Note: This file contains meeting notes and feedback from the MPCA May 9 Technical Discussion of Development of the Wild Rice/Sulfate Study Protocol. Comments are grouped by topic area for the morning discussion of hypotheses, Group A afternoon breakout session, Group B afternoon breakout session, and large group afternoon report-out session.

May 9 Meeting – Morning Discussion of Hypotheses (Large Group)

Biogeochemistry

- Need to understand biogeochemistry and the plant response – these are interrelated, but separate, questions.

Water Level Effects

- Water level changes could flush sulfate/sulfide from the system

Experimental design

- Should look at worst-case scenarios – saturated soils with no flushing, low-iron sediments, so less potential to tie up sulfide, etc.
- Most standards are defended based on controlled experimental conditions. Need a reasonable microcosm to approximate controlled experiment vs numerous variables/trying to explain the distribution.
- Stay focused on "is it sulfate, sulfide, or both?"
- Multifunctional problem – sulfate in combination with other nutrients, organic matter, etc. Test co-occurrence and interconnection. Chain of evidence.

Sulfate/sulfide mechanism of impact

- Natural wild rice grows in slow-moving waters; not a lot of flushing, though some movement is helpful. Natural wild rice grows also in highly stained waters.
- Kinetics of transformations – biotic and abiotic interactions – may be just as important as thermodynamics.
- New hypothesis: Sulfate/sulfide more toxic at lower sediment pH.

Other factors that may affect wild rice/Alternate hypotheses

- In SW (Prairie) Minnesota, other reasons for wild rice impacts: lack of water clarity and perennial competition, especially when water levels do not fluctuate; sulfate/sulfur may not be the issue.
- Color and dissolved organic carbon levels could be important factors to consider. Dissolved organic matter (DOM) export important factor, increase sulfate associated with decreased CDOM (colored dissolved organic matter).

Important chemical (or related) interactions to consider/control for/etc.

- Sulfides found at orders of magnitude lower concentrations. Relative conversion rate – sulfate reduction and oxidation are directly proportional.
- Keep in mind that continued addition of sulfate can change the stoichiometry of the system.
- Amorphous iron more important. Concentrations of amorphous iron are much higher in northern parts of the state.

Site selection/field study considerations

- In northern MN, growing more wild rice than before through water level fluctuation. Study has value as you move west to see why the rice isn't doing as well. Focus on lakes that have been impacted, to figure out what's going on.
- Which lakes to study? Look at healthy lakes, too as a control.

Wild rice as a "system" – life cycle considerations

- Need to understand the wild rice system shading, flow in spring. Wild rice is not just a plant, but its own system; it "self maintains." You can't just focus on understanding the dynamics of one plant, you need to look at the full system. Shading? Straw production? (Helps with competition?) Spring flooding?
- Isolated populations, inbreeding may result in loss diversity, reduced adaptability.

Sampling methodologies

- Sediment sampling – "peeper" method is very time consuming. Profiles vary among lakes and within a lake.
- As an alternative, one could look at microbial profiles – indirect, but may provide information as to "who is where," which can be used to interpret the geochemistry.

Group A – Comments Grouped by Topic

Experimental Design Feedback -- What

Laboratory/greenhouse

- Lab test work well w/metals; algae growth a problem in greenhouse studies as it can affect the results.
- Have done some work with seed germination in petri dishes or hydroponics. Saw some sulfate effects at 1200 – 1500 ppm – corrosion on leaves.
- Like the petri dish/seed testing -- quick (10-14 day) results allows one to react quickly; can look at many variables; don't take up a lot of space – good first approach
- Hydroponic – 1^{st} two elements very clear what is being isolated; spatial resolution is a concern. Some debate of sulfate in the vicinity of roots (how much). Where is the line between lab and field? How do you control variables?
- Hydroponic testing may be problematic and not the most useful testing method. Will be subjected to scrutiny - sulfate not easy to control. Trepidation expressed that if results from hydroponic testing outlined in element A were to show elevated sulfate levels not to be toxic to wild rice then it may jeopardize the continuance of funding subsequent wild rice/sulfate studies to identify the mechanism (sulfide, etc.) – preferred approach would be field study and controlled mesocosm studies.
- Most people don't seem to think direct sulfate toxicity is the mechanism, but rather direct or indirect sulfide effects. Is it worthwhile to do the sulfate experiment (Test A)? Public perception/message is challenging, needs to be managed.
- Need to address perception issues head-on. It would be a mistake if the message/perception after Test A is that "we're done" (if direct sulfate effect not shown). We're not done.
- Need to be mindful of perception but if the science is explained, reporters will get it. Need to educate the press and the public about the study objectives and the study results.
- Concerns come back to money/funding. The reality is that funding gets pulled before things get done. It would be a real problem if funding gets pulled and all that has been completed is a hydroponic study of sulfate. Collaborate not compete. Need to avoid situation of "competing" study results – getting done with studies and they are at odds.
- Hydroponics/sand is the only way to truly test just sulfate.

Field study

- Field study is really important to all of this
- The field is important – can we find places with impacts?
- Also need to avoid field studies being done and not answering the "general mechanism" question – need to parse out the components, either through mesocosms or hydroponics.
- Prioritize the study needs. My view is that water chemistry in the field is most important

Straw proposal-specific feedback

- Recommendation made to start element C this summer – do not wait – do at same time as A & B.
- Start to balloon at element C, but can learn to figure out. A & B plus some of C could help inform field or micro/mesocosm work.
- Repeated over the years A and B and others (C). Field monitoring provides verification.

- Look at A & B as 1-year experiments to eliminate some questions. Has to be a multi-pronged study over several years; repeat field study; also mesocosms with ANOVA or regression analysis. In favor of A & B, also want to get a jump start on other things
- Is there a step between B & C that could control for some elements before the field mesocosm studies are undertaken? Is there a half-way approach between greenhouse and mesocosm studies that should be considered? Growth chambers or cups with three types of sediment to inform mesocosm studies?
- C & D more important that B for replication
- Overall approach of straw proposal is okay generally

Methods feedback/design specifics -- How

Laboratory/greenhouse

- Replication is tough because of genetic variability
- Difficult to grow wild rice hydroponically across its life cycle (because of algae interference) but it can be done. Earlier life stages are easier to grow.

Container mesocosm

- Mesocosom – How you start them is critical as last year's litter is a factor. Root litter decomposes slowly. Wild rice induces its own population cycle due to litter interactions; you get different results in the first year than in subsequent years because in the first year you don't have straw and root litter
- Replication is tough because of genetic variability
- Can use a soil sample in a settling (smaller) field.
- Have used a method to raise plants to near maturity in the field using containers suspended from a rack (less trouble than many mesocosms, essentially between smaller pots and mesococm methods). Also like the idea of "dosing" a paddy in strips.

In-situ mesocosm, whole-lake

- Mesocosom – How you start them is critical as last year's litter is a factor. Root litter decomposes slowly. Wild rice induces its own population cycle due to litter interactions; you get different results in the first year than in subsequent years because in the first year you don't have straw and root litter
- Suggestion made to conduct chemical treatments (gypsum and/or magnesium sulfate additions) within commercial wild rice paddies. Conditions within paddies quite variable
- Replication is tough because of genetic variability
- Samples could be taken adjacent to field experiment. In conventional rice experiments, didn't find much diffusion/edge effects.
- Use of *in situ* barrels/sections of culverts can control certain variables field study tests.
- Specially designed mesocosoms can be floated out into a lake.
- Could be logistically difficult (in-lake mesocosms).
- Every mesocosm is like a lake.
- Potential to divide a lake?

Field study

- How to measure past abundance (wild rice phytoliths) if you know rate of sediment that helps, need to keep past in mind. Field approach has to account for previous conditions, which is difficult if there is not monitoring data.
- Aerial photographic analysis of wild rice stands has limitations – views of wild rice stands from a canoe on the water may judge the stand to be substantial yet air photo analysis may not reflect that fact.
- Lacking a lot of field data, especially for sediment. In field sediment sampling needed to define range of chemical analytes.

Standard considerations

- Keep in mind this is not about creating a new standard, one already exists. Study is about confirming/supporting the existing standard or a change to the existing standard

Other factors affecting wild rice

- Would like to elevate the Null Hypothesis that sulfate is not a causative element, check on competition from other species or ecological effects as causative factors.
- Never seen nitrates in boreal soils in seven years of experiment (except after fire); the nitrate hypothesis is interesting, but not critical – it's a lower priority than variability in dissolved organic carbon
- The wild rice-based sulfate standard is a statewide standard. Elevated nitrate levels present in SW MN and nitrogen-based explosive residues can be found in mine pump out waters. Wild rice is nitrogen deficient, argues for at least monitoring for it so we know where we're at – or go to a high/low nitrate study design. Need background information to properly formulate studies.
- Concentrations will vary – keep nitrate simple
- Why sample for molybdenum (Mo)? Must measure to eliminate as a factor.

General study considerations

- First year answer "Is sulfate a factor?" Keep sulfate as sulfate in the tests – look at performance to see if wild rice can tolerate sulfate. Sand culture/hydroponic studies can be conducted to maintain aerobic conditions to see if SO_4 is actually problematic. Is sulfate and issue or not - yes or no. If sulfate isn't causing an impact, then chase other mechanisms – that informs next steps (lab) threshold question. Problem with mesocosm is there are multiple variables in play.
- Measure as many variables/parameters as you can, rather than eliminate, so you know what's going on if variables change; maintain tight control on pH.
- Experimental design will dictate the statistical methods needed, so leave the statistical methods open in the RFP.
- It is important to use the same seed source in the studies that are conducted.
- Minnesota lakes have genetically diverse populations. Need to control for/address that.
- Need to measure genotype to factor in any difference in sensitivity.
- Sample study areas for genetics??
- Bulk of the study costs is in paying people to do the measurements to discern changes in wild rice growth. Very tedious to measure changes in growth.
- Variables will be hard to measure to low levels.

- This gets back to how we measure health. Seed production, variability are key endpoints.
- Expression of caution about how much information/conclusion is drawn from paddy rice.
- Nitrogen metabolism in an oligotrophic environment? Sounds like something to control for and/or measure, but not something to vary experimentally.
- Measure/analyze as much as you can (within reason) – try to measure sulfur oxidizing bacteria in sediments

Wild rice as a "system" – life cycle considerations

- Articulate what constitutes a "healthy population" of wild rice – this group is in a perfect position to do that.
- Effect may not be acute, may be 2-3 years+ before the effects are felt-need to measure effects in subsequent generations of wild rice plants upon which studies are conducted. Won't get a lot of results in the first year.
- Wild rice population variability influenced by strong maternal effects.
- Take into account 4 + year cycle of wild rice growth. Treatments change the amplitude of the 4-year cycle, not the cycle itself. Can get at that with a ~3-year study that involves planting a gradient of density and measuring changes (due to sulfate and other variables) in the slope of the biomass/change in biomass over time line.
- Certain lakes reported to not exhibit the four-year wild rice growth cycle.
- Wild rice growth in lakes is more cyclical than growth in rivers.
- Do we know where wild rice used to grow? Sediment core sample collection and analysis would be valuable from waters where wild rice used to grow.
- Sample lakes where they tried to plant wild rice.

Where wild rice grows

- Most wild rice lakes have a pH of about 6, paddy wild rice is at about pH 7, so maybe that accounts for differences in sulfate effects between natural stands of rice and paddy-grown wild rice.
- I.D. groups within the communities surveyed to decide which lakes to sample. Hard to know what you have if you don't characterize the groups.
    - Examples; where wild rice grows well and where is doesn't, or where DNR has tried to establish wild rice and it hasn't done well.

Sediment/pore water methods

- Recommendation to measure acid volatile sulfide (AVS) - measure sulfide and associated metals in sediment.

Other

- Need to look at the pH levels and maybe buffering pH.
- pH can easily be controlled in a hydroponic environment.
- pH has a huge effect on iron sulfide compound solubility -- Bi-sulfide less toxic than hydrogen sulfide below pH 7.

- Four-step process involving
  - Historical records – look at lakes for different elements.
  - Mining areas – lakes impacted by sulfate
  - Mesocosm or in-paddy manipulation
- Design an experiment with 1 set of x z y to allow different people measure different things. Bring together a team or teams and conduct a coordinated experiment (NSF LTR method). Experiments like this are best done by building teams. Then coordinate from there with all the same sites/mesocosms.
- Field portion could benefit from a coordinated/team approach, too.
- There is not much vertical stratigraphy in the wild rice root zone. Can still get good age dates, though.
- Employ forensic limnology? Look at sulfate enriched systems with evidence of past wild rice, then paleolimnology could be very informative
  - Ex.: Lake Vermillion? Cores to look at past abundance.
  - The challenge is how you get at sulfate concentrations – hard to get at "paleo-sulfate" – it's not a straightforward analysis, but there are some options.
  - Phytoliths probably most useful for past WR presence/abundance.
- Need to approach this as teams of researchers, rather than individual RFPs

Recommendations Group A

1. Wild rice / sulfur cycle literature review to fill in information gaps.
2. Survey of current landscape—I.D. water bodies to be studied.
3. Form teams to study several methods – i.e. hydroponic team; mesocosm team; outdoor mesocosm team.
4. Lake with multiple experiments (*in situ*)
5. Details worked out by team on variables. Team approach with team members measuring different things at a chosen location in a coordinated, standardized method in order to allow for apples-to-apples comparison of resulting data.
6. PCA survey (20A)?? goes on throughout the study period to account for changes in population (water management, invasives, wildlife, etc., etc., etc.,??)
7. Take advantage of existing/ongoing data monitoring, EPA, USFWS, DNR.
8. Be careful about experiments on lakes (*in situ* manipulations). If *in situ* manipulations are needed, look at seeded sites or impoundments.
9. Hydroponic studies should be included, might inform other aspects. Should always be identified as preliminary step.
10. Water from chosen lake could be used in hydroponic or others studies as an attempt to control variability among various testing approaches.
11. Be very mindful of direct/indirect effects.
12. Frequent communication w/teams.

Group B – Comments Grouped by Topic

<u>Experimental Design Feedback -- What</u>

<u>Laboratory/greenhouse</u>

- Greenhouse and environmental growth chamber experiments good (#A and #B)
- Growth chambers are ok but wonder about multiple cations too complicated to evaluate here
- Toxicity studies start with hydroponics
- Get lab data plus literature
- Controlled studies on different sulfide levels
- Value in greenhouse work – conc. of sulfate that is toxic—good first step; How then do we do the field experiments in a way we can come up with a standard?
- Lab toxicity will need to be extrapolated to natural systems
- Like the hydroponic and container studies
- Need field and experimental component
- Simple (e.g. greenhouse) is good, but a danger to over simplification i.e. genetics. Identify and collect an array of populations (genetically representative, single seed source may be a problem) or test pop on the extremes of sensitivity (extremes of sulfate sensitivity)
- Isolate variables: sulfate studies then sulfide studies
- More emphasis on controlled studies—favor greenhouse and environmental chambers
- Greenhouse and outdoor containers favored
- Field levels first, then greenhouse with sulfide levels

<u>Container mesocosm</u>

- Mesocosms/experiments in containers and buckets do not translate well to field.
- Container studies good
- Mesocosm experiments better manipulate five or six variables
- John Pastor studies supports the research method
- Like the hydroponic and container studies
- Greenhouse and outdoor containers favored

<u>In-situ mesocosm, whole-lake</u>

- Mesocosms very difficult (likely means *in situ*); recommend not doing
- Experiments in lakes not appreciated in any wild rice water bodies by tribes. No in situ experiments on wild rice favored by tribes.
- Mesocosms could represent a set of conditions, but questions how well this represent all the variability found in a natural system
- Doesn't like the mesocosm (in situ) studies-too complicated at this point.
- At some future date whole lake study may be beneficial but this is more like a 10 year undertaking for a long term study
- Mesocosms—will they yield enough insight given their complexity?
- Mesocosm tough to control important variables – less able to manipulate
- Mesocosms (in situ) too risky – too many variables that can wipe out a wild rice crop with loss of entire study year, may not be a worthwhile investment.

Field study

- Field studies important! In situ measurements also important
- Field work: Do seasonal studies to understand changes in sulfate levels, vary environmental conditions over time and track sediment changes.
- Not support long term field work – too many variables, multiplicity of factors
- Field observations result in too many variables.
- Field studies used to consider adequacy of toxicity tests
- Difficult see how field studies get at answers there are so many variables
- Community surveys (ala Moyle) important, survey other plants existing in wild rice beds
- Community studies important– historical information, consider community structure
- Value in greenhouse work – conc. of sulfate that is toxic—good first step; How then do we do the field experiments in a way we can come up with a standard?
- Start with empirical survey approach. Should start this year with more sites the second year; Consider in context of what will be learned from this year field study
- Field scale studies may be very important
- Need field and experimental component
- Visit Moyle's lakes and measure sulfate and sulfide in pore water.
- Field studies – replicating Moyle studies, capture seasonal variation of water and sediment chemistry.
- Need observations at Moyle's sites. For some sites, sample many times during field season—intensive study sites
- Field levels first, then greenhouse with sulfide levels
- Sample Moyle's Lakes again or some subset of those
- Empirical important. Look at relationship between sulfate and sulfide in the environment.

Straw proposal-specific feedback

- C –May be ok but lean toward no
- #D too deep
- #G possibly
- Question #H beyond scope of study needs
- Likes the straw proposal
- Liked #A
- Skeptical of #B – Not sure how it would translate to a study. Need a model to see if it would work. Not sure how it would translate into standard.
- Likes #C – have to be careful about getting representative soils
- #D in lab--shouldn't be done outside (important to the mining industry, esp. winter discharge consideration)
- Likes A, B and C
- Need to understand the sulfide sediment mechanism. D might be interesting but not critical if limited resources
- Likes A,B, C

- D is premature, interesting but not done with limited resources
- A + B important to evaluate plausible mechanisms
- C – Container studies have merit to understand biogeochemistry
  Remove sediment and water from areas that no longer have wild rice and put in container study.
- #A is good
- B+C important next logical steps– what is limiting sulfate reduction? (Focus on this)
- Agrees with the As.
- Field and experimental, both are important.
- Use a weight of evidence approach

Methods feedback/design specifics -- How

Laboratory/greenhouse

- Experimental method must include a system with sediments and with a long enough time to achieve representative chemistry– needs to include sediments

Container mesocosm

- Mesocosm wild rice has multi-year 7-11 boom and bust cycle. At 5-7 years of study would be needed to study well.
- Experimental method must include a system with sediments and with a long enough time to achieve representative chemistry– needs to include sediments

In-situ mesocosm, whole-lake

- Suggested mesocosm size 15 feet diameter
- Insitu – Consider Experimental Lakes Area (ELA) example, large scale sea curtain separating basins of similar geomorphology, (Picture will show effects – Science magazine cover of ELA.) Consider public aspects of studies to –information presentation to various audiences
- Any whole lake experiments should follow mesocosm studies.

Field study

- Field data should cover areas where there is no longer wild rice, chemistry studies
- Conduct controlled field studies with a list of variables
- One state-wide study and standard vs regional
- Multiple visits to field sites to capture variation

Use existing information

- Lots of field work already ongoing. These include stand density and other field data – Effort needed to compile existing field data.
- 1854 Treaty Authority measures stand density and has a method that is repeatable.
- Bands have field data. Sulfate is quite low 2-5 mg/L population.
- Instead of a massive experiment, could look at what has occurred in the past.
- Use published equations to understand sulfate sulfide relationships

Standard considerations

- Is sulfate the right standard? Suspects impact is sulfide
- Could model after conductivity standard standard—weight of evidence approach
- Consider weight of evidence from lab/mesocosm/microcosm studies
- Focus on plausibility of mechanisms in all studies.
- Standards protect use and the potential for use
- For sulfate std. need to link sulfide in sediments to sulfate loading
- Going from observational standard to mechanistic standard
- What work will lead to a sulfate loading rate
- Process of approximation. Goal is to see if the current standard level is at all in line – get handle on whether empirical 10 mg/L sulfate is O.K.

Other factors affecting wild rice

- Wild rice affected primarily by ecological factors; sulfate doesn't make any difference.
- Plant competition complicates issue. Don't study.
- Focus on light regime in lakes with wild rice vs. lake without wild rice, esp. due to algae.
- Plant competition – adapted to low light conditions
- Sees light limitation evaluation as beyond the scope of this study
- Water flow is not that important once plants are established
- Ecological factors (Ag) in western parts of the state

General study considerations

- Can't separate the cultural piece
- Stat. design good
- Do it in a systematic manner
    - Focus on first questions - Is sulfate toxic?
    - Understand whether Sulfate and magnesium co-vary, Mg:Ca important?
    - If sulfate not toxic next concentrate on sulfide
    - Sulfide tricky to control in experiments
    - Need to tie sulfate and sulfide together, recognize pH dependency
    - Need to look at cation interactions with sulfate
    - Mercury, other nutrients, molybdenum, iron, zinc not necessary given study constraints and objectives.
- Be very simple. Ask one question and do it well.
- Focus on sulfate issues
- Do it statistically. Aim for studies of publish quality
- Multi-year studies
- Replication in study design is important
- Co-variables disagreement:
    - Keep it simple vs. more complicated
- Think long term sustainability. Keep studying and adjust based on findings

Wild rice as a "system" – life cycle considerations

- Wild rice management will require a more holistic approach
- Recognizes the long term growth of wild rice is important
- Need to focus in on sulfate levels at various stages of wild rice growth
- Try to I.D. what stage of the plant's life cycle is most susceptible; seed stage, growing up

Where wild rice grows

- Western Minnesota wild rice growth affected by human disturbance
- Flocculent soils are best. Wild rice won't grow in sandy areas/areas with sandy bottoms due to lack of root holding capacity.
- Won't find soft sediments where there is physical scour
- Historical ranges of wild rice included Iowa and Illinois—areas have been altered dramatically by human disturbance

Sediment/pore water methods

- Get idea of variation of sulfide variability in pore water and sulfur in sediment
- Pore water measured with antioxidant buffer fixing and electrode measurement
- Need to characterize microbiology and sediment sulfur cycle, organic matter and sulfide relationship
- More attention to tie it all together. Need methodology to establish pore-water chemistry, which is very important
- Recognize multiplicity of factors influencing sediment sulfide, nitrate, organic matter, iron, etc.

Other

- Recognize changing analytical methods and detections in long term data set evaluation
- Place a bag over seeds to capture
- Number of plants and biomass likely endpoints
- Recognition in studies that sulfide is pH dependent.
- Short time frame for understanding sulfate/sulfide link
- Focus on sulfates and minimize variables 1A, 2A, 3A hypotheses
- Don't know if iron matters

Summary Group B

- Empirical studies
- Divided on whether to do greenhouse or empirical studies first
- Good support for hydroponics—could run sulfate and sulfide simultaneously.
- Mesocosm studies (in situ) does not have as much support, wouldn't allow multi-year effort, difficult to set up, expensive
- Container studies have support
- Need to understand sulfate => sulfide transformation
- Greenhouse will give quick info

Afternoon Report-Out Discussion (Large Group)

- Mesocosm (Part D – field mesocosm) may be interesting, but not needed to review the standard.
- May be useful to continue field survey in the year(s) after the initial preliminary field survey.
- Re-iterated the team approach – one large experiment with design laid out. Researchers do their parts in a coordinated way. And/or one of field containers, looking at different aspects. Ask for RFPs, MPCA pull together teams, or through a University.
- Concerned about any hydroponic work that just looks at sulfate, and no other variables. The work is likely to be misused to suggest the study work is done at the conclusion of that piece.
- Noted that the group didn't talk about periods of susceptibility or risk. Also what is protective – how much protection (to what level). Period of susceptibility may not be in a single year, but across the boom/bust cycle of wild rice – wild rice is most sensitive at the low point of the boom/bust cycle.
    - Another participant noted that is why it's so important to look at a range of lakes.
- Need to be mindful of longer-term impacts.
- Flip side is that controlled studies with one variable is most sensitive to small changes/impacts.
- Stressed organisms are more susceptible to multiple stressors.
- Need both lab portion and natural environment portion. Also noted the variability among wild rice.
- Back to cautionary point about how much we're willing to lose – concerned that managing risk of loss won't be adequately addressed by the studies. The group discussed the concept of the standard being protective of sensitive species like wild rice.