell Erickson [mailto:Erickson.Russell#$epamail.epa.gov]
Sent: Thursday, June 30, 2011 3:33 PM
To: Engelking, Pat (MPCA)
Cc: Dale Hoff; Dave Mount
Subject: Re: Draft Study Protocol for Your Review

Here are comments from Dale Hoff, Dave Mount, and myself:

In general, we found this study protocol to be well conceived and presented. A good context for the work is provided and the general nature of the experiments is good. Because just the general nature of the experiments is given, there are many aspects that cannot be commented on and we would urge further review of any detailed study plans.

However, we do have several concerns:

(1) One concern that merits immediate attention is the 2011 survey work described in Appendix B, and the proposed use of sulfide electrodes to provide in situ measurement of sulfide levels in cores. What type of electrode is involved and how will it be used? Typical sulfide electrodes only measure S=, not HS– and H2S, which are the more predominant and toxic forms. For environmental samples, these electrodes are commonly used to measure total sulfide by raising sample pH to convert the other forms to S=, which is not the case here. The

low levels of S= will make good measurements very difficult, and would require accurate pH measurements to compute the other sulfide species. Is something not explained here?

(2) Table 1 and the associated text regarding the hypotheses might be confusing to the uninitiated reader because various terms and concepts are not adequately explained. For example, hypothesis 1A includes several aspects of the conceptual model that are not really clear – the issue of whether sulfate is toxic or not, whether toxicity occurs at the germination, vegetative growth, or seed production stages, and whether sulfide in the sediments reflects sulfate just during the growing season. Better explanations of this would improve the document, perhaps presenting some of this information in the Methods section.

(3) We would like to strongly urge thorough chemical measurements to characterize exposure conditions for all experiments/surveys, even if this means reducing the number of sites, treatments, etc. Too many ecotoxicology studies suffer from ambiguities regarding such details, which create problems when trying to apply results. For example, we were glad to see attention to sediment coring and sulfide levels at all sites in this season's surveys, and monitoring of sulfide levels in the hydroponic studies with sulfide.

(4) For experiments involving manipulation of sediments, we would also like to urge attention to providing sufficient time to stabilize sediment chemistry and to establishing relevance to field conditions.

(5) For Task A, it is suggested that the hydroponic nutrient solution should be low in sulfate and nitrate because these are often low in the sediments. However, this assumes that nutrient uptake, etc. is strictly root-related, which isn't necessarily true. Also, low nitrate in sediment is associated with high ammonia, which can also serve as a nutrient, so total inorganic nitrogen should not be set too low. Further consideration and care regarding this issue is needed.

(6) For Task A, manipulating multiple cations and anions can lead to a large number of treatments and difficulties with varying factors independently of each other. We support the screening study that was mentioned to determine whether further investigation of any of these factors is needed, but, if so, would suggest additional screening tests to narrow down what chemicals would be manipulated in full dose/response studies.

(7) For Task B, a major concern that the protocol recognizes is the stability of sulfide and how sulfide and certain metals interact. Are

there any contingencies if the monitoring shows sulfide to be too transient in these aerobic static systems? Any thought to flow through systems or some other strategy for maintaining sulfide exposures to plant roots? Also, is there confidence that stabilizing metals with EDTA in the presence of sulfide will support metal bioavailability – are the EDTA complexes actually available, or does EDTA simply buffer free metal, which might still be too low in the presence of sulfide?

(8) For Task C, we would reiterate the need to document sediment conditions and to provide the time and the treatments that make them relevant to what was observed in field surveys. Also, the description here suggests that only germination will be examined. Is this true? More endpoints should be included.

(9) There continues to be an absence of explicit discussion on what work will be done to begin linking surface water sulfate concentrations to sediment sulfide concentrations. If the conceptual model in figure 1 holds true that surface water sulfate is a source for sediment sulfides, then at some point hydrologic geochemical modeling, and its respective data needs, should be considered. There are clear qualitative relationships between surface soil/sediment sulfur concentrations and groundwater/wetland sulfate concentrations. Ultimately, a standard may need to be set by using hydrological modeling to calculate what additional load of sulfate (from a point source) is needed before problems with sulfide begin occurring.

Regarding your specific questions, my opinion is that germination rate, biomass production, and seed production would be the most important metrics, with less importance to quantifying actual photosynthesis rate or plant composition (chlorophyll, elemental analysis).

Regarding task priorities, I think task A and B should be pursued quickly, but as simply as possible to just establish important stressors and their general toxic level, because hydroponic exposure is so different from field exposures (especially with regard to differences between root and leaf exposures).. I would also give immediate and high priority to field surveys involving multiple sites and that include good sediment and water characterization -- to both strengthen the empirical relationship of rice to the nature of the sediment and provide guidance for the container experiments. The final high priority would be the container experiments, provided they are well-guided by sediment characteristics found in the surveys and include a range of endpoints. I would give less priority to intensive field work at a single site and to in situ mesocosms - not that these can't be important, but rather the need for and nature of these tasks would depend on the other tasks and should thus come later..

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From: Nathan Johnson [mailto:nwjohnso#$d.umn.edu]
Sent: Thursday, June 30, 2011 10:12 AM
To: Swain, Ed (MPCA)
Cc: Engelking, Pat (MPCA); raxler#$nrri.umn.edu; ljohnson#$d.umn.edu; Brad Dewey; John Pastor
Subject: Re: Draft Study Protocol for Your Review

Ed, Pat, et al.

Attached are my (11th hour) comments on the draft protocol for wild rice. Most of my comments concern sediment geochemistry aspects of the protocol. I also included a few of my general observations (again sediment geochemistry-related) from a recent trip to wild rice stands.

Thanks for including us in this discussion. I think the guidance document you have prepared will be invaluable to the success of future projects and the way you have consolidated and incorporated feedback has been great. Feel free to contact me if you have any questions about my comments.

Sincerely,

--
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Comments on MPCA Draft Study Protocol for the Sulfate Standard to Protect Wild Rice
Nathan Johnson
UMD Civil Engineering
6/30/2011

(1) Regarding Figure 1:

- I think the arrow showing H2S oxidizing to SO4 via nitrate reduction is a critical one. There could be other mechanisms for sulfide oxidation as well, particularly if the redoxcline moves up into the overlying water due to either groundwater intrusion or depletion of oxygen in the overlying water

(possibly at night when attached algae do not respire in sunlight thereby producing oxygen but decay via cannibalism).

- Not sure how to show it, but the influence of pH could be added as well, at least as an arrow with another species, HS-. Many, many things affect pH and the effect of them all cannot be shown simply on an image like this.

(2) Regarding first year survey & porewater geochemistry:

In appendix B, an attempt to measure sulfide in porewaters is described in the text, but is not included in the table summarizing the analyses to be used. I think it would be great to include this at as many sites as possible and have a few ideas about how to do it in a simple way.

*** Critically, if sulfide is to be monitored systematically during the first year survey sampling, pH MUST be also measured.

In terms of survey sampling, the hydrologic setting of when samples are collected is likely to be important and site-specific. In many cases, sulfate concentrations in surface water are greater following wet periods when sulfate has had less time to be reduced and immobilized to sulfide in a longer groundwater flow path. Although this may be difficult to quantify on a broad scale for a survey-type sampling, potentially water level or recent precipitation, or weather could be recorded so as to inform future sampling at the site.

For “point estimation” statistical methods, care should be taken to ensure that results are widely applicable to different types of wild rice or other relevant conditions which may differ in the field from the laboratory.

(3) Regarding specific tasks:

For tasks B1 to B3, the choice of pH for task B1 & B2 might be informed by task B3. It seems to me that pH below 7 are both relevant to natural setting and most likely to have toxic effects. The sulfide bisulfide question seems important enough that it could render tasks B1 and B2 useless if the correct pH is not chosen. Also, the fact that sulfide speciation is controlled by pH is not a hypothesis worth testing, it is well known and well documented.

I think Task B4 could be critical to validating results of point-estimation, regression-type toxicity testing. Empirical models (which do not delve into underlying mechanisms) such as this are predicated on their basis in a large enough and representative enough sample set. Therefore, having a representative set of data (results from various wild rice populations) could be essential to the success of (or defending the validity of) any correlations/regressions developed in widely predicting toxicity accurately.

Regarding Task C, the extent to which degrading plant litter comprises the matrix that wild rice grows in seems to potentially limit the utility of this study. Although I am not a wild rice expert and this should be validated, it is my initial impression that the relevant geochemic1al setting (top 10cm where roots are) is likely to be dominated by the elemental composition of plan material and less so affected by local geochemistry. Also, might task C attempt to work on hypotheses 5B-5C (Mn/Fe inhibition of sulfate reduction)? Not sure where these “microbial population modification” hypotheses should be addressed. Seems like this is the logical task for it to fall under, but would require serious monitoring of sediment chemistry/biology. Could also be addressed with modeling.

Task D – would add continuous monitoring of oxygen in overlying water to assess effects of seasonal/diurnal variations.

Task F – Seems logical to combine this (if it works) with Task D so that adjacent stands could be monitored heavily during the same growing season in the same geochemical environment. As such, a "research team" approach could foster this large-scale coordination. The management implications of the result of this experiment should be evaluated in light of the fact that different rice populations (or stands) may respond differently due to local species or hydrologic characteristics (the "representativeness" ideas discussed previously).

(4) General impressions concerning sediment geochemistry from visits to wild rice stands:

Three main things struck me during recent visits to wild rice plots (both John Pastor's microcosms and wild rice lakes at FDL).

1) The sediment in which wild rice is growing (particularly the location where the roots are, top 10-15cm) is extremely unconsolidated, probably somewhat homogenized, and almost entirely composed of decaying plant material. Therefore, the local mineralogy may have less importance in defining bulk geochemical conditions that I originally thought. Additionally, there is unlikely to be great vertical variability in solid-phase sediment chemistry (a result of longer-term accumulation), although transient porewater trends may develop seasonally, or even potentially diurnally.
2) The presence of attached algae on plant matter in the overlying water has the potential to change the oxygen concentration in the water column diurnaly and seasonally. This should be considered as the redoxcline could move up and down within the sediment or even cross the sed-water interface.
3) I heard stories about sulfide smells emenating from bubbles in wild rice stands. When we were there last week many bubbles were released when the sediment was disturbed, however no smell of sulfide was present. The presence/absence of sulfide gas in bubbles released from sediment provides strong suggestion that seasonal differences in porewater geochemistry exist. Also, the presence of so much gas could make sampling for these species possible, and could suggest that pH is lower as many sediment gasses (CO2, H2S, CH4) are more volatile at lower pH.

From: MN Cultivated Wild Rice Council [mailto:wr#$mnwildrice.org]
Sent: Thursday, June 30, 2011 9:54 AM
To: Engelking, Pat (MPCA)
Cc: 'Sen.Rod Skoe'
Subject: Sulfate Standard Study Protocol

Hi Pat,

Although we discussed our concerns yesterday, I thought I'd follow-up with a brief email.

We don't believe the preliminary protocol timing is appropriate for cultivated wild rice. Since the purpose of the study is to evaluate the water quality sulfate standard it is important to conduct the sampling at the time our growers bring the water into the paddy from the outside source, and additionally important at the time when the wild rice plant is using the water since it is a water dependent crop (May/June). The sampling months in the preliminary study of mid-July through September couldn't be worse since our paddies don't have water at the time. (Since cultivated wild rice is harvested with modified combines, the growers start drawing down their water late June and complete that process by early- to mid-July.)

I would also express our displeasure in the lack of reference to the Grava 1982 study, specifically the paragraph that states, "Concern about possible detrimental effects of sulfate in the water is based on earlier observations that no large stands of wild rice occur in Minnesota where sulfate concentration in surface

water exceeds 10 parts per million. Sulfate at levels commonly found in Minnesota waters does not injure wild rice. Waters in wild rice fields along the Clearwater River range from 22 to 390 parts per million sulfate. Most river and lake waters in other areas contain less than 10 parts per million sulfate. Wild rice has been grown satisfactorily in experiments at sulfate concentrations of up to 250 parts per million."

There is the option to change the scope of the study to specifically indicate you are studying natural lake and river wild rice, not cultivated wild rice (that approach would be similar to the DNR wild rice management program).

Since we do not believe the timing will provide data to accurately reflect the cultivated wild rice growing system, we will not be identifying growers to participate in the preliminary study. If there is a change in the sampling protocol timing to reflect our growing system we would consider participating (I could likely identify 1-2 growers that may participate if the study is modified; each farm has multiple paddies that could be sampled).

I also asked our plant breeder for his input into the protocol and his suggestions include getting samples from paddies that have been either a) not fertilized with any sulfate-containing fertilizers (like ammonium sulfate), *nor* from paddies that might have been flooded with water that has run through other paddies that were fertilized with fertilizers having sulfate, so as not to bias the samples; or b) *have* been fertilized *consistently* with sulfate-containing fertilizers over a period of time to reflect high-sulfate adapted areas.

We appreciate the opportunity to comment on "The Sulfate Standard to Protect Wild Rice: Study Protocol." Please keep us informed as this study progresses.

Beth Nelson, President

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wr#$mnwildrice.org

From: Peter David [mailto:pdavid#$glifwc.org]
Sent: Wednesday, June 29, 2011 11:08 AM
To: Engelking, Pat (MPCA)
Subject: Re: Extension of time to comment on MPCA's Wild Rice Sulfate Study Protocol until noon on June 30

Hello Pat;
I don't have a lot to add to the draft protocol – just a few varied comments that might contribute something:

I do think it's good that the issue of seed source has been raised. Ideally, I think wild-source seed should be used for studies, and enough collected, mixed to some uniformity and stored to allow for a relatively consistent seed mixture to be used for multiple years of experimentation.

While I am fully supportive of the indoor lab and hydroponic studies that would run only through early growth stages as important first steps, I would note that obviously impacts can also come at later growth stages.   For studies that run through the full life cycle of the plant, seed production, size and viability should be part of the plant metrics measured for impact.

That being said, it should also be noted that seed production and viability can also be affected by pollination, disease and other factors that will be difficult to fully control even in tank experiments. For pollination, it may be wise to surround study tanks with additional rice plants that serve simply as pollinators.

It is also worth noting that in nature, germination and early growth are likely influenced by overwintering conditions. For the indoor lab and hydroponic experiments, it may merit "overwintering" the test seed in the appropriate growth mediums to determine possible impacts on the process of breaking dormancy, etc – and not simply putting the seeds in those mediums at germination.

I don't believe I saw a definition of the "wild rice survival and growth metrics" which will be used, but encourage the use of multiple factors, with a particular emphasis on seed metrics for studies which will reach that end point.

It seems like there may be room for more studies regarding the transference/transformation of sulfate in the water column to sulfide in the sediment/pore water – even absent the presence of wild rice. It would be interesting to determine how quickly this occurs, and to what extent/depth, and does the rate vary seasonally (might it be faster in winter under more anoxic conditions?).

Regarding the preliminary studies (if these are not already underway), I would note the difficulties that may be associated with site selection. In particular, it would worthwhile to investigate the apparent causes of decline in beds with little rice in recent years, as there is a good likelihood that the contemporary declines may be attributable to hydrology changes, carp, or other factors unrelated to sediment chemistry. Similarly, sites with apparently suitable habitat, but without rice may in fact be suitable, but simply were never colonized; it may be good to attempt to grow rice in sediment samples from these sites to determine if there is actually some factor inhibiting rice growth.

I saw that the upper 10 cm of sediment was proposed for analysis, but would note that at least some Wisconsin sites, it appears roots extend to greater depths into the sediment. Where rice plants are present, it may be worthwhile to pull several plants to determine typical root length, and base the depth of sediment sampling on what is observed.

Finally, I would note that certain measurements suggested for study in the preliminary field survey – such as water depth, temperature, oxygen, etc – may have little to offer in this study, as any impacts they may have are unlikely to be captured in a single measurement – quite possibly taken at a relatively non-critical time of the year. (As an aside, even something as simple as depth can be a bit tricky to measure in the soft sediments where rice often grows; where rice grows, we use an index to depth, measuring from the surface of the water to the top of the first roots.) In addition, there is likely to be a lot of variability in these measurements, as field sampling, out of necessity, will have to occur over a fairly wide time period.

I would recommend that a photo be taken of a representative plant (uprooted, dated, and with a scale) from each study site to provide some insight into relative plant robustness, root development, etc .

I hope comments were worth reading. I look forward to hearing what other comments you receive.

Cheers - pd

From: Robert Pillsbury [mailto:pillsbur#$uwosh.edu]
Sent: Tuesday, June 28, 2011 1:48 PM
To: Engelking, Pat (MPCA)
Subject: Comments on wild rice Draft Study Protocol

Here are some quick comments concerning the wild rice Draft Study Protocol.

-The Outdoor Container Mesocosm-type experiments concern me a bit since they may probably be looking at longer growth phases of wild rice yet the rice will still be grown in rather artificial conditions. Rice does not often grow in stagnant water and this may provide additional stress to the rice. May consider also growing rice in good in situ conditions as an extra control for whatever container effect that might exist.

-Experiments designed to be examined by regression analysis should be stronger.

-The rice survey may also want to talk into account existing surveys and (when practical) attempt to be comparable with those data sets as well.

-For the wild rice survey, will other macrophytes also be measured? If so, it would be best to estimate densities both within the wild rice beds and adjacent to wild rice beds. Other plants growing within wild rice beds may be responding to the dominance of the wild rice rather than acting as an indicator of water quality. Plants growing outside of the rice beds will not have this problem.
-For experiments on wild rice seed/germations.
-may want to compare germination rates of in situ grown seeds as yet another control to see if growing seeds in the lab has an effect of germination rates.
-wild rice seeds have a staggered germination. Suggest trying to germinate many seeds at once and selecting seeds from that group that have all germinated at the same time. Otherwise you are introducing a lot of variability into your experiments.

-Will you examination of different sediment types include sand? I did not see this specifically mentioned yet I have seen a fair number of rice stands growing in sand (although not typical) - but they be most susceptable to stress.

-During the wild rice survey, how will the sample areas (quadrants?) be determined?
I suggest trying to locate the area with the densest stands of wild rice on each lake and sample there. Visually this is easy to do and it gives you the optimal set of environmental variables for rice in that lake (which is something we would like to know). Sampling a transect across densities will introduce more variation but will also be hard to interpret.

Best of luck!
If you would like me to elaborate on any of these suggestions please let me know.
--Bob Pillsbury

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-----Original Message-----
From: Bill Arnold [mailto:arnol032#$umn.edu]
Sent: Tuesday, June 28, 2011 10:32 AM
To: Engelking, Pat (MPCA); Amy Myrbo; Daniel Engstrom
Subject: Re: Extension of time to comment on MPCA's Wild Rice Sulfate Study Protocol until noon on June 30

Hi Pat,

My major comment in looking through the protocol is that the
description of sulfide chemistry is overly simplistic. If there is any
elemental sulfur present, polysulfides will form. These can be

important components of the sulfur speciation chemistry at pH values above 8 (so much so, that bisulfide concentrations decrease with no corresponding increase in S(2-)). This is important because polysulfides are much more reactive (both as nucleophiles and as reductants) that HS-. Relevant references are

Kamyshny, A.; Goifman, A.; Gun, J.; Rizkov, D.; Lev, O., Equilibrium distribution of polysulfide ions in aqueous solutions at 25 degrees C: A new approach for the study of polysulfides equilibria. Environ. Sci. Technol. 2004, 38, 6633-6644.
and
Shea, D.; Helz, G.R., The solubility of copper in sulfidic waters - sulfide and polysulfide complexes in equilibrium with covellite. Geochim. Cosmochim. Acta 1988, 52, 1815-1825.

We have the capability to measure sulfide and polysulfide in my lab in collected field samples if it is needed/desired (I have talked to Dan and Amy about this). Polysulfides may also need to be considered in the hydrponic/lab experiments.

Best regards,
Bill

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William Arnold, Ph.D., P.E.
Joseph T. and Rose S. Ling Professor
Department of Civil Engineering
University of Minnesota
http://personal.ce.umn.edu/~arnold

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From: John Pastor [mailto:jpastor#$d.umn.edu]
Sent: Monday, June 27, 2011 3:44 PM
To: Engelking, Pat (MPCA)
Cc: raxler#$nrri.umn.edu; ljohnson#$d.umn.edu; Nathan Johnson; Brad Dewey
Subject: Re: Draft Study Protocol for Your Review

Dear Pat, Ed, and colleagues at MnPCA,

Attached please find my comments on your draft protocol. This document was superb and I give a few suggestions based on my experience. I also attach two papers to which I refer in my comments.

Please let me know how else I can help.

Best regards,

John Pastor

[PCA note: The following were attached to the comment:
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Timmer VR, Stone EL (1978) Comparative foliar analysis of young balsam fir fertilized with nitrogen, phosphorus, potassium, and lime. Soil Science Society America Journal. 42:125-130

Unpublished manuscript obtainable from John Pastor: Sims, L., J. Pastor, T. Lee, and B. Dewey. Nitrogen, phosphorus, and light effects on reproduction and fitness of wild rice.

Unpublished manuscript obtainable from John Pastor: Sims, L., J. Pastor, T. Lee, and B. Dewey. Nitrogen, phosphorus and light effects on growth and allocation of biomass and nutrients in wild rice.]

June 27, 2011

Dear Pat, Ed, and colleagues at MnPCA,

Thank you for sending the Draft Study Protocol for a Sulfate Standard to Protect Wild Rice, which grew out of our meeting on May 9. Let me congratulate you on producing a superb document which successfully summarizes and encompasses the very large amount of advice which we all gave you. This is really superb.

Here are some comments on your two queries, based on my experiences in doing experiments with wild rice. I also attach two papers which I refer to below which may also be of some help.

1. What are your recommendations on which metrics would be best to include in the protocol from the list of response metrics on p. 14?

   The metrics are listed on p. 15 (not p. 14 in my copy): seed germination rate; photosynthetic rate; leaf elongation rate; chlorophyll fluorescence; live and dead biomass; biomass of leaves, shoot, and roots; biomass per square meter; seeds per plant; mean seed weight; elemental concentrations in leaves and/or seeds (C, N, P, S, Fe, Zn, Cu, Mo).

   Of these, the highest priority should be the end-of-season biomass measurements. These integrate all other processes such as photosynthesis and leaf elongation. We harvest 8 plants per year from our mesocosm (stock tank) containers, including seeds and roots, and then calculate biomass per m2 by multiplying the weight of the roots, shoots, and seeds by plant density. We have measured photosynthesis, but this is tricky and time consuming with a LiCor because it can be done only on either a blue sky or over cast day (not partly cloudy when light conditions are changing) and only between 9AM and about 1 at the very latest. We did not find it added much beyond end-of-season growth. Similarly for chlorophyll florescence. Leaf elongation is not very useful – the leaves elongate very rapidly (within a matter of days). If you want to measure something over the growing season, I would recommend height growth – this gives a nice growth curve. When measuring mean seed weight, it is important to distinguish between filled seeds, unfilled seeds, and seed which were filled by eaten by riceworm (as evidenced by being empty and with a small exit hole in the side). The biomass measurements are tedious but worth more than the other measurements and will be the most easy to explain to the public. The elemental concentrations could be determined by the State Plant and Soils Analysis Lab for about $10-12 per sample. If you couple this with plant weight, there is a nice procedure for determining whether a nutrient is limiting, taken up in luxury amounts or toxic (Timmer and Stone 1978). We have used this technique to determine if N or P is limiting to plants (Sims et al. submitted a). Both papers are attached with these comments.

2. If we have to prioritize among Tasks A through E (or F), which are most critical/or the most critical variations to do in the next two years?

   I think it is absolutely critical that we get data in two years which can be collected without any technical difficulties, which show what the patterns are in wild rice growth and water chemistry in the field, and which begin to determine the mechanisms by which wild rice interacts with sulfate. Unless we have some clean data from measurements that can be defended to the public, this effort may go nowhere.

   I would therefore recommend coupling the field surveys, and especially expanding the 2011 Preliminary Field Survey (Task E) with container mesocosm experiments (Task C). The public will want to know what is happening in the field, so Task E is very important. It can also help with the design of any experiment by helping us set ranges on sulfate concentrations in waters, organic matter concentrations in sediments, etc. I highly recommend resampling at least some of John Moyle's lakes to make a connection with that work, since Moyle's data is what the current regulations are based on. Some of these lakes can be selected for more intensive field study (Task D).

   But the field survey will not be able to easily determine cause and effect. Of all the other tasks, the safest one would be the container experiments such as we have been using. I notice that of all the methods, you list 8 hypotheses that can be tested using container experiments, which is more than any other method. It would seem that these return much data for the money. We have been using these very successfully (Walker et al. 2010) to test other hypotheses about wild rice growth. The population densities, growth and biomass, seed yield, and bulk sediment characteristics in our container mesocosms are very similar to wild rice lakes. Of course, the sediment must be transported to the containers and during the first year any stratigraphy or profiles will be lost, but given that the sediments are so loose and flocculent, I doubt there is much fine stratigraphy in the lakes anyhow.

   Containers can also be used to test and perfect other methods such as pore water samplers, etc which can then be deployed in the lakes. In fact, Nate Johnson will be doing that this year in our container experiments. We have also installed an additional thirty containers this spring identical to those in Walker et al. (2010) for experiments on the effects of sulfate additions on wild rice growth. Sediment from a wild rice lake was added and seeds were planted to yield 30 plants per container. There will be six replicates each with sulfate concentrations of 0, 50, 100, 150, and 300 mg/l. We can use the data from these treatments, especially the standard deviations, to help design the proposed container experiment, for example by calculating sample sizes required to detect significant differences of XX g/m^2 in productivity, etc.

   In situ mesocosms would be risky because of the reasons listed on p. 20 but also because of the logistic difficulties in getting these mesocosms in place. How will they be deployed – from a boat? From a canoe (not likely)? How will they be anchored in the sediment? Etc. However, it may be worthwhile doing a pilot experiment the first two years to work out some of the bugs for this approach.

   The greenhouse experiments, including the hydroponic experiments, would be some help, but probably not as useful as the field surveys and the container experiments. Although I've done them, I always worry about the applicability of greenhouse experiments to field conditions. The containers at least represent some aspect of field conditions (populations of plants, outdoor conditions) but also allowing some aspect of control of variables (water levels, sediment chemistry, etc) to increase ability to detect effects of sulfate. We have also occasionally had trouble with

seedling mortality in the greenhouse because of some sort of damping off, but Raymond Porter may have better advice about this.

I would also encourage you to explore putting together research teams to address specific questions or approaches. There could be one team for lake sampling and another for container experiments, for example. It is imperative to coordinate all measurements and treatment protocols. This coordination would be much easier in teams rather than in individual proposals which would come in response to a general RFP. This is the approach that LTERs, BOREAS, and other large campaign style research projects have taken. It would in the end probably also be cheaper because it would reduce duplicative installations, etc.

I hope these comments are of some help to you. Please let me know if there is anything else I can help with, and please let me know what you decide.

Best regards,

John Pastor
Professor

From: Bethel [mailto:bander#$northlc.com]
Sent: Monday, June 27, 2011 2:35 PM
To: Engelking, Pat (MPCA)
Subject: Wild rice research protocol

Pat, thank you for the opportunity to involved in this important process. Please find attached, my comments on the wild rice research protocol.
Thanks again. Len Anderson

Text from attached Word document:

Review of MPCA'S draft *Sulfate Standard to Protect Wild Rice: Study Protocol*

I would like to commend the MPCA for the work that has already gone into understanding the relationship between sulfate and wild rice. I recognize good scholarship in identifying the significant holes in our present understanding of the topic.

I have some feedback first on "which would be best to include in the protocol from the list of response metrics on p. 15". Of course it would be best to measure all of these metrics, but if that is not possible, I think the following are most appropriate.

1. Seed germination rate
2. Leaf elongation rate. This can be done in the field along with porewater analysis.
3. Biomass per square meter. This can be done in the field along with porewater analysis.

Next, I would like to recommend the following order to prioritize tasks A through F.

First; Letter D Intensive field study of natural wild rice stands. This would follow up immediately on the Preliminary Field Study and then guide which tasks and response metrics should be started the next year. With limited time and resources, the most efficient way to understand "the chemical nature of porewater that wild rice roots encounter in sediments" is to measure those parameters in the field.

Second; Letter C. Effects of sulfate across differing sediment types. However, I would recommend *in situ* mesocosms. I see no problem adding minute quantities of sulfate to small natural systems. To put it in perspective, at the Forbes taconite plant on the St Louis River, they add 14,260 Kg/day of sulfate and that is allowed year after year.

Third; Letter A Effect of sulfate and cations on wild rice.

Fourth; Letter B Effect of sulfide on wild rice.

1. I would object to using EDTA *in situ.*

4. The effect of seed source on vulnerability of wild rice. However, only if intensive field studies of natural wild rice stands is done first, would you have a clue about which genetic variants are worth further investigation.

Fifth; Letter F Effect of elevated sulfate utilizing *in situ* mesocosms

Sixth; Letter E Additional field survey. Probably using different monies, this should go on for a decade, because of the normal oscillations in wild rice populations.

In general, you will notice that I rate tasks that are various forms of "bench" work lower than tasks involving field work. It is always wise to remember the Bois Forte admonition in "Sulfate and Sulfide Residuals in Water and Sediment, Sandy River and Pike River, Fall-Winter 2000", where they said, "Laboratory evaluations carried out recently (Lee,1999) suggest that wild rice plants can withstand substantially high levels of dissolved sulfate (thousands of ppm) without detrimental impact to plant growth. While these laboratory results assuredly are reported with a substantial degree of confidence, great care must nonetheless be used in interpreting these results since such studies are again inherently removed from the natural interactions that occur under field conditions. These thus cannot be construed to be reflective of optimum wild rice plant nutrient requirements in the water system." Remember, if you study many stands of different genotypes, they will be reflecting the impacts of all the complex sediment biochemistry that has impacted that stand over the last several decades. That cannot be done with "bench work".

In addition, I would like to evaluate your proposed hypotheses A, B, and C. I would recommend elevating Priority "C" hypotheses so they are used to help determine which of the A and B hypotheses are actually addressed. Then use information from Priority "C" to inform the design of the research so that some of the work will be helpful for understanding sulfate impacts on both wild rice and mercury methylation.

We are so fortunate in having two renowned and sophisticated researchers working for the state of Minnesota for decades on sulfate and methyl mercury and impacts on wild rice. I am of course referring to Mike Berndt of the MDNR and Ed Swain of the MPCA. They should be charged with the responsibility of fine tuning each research plan so that, where possible, it will shed light on sulfate impacts on both wild rice and methylation. These are tax dollars being spent and it is imperative that we get the most "bang for our buck".

Finally, since these are our tax dollars being spent, I would recommend, for the sake of transparency, that you revisit the paragraph titled "Tasks for testing hypotheses". Instead of saying "no schedule is presented", I think it would be good public policy to ask for an interim report. Just as the Preliminary Field Study report is required by January 31 of 2012, I think it reasonable to have preliminary reports on all of the funded tasks to be filed by January 31 of 2013.

Respectfully submitted. Len Anderson

From: Amy Myrbo [mailto:amyrbo#$umn.edu]
Sent: Sunday, June 26, 2011 9:46 PM
To: Engelking, Pat (MPCA)
Subject: Re: Draft Study Protocol for Your Review

Hi Pat -

The below comments are a bit of a mish-mash:

The last two paragraphs on p 5 seem contradictory. The point that iron can act to either + or - sulfide

toxicity is elaborated and clarified later in the document, but at this point the distinction is unclear.

p 6, paragraph beginning "It is conceivable . . .", missing a word, e.g., "grow" after "aquatic plants."

Next paragraph, "or exacerbated" might be added after "mitigated," unless it is known that other biogeochemical reactions would only have moderating effects.

p 10, is it possible (or necessary?) to tell the difference between hypotheses 4B and 5A? I.e., to distinguish, in a nitrate-rich system, between non-production of sulfide and reoxidation of sulfide to sulfate? In the case of 4B being important, seems like sulfide presence prior to reoxidation might have an effect on the system.

p 19, Last item in B3, reword to ". . . that sulfide speciation as controlled by pH is an important factor."

p 19, Criteria for choice of sites for intensive field study? Accessibility (near/in a field station such as Itasca)? Natural-ness? Existing monitoring and/or records? Extreme vs middle-ground values of sulfate, wild rice abundance?

Fig. 3D is either missing the 50 ppm contour between 10 and 150 ppm contours, or 50 ppm is mislabeled in the legend.

As for your specific questions:

1. I'm not qualified to address this question.

2. B and its variantsmay be the most critical, although as discussed on May 9, A needs to be tested if it is to be dispatched with - as many of those present seem to think it should be (and it should be comparatively easy to test). C is the one that I believe may provide the most definitive answers, but the number of permutations (and thus containers) is high. D should be highly informative as well. E is somewhat unpredictable without knowing the results of the preliminary field survey, but I would guess that 50 sites is too few to fully characterize the diverse water bodies across Minnesota. I am not sure that in situ mesocosms would provide information that could not be gained from containers and intensive field sampling, and would be complicated (providing water flow, avoiding bird perching, etc.) It's a difficult decision, but if I had to rank them in terms of how I think that they will inform the study, I would rank them CBDEAF.

Thanks very much,
Amy
--

Amy Myrbo, Ph.D.
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Pat & Ed

Here are my comments - inserted throughout the document as text edits and "balloon" comments.

I think you did a superb job in getting a quality draft experimental design done for an extremely difficult scientific issue. I also appreciate the a-political way you folks assembled a panel to help do this.

I'm cc-ing my comments to Nate Johnson, John Pastor, and Lucinda Johnson because though we are all up in Duluth in theory, we've not been able to sit down together to brainstorm a review together.

Cheers....Rich
--
"It all comes down to your water"

Richard Axler, PhD raxler#$nrri.umn.edu
Senior Research Associate - Limnology/Water Quality
Natural Resources Research Institute, U. of Minnesota-Duluth
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(218)720-4316 /4279 (voice); 720-4328 (FAX)

[PCA note: Comments were pasted into pdf of the Protocol, and are pasted below]:

page 14
My thought was that seed germination versus sediment "type" could be tested in small containers in growth chambers, a greenhouse or perhaps in outdoor stock tanks a la John Pastor's set up. Since only small volumes are needed for germination, the tanks could be used essentially as outdoor growth chambers. I like the idea of doing experiments in natural light and ambient temperatures although you run the risk of unusual weather screwing it all up. Or birds, or the fact that water depth and color are not the same as in a natural wetland. So it might make more sense to do the germination vs sediment type experiments sequentially with the hydroponic experiments in under the same "artificial" conditions - in a plant growth chamber with controlled lighting or in a greenhouse where temperature is controlled via water baths but light is closer to ambient.

P 17:

I suggest adding:
1. some measurements of DO as confirmation - silly though this might seem

2. It would also be useful to include an EDTA treatment to (hopefully) show that it has no effect (see Part B)

p. 18
I think it should be mandatory to include at least "some" sulfide treatment(s) in Part A. These would be also included in Part B, although expanded to include a wider range of sulfide values perhaps, for confirmation/replication

I think that this would depend on the metals concentrations in the growth medium. How would you knowis realistic? I thought you needed rather high available metal concentrations (Fe and Mn presumably) to tie up high sulfide concentrations.

p. 19

2 or at max 3 pH's
Good idea! Perhaps even 3 seed sources to allow for a gradient of some sort. I don't think you can do toooo many of these simple experiments and they will be relatively inexpensive, at least for germination.
C. ..."Container Mesocosms"

1. Since these are just germination experiments versus sediment "type" - I would think they could be carried out in small containers (i.e. pint size perhaps) - I would call these microcosms, not mesocosms.

2. Perhaps set this up first cut as 3-4 sediment types based on organic matter (%AFDW) and save an aliquot dry material for potential TOC analysis (and perhaps %N and %P at some later time if funding can be secured)

3. I cannot see how nitrate can be mentioned for sampling or as a treatment a treatment w/o also including ammonium; and P will need at least 2 fractions - ortho-P and dissolved-P.

4. Presumably these are porewater metal measurements. Good to characterize them, but Fe and Mn will need to be carefully sampled and "fixed" to avoid redox effects on soluble fractions in porewater

5. Not sure is this set of experiments is where there is a need for microelectrode measurements although it could be informative and perhaps easier than in mesocsoms or in the field for sure.

6. These experiments could be set up in a growth chamber or greenhouse for convenience OR they could be set up in subdivided stock tanks (i.e. Pastor mesocosms) in the field to provide better realism in terms of ambient temperature conditions and light (to some extent). The tanks would simply be used as support structures and either smaller mesocosms are just set inside them or each tank could be filled with a single sediment type and then partitioned into 20 or 30 compartments that might be amended. The down side is that birds or severe weather could trash the experiment unexpectedly.
D. Since the germination in sediment expts can be done (perhaps) in smaller containers, I envisioned Part D. to be the next size up - i.e. the John Pastor stock tank mesocosms where a smaller set of experiments, informed by Part C results, would allow for germination (do you get the same result as in the microcosms?) and then a suite of maturing plant metrics such as annual vegetative production, seed production, physiological condition. yada yada yada. If John still has sediment from his grad student Lee Sim's work in the mesocosm's,as well as active systems, these could be incorporated into the experimental design by doing additional sampling in them.

Note- at least some thought needs to into wherever sediment nutrients in less organic sediment treatments will be sufficient to support plant growth for a full seasonal cycle without enrichment from the start. A really messy thought if true.

Something like 3-4 sediment %OM x 6 SO4 concentrations (10, 25 50, 100, 250 ppm) = 18-24 cosms x 2 or 3 reps. Or partition in half each cosm to add some level of replication.

p. 20

E. I forget how many sites Moyle sampled but it seems important to me to try and re-sample all the ones that still exist. For this project as well assessing wetland condition changes in MN from a broader perspective.

From: lucindabjohnson1#$gmail.com [mailto:lucindabjohnson1#$gmail.com] **On Behalf Of** Lucinda Johnson
Sent: Wednesday, July 20, 2011 2:12 PM
To: Engelking, Pat (MPCA); Swain, Ed (MPCA)
Cc: raxler#$nrri.umn.edu
Subject: Re: Extension of time to comment on MPCA's Wild Rice Sulfate Study Protocol until noon on June 30

Dear Ed and Pat;

I'm guessing you are just now thinking about getting back to work after the shut-down. I hope it was not too stressful for you.

I just found my marked up copy of the report after letting it get buried on my desk. I hope it's not too late to accept comments.

I echo Rich's comment about the quality of the document. It is superbly written, and well-thought out. Kudos to the writing team.

I won't rehash Rich's comments, but may reiterate some that I think are particularly important. See attached doc.

Regards,

Lucinda Johnson

Lucinda B. Johnson
Center Director
Natural Resources Research Institute
University of Minnesota
Duluth, MN 55811
218 720-4251

p. 5

need to clarify what it means to poison iron-containing compounds.

p. 6
is there a word missing... "in which aquatic plants grow":

organicrich
sediment in which aquatic plants can be directly toxic to a plant rooted in the
sediment, a mechanism described by Koch et al. (1990). The increased loading of sulfate
has been hypothesized to alter the aquatic plant species composition of wetlands (e.g.

Smolders et al. 2003, Li et al. 2009).

Table 1 (page 9):
5E. Water movement prevents oxygen depletion in sediments, limiting sulfide production\

Seems to be there is a tight linkage between O2, temp, (+/-flow), and OM that ends up controlling sulfide production. How can you test one without the other?

p. 15: response metrics:

this is a great list. is it all practical and feasible?

What about listing the environmental covariates as well... water column physical - chemical; OM (composition, AFDW?); sediment: texture, pH, nutrients, etc.; aquatic macrophytes; aquatic invertebrates; flow regime; water depth; water clarity;

Also- I wonder about ecosystem-level responses- food web? metabolism?

P 17:

What does this mean: It is anticipated that any *in situ* mesocosm experiments
would be installed in locations where the sediment and surface [water??] had been characterized

prior to installation.

P 18:

I suggest that these intensive studies be conducted at a range of sites (5-6) that represent the climatic and ecoregional variation across which WR occurs.

Use these sites to understand some of the seasonal responses as well as the growing season responses.

Also need to understand the prevailing cycle for the wild rice at those sites so that you capture the WR endpoints for populations that are in a similar point in the multi-year cycle. The tribes are probably the only ones that have the appropriate data to show where the site is in the cycle.

It is at these sites that some of the ecosystem-level analyses could be included. Depending on the locations, you might be able to fund some student work on those topics.

From: Lawrence Baker [mailto:baker127#$umn.edu]
Sent: Friday, June 24, 2011 12:46 PM
To: Engelking, Pat (MPCA)
Cc: Tomasek, Mark (MPCA)
Subject: Comments from MCEA prepared by Larry Baker

Pat:

Attached are my comments, on behalf of MCEA, regarding the Sulfate Standard Protocol.

If you have any questions, please do not hesitate to call.

--

Lawrence A. Baker, Ph.D.
Research Professor
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Comments of the Minnesota Center for Environmental Advocacy on "Sulfate Standard to Protect Wild Rice: Study Protocol, June 6, 2011."

Prepared and submitted by Lawrence A. Baker, Research Professor, Department of Bioproducts and Biosystems Engineering, University of Minnesota.

Comment on the biogeochemistry of sulfate.
In weighing the merits of various types of experiments, from hydroponic bioassays to in-situ mesocosms, it is important to consider the likelihood that sulfate is not directly toxic (at least, not at levels found in most MN waters), but exerts its effect on wild rice ecosystems via biogeochemical transformations of sulfate. These reactions convert sulfate to sulfide, which is directly toxic to plant roots; this conversion also alters the chemistry of sediments and the overlying water, especially for reactions involving iron (Fe) and phosphorus (P).

Because sulfate undergoes important reactions in sediments, the rate of these reactions (especially sulfate reduction) is important. Because of this, the design of mesocosm experiments should vary two parameters that influence sulfate reduction rates: (1) the supply of sulfate; and (2) the supply of organic matter (OM). The reason for the latter is that sulfate reduction, a bacterial reaction, requires a supply of OM (bacterial food). In wild rice beds that do not receive large amounts of organic matter in water (as BOD), decaying wild rice itself is probably the dominant supply of organic matter to sediments.

To illustrate this interaction, consider experiments that we conducted to determine factors limiting denitrification rates in wetlands (Ingersoll and Baker, 1998). This experiment is relevant because it also involved a reduction reaction involving an electron acceptor (nitrate), hence the findings are relevant to understanding rate limitations for sulfate reduction and can be used to guide experimental designs to develop a wild rice sulfate standard.

In our sediment-water microcosms, we added OM in the form of dried cattails, which we "fed" to the microcosms once a week, at three different rates, thereby varying the OM supply rate. To vary the nitrate supply, we supplied nitrate-enriched (10 mg/L) water to the microcosms at three different flow rates. We then measured nitrate in the inflow and outflow of the microcosms for several months and calculated % removal rates. Our experiment showed that the rate of denitrification was controlled by the ratio of organic matter supply (the chopped-up cattails, in g C/day) and the nitrate supply (as g N/day). When the C: N ratio

was low, the percentage loss of nitrate was low (see Figure 1). As the C:N ratio increased, the percentage loss of nitrate increased, reaching about 90% loss at a C:N ratio of 5:1.

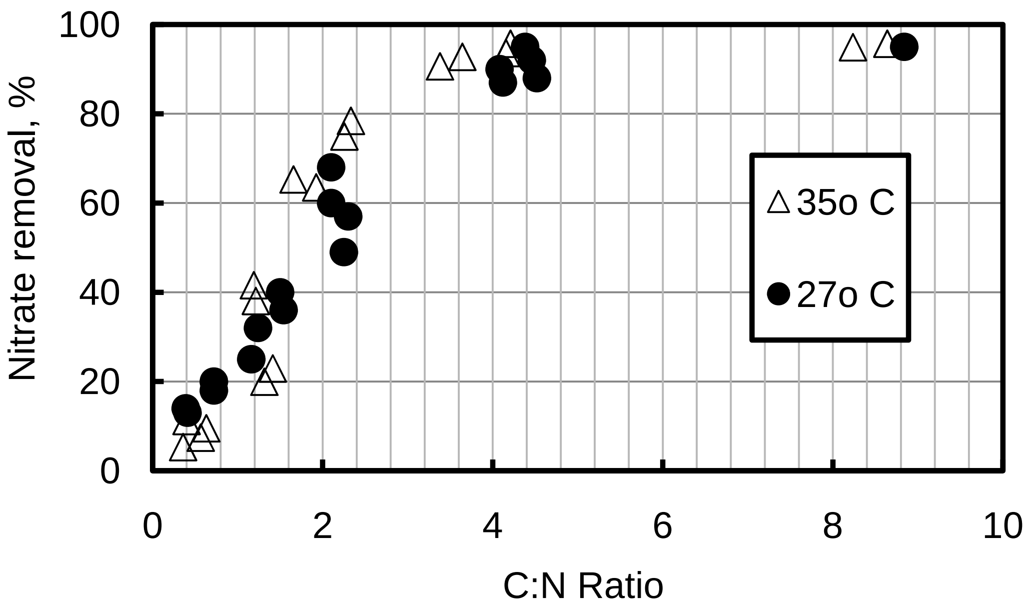

Figure 1. Relationship between C:N ratio (wt:wt) and percentage of nitrate removed in laboratory microcosms. From Ingersoll, T., and L. Baker (1998) Nitrate removal in wetland microcosms. Water Research 32: 766-684.

What this says is that denitrification is limited by OM supply, until a ratio of 5 parts of C to 1 part N (wt:wt) is achieved. Because the denitrification and sulfate reduction are biogeochemically similar, one would expect the same type of interaction between sulfate supply and OM supply, though the saturation point (C:S ratio) may be different (e.g., not necessarily 5:1).

In a wild rice bed, sulfate supply to the sediment surface would depend on both sulfate supply and OM supply. In a mesocosm experiment, sulfate supply could be controlled by adding small aliquots of a concentrated sulfate solution, or by adjusting sulfate supply by flow rate control (as we did in our microcosm experiment). The supply of OM to sediment surfaces would almost certainly be controlled by the deposition of wild rice stems and leaves to the sediment surface, "feeding" the sulfate reducers.

Hence, I suggest that mesocosm experiments include the effects of sulfate supply and OM supply. The supply of OM to the sediment surface could be measured in small quadrants within larger mesocosms.

What I would predict is that the impact of sulfate (as expressed through sulfide buildup in porewater) would increase in relation to the C:S ratio, up to a point. Thus, a given supply of sulfate may have little effect if the OM supply is low (e.g., in a sparse wild rice bed), but greater when the OM supply is high (in a dense wild rice bed). For a wild rice bed of given density, the production of sulfide would depend on the sulfate supply, which would be a function of both sulfate concentration and flow. For a given sulfate concentration, I would predict greater sulfide toxicity with increasing flow rate, up to the point that the optimum C:S ratio is achieved.

Beyond the effect of sulfide on roots (direct toxicity), sulfide also controls the cycling of iron (Fe) and, in some cases, phosphate (PO4)(see especially Lamers et al., 2002). In some cases, addition of sulfate results in higher porewater sulfide, which binds readily with Fe. In situations where sediment PO4 is bound to Fe, the reaction of Fe with S may release PO4 (see Figure 4, "TH" wetland).

In large wild rice beds, the release of PO4 may trigger eutrophication, resulting in more algae growth, which could possibly shade young wild rice shoots, reducing their growth. I do not know of a demonstration of this hypothesis, but the idea is well developed in the context of "flipping" of shallow lakes, a situation in which alternating dominance by macrophyes and planktonic algae shifts over the period of years.

In summary, biogeochemical theory and many empirical studies predicts that the impact of sulfate on wild rice beds will be complex, involving interactions of sulfate, OM, Fe, and P.

Comments on Appendix B (preliminary study for 2011):

Based on previous studies (e.g, van der Welle 2007; Lamar et al. 2002 and what we generally know about sulfide toxicity), it is very likely that the effect of sulfate on wild rice will occur via the interaction between H2S, pH, and Fe2+ in the porewater environment. Although measurement of porewater sulfide is proposed, there are no proposed analyses of in-situ pH or iron (any species). To get an idea of sulfide toxicity to roots (the most likely mechanism for plant toxicity), it will be very important to measure total sulfide, in situ pH, and Fe. The first two measurements will allow calculation of sulfide species (H_2S, HS^-, and S^{2-}). Porewater iron (presumably $Fe^{2+)}$ will allow an assessment of whether iron is controlling porewater sulfide.

Curiously, there is no mention of any metric of wild rice condition other than "visual" (Table 2). Certainly there will have to be some measurement – stand density, nutrient content of leaves, condition of plants, etc. - in order to make any sense of the chemical data. Flow should also be measured, even if it is just a "tennis ball" measurement, for reasons discussed above.

Also, the interpretation would benefit from a careful description of each wild rice site. For example, what is the increased source of sulfate to the wild rice beds identified as having "sites receiving elevated sulfate"? Can the increase in sulfate be quantified? Is the source sewage, mine discharge, or something else? How well can this be documented? Because the impact of sulfate depends upon many factors, it is important to understand these factors as well.

Comments on section A. Effects of sulfate and cations on wild rice.

This section is vague, calling for varying both cations and sulfate, and implicitly (because all cations are associated with anions), a range of anions (e.g., Cl). This could result in far too many experimental treatments to be practical.

Instead, I suggest using only a few media: the well-standardized Hoagland's solution, and one or two "typical" compositions of wild rice waters, developed using data collected from the field study in 2011. Sulfate could then be varying using Na_2SO_4, perhaps using NaCl as a "sodium" control. No range is indicated, but I suggest going to very high concentrations initially, perhaps even 1000 mg SO4/L, to be certain that an effect level is reached (bearing in mind that the evidence for direct sulfate toxicity in wild rice is weak).

The details of this experiment are quite limited – would the plants be started from seed, how would they be transplanted and grown in a hydroponic environment, etc.?

Comment on section B. Effect of sulfide on wild rice.

In this experiment, the wild rice would have to be grown in a two-phase environment, with roots in a liquid media and the stems in air. This is often done by putting the plants through a rubber stopper, which is then placed into a flask (see Li et al and others). Anaerobic conditions would have to be maintained, for example, by purging with N_2. These steps are important because sulfide is easily oxidized and/or volatilized. Li et al. actually changed media frequently to maintain sulfide levels.

Sulfide toxicity will depend on pH (B3). I think B2 is unrealistic – the role of Fe and other metals would be more realistically evaluated in mesocosm experiments, which represent more natural conditions.

Comment on section C. Mesocosm studies.
Because of the complexity of potential sulfate effects on iron and nutrient chemistry, mesocosm experiments will likely yield the most useful results.

One particular advantage of using "container" mesocosms is that the University of Minnesota – Duluth has been conducting these types of experiments for several years. This means that they have an established facility (with power, a site with controlled access, and presumably a nearby lab), plus experienced investigators. Given the complexity of mesocosm experiments, this is a huge advantage.

I suggest starting these immediately, in conjunction with hydroponics experiments, not after, because they will almost certainly be needed anyway.

It would be difficult to vary the "Fe, sulfur, and organic matter" of sediment directly, except for perhaps OM. Instead, I suggest using one or two key sediment types that normally support wild rice. Then, vary OM supply using wild rice straw; sulfate either by adding small aliquots of a high sulfate solution (to keep sulfate at a constant concentration), or by varying flow and sulfate concentration simultaneously (for example, 10 mg/L SO4 fed at 10 cm/day and 20 cm/day). As discussed above, I would expect sulfide formation, and therefore root toxicity, to depend on both sulfate supply and OM supply.

The mesocosm study should include extensive measurements of porewater, with a focus on the S-Fe-pH linkage, and should extend for at least an entire season.

Finally, it would be important to measure sulfate, total and dissolved, P, nitrate, etc. in the overlying water.

D. Intensive field study
While I can certainly appreciate the concern over in-situ mesocosm experiments, this is a well tested methodology for understanding sediment-water interactions in the context of lake acidification, macrophyte growth, and eutrophication. The main advantage of in-situ mesocosms is that the use of undisturbed sediments and "natural" wild rice beds, making the results more credible, verifying results from container mesocosms. If the effects of sulfate on wild rice growth are profound (e.g, major die-off vs. healthy growth), the results can be visually striking, which can be useful for communicating results to the public.

From: David Zentner [mailto:dzentner#$charter.net]
Sent: Thursday, June 23, 2011 10:33 AM
To: Engelking, Pat (MPCA)
Cc: Bethel; alphaflipr#$gmail.com; 'Curt Leitz'; Bob Tammen; Martha Minchak; Gary Meier; 'Bob Hedburg'; rph; Scott & Phyllis Mead
Subject: wild rice / sulfate standard

Mr. Engelking;

I am extremely interested in the work you are doing regards the protocols approaching the wild rice / sulfate standards. Many of us do appreciate your diligence, willingness to work with those among us who have intense interest in protecting and in restoring wild rice in Minnesota.

I am not going to attempt to speak to the issues around the science of wild rice stand survival and prosperity. That does appear to me to be well covered by others well qualified to provide guidance for this process. I will, however, briefly share some thoughts about what I consider to be "background realities". I believe that while getting the science right is fundamental to creating a standard that is adequate to protect and restore wild rice; recognition that people and budgets and politics are a very important element that needs integration as the protocol is finalized and the study commence' is of more than passing importance!

1. I question whether using what are called narratives is wise. I like others feel that a very strong numerical standard meets the need to provide strong protections, and, allow regulatory administration that can be relied upon to be consistently applied. There may be cases where rigid application is unwarranted as we've all witnessed in our lives at times. As I understand the narrative process, it seem logical to envision an endless process that MPCA etc. is unlikely to be equal to tasking effectively. Observe the lack of enforcement of existing regulations. Wild rice is an example. Allowing a major taconite company to operate a tailings basin without a permit another. Water withdrawal from a trout stream on the Minnesota North Shore significantly in excess of the terms of the permit without any "direct" enforcement of permit terms is another.
2. Politics. Our legislative and executive branches have given ample indication over time that they will be open to the influence of very strong special interest to the extent of interfering with the best interest' of long term resource stewardship. The existence of a science based strong numerical standard may be the best defense that could prevent micro management and legislative end runs.
3. Budgets. We've witnessed thirty plus years of budget decline for many natural resource agencies even before adjusting for inflation. How then with fewer personnel can one realistically believe that a standard fashioned around a complex set of interface between the permit holder and the regulatory process be done in a timely, adequate fashion, and, all the while achieve the goal of protecting and, restoring our wild rice?
4. In the decades of advocating as a volunteer on behalf of our natural resources, over and over again, business and industry has pleaded for a level playing field, one that is based on sound science, one that is applied in common sense fashion of course, and also applied evenly.
5. Over and over back in the late 60's and the 70's I heard threats that if the CWA passed, if this or that legislation was enacted , business would suffer, jobs would be lost. Claims were made that the economy over-all would decline. Little of that happened. In fact, the economy in the decades the followed PL92-500 etc. would be described today as very robust compared to today's enormously challenging economic environment. I would add that the "today" we face has been accompanied by a steady reduction in public agency budgets, and in the regulatory approach state and federal agencies have been permitted to apply over broad subjects (environment-anti- trust-financial industry etc.)

6. Obviously I would greatly resist a numerical standard that fails the litmus test of good science, and the recognition of the variables in which our major stands of wild rice are found. But, I do have to trust the science you are about to embark upon, and, hope that politics allows it to work.

In closing I must admit that I do realize that your job must focus on construction of appropriate protocols, seeing the good science is done, and bringing in the best recommendation possible. Your primary focus is not based on current events in the commons. Having said that, it would be very un-wise to complete this project without integrating operational effectiveness and current events into the equation. History is very relevant. Many natural resource decisions are not science based unfortunately.

I was a private business person for over four decades. I understand the values of enterprise, good business environments, good relationships between and among private sector and public. I have chafed at overly redundant regulations that result in little if any value to citizens, yet reduce productivity and auger up cost'. I know the story, have been there, experienced it. Positive regulatory reform for all is just that, very desirable. Yet, I do not believe that is what we are experiencing today. We are much, much nearer to "throwing-out-the baby-with-the bath-water!

Thank you.

Dave Zentner
Past National President
Izaak Walton League of America
2116 Columbus Ave
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June 23, 2011
Pat Engelking (pat.engelking#$state.mn.us)
Water Assessment and Environmental Information
Minnesota Pollution Control Agency
520 Lafayette Road North
St. Paul, MN 55155-4194
RE: Review of Wild Rice/Sulfate Study Protocol

Dear Ms. Engelking:
I represent WaterLegacy a Minnesota non-profit organization formed to protect Minnesota's water resources and the communities that rely on them. Thank you for the opportunity to comment on the June 6, 2011 Draft Study Protocol for Minnesota's Sulfate Standard to Protect Wild Rice ("Wild Rice/Sulfate Protocol").
First, WaterLegacy appreciates the Minnesota Pollution Control Agency ("MPCA") commitment to new research and to rigorous collection and assessment of existing data to ensure that Minnesota's water quality standards protect natural stands of wild rice. Our feedback regarding priorities is summarized below:

1. WaterLegacy would reiterate our view that it is a high priority to conduct comprehensive collection and analysis of historical and existing data relating water chemistry and natural stands of wild rice, including data from John Moyle, the Minnesota Department of Natural Resources ("MDNR"), Minnesota tribes and any existing academic field studies. (Wild Rice/Sulfate Protocol, p. 2). We would suggest that mapping and information technology expertise be used to correlate the information and make it as useful as possible both to demonstrate the basis for the current water quality standard and to allow comparison with new field research.

2. WaterLegacy would state that our highest priorities for the Wild Rice/Sulfate protocol are Survey Field Sampling, Intensive Field Sampling and Additional Field Survey of natural wild rice stands. (Wild Rice/Sulfate Protocol, Tasks D and E, pp. 19-20 and Appendix B). WaterLegacy supports a strong role for Minnesota tribes in identifying appropriate locations for field work, and supports the MPCA's commitment to ensure that Study methods are consistent with tribal scientific methodology. (Wild Rice/Sulfate Protocol, p. 15). Although we believe that identification of the mechanisms of action by which sulfate chemistry in the water column or sediments impairs wild rice may be useful to support water quality standards, WaterLegacy would caution that there are many highly toxic chemicals for which the mechanism of toxicity is poorly or incompletely understood. We would suggest that the Wild Rice/Sulfate Protocol focus primarily on what restrictions are needed to protect the designated use and only secondarily on the applicable mechanistic model.

3. WaterLegacy's second research priority for the Wild Rice/Sulfate protocol is container mesocosm research. (Task C, Wild Rice/Sulfate Protocol, p.16). This research would allow manipulation of variables in sediments and chemistry pertaining to wild rice without disrupting natural stands of wild rice. As with the Survey Field Sampling, Intensive Field Sampling and Additional Field Survey discussed above, comparability of data and methods with past and existing research would be recommended.

4. To the extent hydroponic or other greenhouse laboratory work (Tasks A and B, Wild Rice/Sulfate Protocol, pp. 17-19) might be helpful to confirm mechanisms of action by which sulfates and sulfides impair wild rice, WaterLegacy recommends both that this portion of the protocol should be a lower priority and that no data from bench testing should be available or released prior to the completion of assessment of existing field data, completion of Intensive Field Sampling and analysis of mesocosm research. This recommendation is not only important from a communications perspective, but to ensure that funds are not expended or withdrawn before the research is done that is most critical to protect the natural wild rice resource.

5. Finally, although studying sulfate impacts on mercury methylation is very important to WaterLegacy and our members, we recognize that this issue is appropriately separate from protection of wild rice. WaterLegacy would strongly recommend that the MPCA seek or allocate funding from other sources, perhaps from the St. Louis River mercury TMDL, to address the questions identified as priority "C" hypotheses in the Wild Rice/Sulfate Study Protocol. (Wild Rice/Sulfate Protocol, p. 12). WaterLegacy would be interested in meeting with the MPCA to develop strategies to ensure that vital research on sulfates and mercury methylation can also be conducted.

WaterLegacy does not have sufficient expertise in conducting wild rice research to provide recommendations on the specific metrics that should be used to measure health or impairment of wild rice. (Wild Rice/Sulfate Protocol, p.15). We'd defer to tribal scientific expertise on

this issue, but we'd suggest that any studies (such as hydroponic or greenhouse studies) that do not permit use of the multiple metrics available in field research be described to reflect the limits in scope and comparability of such data.
Please feel free to contact me if you have any questions regarding WaterLegacy's recommendations or perspectives regarding the Wild Rice/Sulfate Protocol.

Sincerely yours,
Paula Goodman Maccabee
Counsel for WaterLegacy

From: Steve Grattan <srgrattan#$ucdavis.edu>
Subject: Re: Draft Study Protocol for Your Review

Dear MPCA Staff,

First I want to start off by saying thank you for inviting me to the MPCA wild rice workshop last month. It is evident that the MPCA invested a lot of time and did an outstanding job preparing for the meeting which helped lead and focus the discussion of experts. Below is my response to the two questions you mentioned below.

1. The are a number of critical response metrics that need to be made. These metrics should be related to germination, vegetative growth and reproductive responses of wild rice to the variables tested. In terms of germination, metrics should be rate of germination and percentage of germination over time. Also, it would be valuable to have some biomass parameters and tissue ion concentrations. In terms of vegetative growth, these could be non-destructive measurements (number of plants per area, leaf elongation rates, number of leaves, photosynthesis, stomatal conductance, reflectance measurements, carbon isotopic ratios, etc.), or destructive measurements (leaf area, biomass of roots, stems, leaves, tissue ion analysis, etc.). In terms of reproductive growth, a complete yield component assessment should be made (number of tillers plant, seed wt per tiller, individual seed wt, number of days to flowering, etc.) in addition to partitioning biomass into different organs, tissue ion analyses, etc. Other measurements may be appropriate and added as the study progresses.

2. In terms of task order, I think the first 2 years tasks A and B are essential. Then task C could be done. Task D would only be beneficial after a lot is learned from tasks A and B. That way the metrics and timing of measurements can be established knowing what mechanisms and processes to evaluate. I would hold off on D and extend research on A and B until many of the burning questions are answered and mechanisms worked out. I think task E would also benefit from what is learned in taskes A and B. I like task F if it can be worked out in this freeze-thaw environment. Since SO4 is the parameter being evaluated, I can't understand why some investigators object to it being added to in-vivo "rings" or "mesocosms"? It is not a 'toxic' pollutant but rather a naturally occurring ion. The contribution to small rings is negligible to that in the surrounding environment.

Other comments....

Task A studies are pretty straight forward. Task B studies are going to be more difficult than those for A. Sulfide is very toxic so if done in controlled studies, it would have to be done smaller scale and maybe in a fume hood. A study of this kind would go before B1/B2. I would have to do some discussion with colleagues before designing this in detail. For B1 studies, the EDTA chelate seems logical...but there are other chelates too. This is where the field sampling would be useful to get a range of naturally occurring concentrations so treatments can be set to include treatments that are far below and well above that in the

field. B3 is also logical but maybe it has to be done at the same time with that in B1 and B2 and not necessarily waiting for the outcome of B1 and B2.. Changing pH may cause one metal to behave differently than the others.

Best regards

Steve Grattan
UC Davis
==

From: Wagener.Christine#$epamail.epa.gov [mailto:Wagener.Christine#$epamail.epa.gov]
Sent: Tuesday, June 14, 2011 3:13 PM
To: Swain, Ed (MPCA)
Cc: Blaha, Gerald (MPCA)
Subject: Fw: Draft [MN Wild Rice] Study Protocol for Your Review

Hi Ed,

Have you given thought to the issue presented below, that of copper toxicity when present with sulfate? I understand we are trying to keep the study focused on sulfate but there are many confounding issues, as we all know.

Chris

Christine M. Wagener, PhD
312-886-0887
U.S. EPA, Region 5
77 W. Jackson Blvd.
WD/WQB - 16J
Chicago, IL 60604
----- Forwarded by Christine Wagener/R5/USEPA/US on 06/14/2011 03:09 PM -----

From: John Dorkin/R5/USEPA/US
To: Christine Wagener/R5/USEPA/US#$EPA
Cc: Brian Thompson/R5/USEPA/US#$EPA, David Pfeifer/R5/USEPA/US#$EPA, Robie Anson/R5/USEPA/US#$EPA, Thomas Poleck/R5/USEPA/US#$EPA
Date: 06/08/2011 11:46 AM
Subject: Re: Fw: Draft [MN Wild Rice] Study Protocol for Your Review

[PCA note: A pdf was attached that is essentially identical to http://www.mass.gov/agr/pesticides/aquatic/docs/copper.pdf]

From: Goldberg, Sabine [mailto:Sabine.Goldberg#$ARS.USDA.GOV]
Sent: Thursday, June 09, 2011 2:33 PM
To: Engelking, Pat (MPCA)
Subject: RE: Draft Study Protocol for Your Review

1. What are your recommendations on which metrics would be best to include in the protocol from the list of response metrics on p. 14?

Using the list of response metrics on p. 15: live biomass, mean seed weight, seed germination rate

2. If we have to prioritize among Tasks A through E (or F), which are most critical/or the most critical variations to do in the next two years?

   A, B1, B2, B3, C, D

Sabine Goldberg
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[End of comments]