Technical Analysis of Wild Rice Research

Prepared by
Minnesota Chamber of Commerce

February 2014

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1.0 Executive Summary

In 2011, the Minnesota Legislature enacted the Wild Rice Rulemaking and Research law, which provided the Minnesota Pollution Control Agency (MPCA) with funding to assess the effects of sulfates and other substances on the growth of wild rice.[1] The legislation also created a Wild Rice Standards Advisory Committee, on which representatives from Minnesota Chamber of Commerce (Chamber) members and staff serve and which is responsible for reviewing MPCA's research results. In January 2014, researchers submitted wild rice data collected since 2011 to MPCA, which the agency is now reviewing. To assist the agency's review, the Chamber prepared a detailed technical analysis of MPCA's research. The Chamber committed to that engagement because of the importance of the standard as a statewide issue, with major ramifications for industry, municipalities and other dischargers.

The Chamber's analysis of the MPCA research is in three parts:

- A critical analysis of each MPCA study (Section 2);
- An integration of the data from each study (and other credible, public information) to address MPCA's goals and primary hypotheses (Section 3); and
- Responses to the regulatory questions posed by MPCA (Section 4).

The Chamber's analysis was prepared by a team of scientists and policy experts holding post-graduate degrees and possessing decades of applied experience in aquatic toxicity assessment, water resources, soil science, rice nutrient dynamics, forest resources and genetics, chemical engineering, statistics, salinity effects on plants, and federal and state environmental permitting and rulemaking.

Based on its analysis, the Chamber makes the following recommendations regarding the three regulatory questions posed by MPCA (shown in italics below).[2]

- *Does the scientific evidence indicate that the standard should go up or down and, if so, generally by how much?*

Given the high concentration of sulfate necessary to adversely affect the growth of wild rice, as well as the lack of a relationship between wild rice growth and surface water sulfate concentrations demonstrated in MPCA-sponsored field surveys, a sulfate water quality standard is unnecessary.

However, if MPCA determines there is a need for a sulfate water quality standard, the standard should be increased to 1,600 mg/L in surface water at the location where wild rice is present. Two sulfate hydroponics studies support such an increase. Moreover, MPCA-directed field surveys and private surveys document observations in waters with sulfate concentrations above 800 mg/L and 1,000 mg/L at the location where wild rice is present.

The studies show that sulfide in sediment water[3] only affects wild rice at very high levels, only in lakes in the western and southern portions of the state, and only where iron concentrations in sediment water are very low. See Section 4 of the report.

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[1] 2011 Minn. Laws, 1st Special Session, Ch. 2, Art. 4, Sec. 31
[2] Wild Rice Sulfate Study Summary and Next Steps, MPCA December 2013
[3] Technically referred to as "porewater"

○ *Should there be a different standard for lakes/wetlands, or streams, or paddy rice?*

No. At an appropriately set standard of 1,600 mg/L, there is no need for a separate stream or lake standard. MPCA has conducted no research on wild rice in wetlands.

○ What more may be said about the *period when the rice may be susceptible to high sulfate*?

Wild rice is not susceptible to sulfate concentrations in overlying water between senescence (early September) through seed germination and early stages of seedling growth (mid-April). As a result, any sulfate water quality standard should not apply between early September and mid-April. If MPCA determines to impose a 1,600 mg/L sulfate water quality standard at locations where wild rice is present, that standard should apply only during the wild rice growing season (mid-April through early September).

2.0 Introduction

2.1 Overview of Minnesota Chamber of Commerce Analysis

The Chamber's analysis of MPCA Wild Rice Research is divided into three parts:

- A critical review of each study;
- An integration of the data from each study (and other credible, public information) to address the agency's goals and primary hypotheses; and
- Recommendations to answer the regulatory questions posed by the agency:
    - o *Does the scientific evidence indicate that the standard should go up or down and, if so, generally by how much?*
    - o *Should there be a different standard for lakes/wetlands, or streams, or paddy rice?*
    - o *What more can be said about the "period when the rice may be susceptible to high sulfate?* [4]

These comments were prepared by the Chamber with significant contributions by a team of individuals with significant training and experience in a variety of fields related to wild rice, plant toxicology and other disciplines. See Section 5.0 for more detail.

2.1.1 Analysis of MPCA Wild Rice Research

The Chamber provides an analysis review of each of the studies, to answer the two most pertinent questions posed by the MPCA at the February 3, 2014 Advisory Committee Meeting:

- *Are there concerns about the methods?*
- *Are there concerns about data quality?*[5]

In addition, the Chamber poses and provides its opinion to a third question:

- *What conclusions can be drawn from the study?*

In general, there were few concerns about the methods used in the following studies:

- Field survey;
- Sulfate hydroponics studies;
- Seed germination and mesocotyl growth portion of the sulfide hydroponic studies;
- Root zone geochemistry studies; and

[4] Wild Rice Sulfate Study Summary and Next Steps, MPCA, December 2013
[5] Input on Study Reports, MPCA, February 2014

- Temperature dependent diffusion rate studies.

Data and conclusions from these studies should be used to draw conclusions and to address the regulatory questions posed by the MPCA.

There were serious concerns with the methods used in the following studies:

- Juvenile seedling portion of the sulfide hydroponic studies; and
- Mesocosm (outdoor container) studies

Data from these studies should not be used to draw conclusions because of serious concerns about the methodology, and should not be used to address the regulatory questions posed by the MPCA.

In general, insufficient statistical analyses were provided in the MPCA's reports; additional analyses are provided by the Chamber here.

Conclusions from all the studies are discussed in Section 2.

2.1.2 MPCA's Goals and Primary Hypotheses – Chamber's response

The MPCA set the following goals for all the studies:

> *The goal of the Wild Rice Sulfate Standard Study is to enhance understanding of the effects of sulfate on wild rice and to inform a decision as to whether a revision of the wild rice sulfate standard is warranted.*[6]

The MPCA also notes that the studies need to describe:

- *The effect of sulfate on wild rice;* and
- *The effect of sulfide on wild rice*

Based on the data in the MPCA studies, other credible studies and the Chamber's analysis, the Chamber concludes that there is no correlation between sulfate in surface water and wild rice growth, and sulfate does not affect wild rice growth except at very high concentrations (e.g., in excess of 1,600 mg/L). Effects from very high concentrations of sulfate are likely caused by osmotic stresses resulting from high concentrations of any salt, including sulfate.

Based on the data in the studies, other credible studies and the Chamber's analysis, the Chamber concludes that there is a correlation between sediment porewater sulfide and wild rice growth, but that porewater sulfide does not affect wild rice seed germination or mesocotyl growth except at very high levels (e.g., above 2.4 mg/L (above ~90 μM), only in lakes in the western and southern portions of the state, and only where iron concentrations in porewater are very low (e.g., less than 1 mg/L (~ 2 μM).

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[6] Wild Rice Sulfate Study Summary and Next Steps, MPCA, December 2013

The MPCA also proposed the following hypotheses:

- *wild rice can be impacted by sulfate via the conversion of sulfate to sulfide in the rooting zone of the plants* and
- *iron may mitigate the effects of sulfide production in the rooting zone of the sediment*[7]

The MPCA has acknowledged that no single study can elucidate these hypotheses; rather, an integration of the data from all of the studies (and perhaps other sources as well) is necessary to provide sufficient data to support these hypotheses.

Based on the data in the studies, other credible studies and the Chamber's analysis, the Chamber concludes that the first hypothesis may be partially correct. In the absence of sufficient iron to precipitate dissolved sulfide, sulfate diffuses into the rooting zone of aquatic plants and is converted to sulfide. However, in all streams and most lakes in Minnesota, there is ample naturally occurring iron to precipitate dissolved sulfide. Only very high levels of porewater sulfide (e.g. greater than 90 µM) have been shown to impact wild rice plant growth.

Based on the data in the studies, other credible public studies and the Chamber's analysis the Chamber concludes that the second hypothesis is strongly supported by the useable research and other credible evidence. In all streams and in the majority of the lakes surveyed, there is more than sufficient porewater iron to prevent the accumulation of soluble sulfide in porewater to any significant concentration. The few lakes where high sulfide was observed are lakes that reflect the parent material and groundwater in which they are located, and tend to be lower in porewater iron.

These conclusions are further discussed in Section 3.

2.1.3 Chamber's Recommendations for Revisions of the Water Quality Standard for Protection of Waters Used for the Production of Wild Rice

The data developed in the studies and the statistical analysis herein demonstrate that the water quality standard for the protection of waters used for the production of wild rice should be revised, based upon the integration of the data provided by the MPCA's research, and other credible, public information. The Chamber recommends the following with respect to the key questions posed:

- *Does the scientific evidence indicate that the standard should go up or down and, if so, generally by how much?*

The scientific evidence indicates the standard should go up. Multiple observations of wild rice have been made in waters with concentrations well above the current 10 mg/L water quality standard, both in the University of Minnesota Field Survey and in private surveys.

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[7] Id.

Given the high concentration of sulfate needed to have an effect on the growth of wild rice (see below), and the fact the field survey showed no relationship between wild rice coverage and surface water sulfate concentrations, a sulfate water quality standard is unnecessary.

However, if the MPCA decides that there is a need for a sulfate water quality standard, the standard should be increased to 1,600 mg/L sulfate at the location where wild rice is present. In accordance with EPA guidelines and state procedures for establishing water quality regulations, two sulfate hydroponics experiments were conducted producing results which support this conclusion. Moreover, MPCA-directed field surveys and private surveys document observations in waters with sulfate concentrations above 800 mg/L and 1,000 mg/L at the location where wild rice is present.

The studies show that sulfide in sediment water[8] only affects wild rice at very high levels, only in lakes in the western and southern portions of the state, and only where iron concentrations in sediment water are very low. See Section 4 of the report.

- *Should there be a different standard for lakes/wetlands, or streams, or paddy rice?*

No. At an appropriately set standard of 1,600 mg/L, there is no need for a separate stream or lake standard. MPCA has conducted no research on wild rice in wetlands.

- *What more can be said about the period "when the rice may be susceptible to high sulfate"?* [9]

For clarification, the exact language of the current rule is:

> *… during periods when the rice may be susceptible to damage by high sulfate levels*[10]

The Chamber assumes the MPCA's question is a short-hand restatement of the rule. In the Chamber's analysis, the Chamber will use the rule precisely as currently drafted. Wild rice is not susceptible to any concentration of sulfate in overlying water between senescence (early September) through seed germination and early stages of seedling growth (mid-April). There should be no sulfate water quality standards applicable during those times. The 1,600 mg sulfate/L water quality standard (at locations where wild rice is present) should apply only during the growing season of wild rice (mid-April through early September).

These recommendations are further discussed in Section 4.

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[8] Technically referred to as "porewater"
[9] Wild Rice Sulfate Study Summary and Next Steps, MPCA, December 2013
[10] Minn. Rules 7050.0224, Subpart 2

2.2 Overview of MPCA Research

In order to avoid constant cross referencing of each of the studies, a succinct synopsis is provided for each study. These synopses are taken from each study and listed as direct quotes when appropriate. Indented italics are used to denote quotations. Foot notes are provided either before the quotations (when citing a study for example) or at the end of the quotations. Additional text is provided by the Chamber to provide additional background and context for each study.

2.2.1 Field Surveys

Under contract from the Minnesota Pollution Control Agency (MPCA), the University of Minnesota conducted a survey of water bodies across Minnesota in the summers of 2011, 2012, and 2013, to assist the evaluation of the State's sulfate water quality standard to protect wild rice waters (also known as the "wild rice sulfate standard"). This activity is referred to as the "field survey" (or "2011 survey," "2012 survey," and "2013 survey") is intended to provide:

- information to the MPCA about correlations between wild rice presence and environmental parameters; and
- data collected in a comparable way at field sites and in mesocosms.

The 2011 survey was a preliminary effort to collect initial data on wild rice stands and to develop the methods for larger field surveys in 2012 and 2013.

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

Site selection was conducted in close collaboration with MPCA personnel. Seventeen sites were selected as "multiple visit" sites that were visited three to five times between May and September 2013, while 19 sites were sampled one time. Sites included lakes, rivers, wetlands, and cultivated wild rice paddies, and were selected based on information provided by stakeholders, and on data on the chemistry and distribution of wild rice waters and other shallow water bodies.

For statistical purposes of investigating the hypothesized relationship between sulfate and wild rice growth, the team sought sites with a range of values in both parameters (i.e., low sulfate/low wild rice, low sulfate/high wild rice, high sulfate/low wild rice, and high sulfate/high wild rice). The team also attempted to sample widely across the state, and to sample sites that had a history of past or present drainage of high-sulfate waters into wild rice waters, as well as sites with no known history of high sulfate waters.

2.2.2 Hydroponics experiments

This report focuses on the hydroponics experiments to determine the effect of elevated sulfate and sulfide on wild rice growth and development[11]

> *In order to determine whether sulfate or sulfide have toxic effects on wild rice growth, it is instructive to perform hydroponics experiments where the chemistry of the growth solution and the presence of sulfate or sulfide can be controlled precisely. Accordingly, we began a series of hydroponics experiments to test the effects of sulfate and sulfide on wild rice seed germination and juvenile growth of seedlings under highly controlled conditions in growth chambers*[12]

2.2.2.1 Sulfate hydroponic experiments

> *The selected seeds were placed into each of six numbered plastic cups to total fifty seeds each, then randomly assigned and transferred to each of six 1-pint Mason jars containing six sulfate treatment levels of 0, 10, 50, 100, 400, or 1600 mgSO_4 · L-1*
>
> *The experiment proceeded in a growth chamber at 20 ˚C in the dark. The solutions were exchanged with fresh solution of the appropriate treatment concentration every three days. Solution pH was measured both on the initial and exchanged solutions. The germinated seedlings were harvested after 11 days.*[13]

Hydroponic techniques were used to test growth of juvenile wild rice seedlings under aerobic conditions subject to various concentrations of sulfate. Seedlings from germinated seeds from Little Round Lake were used.

Each tube was considered a replicate for the corresponding test concentration. One seedling chosen as described above was placed with forceps into each Kimax tube, which was then filled with modified 1/5 strength Hoagland's solution and an appropriate amount of sulfate.

The tubes were placed in an environmental growth chamber with lamps of maximum light intensity of 800 or greater µmol m-2 sec-1 (measured 6 inches below the lamps) produced by either fluorescent lamps or an LED light system. Tests were performed under a 16h: 8h light: dark photoperiod. Temperature was maintained at 21˚C during lighted periods and 19˚ C during dark periods, and relative humidity was maintained at 85%. Plants were harvested after 10 days.

2.2.2.2 Sulfide hydroponic experiments

> *The techniques used here were the same as for the germination trials under various sulfate concentrations, except that extra care was necessary to ensure anaerobic conditions. Fifty conditioned seeds were placed in 700 mL borosilicate glass jars capped using phenolic screw caps*

[11] Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor, University of Minnesota Duluth, December 2013
[12] Id.
[13] Id.

with chlorobutyl septa 5 mm thick. The 1/5 Hoagland's nutrient solution was deoxygenated with purified nitrogen before being added to the bottles.

The target concentrations were 0, 3, 10, 30, and 90 µM sulfide. The bottles were placed in a growth chamber in continuous darkness 20° C ± 1°C. Solutions were exchanged every two days. After 11 days, the germinated seeds were harvested.[14]

It is critical to note that the fully-enclosed test system would have severely limited gas exchange during wild rice life stages that would not be exposed to anaerobic conditions.

2.2.3 Outdoor Container Experiments

Sulfate amendments to tank mesocosms similar to those used by Walker et al. 2010 began in Summer 2011 and continued through 2012 and 2013 at five different amendment levels: control (~10 mg sulfate /L), low (50 mg sulfate / L), medium-low (100 mg sulfate / L), medium (150 mg sulfate / L), and high (300 mg sulfate / L). Water and sulfate levels in mesocosms were maintained by weekly sampling and appropriate additions of well water (~10 mg/L sulfate) and sodium sulfate stock solution to account for rain water dilution, evaporation, and flux of sulfate into sediment. During the fall of 2012, overlying water sulfate levels were not maintained actively after plant senescence in September, and sulfate amendments did not begin again until June 2013.

The outdoor containers with sediment from lake bottoms over sand were used to determine the response of wild rice to a range of sulfate concentrations in the surface water, and associated sulfide in the rooting zone, across the growing season.[15] *The experiments were established in 2011 and run through 2012. However, only data from 2013 are available, and no initial conditions were measured. Also, in 2013, there was significant seedling mortality in all tanks after thinning but before the floating leaf stage*[16]*, which not only precluded sampling of individual plants, but also casts doubt about the viability of the plants being tested and their response to the sulfate treatments.*

Additional sulfate was added to tanks to reach nominal sulfate concentrations in the overlying water of 0, 50, 100, 150 and 300 mg/L sulfate. A variety of biological endpoints, including seed weight, number of viable seeds, and plant biomass were measured at the end of the growing season and the results of each treatment compared to one another.

It is critical to note that the mesocosm systems used contained sediment with a past history of wild rice growth in sulfate exposures in overlying waters. Key sediment components such as iron and various trace nutrients were not replaced nor were fresh sediments added to the test systems prior to initiation of the experiments. Thus, the mesocosm test system was likely limited in its ability to provide plant nutrients and

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[14] Id.

[15] Effects of enhanced sulfate concentrations on wild rice populations: results from a mesocosm experiment John Pastor, University of Minnesota Duluth, December, 2013

[16] Id.

would have been deficient in the iron necessary to complex any dissolved sulfide naturally generated in sediment porewater.

2.2.4 Root Zone Geochemistry

2.2.4.1 Outdoor Containers

During the summer of 2012, passive porewater equilibrators (peepers) were deployed to collect depth-profiles of samples approximately monthly in duplicate control, low (50 mg/L), medium (150 mg/L), and high (300 mg/L) sulfate mesocosms.

During the summer of 2013, peepers were again deployed monthly at the four sulfate treatment levels. Once a month from May to September, duplicate peepers were deployed within an individual tank at each treatment level. One peeper was inserted in the plant-free zone and the other peeper was located near the center of the tank where wild rice was allowed to grow. Sediment cores were extracted monthly during peeper retrieval in locations coincident with peepers, and sectioned into intervals consistent with peeper well spacing.[17]

2.2.4.2 Field sites

In an attempt to characterize seasonal variability of porewater geochemistry in a field setting, peepers were also deployed at two field sites: one river site, Second Creek (47.52042 -92.1925), and one lake site, Sandy Lake (47.61872 -92.59314) during the summer of 2013. Both sites have sulfate concentrations in the overlying water elevated above regional background levels (150 to 700) mg/L in the overlying water [and as high as 838 mg/L sulfate] in contrast to regional background concentrations of less than 10 mg/L. Both sites have potential for groundwater flow due to coarse sediment and regional hydraulic gradients imposed by human alterations of the landscape. At each site, duplicate peepers were deployed and collected monthly: one in an area of coarser sediment and one an area of finer, organic sediment. The solid phase was also characterized monthly at these field sites, but was limited spatially to an analysis of the homogenized top 10 cm of sediment.[18]

2.2.5 Sediment Incubation Experiments

Experimental sediments were retrieved from two locations within the St. Louis River watershed. The Partridge River (PR) sampling location is near the headwaters of the St. Louis River on the East-central portion of the Mesabi Iron Range in Northern Minnesota (Figure 2-1). The Partridge River site provided high organic sediment from a slow-moving part of a sulfate-impacted river where wild rice had been observed in recent years. The second sampling location, North Bay (NB), is near the tail waters of the St. Louis River, approximately 15 km upstream from the entrance into Lake Superior in the St. Louis River Estuary. The North Bay site, a protected bay away from the main channel, provided a lower organic sediment from a location where rice had also been observed in

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[17] Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms, Nathan Johnson, University of Minnesota Duluth, December 2013
[18] Id.

recent years. In January 2013, approximately 50 L of sediment was recovered from the top 10 cm of the river beds, transported back to the University of Minnesota – Duluth, and homogenized.[19]

Figure 2-1 Sediment Sample Locations[20]

Microcosms consisted of polycarbonate plastic tubing with a sealed bottom and Rhizon® soil moisture samplers fixed and sealed at varying depths along microcosm's profile to extract water from the pore space of the sediment, (Seeberg-Elverfeldt et al., 2005). The Rhizon® samplers were used to take 3 mL samples of porewater at specific time points throughout the experiment for the monitoring of anion transport within the sediment. Homogenized sediment was transferred to microcosm tubes, and gently consolidated by the use of a vibration table to minimize settling during the experiment. Fresh site water was placed over the sediment and the microcosms and allowed to equilibrate to lab conditions for three weeks prior to the beginning of experiments.

An aeration system was also included to provide oxygen and mixing to the overlying water. The microcosms were incubated in dark, temperature-controlled conditions throughout the experiment to minimize disturbances and to eliminate variables such as photosynthesis. Triplicate microcosms were constructed for each temperature. Three microcosms filled with North Bay Sediment were incubated at 4.5 °C for the duration of the experiment and three identical microcosms were incubated at 23 °C. An analogous set of microcosms were constructed and incubated using sediment from the Partridge River.

The experimental portion of the study occurred in three phases to analyze the flux of sulfate and several inert, tracer anions across the sediment-water interface. Water overlaying the sediment in each microcosm was continuously mixed and aerated to eliminate chemical gradients near the sediment-water interface in an effort to mimic conditions in a shallow natural stream that might receive sulfate-enriched discharges.

[19] Id.
[20] Id.

After an initial three-week equilibration period, Phase I began with a chloride spike (30 mg/L) into the surface water of the all of the microcosms. During Phase I, the sulfate concentration in the overlying water was monitored weekly, and was replaced when necessary to maintain concentrations similar to those experienced in the field.[21]

Phase II, the sulfate loading phase intended to mimic the onset of a sulfate discharge, was initiated by replacing the overlying water with fresh site water spiked with sodium sulfate (approximately 300 mg/L as sulfate) and sodium fluoride (16 mg/L as fluoride) and no chloride (Table 2 of Outdoor Containers report). Sulfate was spiked at the outset of Phase II and re-spiked three times throughout the 11 week loading phase.

Phase III, the recovery phase, was initiated by replacing the overlying water with fresh site water spiked with sodium bromide (20 mg/L as bromide; no additional chloride, sulfate, or fluoride) which was maintained for 9 weeks. The sulfate recovery phase was designed to simulate the end of sulfate-elevated discharge to surface waters, with the goal of determining how long sediment in a river system may be exposed to residual sulfate after sulfate concentrations in the overlying water are reduced. In between each phase was a three-day interlude in which only clean, unamended site water was present before starting the next phase.

To maintain target experimental conditions, surface water samples were collected and analyzed weekly from all replicate microcosms. The overlying water was changed as often as necessary to maintain the ion concentration gradient between the sediment and water or as otherwise deemed necessary. The amended overlying water used during incubations was retrieved from the same locations where the sediments were obtained.[22]

[21] Id.
[22] Id.

3.0 Technical Analysis of MPCA Wild Rice Research

3.1 Overview

For each study, the Chamber considered two of the questions posed by the MPCA at the February 3, 2014 Advisory Committee meeting:

Are there concerns about the methods?

Are there concerns about data quality?

The Chamber asks one additional question for each study:

What conclusions can be drawn from the study?

3.2 MPCA's Field Surveys

3.2.1 Are there concerns about the methods?

Yes. Water bodies were not selected randomly, but were selected based upon the presence of wild rice or the "expected" presence of wild rice. This limits the robustness of any statistical analyses. Given redox potential was not measured, it is difficult to make detailed geochemical predictions on the data.

3.2.2 Are there concerns about data quality?

Yes. The limited scatter plot analyses did not elucidate statistical relationships between surface water sulfate and sediment porewater sulfide and the presence or absence of wild rice. A thorough statistical analysis of the data is undertaken in Section 3.3, where data are integrated from all studies.

3.2.3 What conclusions can be drawn from the study?

Because statistical analyses of the field surveys by the MPCA were lacking, the Chamber undertook its own statistical analyses. Our analyses concluded that there is no statistically significant correlation between surface water sulfate concentrations and wild rice cover. There is, however, a weak correlation between porewater sulfide concentrations and wild rice cover.

Recognizing that a large number of chemical and physical parameters were measured during the field survey, an attempt was made to determine whether other variables may impact the presence or absence of wild rice. Since the updated data set was not supplied by the MPCA until January 29, 2014 the Chamber could not analyze all parameters for significance.

A binary logistic regression was conducted on field data from 2012-2013 to determine whether other redox-related water quality parameters, organic matter, or macronutrients may influence the presence vs. absence of wild rice across the sampling sites. The resulting statistical model identified porewater total sulfide, sediment total sulfur, and sediment percent total organic carbon as having statistically significant predictive value for the presence vs. absence of wild rice.

Overall, the results of binary logistical regression models of different components indicate that these three parameters are likely to be predictors of wild rice presence vs. absence, and that sediment porewater iron's contribution to predicting wild rice presence vs. absence occurs indirectly, via porewater total sulfide. In contrast, surface water sulfate concentration does not have any significant predictive value for wild rice presence vs. absence in this field study.

See Section 3.3 for a statistical analysis of this data, as part of the data integration discussion.

3.3 MPCA's Hydroponics experiments

3.3.1 MPCA's Sulfate Hydroponic Experiments

3.3.1.1 Are there concerns about the methods?

Yes, there are a few. Transfer of seedlings in these experiments likely increased their sensitivity to osmotic stress, i.e. sulfate or any other elevated ion that would have been present. Evidence of transfer stress was present in the method development testing conducted prior to these experiments. While the composition and concentration of the base nutrient solution differed, results of the University of Minnesota Duluth experiments are in general agreement with the results obtained in a separate study by Fort Environmental Labs, Inc.[23]

3.3.1.2 Are there concerns about data quality?

Data quality appears to be good, and sufficient replicates were taken to allow meaningful statistical analyses.

3.3.1.3 What conclusions can be drawn from the study?

Dr. Pastor concludes:

> *Sulfate did not affect either seed germination or seedling growth other than a slight depression of root lengths at extremely high concentrations (1,600 mg SO4 · L-1). These high concentrations, while possible in nature, are not likely to be common.*[24]

The data and statistical analysis support this conclusion. The slight depression of root lengths at the highest sulfate concentration was statistically significant only when compared against 50 mg SO_4/L (Analysis of Variation (ANOVA) followed by Tukey's *post hoc* separation of means).

The Fort Environmental Labs[25] study found a No Observed Effect Concentration (NOEC) value of 5,000 mg/L sulfate for the majority of plant endpoints evaluated. The Fort Labs study followed Good Laboratory

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[23] Definitive Hydroponics-Based Wild Rice (*Zizania palustris*) Sulfate Toxicity Testing, Fort Environmental Laboratories, December 2013

[24] Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor, University of Minnesota Duluth, December 2013

Practices.[26] Fort Labs also followed the US EPA Guidance for ecological effects testing.[27] The conclusions reached in that study include:

> *Results from the present 00325 study indicated that exposure of developing wild rice to sulfate generally did not induce an adverse response at concentrations ≤2,500 mg sulfate/L at study day (SD) 10 (Table 1) and ≤5,000 mg/L at SD 21. For example, the no observed effects concentration (NOEC) for three of the ten SD 10 NOEC values were 2,500 mg/L sulfate or lower, and seven of ten SD 10 NOEC values were 5,000 mg/L sulfate. For SD 21, eight of ten concentration endpoints exhibited NOEC values of 5,000 mg/L sulfate, indicating that sulfate was generally not toxic at the highest concentration that could be tested within the limits of solubility of the salts.*[28]

While seedlings were not adversely affected in the Fort Environmental Lab study up to and including exposures of 2,500 mg/L sulfate, the Chamber was concerned that exposures exceeding 1,600 mg/L may, like any ion, pose a salinity stress (osmotic stress) potentially adversely affecting wild rice during its life cycle (at least the portion of life cycle tested). This conservative assessment assumes that wild rice is salt sensitive and that exposure exceeding 1,600 mg/L could potentially impose an abiotic stress.

3.3.2 MPCA's Sulfide Hydroponic Experiments

The sulfide hydroponic experiments consisted of two parts:

- Range finding experiments on seed germination and mesocotyl growth and
- Range finding and "definitive" or additional testing on juvenile seedlings.

The experiments on seed germination and mesocotyl growth were range-finding experiments, and not definitive toxicity experiments. However, because there was no impact observed on those life stages, MPCA researchers determined that definitive toxicity experiments on these plant life stages were

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[25] Dr. Fort has over 27 years of experience working with the Federal Insecticide Fungicide and Rodenticide Act (FIFRA), Toxic Substances Control Act (TSCA), and most recently adaptations of FIFRA to the FQA (1996). Graduating with a B.S. in Biology- Chemistry from Southwestern College and a M.S. and Ph.D. in Zoology from Oklahoma State University, Dr. Fort has served as Study Director on 25 GLP studies involving a variety of plant and animals exposures. He assists private clientele and governmental agencies with interpretation of toxic substances (TSCA) and pesticide regulations (FIFRA), and most recently the Registration, Evaluation, Authorisation and restriction of Chemical substances program (REACH) program in Europe. Dr. Fort has worked on various pesticide risk assessments on behalf of both government and manufacturers, and has an extensive knowledge of the ecotoxicology and general health risks of pesticides in the workplace and environment. Dr. Fort directed several large studies involving the development of a multi-generational study of mysids and reproductive studies of freshwater amphipods and marine copepods. Dr. Fort has directed 11 large vascular plant phytotoxicity studies involving both traditional soil and hydroponic exposures.

[26] USEPA, Toxic Substance Control Act (TSCA), Good Laboratory Practice Standards, 40 CFR Part 792, Chapter 1, Subchapter R, 1989

[27] Ecological Effects Test Guidelines OCSPP 850.4100: Seedling Emergence and Seedling Growth US EPA 712-C-012 January 2012

[28] Definitive Hydroponics-Based Wild Rice (*Zizania palustris*) Sulfate Toxicity Testing, Fort Environmental Laboratories, December 2013

unnecessary. The experiments on juvenile seedlings consisted of both range-finding and "definitive" studies designed to better define the toxic threshold of sulfide to wild rice seedlings.

3.3.2.1 Are there concerns about the method?

For the range finding experiments on seed germination and mesocotyl growth, there were very few concerns. The placement of seeds, the light regime and temperature controls seem reasonable. While exposure of seeds and mesocotyls to anaerobic conditions may be appropriate, there is some concern about whether the entire mesocotyl would be exposed to totally anoxic conditions. Given that wild rice reseeds itself by falling to the sediment, it is unlikely that the entire mesocotyl is always exposed to anoxic conditions. It is very likely that a larger juvenile seedling would not be exposed to anoxic conditions.

Wild Rice seeds are quite thin and light. Seed used in the hydroponic experiments is approximately 2 cm in length and < ½ cm in width.[29] When this seed falls to the sediment it will likely remain in the top 1 – 2 centimeters. Cultivated wild rice is generally planted at depths of 1-3 inches (2.5 to 8 cm).[30] Seedlings will not emerge when planted deeper than 3 inches.[31] During the sulfate seed germination and mesocotyl growth experiments, the mesocotyls grew to lengths of 11.8 to 13.5 cm, while during the sulfide seed germination and mesocotyl growth experiments, the mesocotyls grew to lengths of 7.5 to 9.3 cm.[32] In both cases, the tops of the mesocotyls would not be in sediment.

In addition, the top portion of the sediment is likely not anoxic, due to diffusion of oxygen, sulfate and other oxygenated compounds in the upper few cm of the sediment.[33] Dr. Johnson noted very low levels of sulfide in the upper 3 inches (8 cm) of the sediment in the mesocosms (outdoor container) studies[34], and very low to non-detectable sulfide concentrations in the root zone geochemistry of the two field sites in May and June.[35]

The juvenile seedlings used in the second portion of the sulfate hydroponics experiments began as 1-2 cm mesocotyl seedlings, and grew to 11 to 12 cm seedlings by the end of the test (10 days).[36] Plant development of the shoot portion (following mesocotyl growth) would require oxygen for photosynthesis; shoot parts of the plants would not be exposed to anoxic conditions, either in the upper sediment or in the overlying water. Therefore, exposure of juvenile seedlings to anoxic conditions is inappropriate.

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[29] Wild Rice Juvenile Seedling Growth Test: Anoxic Conditions Experimental Method for Hydroponic Sulfide Toxicity Testing in Anoxic Conditions Using *Zizania palustris* (Wild Rice) as Test Organism, John Pastor, University of Minnesota Duluth, November 2013, Image 4.

[30] Wild Rice E.A. Oelke1, T.M. Teynor, P.R. Carter, J.A. Percich, D.M. Noetzel, P.R. Bloom, R.A. Porter, C.E. Schertz, J.J. Boedicker, and E.I. Fuller, University of Minnesota Alternative Field Crops Manual, December 1997

[31] Id.

[32] Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor, University of Minnesota Duluth, December 2013. Tables 2 and 11

[33] Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms, Nathan Johnson, University of Minnesota Duluth, December 2013 Appendix B.

[34] Id.

[35] Id.

[36] Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor University of Minnesota Duluth December 2013

There are a multitude of concerns with the design of this experiment. First, in this experiment, juvenile seedlings were germinated in an aerobic environment (open vessels) and then transplanted to an anaerobic environment (closed vessels). Seedling transplantation may have resulted in stress to all portions of the plant. Furthermore, if seedlings were photosynthesizing and producing oxygen, sulfide concentrations may have been influenced by this plant process. Dr. Pastor noted this in his report:

> *Although the solutions for each of these experiments were exchanged every two days, the initial sulfide concentrations declined during those two days, perhaps because of the production of oxygen by the photosynthesizing plants.*[37]

Finally, placing plant parts adapted to photosynthesis in an aerobic environment in an anaerobic environment may have resulted in additional stresses and increased susceptibility to effects from sulfide exposure.

3.3.2.2 Are there observations about data quality?

The data were presented clearly, especially for the germination and mesocotyl growth portions of the experiment. Statistical analysis of the data included tests for normality of distribution, a one-way ANOVA, and post hoc Tukey's means separation tests.

3.3.2.3 What conclusions can be drawn from the study?

Dr. Pastor draws the following conclusions:

> *Enhanced sulfide under anaerobic conditions did not affect germination of seeds ($p > 0.10$, Table 9), mesocotyl weights ($p > 0.10$, Table 10), or mesocotyl lengths ($p > 0.10$, Table 11) in a rangefinder test at nominal exposure concentrations of 0, 3, 10, 30 and 90 µM sulfide (see Appendix 7 for raw data and statistics). The rangefinder test was repeated, and because the same results were observed, we did not proceed with any further tests.*[38]

Exposures up to 90 µM sulfide in a hydroponic experiment did not affect wild rice seed germination or mesocotyl length. While exposures on seed germination and mesocotyl growth were conducted appropriately, experiments conducted on juvenile seedlings were not. Concerns were raised above that the entire seedlings (root and shoot) were exposed to sulfide treatments during the test. The plant parts above the root-shoot node (crown) would not grow in anaerobic conditions where sulfide would form in the field. Rather the shoot emerges into the aerobic water above the sediment; sulfide production does not occur under aerobic conditions. Since shoot parts were more sensitive to sulfide than root parts, developing an upper sulfide limit based on shoot sensitivity is not appropriate given the highly inappropriate exposure regime for the plant shoots. Therefore, the Chamber recommends that results from this test not be used to inform or develop water quality regulations. The highest observed porewater sulfide concentrations in the field surveys were observed in Bean Lake in Becker County (sulfide

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[37] Id.
[38] Id.

concentration 16 mg/L) and Lady Slipper Lake in Lyon County (one measurement at 14.8 mg/L, other measurements below 10 mg/L). These concentrations are the equivalent of approximately 500 μM sulfide, more than five times the NOEC from the sulfide hydroponic studies.

Three other lakes had maximum porewater sulfide concentrations near the 90 μM NOEC:

- South Geneva Lake in Freeborn County (99 μM)
- Sandy Lake in St. Louis County (96 μM) and
- Rice Lake in Stearns County (93 μM).

It must first be stressed that the 90 μM NOEC observed in the sulfide hydroponic studies reflects a no effects concentration, and the sulfide concentration above 90 μM where effects would occur is unknown. While it is not known whether slightly higher concentrations of porewater sulfide could result in effects on wild rice, these three lakes, sulfide concentrations are within 10% of the NOEC value. Given the variability associated with biological endpoints, it is likely that the concentrations observed are within the margin of error of the experiment.

Table 3-1 shows that several of these lakes had multiple samples, with multiple concentrations of sulfide. Both Lady Slipper and Sandy Lake ranged over an order of magnitude in sulfide concentration. Thus, the highest concentrations observed may be temporal in nature, varying widely over time.

Table 3-1 Lakes with Multiple Sample Dates

LacCore field ID	Class	Site name	DNR/State ID	County	Site type (Lake/ Stream/ Paddy)	Wild Rice presence (yes/no)	Date	Pore-water Total Sulfide (TS, mg S/L)	Porewater Fe (µg/L)
FS-85	4 Survey	Bean	03-0411-00-201	Becker	L	no	8/21/2012	16.00	50
FS-79	5 Survey Duplicate	Lady Slipper	42-0020-00-203	Lyon	L	no	7/27/2012	1.63	1,320
P-55	1 Pilot Survey	Lady Slipper	42-0020-00-204	Lyon	L	no	9/22/2011	14.84	638
FS-78	4 Survey	Lady Slipper	42-0020-00-202	Lyon	L	no	7/27/2012	1.68	98
FS-184	4 Survey	Rice	73-0196-00-216	Stearns	L	no	7/30/2012	2.97	25
FS-345	6 Survey 2nd Year	Rice	73-0196-00-216	Stearns	L	yes	8/7/2013	2.08	< 10
FS-251	4 Survey	Sandy-1	69-0730-00-203	St. Louis	L	yes	9/21/2012	0.12	12,500
FS-306	7 Survey Seasonal	Sandy-1	69-0730-00-203	St. Louis	L	no	6/11/2013	0.09	49,300
FS-305	7 Survey Seasonal	Sandy-2	69-0730-00-204	St. Louis	L	no	6/11/2013	1.08	82
FS-321	7 Survey Seasonal	Sandy-1	69-0730-00-203	St. Louis	L	no	7/9/2013	0.19	34,200
FS-320	7 Survey Seasonal	Sandy-2	69-0730-00-204	St. Louis	L	no	7/9/2013	3.08	4,670
FS-348	7 Survey Seasonal	Sandy-2	69-0730-00-204	St. Louis	L	yes	8/13/2013	0.31	6,570
FS-349	6 Survey 2nd Year	Sandy-3	69-0730-00-205	St. Louis	L	no	8/13/2013	0.07	12,600
FS-382	7 Survey Seasonal	Sandy-1	69-0730-00-203	St. Louis	L	no	9/17/2013	0.14	29,500
FS-381	5 Survey Duplicate	Sandy-2	69-0730-00-204	St. Louis	L	yes	9/17/2013	0.03	23,900
FS-380	7 Survey Seasonal	Sandy-2	69-0730-00-204	St. Louis	L	yes	9/17/2013	0.03	23,900

Based on the data in the above table, there is no relationship between sediment porewater sulfide concentrations and the presence of wild rice.

It is interesting to note that while no wild rice was observed on the following lakes:

- Bean (1 sample)
- Lady Slipper (3 samples)
- South Geneva (1 sample)

Wild Rice was found in two of the lakes:

- Sandy Lake (9 samples (+ 1 duplicate), wild rice found in 3 samples)
- Rice Lake (2 samples, wild rice found in 1 sample)

While wild rice was not found when the highest sulfide readings were observed, wild rice was found later in the season, sometimes only a month after the highest sulfide concentration was observed.

It is also interesting to note that the following lakes are listed in the DNR's 2008 survey of wild rice waters[39]:

- Bean
- Sandy
- Rice

(Note that a Geneva Lake in Freeborn County is listed in the DNR report, but it has a different Lake ID number than the one sampled in the field survey.)

Bean and Rice Lakes are listed without any estimated wild rice coverage. Sandy Lake is listed as having 100% wild rice cover. However, this is at odds with the field survey observations[40] and recent observations by Bois Forte, which found wild rice with only scattered locations with wild rice plants.[41]

Although Sandy Lake had one sulfide observation greater than 90 µM, and one at 41 µM, the other observations were less than 10 µM. The value at 90 µM is not statistically different than the rest of the observations when the data were analyzed on a log normal distribution[42]. The median concentration observed including the two high values is 4 µM. It is inaccurate to state that Sandy Lake routinely has sulfide porewater concentrations greater than 90 µM. It is more accurate to say that Sandy Lake occasionally has high sulfide concentrations. It should be noted that Sandy Lake was sampled in three

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[39] Natural Wild Rice In Minnesota, Department of Natural Resources, February 2008

[40] Wild Rice Sulfate Standard Field Surveys 2011, 2012, 2013: FINAL REPORT, Amy Myrbo, University of Minnesota, December 2013

[41] Sandy Lake and Little Sandy Lake Monitoring (2010-2013), Bois Forte Reservation Technical Report 13-06, December 2013

[42] Environmental data frequently follow lognormal or right-skewed distributions (e.g., Gilbert 1987 – Statistical Methods for Environmental Pollution Monitoring)

different locations as shown in Figure 3-1. The two highest readings occurred at the Sandy-2 sampling location. Again, the lake sulfide concentrations are compared to the no effects concentration appropriately derived in the sulfide hydroponics test evaluating plant endpoints typically exposed to anaerobic environments. The toxic concentration of sulfide to these plant life stages may be well in excess of 90 µM.

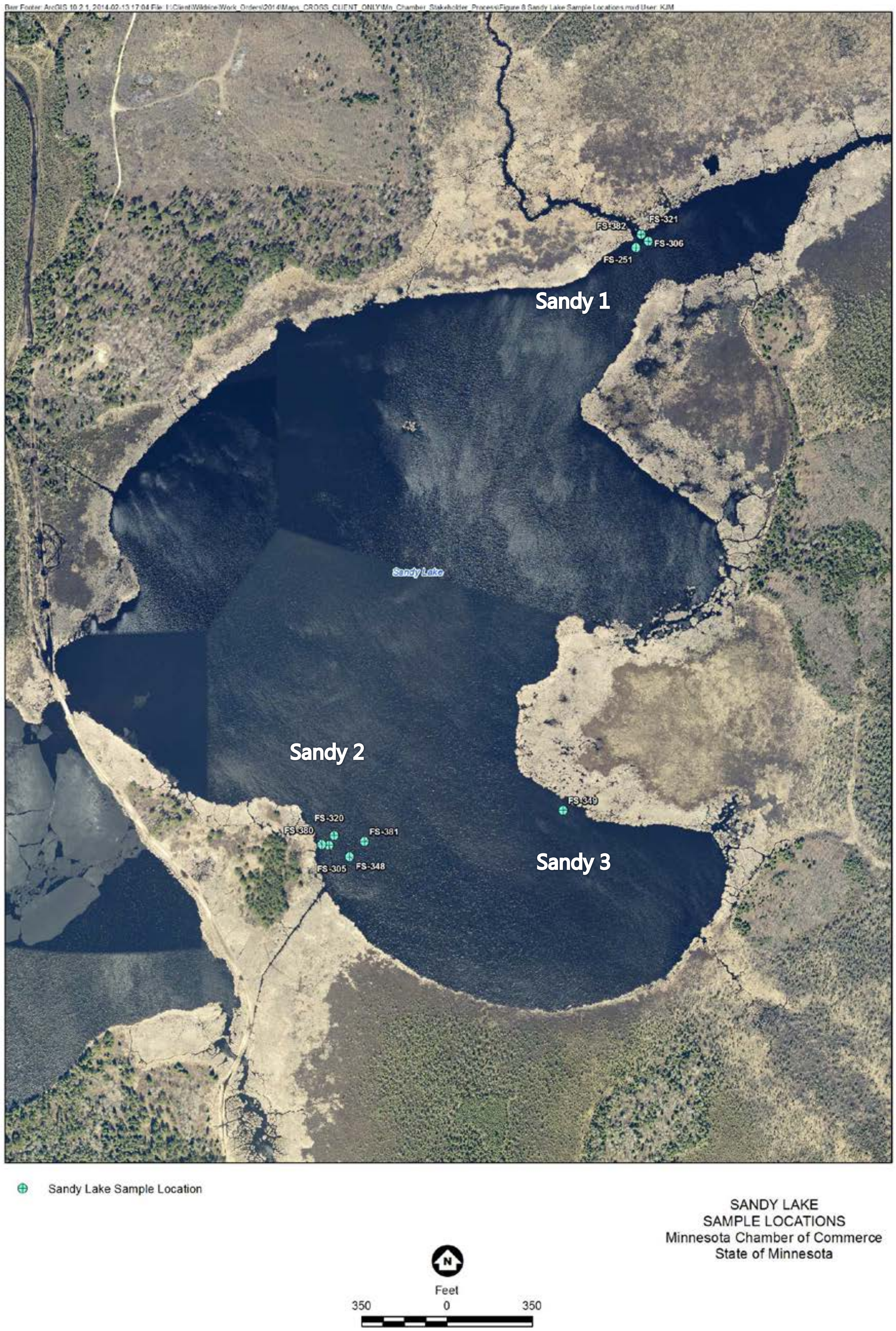

Figure 3-1 Sandy Lake Sample Locations

The lack of wild rice presence during the high sulfide (96 µM on July 9, 2013) observation is not clearly due to the porewater sulfide concentration. A month later, the sulfide concentration had dropped by an order of magnitude and wild rice was present. A month after that (September 2013) the sulfide concentration had dropped by another order of magnitude and wild rice was also present.

Fluctuating water levels likely confound the chemical analysis results (and perhaps other variables not measured). Sandy Lake is a very shallow lake, with a maximum depth of three feet,[43] and is subject to fairly wide fluctuations in water level. The Bois Forte study measured water levels bimonthly approximately every two weeks from May through early November each year in 2010-2013.[44] Water levels in 2013 varied by nearly 10 inches between May and November. Between June and July there was an increase of nearly 6 inches in water depth, or an increase of approximately 16 percent. While the highest sulfide reading at Sandy 2 occurred during June 2013, the month prior to the highest sulfide reading it is not clear whether water level fluctuations and sulfide levels are related or are simply confounding results.

Based on this analysis, Sandy Lake should not be considered as a lake with porewater sulfide greater than 90 µM. Bean and South Geneva lakes were only sampled once. Porewater sulfide from those samplings was greater than 90 µM. Even though Lady Slipper and Rice lakes have other measurements that are less than 90 µM, the other concentrations are sufficiently high that they will also be considered as lake with porewater sulfide greater than 90 µM. Again, despite periods of time when porewater concentrations exceed 90 µM, both Rice and Sandy lakes were observed to have wild rice present.

The four lakes with porewater sulfide concentrations greater than 90 µM are located in the Prairie Parkland ecological province or on the border between that province and the Eastern Broadleaf Forest (Figure 3-2). The significance of this finding is discussed further in Section 3.

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[43] MN DNR topographic map at http://files.dnr.state.mn.us/lakefind/data/lakemaps/c0826010.pdf and MPCA water quality database at http://cf.pca.state.mn.us/water/watershedweb/datasearch/waterUnit.cfm?WID=69-0730-00

[44] Sandy Lake and Little Sandy Lake Monitoring (2010-2013), Bois Forte Reservation Technical Report 13-06, December 2013

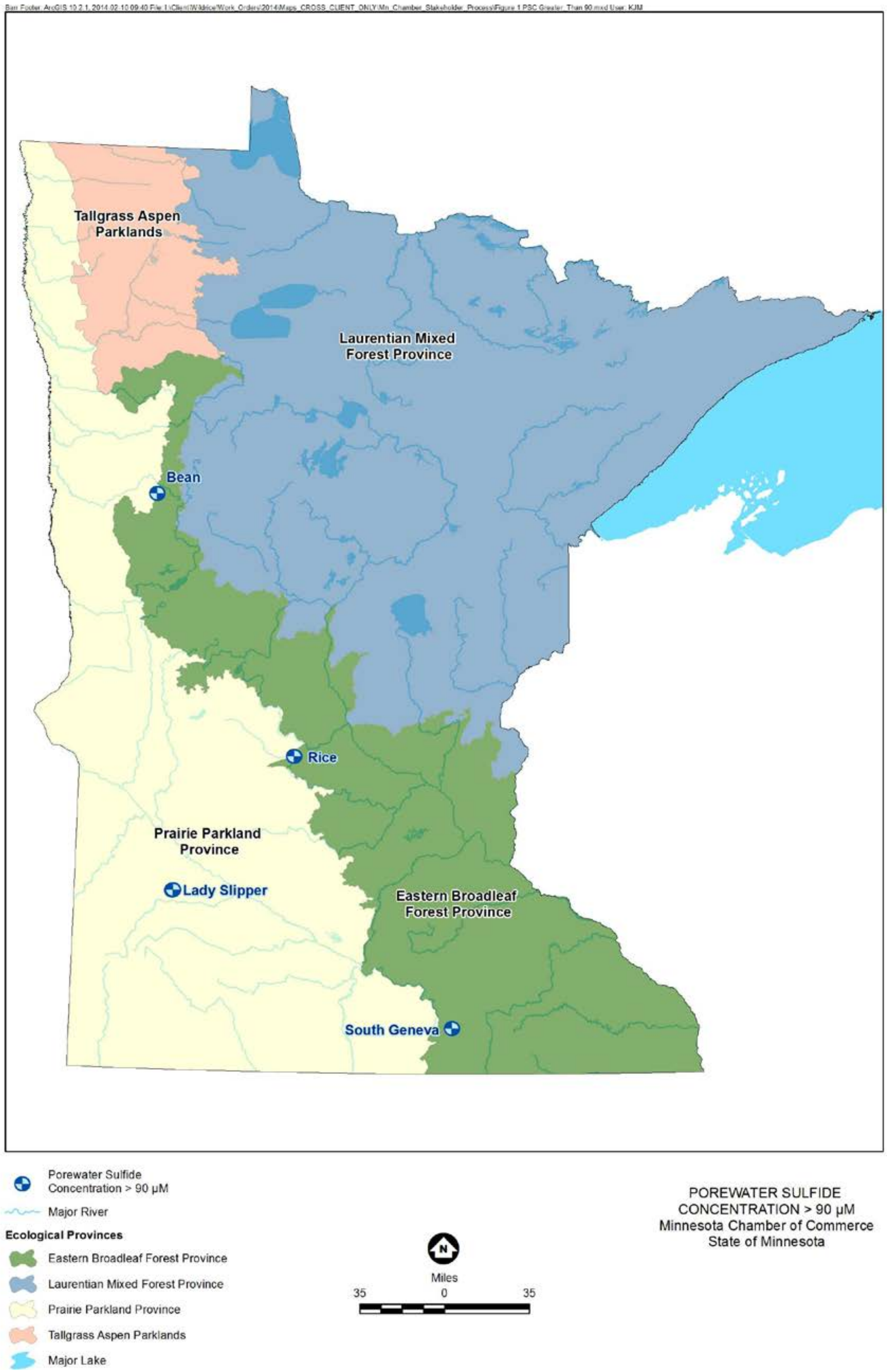

Figure 3-2 Porewater Sulfide Concentration >90 µM no effects concentration

3.4 MPCA's Outdoor Container Experiments

The MPCA's outdoor container experiments were seriously flawed, and no conclusions should be drawn from them. First, only one year's raw data were included in the appendices, making it impossible to analyze results from previous years' experiments. As a result of omitting data from years 2011 and 2012, it is not possible to know initial container conditions, including baseline sediment, porewater, and surface water physical conditions and chemical concentrations. Second, the containers are hydrologically isolated, preventing infusion of groundwater carrying iron or other constituents (e.g., plant micronutrients) that would be present in the natural environment. Nutrient depletion may also have occurred over time (without replenishment). Third, the mesocosms had been used in other experiments prior to initiation of testing in 2013. As a result, their history of sulfate exposure is unknown. The systems appear to be aged and to be potentially depleted of micronutrient prior to their use in 2013. Finally, in 2013, Dr. Pastor reported significant seedling mortality following thinning. As discussed by Dr. Pastor, seedling mortality may have been influenced by removal of five plants per tank in years 2011 and 2012 (one sixth of the population) resulting in depletion of the seed bank for future population growth.[45] In 2013, decreases in total plant biomass were not significantly correlated with increases in sulfate concentration.

3.4.1 Are there concerns about the methods?

Yes. Unlike the hydroponic experiments conducted by Dr. Pastor and Fort Labs, no test acceptability criteria were established to determine whether the test data were acceptable. In Dr. Pastor's sulfate hydroponic experiments, the following test acceptability criteria are established:

> *Tests were deemed acceptable if: 1. At least 90% of control juvenile seedlings were living at test termination; 2. Mesocotyl length of juvenile seedlings from control exposures were at least 5.0 cm at the end of the 10 day duration of growth; and 3. Control juvenile seedlings did not indicate any visible phytotoxic or developmental symptoms at any time during the test and the controls grew. See Appendix 2 for more details.*[46]

Dr. Pastor's sulfide hydroponic experiments had similar test acceptability criteria:

> *Tests were deemed acceptable using the same criteria as described above for the tests of sulfate on germination. See Appendix 3 for more details.*[47] (for seed germination and mesocotyl growth)

The Fort Environmental Labs study applied more rigorous test acceptability criteria (Table 3-2):[48]

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[45] Effects of enhanced sulfate concentrations on wild rice populations: results from a mesocosm experiment, John Pastor, University of Minnesota Duluth, December 2013
[46] Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor, University of Minnesota Duluth, December 2013
[47] Id.
[48] Definitive Hydroponics-Based Wild Rice (*Zizania palustris*) Sulfate Toxicity Testing, Fort Environmental Laboratories, December 2013

Table 3-2 Fort Labs Hydroponic Studies Acceptability Criteria

Criterion	Acceptable Limits	Criterion Passed? (d21 value, if applicable)
Control activation	95%	√ (100%)
Control mesocotyl emergence	≥30%	√ (38.3%)
Control survival	≥90%	√ (100%)
Positive control (BA) phytotoxicity	≥80%	√ (100%)
pH	6-7.5 in all replicates of control and treatments	√ (within range)
Water temperature	21° ± 2°C (day), and nightly, 12 ± 2°C (night) in all replicates of control and treatments	√ (within range)
Sulfate concentration	Inter-replicate CV ≤20% for control and treatments for individual measurement set (Study Day 0, 10, and 21)	√

No test acceptability criteria were established for the outdoor container studies. [49] Significant but undefined mortality occurred in 2013 across all concentrations, including controls. High mortality is indicative of a test system unable to support healthy plants absent the presence of the test variables (i.e., increased sulfate). In most laboratories, the results would subsequently be qualified or rejected as unreliable, especially given the poor rate of control survival (i.e. 15 percent in 2013). Although not directly applicable, an attempt was made to compare the results of the outdoor container study to the test acceptability criteria for the hydroponics study. That comparison is provided in Table 3-3.

[49] Effects of enhanced sulfate concentrations on wild rice populations: results from a mesocosm experiment, John Pastor, University of Minnesota Duluth, December 2013

Table 3-3 Outdoor Container Study Acceptability Criteria

Hydroponic Experiment Acceptability Criteria	Outdoor Container Study – Criteria Passed?
At least 90% of control juvenile seedlings were living at test termination	Fail – less than 15% of control seedlings survived
Length of juvenile seedlings from control exposures were at least 5.0 cm at the end of the 10 day duration of growth;	Passed. Initial seedling stem and leaf length was 6.1, 6.6 and 6.8 cm. Final control seedling stem and leaf length were 10.1, 11.4 and 12.9 cm
Control juvenile seedlings did not indicate any visible phytotoxic or developmental symptoms at any time during the test and the controls grew.	Passed in part, unknown in part. Control seedlings grew (see above). Phytotoxic or developmental symptoms of controls were not reported.

Based on Dr. Pastor's criteria for the hydroponic experiments, the outdoor container studies do not pass the acceptability test.

The test design did not include groundwater or surface water recharge. Instead, the water levels were maintained by intermittent additions of well water or precipitation, and water quality was infrequently monitored. Although well water is considered ground water, it does not have the same chemical composition as shallow groundwater that would be in contact with water bodies in nature. Without nutrient and iron infused recharge, this experimental design more closely resembles a seasonal pond or pothole, where wild rice may not grow or grow as well as in a natural setting. The test design likely stressed the entire wild rice population and made the results questionable. Conditions with no groundwater infusion, and no inflow or outflow carrying additional nutrients are important constraints that confounded results.

It appears that the tanks were nutrient deficient including iron and perhaps other limiting trace metal nutrients. As discussed by Dr. Johnson in *Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms*, in hydrologically isolated mesocosms without the delivery of iron, it is likely that sulfide would build up. Without the benefit of measurements of initial conditions and data from previous years' experiments, no one can analyze the 2013 results. Similarly, without the benefits of measurements of initial conditions, no one can determine whether sulfide build up (unprecipitated by iron) that occurred or other substances (or lack of other substances) affected the test organisms. It may be that the third year of testing (2013) was a part of the normal life cycle of wild rice. Dr. Pastor notes:

Delays in the release of nitrogen from these litters in subsequent years may be responsible for the population oscillations of 3-5 year periods often seen in wild populations (Pastor and Walker 2006, Walker et al. 2010, Hildebrandt et al. 2012).[50]

The Great Lakes Indian Fish and Wildlife Commission also note;

Rice abundance can vary widely from year to year, especially on the most "lake-like" beds. The rule-of-thumb for lake beds: A typical four year period will include a bumper year, two fair years, and a bust year.[51]

The results between 2011 and 2013 may have been simply part of the natural low-density cycle of wild rice, caused, perhaps, by delays in release of nutrients from the litter.

Measured sulfate concentrations in the overlying water fluctuated considerably; therefore, assigning precise sulfate concentrations to each treatment was difficult. From the study, and from an analysis of the data provided for 2013, each treatment only achieved the nominal treatment level once, sometime in June 2013. Concentrations were less than the nominal treatment goal at all other times during the experiment.

Based on all of these considerations, the effects of sulfate or sulfide on wild rice could not be evaluated. The study should not be relied upon to inform or develop water quality regulations.

3.4.2 Are there concerns about data quality?

Only one year (2013) of data was made available, so verification of additional data cannot be conducted. Additional statistical analysis should be conducted, including ANOVA. Results from regression analysis do not allow for a comparison of means of multiple samples.

3.4.3 What conclusions can be drawn from the study?

Given the serious concerns with the methodology, this study cannot be used to inform or develop water quality regulations. In particular, the MPCA cannot rely upon the presentation and analysis of data from 2011 and 2012 if that data is not publicly available.

3.5 MPCA's Root Zone Geochemistry

3.5.1 Are there concerns about the methods?

This study consisted of two parts:

- Root zone geochemistry studies of the outdoor containers; and
- Root zone geochemistry studies of two field sites (one stream, one lake).

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[50] Id.

[51] Wild Rice Ecology-Harvest-Management, Great Lakes Indian Fish and Wildlife Commission, undated

As discussed above, the outdoor container study did not produce acceptable results, based on the condition of the containers, so the data from the root zone geochemistry study of the outdoor containers cannot be used.

The results of the root zone geochemistry study of the two field sites (with two different substrate sites at each field site) is useful, and can help provide data on sulfate to sulfide transformations. The field sites for the root zone geochemistry studies were chosen as ones where wild rice was observed in the field surveys; however it was not clear from the report that wild rice was growing in the precise locations where the peepers were deployed.

3.5.2 Are there concerns about data quality?

Only limited chemistry data were collected (as opposed to that collected in the field study, for example), limiting the types of geochemical models that can be developed. Other substances (e.g. manganese, nitrate, phosphorus) could also precipitate or tie up sulfide, and sediment interactions are much more complex than those measured in this experiment. The geochemical model is not sufficiently robust to address sediment complexity or be used as a predictive model to revise the wild rice sulfate standard. The geochemical model is also of limited utility because it was derived from an experimental approach that was not mass-balanced with respect to sulfur loading.

3.5.3 What conclusions can be drawn from the study?

Dr. Johnson reaches the following conclusions:

> *Sulfide concentrations in sediment pore fluids were almost always less than 10 μM (compared with 1000 – 7800 μg/cm^2 sulfate in the overlying water), and were often below the method reporting limit of 0.7 μM. The steep gradients of sulfate, sustained throughout the summer, and a lack of buildup in porewater sulfide indicate a consistent removal mechanism for sulfur in sediments. Iron concentrations at field sites were frequently in excess of 500 – 1,000 μM and precipitation of sulfide as iron sulfides provide a likely explanation for the low dissolved sulfide concentrations.*[52]

The Chamber agrees with these conclusions.

3.6 MPCA's Temperature Dependent Diffusion Rate Studies

3.6.1 Are there concerns about the methods?

This study must be considered an exploratory study of the fate and transport of sulfate into and out of sediments, and not a definitive test. Only two sediments were analyzed, limiting conclusions about other sediment characteristics. Sediments could not be extracted whole, but were homogenized prior to testing. The homogenization would have altered basic sediment chemical characteristics by exposing anaerobic sediments to oxidizing conditions.

[52] Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms, Nathan W. Johnson, University of Minnesota Duluth, December 2013

3.6.2 Are there concerns about data quality?

Yes, first one of the sediment samples had significant quantities of sulfate in the porewater. Second, there were difficulties in achieving equilibrium. Third, there were difficulties in defining the water/sediment interface.

3.6.3 What conclusions can be drawn from the study?

Dr. Johnson draws the following conclusions:

> Negligible dissolved sulfide was generated in sediment pore waters despite the 300 to 400 mg/L sulfate in overlying waters for 11 weeks.[53]
>
> Sufficient sediment iron clearly eliminates the build-up of dissolved (toxic) sulfide in sediments. Increases in soluble sediment iron concentrations during the test indicate iron will not be depleted.[54]

The Chamber agrees with Dr. Johnson's conclusions that with sufficient naturally occurring porewater iron concentrations, there will negligible accumulation of porewater sulfide concentrations.

3.7 Summary

Given the high concentration of sulfate needed to have an effect on the growth of wild rice (1,600 to 2,500 mg/L in the hydroponic experiments), and the MPCA's field survey showed no relationship between wild rice coverage and surface water sulfate concentrations, a sulfate water quality standard is unnecessary.

However, if the MPCA decides there needs to be a surface water standard, the standard should be 1,600 mg/L sulfate where wild rice is present. Two sulfate hydroponics experiments were conducted producing results support such an increase[55]:

- 1,600 mg/L (Sulfate did not affect either seed germination or seedling growth other than a slight depression of root lengths at extremely high concentrations (1,600 mg SO4 · L-1). – not statistically significant)[56]

- 2,500 mg/L(The no observed effects concentration (NOEC) for three of the ten Study Day 10 NOEC values were 2,500 mg/L sulfate or lower, and seven of ten SD 10 NOEC values were 5,000

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[53] Temperature Dependent Diffusion Rates of Sulfate in Aquatic Sediments. Nathan W. Johnson, Will DeRocher, University of Minnesota Duluth, December 2013
[54] Id.
[55] In accordance with EPA guidelines and state rules for establishing water quality regulations, hydroponic testing is required. See Minn. Rules 7050.0218 and "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses," USEPA, Office of Research and Development, Environmental Research Laboratories, Duluth MN; Narragansett, RI, Corvallis, OR, 1985
[56] Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor University of Minnesota Duluth, December 2013

mg/L sulfate. For Study Day 21, eight of ten concentration endpoints exhibited NOEC values of 5,000 mg/L sulfate, indicating that sulfate was generally not toxic at the highest concentration that could be tested within the limits of solubility of the salts.[57])

While seedlings were not adversely affected in the Fort Environmental Labs study up to and including exposures of 2,500 mg/L sulfate, the Chamber is concerned that exposures exceeding 1,600 mg/L may, like any ion, pose a salinity stress (osmotic stress) potentially adversely affecting wild rice during its life cycle. This conservative assessment assumes that wild rice is salt sensitive and that exposure exceeding 1,600 mg/L could potentially impose an abiotic stress. Exposures up to 90 µM sulfide in a hydroponic experiment did not affect wild rice seed germination or mesocotyl length.

The juvenile seedling sulfide hydroponic studies and the outdoor container study raised serious concerns about the methodology. The entire seedlings (root and shoot) were exposed to sulfide treatments during the test; plant parts above the root-shoot node (crown) would not grow in anaerobic conditions where sulfide would form. Large seedling die-off occurred in 2013 for reasons that are not clear. Since MPCA did not provide the data from 2011 or 2012, it is not possible to evaluate initial tank conditions. Data from these studies cannot provide useable data for determining the relationship between surface water sulfate, porewater sulfide porewater iron and wild rice growth.

[57] Definitive Hydroponics-Based Wild Rice (*Zizania palustris*) Sulfate Toxicity Testing
Fort Environmental Laboratories, Inc., December 2013

4.0 MPCA's Goals, Primary Hypotheses - Chamber's analysis

4.1 Overview

In Section 3, the Chamber reviewed each of the MPCA's studies to determine whether the studies could be used to address the questions posed by the MPCA at the February 3, 2014 Advisory Committee Meeting:

- *Are there concerns about the methods?*
- *Are there concerns about data quality?*[58]

In addition, the Chamber reviewed each MPCA study to determine whether the studies could provide scientifically valid conclusions.

In this section, the Chamber integrates all of the studies, along with other public, credible data to address the goals and hypotheses posed by the agency.

The MPCA sets the following goal of all of the studies:

> *The goal of the Wild Rice Sulfate Standard Study is to enhance understanding of the effects of sulfate on wild rice and to inform a decision as to whether a revision of the wild rice sulfate standard is warranted.*[59]

The MPCA also notes that the studies need to determine:

> - *The effect of sulfate on wild rice and*
> - *The effect of sulfide on wild rice*
>
> *Note: To inform the wild rice sulfate standard review, an important aspect of this analysis will be determining what concentrations of sulfate and sulfide are protective of wild rice, which may be different than the concentrations at which effects are observed in the study results.*

The first step is to determine how studies can inform the effects of sulfate and sulfide on wild rice. The Chamber notes that the United States Environmental Protection Agency (US EPA) specifies methods to set water quality standards for aquatic plants:

[58] Input on Study Reports, MPCA, February 2014
[59] Wild Rice Sulfate Study Summary and Next Steps, MPCA, December 2013

The Final Plant Value should be obtained by selecting the lowest result from a test with an important aquatic plant species in which the concentrations of test material were measured and the endpoint was biologically important[60].

Given the very low toxicity of sulfate to wild rice as confirmed in two independent studies, the MPCA also assumed that it was likely that sulfate by itself would not impact wild rice, and proposed the following hypotheses:

- *wild rice is impacted by sulfate via the conversion of sulfate to sulfide in the rooting zone of the plants* and
- *iron may mitigate the effects of sulfide production in the rooting zone of the sediment*[61]

The MPCA has acknowledged that no single study can prove the hypotheses; rather, an integration of the data from all of the studies (and perhaps other sources as well) may demonstrate the veracity of the hypotheses.

Based upon the field surveys and hydroponic sulfate studies there is no correlation between sulfate in surface water and wild rice growth; sulfate does not affect wild rice growth except at very high levels (e.g., 1,600 mg/L) (which effects are likely caused by osmotic stresses brought on by high concentrations of any salt, including sulfates).

Based upon the field surveys and hydroponic sulfide studies and the data analysis presented here, there is a correlation between sulfide in porewater and wild rice growth, but that porewater sulfide does not affect wild rice seed germination or mesocotyl growth even at very high concentrations (e.g., above 2.4 mg/L or ~90 µM). Only lakes in the western and southern portions of the state have sulfide levels exceeding 90 µM, and only where iron concentrations are very low (e.g., less than 1,000 µg/L ~ 2 µM).

The data analysis presented here indicates that the first hypothesis is only partially supported by the useable research provided by the MPCA and other credible, public evidence. There is evidence that sulfate in the overlying surface water diffuses into the sediment and is converted to sulfide in the rooting zone of the plants (porewater), but only in the absence of sufficient iron to precipitate the sulfide. However, in all streams and most lakes in Minnesota, there is ample iron to precipitate the sulfide. Where there is no discharge of sulfate to the water body, the sulfide in porewater is dependent on other factors such as parent soil material in which the lake is located, which is in turn affected by a number of climatic, geologic and biologic factors.

The second hypothesis is strongly supported by the useable research and other credible, public evidence. In all streams and in the majority of the lakes surveyed, there is more than sufficient porewater iron to

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[60] Guidelines for Deriving Numerical National Water Quality Criteria for the Protection Of Aquatic Organisms and Their Uses, US EPA**,** PB85-227049. December 2010

[61] Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms, Nathan W. Johnson, University of Minnesota Duluth, December 2013.

prevent the accumulation of sulfide in porewater to any significant concentration. The few lakes where high sulfide is observed are lakes that reflect the parent material and groundwater in which they are located, and tend to be lower in porewater iron.

4.1.1 Effects of Sulfate and Sulfide on Wild Rice

From the scatter plots presented in the field survey report, there is no statistically significant correlation between surface water sulfate and wild rice cover. Taking the data as presented (e.g. untransformed), the probability of fitting a linear (p=0.375), quadratic (p=0.110), and cubic (p=0.179) relationship to the data indicate that there is no significant relationship between surface water sulfate and wild rice cover. Viewing the data in three dimensions (plant cover, surface water sulfate, and porewater iron) shows that there is no correlation between surface water sulfate and wild rice growth (Figure 4-1).

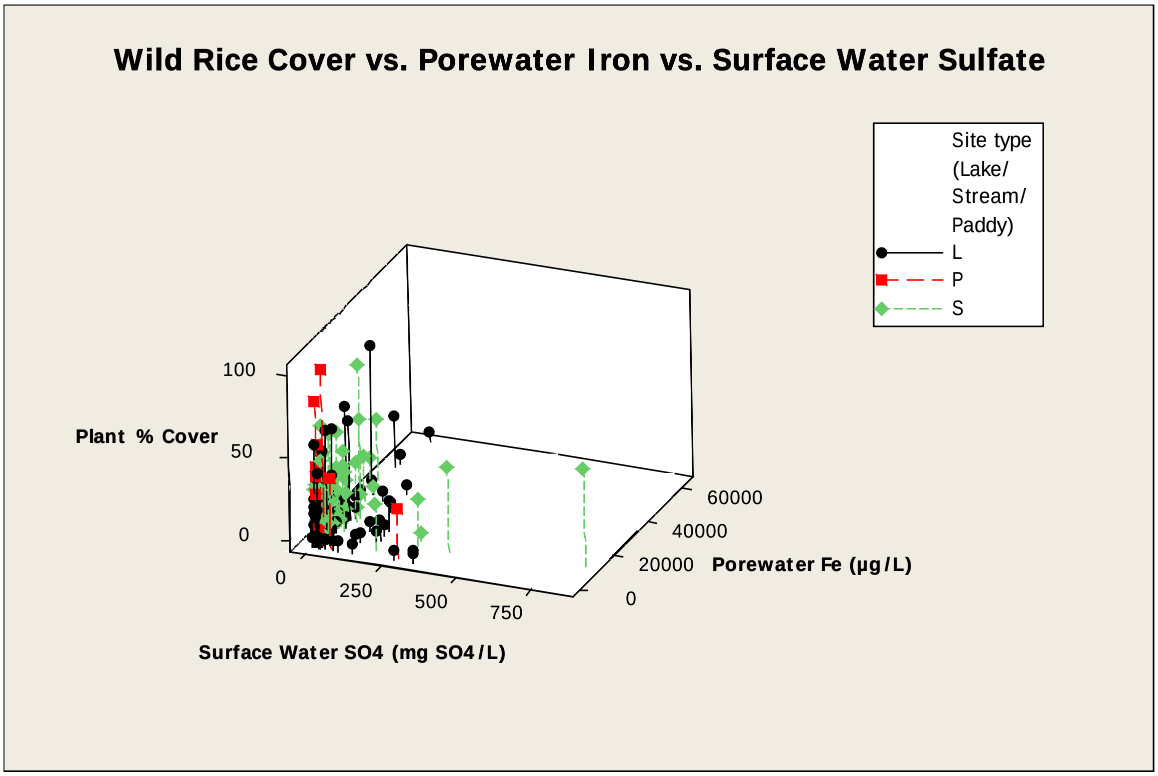

Figure 4-1 Wild Rice Cover vs. Porewater Iron vs. Surface Water Sulfate

There is, however, a statistically significant correlation between porewater sulfide and wild rice cover. Taking the sulfide data as presented (e.g. untransformed), the probability of fitting a linear (p=0.115), quadratic (p=0.066), or cubic (p=0.144) relationship to the data is not significant[62]. However, if the highest outlier (Bean Lake, porewater sulfide = 16.0 mg/L (500 µM) is removed, a quadratic (p=0.088), and cubic (p=0.166) relationship is not significant, but a linear relationship is (p=0.028). The linear regression

[62] In keeping with standard practice, $p \leq 0.05$ is taken as statistically significant.

was y = -6.401 x +16.90; adjusted R^2 is 2.1% (x-intercept is 2.64 mg S/L) (Figure 4-2). Also, despite the statistically significant linear relationship between sediment porewater sulfide and wild rice cover, the very low R^2 value (correlation coefficient) indicates that very little data variability is accounted for by the regression. Thus, other factors play a more important role in the presence of wild rice.

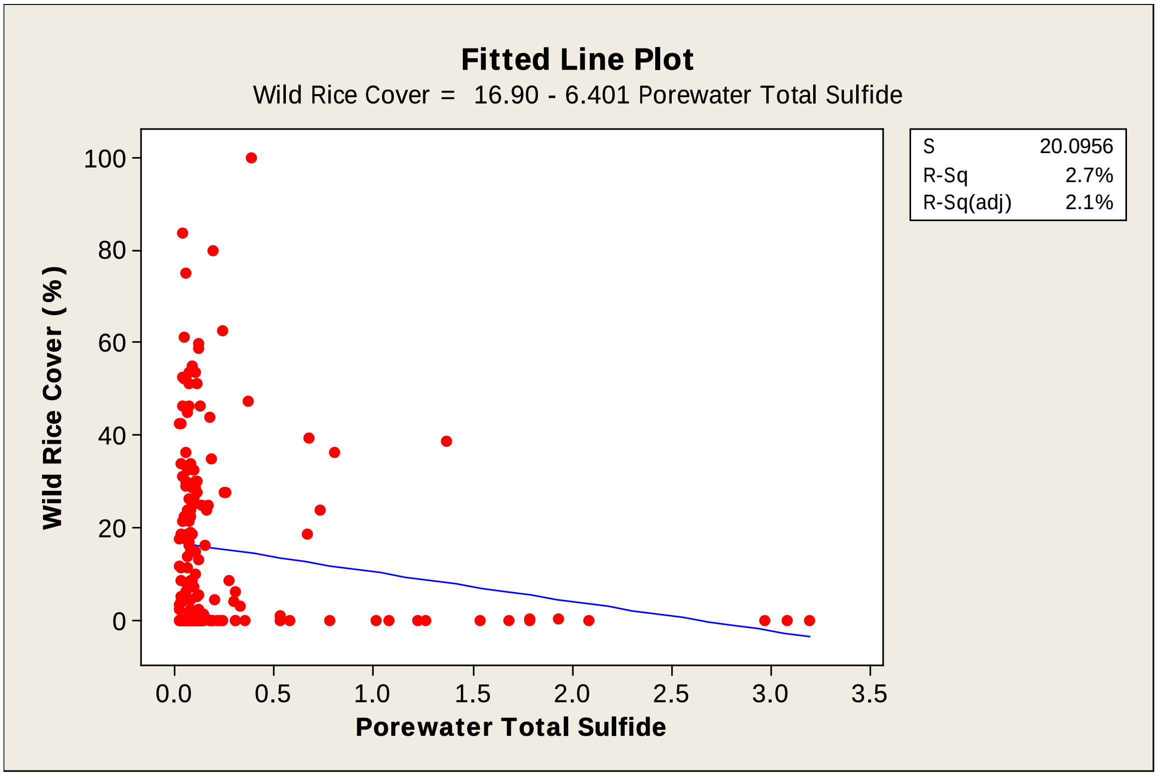

Figure 4-2 Fitted Line Plot

The significance of the x-intercept is that one would expect that when the porewater sulfide is greater than 2.64 mg S/L (83µM), one would expect that wild rice would not be present. Three lakes (no streams or paddies) had porewater sulfide concentrations greater than 2.2 mg S/L (83 µM), plus two outlier lakes (Bean Lake, as noted above and one of the samples at Lady Slipper Lake). None of these lakes had wild rice present in any of the surveys. Given the poor correlation coefficient for the relationship between wild rice cover and porewater sulfide, the resulting 83 µM sulfide intercept (the concentration at which wild rice would likely not grow) should only be considered an approximate sulfide concentration potentially limiting wild rice growth under some field conditions. A better value to use is the value derived from the sulfide hydroponic studies – 90 µM as the No Observed Effect Concentration (NOEC).

Wild rice cover was plotted (Figure 4-3) as a three-dimensional space with porewater iron and porewater sulfide.

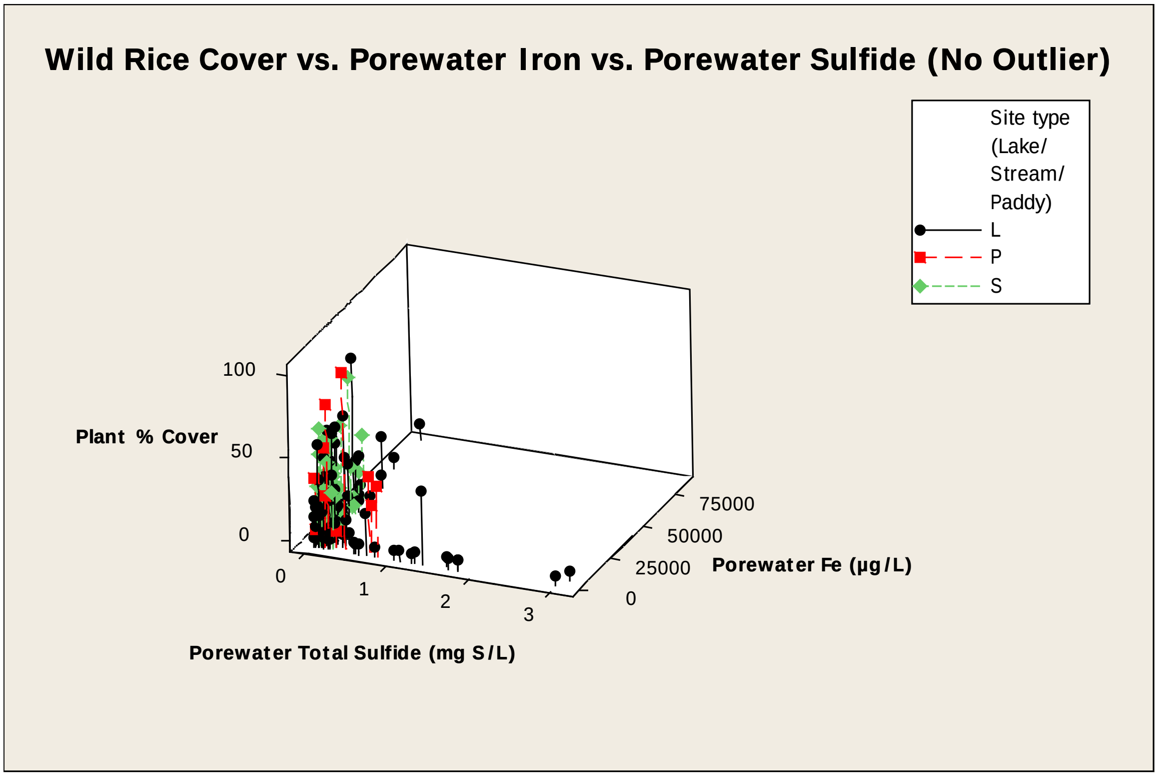

Figure 4-3 Wild Rice Cover vs. Porewater Iron vs. Porewater Sulfide (no Outlier)

Because the updated field survey data were not available until January 29, 2014, only the relationships between wild rice growth, sulfide and iron could be explored fully in order to meet the MPCA's timeline for a preliminary determination.

4.1.2 MPCA caveats regarding sediment interactions and limitations on research

Sediment interactions of sulfur are extremely complex, involving physical interactions (e.g. diffusion) between sediment and overlying water and between sediment and groundwater, chemical interactions (e.g. precipitation of dissolved sulfide) and biological interactions (e.g. conversion of sulfate to dissolved sulfides, precipitation of sulfides by iron (and perhaps other substances), the role of nutrients in these and other pathways). Other component interactions are occurring simultaneously. The MPCA attempted to illustrate these interactions in Figure 4-4.

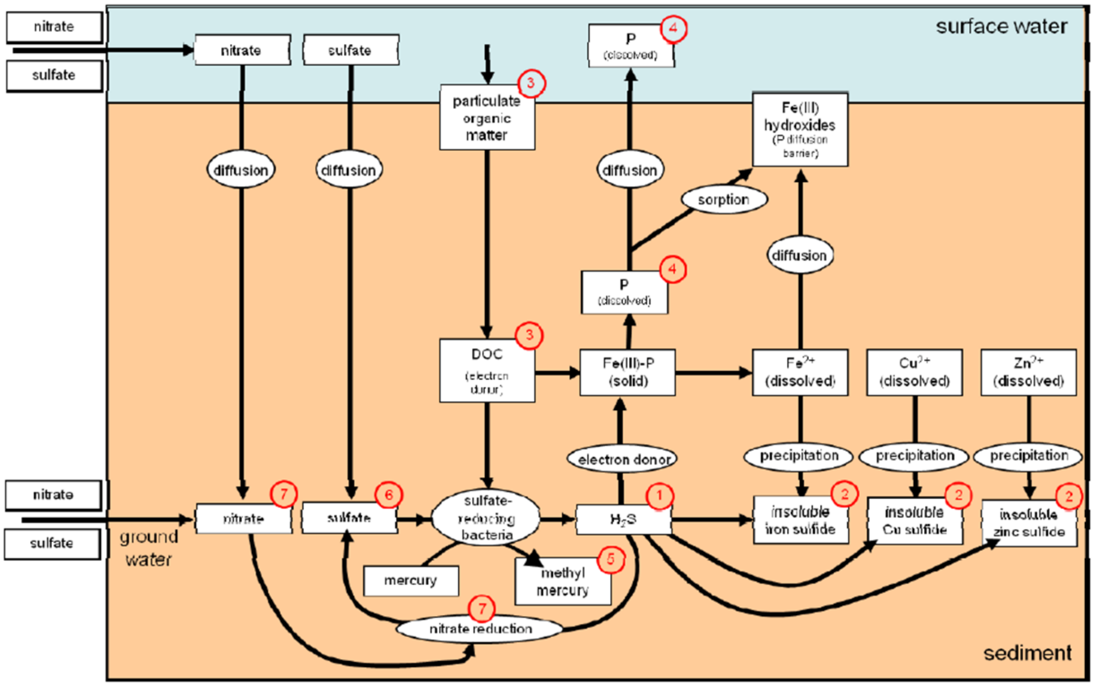

Figure 4-4 Sulfur interactions in wetland sediments that might affect wild rice growth

The research conducted by the MPCA only began to explore the sulfur interactions, and failed to explore interactions of other components, nor were the relationships between sulfur and other components explored.

4.1.3 Integration of Data – Sulfate Water Quality Standard

First, the Chamber attempted to integrate the data from the studies and from other publicly available data to answer two of the regulatory questions posed by the MPCA:

- Does the scientific evidence indicate that the standard should go up or down and, if so, generally by how much?
- Should there be a different standard for lakes/wetlands, or streams, or paddy rice?

4.1.4 Integration of Data – Period when "rice may be susceptible to high sulfate"

Secondly, the Chamber attempted to integrate the data from the studies and from other publicly available data to answer the third regulatory question posed by the MPCA:

What more can be said about the "period when the rice may be susceptible to high sulfate?

4.2 Integration of Data – Sulfate Water Quality Standard

First, the data from the field survey were analyzed to determine whether they support the MPCA's two primary hypotheses – that wild rice is impacted by sulfate via the conversion of sulfate to sulfide in the rooting zone of the plants and iron reduces sulfide production in the rooting zone of the sediment by precipitating it out of solution as iron sulfide.[63]

It is also noted that several mining companies have been requested or required to undertake wild rice field surveys. These surveys have been conducted using methods similar to those used in the field survey conducted by the University of Minnesota. Surface water grab sampling near wild rice stands was carried out as part of the private surveys, but not for the extensive list of analytes conducted by the University. All areas of wild rice identified in these surveys had surface water sulfate concentrations greater than 10 mg/L; the highest observed (1,040 mg/L) was in an area also monitored by the University as having 838 mg/L sulfate.

A list of the surveys which have been submitted to the MPCA is included in Appendix A. The MPCA should incorporate the results of these surveys in their regulatory deliberations as further evidence of wild rice growth at surface water sulfate concentrations above 10 mg/L.

4.2.1 Confirmation of Hypotheses – Integration of Data

A relationship between sulfide, sulfate and iron was developed from the field survey data for lakes and streams data (paddies not included). That relationship is given in Equation 4-1:

Equation 4-1

$$\text{Porewater Sulfide (mg/L)} = 6.42 * \text{Surface Water Sulfate (mg/L)}^{0.00427} * \text{Porewater Iron } (\mu\text{g/L})^{-0.445}$$

Or

$$\text{Sulfide} = 6.42 * SO_4^{0.00427} * Fe^{-0.445}$$

This relationship had an r^2 between observed and predicted sulfide of 0.595, and was significant (*prob. <0.001)*, (Figure 4-5). The relationship was derived from 198 observations. Forty Six (46) observations with missing sulfide sulfate or iron data were eliminated from the original data set. Two (2) sulfide outlier values were eliminated, as were all true duplicates. Values which were reported as less than detection limits (e.g., < values) were included at the detection limit.

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[63] Id.

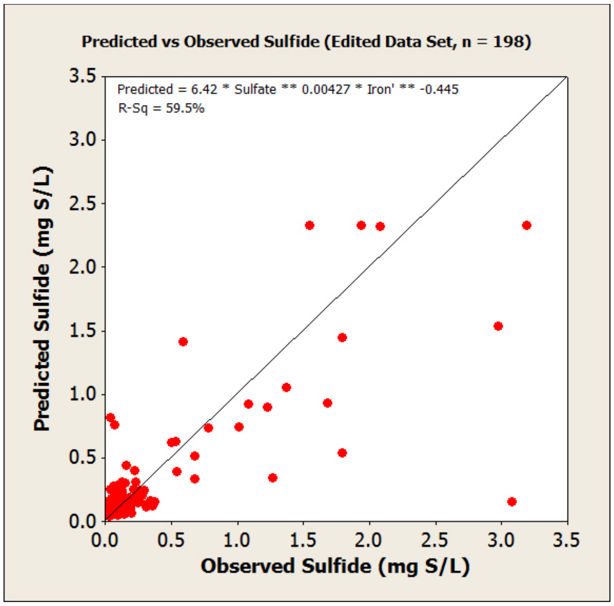

Figure 4-5 Relationship between observed sediment pore water sulfide in lakes and stream sediments and that predicted by a non-linear relationship with surface water sulfate and sediment pore water iron as predictor variables, n = 198 (1:1 line indicated).

The explanatory power of this equation is surprisingly high considering that the data represented 198 samples from uncontrolled natural systems (lakes and streams) and the samples were collected over a period of three years during varying times of the growing season.

Put simply, if there is sufficient iron available in the sediment; it will tie up any sulfide generated in the sediment, making it biologically unavailable to wild rice. There is such a small exponent on the sulfate concentration (two orders of magnitude lower than the exponent on the iron concentration) that the surface water concentration is nearly inconsequential. In fact, nearly the same amount of variability can be explained by a power formula using only iron and the field survey data:

Equation 4-2

$$\text{Sulfide} = 6.51 * \text{Fe}^{-0.446}$$

This relationship had an r^2 between observed and predicted = 0.595 and was significant (*prob. <0.001)* Figure 4-6.

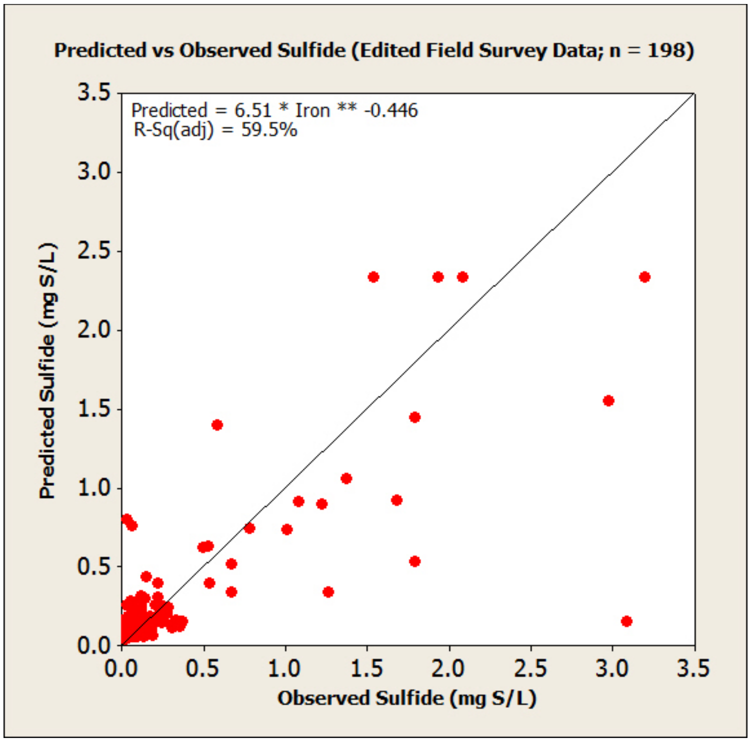

Figure 4-6 Relationship between observed sediment pore water sulfide in lakes + stream sediments and that predicted by a non-linear relationship with only sediment pore water iron as a predictor variable, n = 198 (1:1 line shown)

The relationships in Equation 4-1 and Equation 4-2 do not explain all of the variability observed in the field, but they do account for a large portion of that variability, and amply demonstrates that the MPCA's hypothesis was well founded with respect to the role of iron in mitigating the presence of dissolved sulfide. The hypothesis that surface water sulfate is a key determinant in the formation of sediment porewater sulfide is not supported.

In other words, Equation 4-2 confirms one of the primary hypothesis:

> *..., if elevated sulfate has a negative effect on the growth of wild rice, it is mediated through the formation of hydrogen sulfide in the rooting zone of wild rice, and that elevated iron would mitigate the toxicity of the sulfide by forming insoluble iron sulfide compounds*[64].

The derivation of these relationships (Equation 4-1 and Equation 4-2) is provided Appendix B.

4.2.2 Alignment of Hydroponic Experiments

Next, the Chamber reviewed the Hydroponic Experiments to see whether the data were consistent with the relationship developed from the field surveys.

[64] Wild Rice Sulfate Study Summary and Next Steps, MPCA, December 2013

4.2.2.1 Sulfate Hydroponic Experiments

The sulfate hydroponic experiments demonstrated that sulfate was not toxic to wild rice plants at any life stage tested, even at extremely high sulfate concentrations. The University of Minnesota Duluth study found no impacts up to 1,600 mg/L, while the Fort Environmental Labs study found LOECs of 5,000 mg/L sulfate and > 5,000 mg/L sulfate for the full 21 d exposure period, depending upon the biological endpoint. None of the field surveys showed sulfate concentrations near these levels. The statistical analysis of the field data showed that there was little, if any, correlation between porewater sulfide and surface water sulfate.

These conclusions are consistent with the relationship developed from the field surveys. The amount of sulfate in the overlying surface water had little influence on the amount of porewater sulfide, and sulfate by itself does not impact the growth of wild rice.

4.2.2.2 Sulfide Hydroponic Experiments

The portion of the sulfide hydroponics experiments that addressed seed germination and mesocotyl growth demonstrated that sulfide was not toxic above 90 µM sulfide. That concentration can be considered a No Observed Effects Concentration or NOEC value.

Only a few of the lakes (no streams or paddies) in the field survey had sediment sulfide concentrations greater than 90 µM (Figure 4-7).

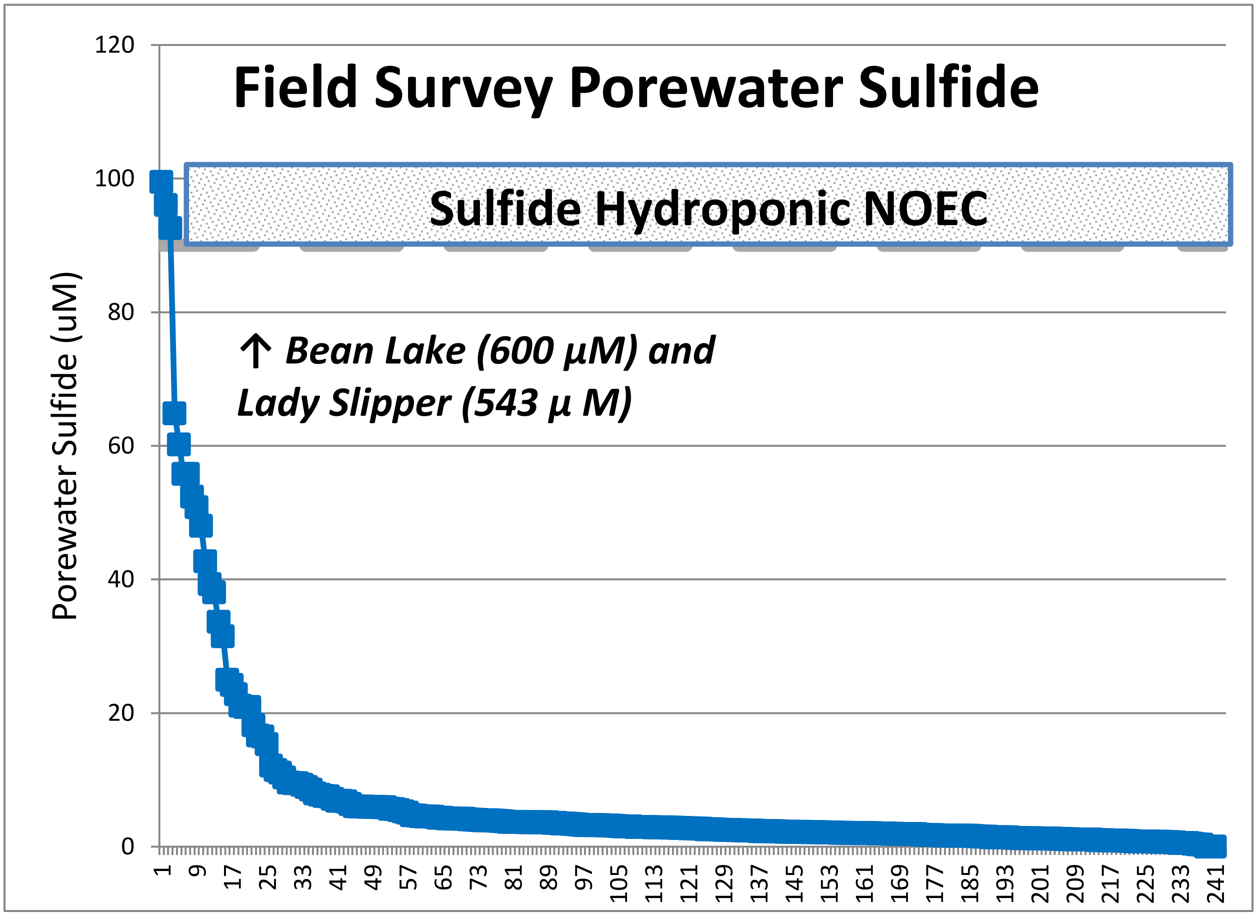

Figure 4-7 Field survey porewater sulfide (rank ordered largest to smallest) vs NOEC from sulfide hydroponic experiments

These include the two outlier lakes:

- Bean (Becker Co – 600 µM)
- Lady Slipper (Lyon Co – 543 µM) and

as well two others:

- South Geneva (Freeborn Co – 99 µM)
- Rice (Stearns Co – 93 µM)

Recall that one of 10 observations from Sandy Lake in St. Louis County had sulfide porewater concentrations greater than 90 µM. Because only one of ten observations was greater than90µM, the Chamber determined, based on the weight of evidence approach, that Sandy Lake did not consistently have sulfide porewater concentrations greater than 90 µM. See Section 2.3.2.3 above.

The location of these lakes is shown in Figure 3-2.

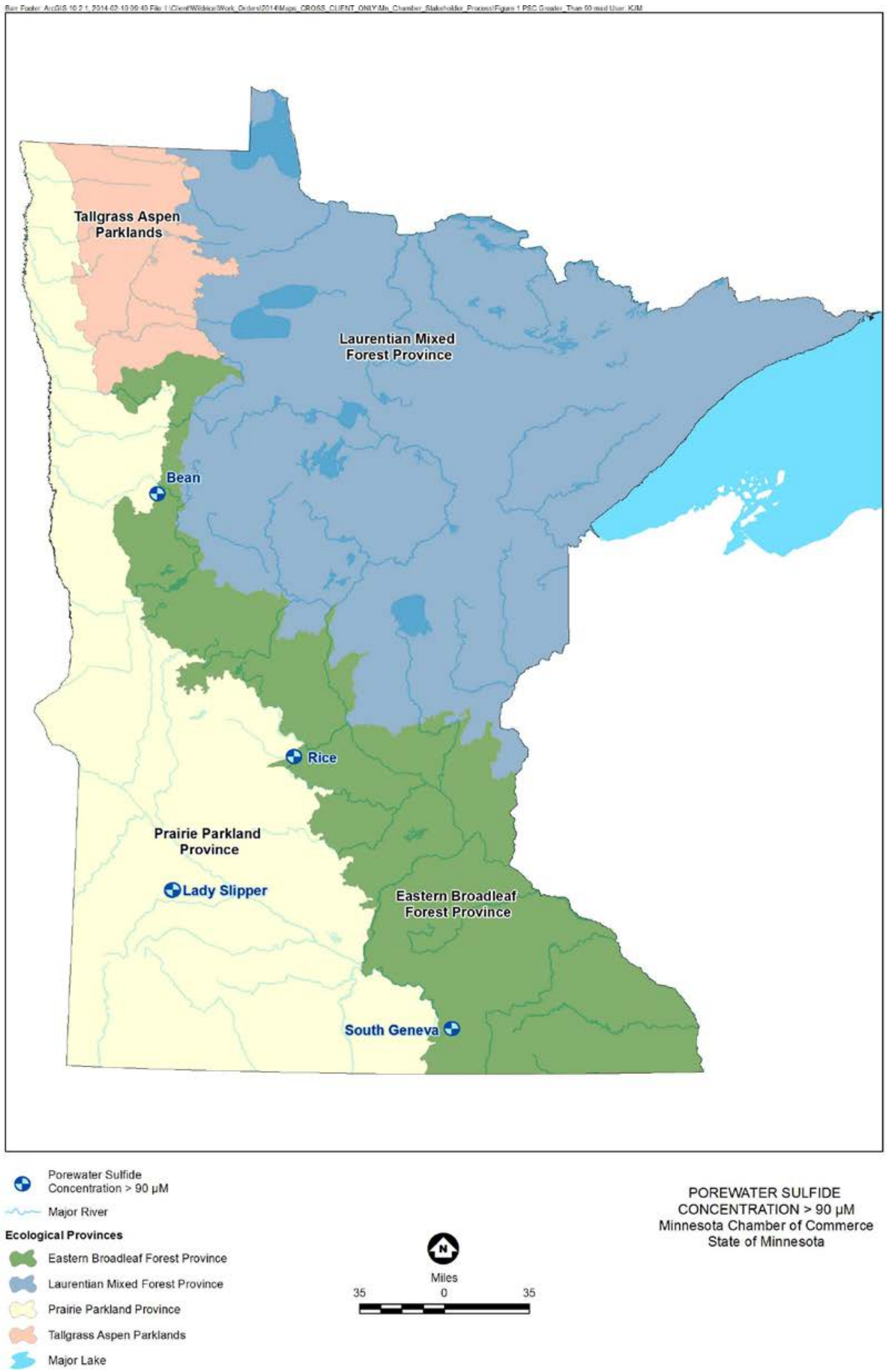

Figure 3-2 Porewater Sulfide Concentration >90 µM no effects concentration

These lakes are all located in the Prairie Parkland Ecological Province or on the border of the Prairie Parkland and Eastern Broadleaf Forest Ecological Province.

Table 4-1 summarizes the sulfide, sulfate and iron data from the field survey. Note that none of these observations were associated with the presence of wild rice.

Table 4-1 Lakes with Sulfide >90µM

Lake Name	Inventory Number	County	SO4 (mg/L)	Sulfide (µM)	Fe (µg/L)
Bean	03-0411-00	Becker	85	500	50
Lady Slipper(1)	42-0020-00	Lyon	108	463	638
South Geneva	24-0015-00	Freeborn	14	99	< 10
Rice(1)	73-0196-00	Stearns	3	93	25

1 Multiple samples were taken at these lakes. Only the sample set with porewater sulfide concentrations >90µM are shown.

These four lakes have very low porewater iron concentrations, with three in the lowest five percentile of iron concentrations and one (Lady Slipper) in the lowest 10 percentile. The data from these four lakes are consistent with the results of the sulfide hydroponic studies.

The Minnesota Department of Natural Resources (MN DNR) lists two of the lakes as part of their Natural Wild Rice in Minnesota Inventory:[65]

- Bean (but no wild rice acreage indicated)
- Rice (but no wild rice acreage indicated)

It should be noted that the "Geneva" Lake listed in the DNR inventory is North Geneva Lake, a different lake, which has much lower porewater sulfide, but no wild rice was found during the most recent survey.

Next the size, depth, and clarity of these lakes are explored in Table 4-2.

Table 4-2 Lakes with Sulfide >90µM Lake Properties

Lake Name	Inventory Number	County	Acres	Littoral Area (acres)	Maximum Depth (ft)	Water Clarity (Secchi Disk – ft)
Bean	03-0411-00	Becker	14	14	9	-
Lady Slipper(1)	42-0020-00	Lyon	286	286	11	1.7
South Geneva	24-0015-00	Freeborn	2,214	2,214	8	0.2
Rice(1)	73-0196-00	Stearns	1,509	958	41	3.6

1 Multiple samples were taken at these lakes. Only the sample set with porewater sulfide concentrations > 90 µM are shown.

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[65] Natural Wild Rice In Minnesota: A Wild Rice Study. Minnesota Department of Natural Resources, February 2008

All four are very shallow lakes, likely subject to winter fish kills (when the dissolved oxygen levels drop to levels too low to support fish in the lake). While not all four lakes had information on fish kills, the information from the MN DNR LakeFinder website for Lady Slipper Lake might be typical of such shallow, prairie pothole lakes:

> *Both partial summer kills and winterkills have occurred in Lady Slipper, but these have not been documented since the late 1990's. However, a winterkill assessment was conducted during late April of 2010 for Lady Slipper Lake.*[66]

And

> *Oxygen levels in Lady Slipper were near 13 ppm during late December of 2009, 3 ppm during mid-January of 2010, 1.7 ppm by mid-February of 2010, 1 ppm during late February of 2010, and 15 ppm by mid-March of 2010. The aeration system was started during mid-January of 2010 on Lady Slipper. One surface aerator quit during late February.*[67]

Similarly the information for Rice Lake from the MN DNR LakeFinder website for Rice Lake may also be typical for such shallow prairie pothole lakes in agricultural areas:

> *Nutrient runoff enters Rice from agricultural row crops, feedlots/pasture areas, city storm sewer, and lake residential sources. Water clarity was poor during mid-July of 2012 (secchi=3.0 feet). Dissolved oxygen levels were less 1 ppm below 18 feet deep during the survey. Low water levels due to drought conditions and high summer air temperatures were the norm during the 2012 summer. Nutrient levels (total phosphorus=0.049 ppm, chlorophyll a=36.3 ppm) were moderately high during June of 2007.*

Finally, carp are known to harm wild rice by uprooting the wild rice plants and consuming seeds from the sediment.[68] Rice Lake was noted as having carp in the MN DNR FishFinder website.[69]

4.2.3 Alignment of Root Zone Geochemistry Experiment

The Chamber reviewed the Root Zone Geochemistry Experiments to see if the data presented there aligned with the relationship developed from the Field Study. Dr. Johnson concludes that root zone geochemistry supports the theory of sulfide being mitigated by sediment iron, and this conclusion is consistent with Equation 4-1 and Equation 4-2:

> ***This oversaturation of porewaters with iron and sulfide highlights the critical role of ferrous iron in controlling dissolved sulfide in porewaters*** *and indicates that the precipitation of solid*

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[66] MN DNR LakeFinder interactive website: http://www.dnr.state.mn.us/lakefind/index.html
[67] Id.
[68] Effectiveness of Temporary Carp Barriers for Restoring Wild Rice Beds in Upper Clam Lake: 2010 to 2013, Freshwater Scientific Services, October 2013
[69] MN DNR LakeFinder interactive website: http://www.dnr.state.mn.us/lakefind/index.html

phase iron sulfides in surficial sediments represents a sink for removing dissolved iron and sulfide from sediment porewaters.[70] (emphasis added)

Thus, while sulfate can diffuse into sediment and can be converted to sulfide, accumulation occurs only where there is insufficient iron in the porewater. With only a few exceptions, ample naturally occurring iron is present to precipitate dissolved sulfide and prevent i accumulation in porewaters. The mean porewater sulfide concentration observed in the field surveys was 3.9 µM.

4.2.4 Alignment of Temperature Dependent Diffusion Rate Studies

Dr. Johnson concluded in his study of transport and reaction of sulfate between overlying waters and sediment:

> *Based on the geochemistry of the sediments used for the present study* ***where iron concentrations of 200 – 1000 µM were observed, low sulfide concentrations could be expected*** *(Van Der Well et al. 2007), based on the formation of insoluble iron sulfide compounds. The initial hypothesis for the present study was that a decrease in porewater iron would be observed during the loading phase of the study as iron sulfide was formed; however, the sulfate exposure portion of this study was not long enough to allow a measurable titration of the high iron content (including solid phase) of the sediment and the appreciable accumulation sulfide in the porewaters"*[71] (emphasis added)

And:

> *Though porewater sulfide was measured initially and at the end of each phase****, a quantifiable rise in dissolved sulfide was not observed over the course of this nine-month study****. A similar study conducted by Van der Well et al. 2007, observed a strong negative relationship between iron and sulfide concentrations within sediment porewaters. Their research was conducted over the course of 21 months and utilized in situ testing within a peat meadow"* [72](emphasis added)

Dr. Johnson's research supports the MPCA's hypothesis that:

- *iron may mitigate the effects of sulfide production in the rooting zone of the sediment*[73]

4.2.5 Alignment with geology and geochemistry

According to the Minnesota Geological Survey (MGS), groundwater in the Quaternary deposits (e.g., shallow groundwater in soil, as opposed to groundwater in sedimentary or metamorphic bedrock) is chiefly glacial in origin.[74]

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[70] Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms, Nathan W. Johnson University of Minnesota Duluth December 2013
[71] Temperature Dependent Diffusion Rates of Sulfate in Aquatic Sediments Nathan W. Johnson, Will DeRocher, University of Minnesota Duluth, December 2013
[72] Id.
[73] Id.

Areal variations in the chemical characteristics of the groundwater are controlled by mineralogical composition of the Quaternary deposits, the length of time the water remains in contact with the glacial materials, climatic factors (especially precipitation and temperature which influence evapotranspiration), and physiography.[75]

Each of these factors which influence the Quaternary deposits and the water which runs through it are explored below.

4.2.5.1 Glacial origin of Quaternary Deposits

The most recent glacier to cross the state was the Des Moines lobe. About 14,000 years ago, this ice extended through the Red River lowland in northwestern Minnesota south to Des Moines, Iowa. Des Moines lobe till is gray to brown and is distinctive because it contains Cretaceous shale imported from North Dakota and Canada.[76] A map showing the extent of the Des Moines lobe is shown in Figure 4-8. Note that all of the lakes with high (> 90 µM) sulfide are on or near deposits of the Des Moines lobe.

This is significant in terms of groundwater chemistry because the groundwater takes up the minerals in the glaciated deposits. From the MGS:

Toward the west, the groundwater quality gradually changes from the calcium-magnesium carbonate type to the calcium magnesium and sodium-potassium sulfate and chloride types. This occurs because the glacial deposits of western Minnesota contain a high fraction of pulverized ***Cretaceous shale, which contains gypsum and disseminated crystals and nodules of iron sulfide****. The shale also retains absorbed sodium and potassium ions on its constituent clay minerals. Fragments of shale, incorporated in the glacial drift, have high exchange capacity and readily give up sodium and potassium ions for calcium and magnesium ions in the circulating water.* ***High concentrations of sulfate are caused by direct leaching of sulfate minerals, such as gypsum, and by oxidation of sulfide. In areas of increasing sulfate concentration total dissolved solids increase to more than 1000 ppm****. Similar changes occur with depth, because Cretaceous shale makes up more of the glacial drift near the bottom of these materials than it does near the top.*[77] (emphasis added)

In contrast, the eastern part of the state was largely influenced by the Superior and Rainy lobes. "Till from the Superior lobe is distinctly red in color and contains rocks derived from the Superior basin—red sandstone, shale, and agates."[78] The red color, of course is due to iron. In addition, "sulfate concentration

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[74] Major Constituent Chemistry of Selected Phanerozoic Aquifers in Minnesota, Roman Kanivetsky, Minnesota Geological Survey 1986
[75] Id.
[76] Minnesota at a Glance: Quaternary Glacial Geology. Minnesota Geological Survey, B.A. Lusardi, 1994; revised March 1997
[77] Major Constituent Chemistry of Selected Phanerozoic Aquifers in Minnesota, Roman Kanivetsky, Minnesota Geological Survey 1986
[78] Minnesota at a Glance: Quaternary Glacial Geology. Minnesota Geological Survey, B.A. Lusardi, 1994; revised March 1997

in waters of eastern Minnesota is low, because most of the readily soluble sulfate minerals have been leached.[79]

Figure 4-8 shows the highest sulfide sediment lakes (those greater than 90 μM). As can be seen in Figure 4-8 all these lakes are in or near the deposits put down by the Des Moines Lobe of the Wisconsin Era glaciation.

[79] Major Constituent Chemistry of Selected Phanerozoic Aquifers in Minnesota, Roman Kanivetsky, 1986

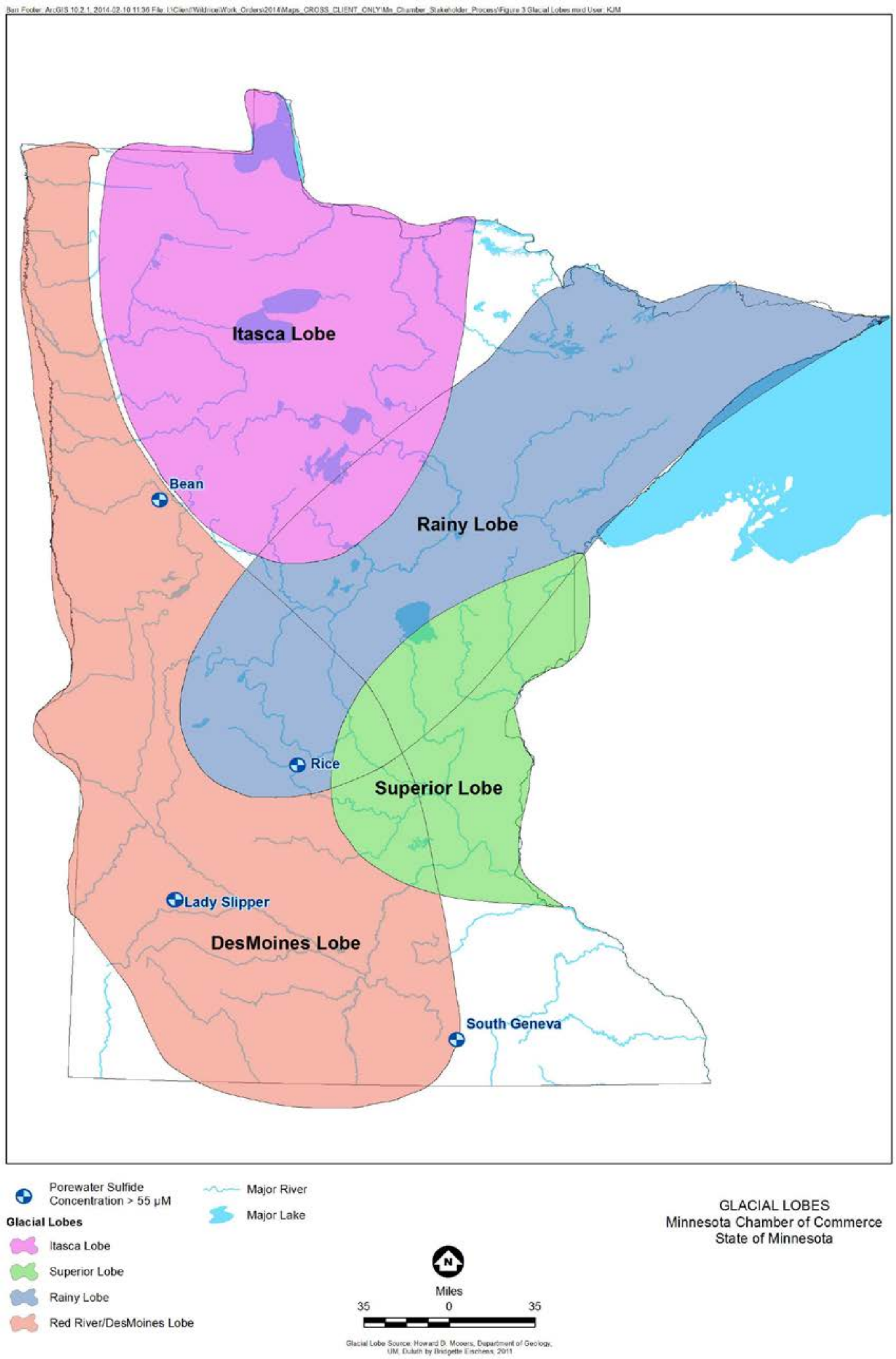

Figure 4-8 Glaciation in Minnesota (USGS)

4.2.5.2 Climatic factors

Minnesota can be thought of as lying astride the Precipitation – Evaporation (P-E) divide (Figure 4-9). To the east of this divide, precipitation exceeds evaporation, while west of the divide, the opposite is true. This is significant because it explains how, over thousands of years, minerals either leached out of soils or remain as a source for groundwater.

> *Water in contact with Rock that contains high soluble gypsum or anhydrite acquires calcium and sulfate ions from these minerals.*

Once dissolved:

> *sulfate [is] reduced to sulfide in reducing conditions in MN groundwater.*
>
> *South of the Minnesota River, direct leaching of sulfate minerals and oxidation of sulfide change the water from the bicarbonate to the sulfate type*[80]

Figure 4-9[81] shows the highest sulfide sediment lakes (those greater than 90 μM). As shown in Figure 4-9 most are on the semi-arid side or in the transition of the climatic divide. The two highest sediment sulfide concentrations of 600 μM (Bean Lake) and 543 μM (Lady Slipper Lake) were observed in net-zero or negative precipitation minus evapotranspiration zones.

[80] Id.

[81] Minnesota's Water Supply: Natural Conditions and Human Impacts, MN DNR, September 2000

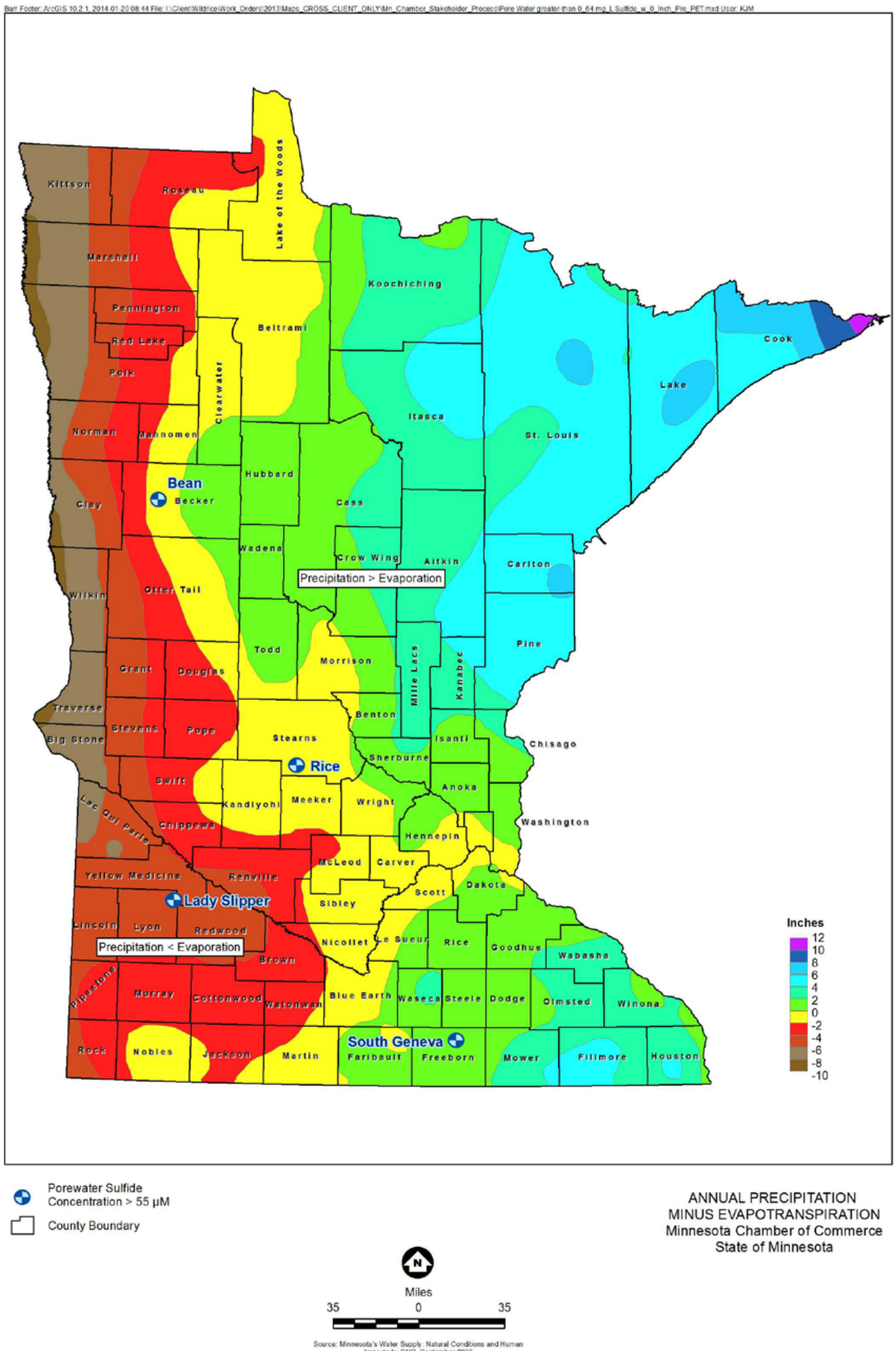

Figure 4-9 Annual Precipitation Minus Evapotranspiration

4.2.5.3 Groundwater Provinces

Figure 4-10 shows the highest sulfide porewater lakes (those greater than 90 μM). As shown in Figure 4-10, while the lakes are in a number of groundwater provinces, those groundwater provinces are wholly or partially within the Des Moines glaciation lobe. The soils within these groundwater provinces tend to be high in sulfates and low in iron, similar to the porewater observed in these lakes.

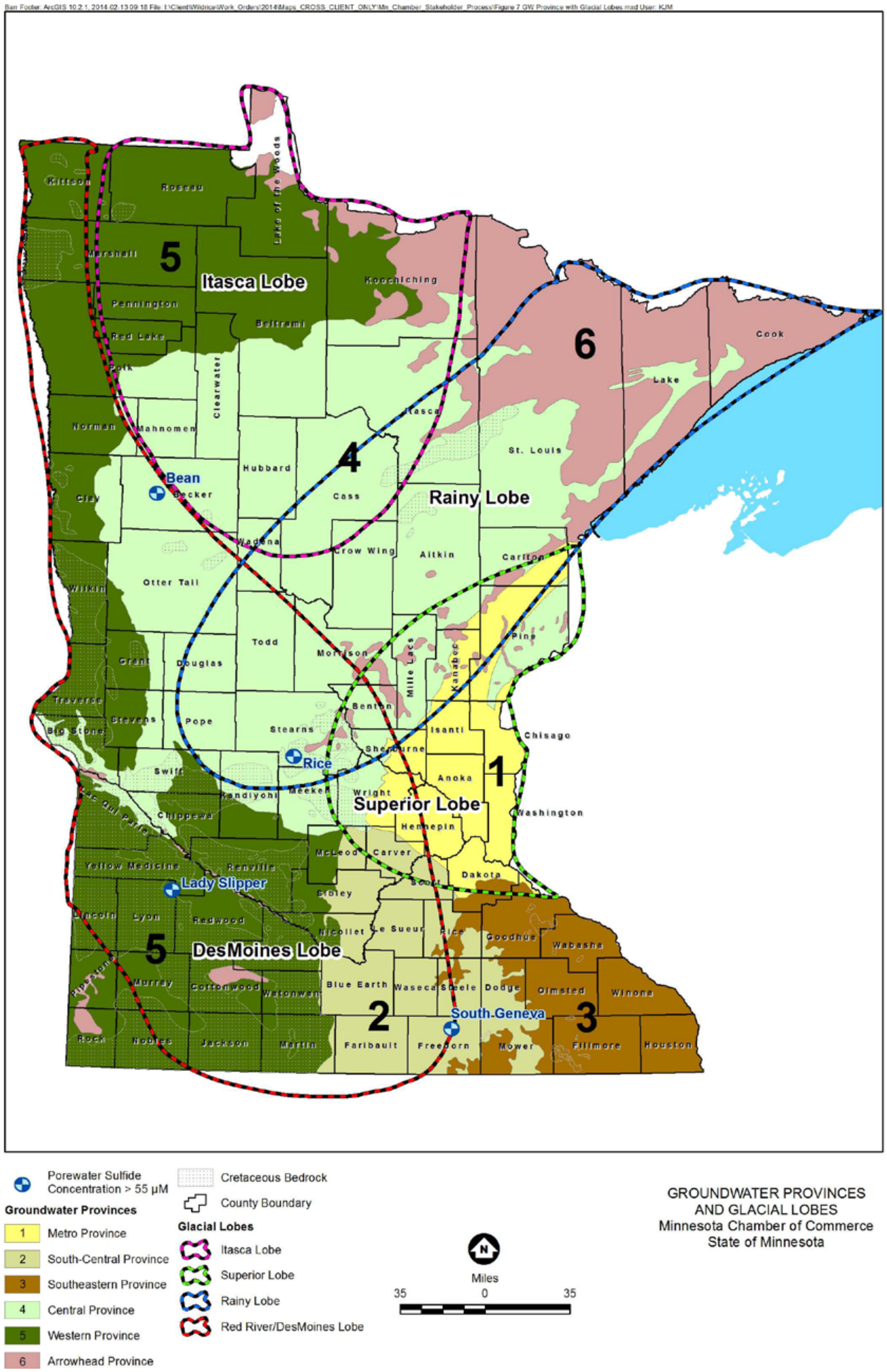

Figure 4-10 Groundwater Provinces

4.2.5.4 Ecological Provinces

Figure 3-2 shows the highest sulfide sediment lakes (those greater than 90 μM). As shown in Figure 3-2, all are in the Prairie Parkland province in the transition from the Eastern Broadleaf Forest and the Prairie Parkland province. The original surface vegetation reflects the combination of the glacial origins of soils, the groundwater interaction with those soils, the climate and other factors. Since the mid-1800's, wholesale conversion of the landscape to agriculture has resulted in increased application of nutrients, and increased runoff of sediments and nutrients to lakes and streams.

Figure 3-2 Porewater Sulfide Concentration >90 µM no effects concentration

The "Moyle's Isopleth"

Moyle's Isopleth denotes a line that Dr. John Moyle drew based on his field observations that abundant wild rice and low sulfate levels were primarily found north and east of this line, whereas less abundant or no wild rice and high sulfate levels were primarily found south and west of this line. Figure 4-11 shows the highest sulfide sediment lakes (those greater than 90 µM) and all are near the Moyle's isopleth, which is the area where wild rice was not found.

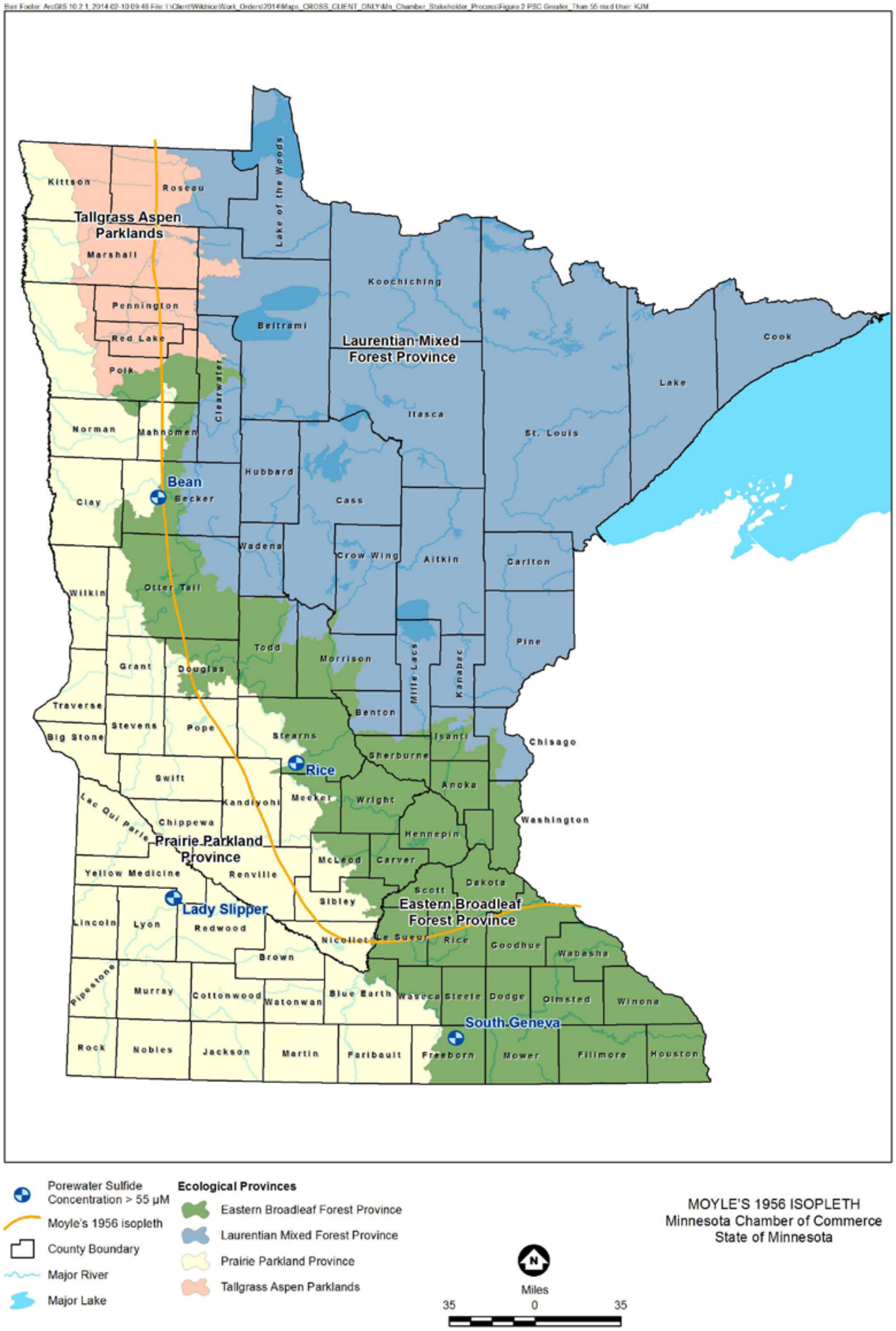

Figure 4-11 Moyle's 1956 Isopleth

Dr. Moyle had limited tools at the time of his observations (mid- to late 1940s). He could not measure sulfide, could not measure iron at the low levels found in porewater, and probably could only measure bulk sediment properties. He did measure sulfate, and made observations about wild rice.

"

"Moyle's Isopleth" reflects a line dividing:

- Semi-arid from semi-humid climates
- Soils with high sulfate and lower iron content from soils with low sulfates and high iron content
- Nutrient rich prairie topsoil (Prairie Parkland Ecological Province) from nutrient deficient forest topsoil (Forest Ecological Province)

Lakes which are to the west of the "Moyle's Isopleth" may be waters which do not support the production of wild rice, not because of sulfate concentration, but because of the ecosystem, soil and climate in which they were formed and in which they exist today.

4.2.6 Summary

One of the MPCA's primary hypotheses is borne out by the data from all of the studies. A relationship between porewater sulfide, surface water sulfate, and porewater iron is described by Equation 4-1:

Equation 4-1

$$\text{Sulfide} = 6.42 * SO_4^{0.00427} * Fe^{-0.445}$$

In fact, nearly the same amount of variability can be explained by Equation 4-2 using only iron and the field survey data:

Equation 4-2

$$\text{Sulfide} = 6.51 * Fe^{-0.445}$$

These relationships were used to predict the expected porewater sulfide concentrations under a number of permutations and combinations of porewater iron and surface water sulfate. We used Equation 4-1 to estimate sulfide concentrations at the 5th, 25th, 50th, 75th and 95th percentiles of iron distributions for the edited data set from the field survey (n = 198) and plotted these data against various surface water sulfate concentrations of interest.

We selected a range of sulfate values that could help illuminate the question of whether the current surface water standard should be modified. The values for sulfate were 1.2 mg sulfate/L representing the 25th percentile in the data set (Figure 4-12), 838 mg sulfate/L representing the maximum observed in the data set (Figure 4-13), and 1,600 mg sulfate/L representing the NOEC observed in the hydroponics study (Figure 4-14). In the case of iron, the concentrations shown in each figure are 90, 2.79, 6.65, 13.9, and 33.3 µg iron/L in sediment pore water respective to the percentiles listed above. Results are shown in Figure 4-12, Figure 4-13, and Figure 4-14.

Equation 4-1 is the best tool for predicting the concentrations of porewater sulfide in sediment. Equation 4-1 and the associated figures below integrate data from the field survey, the porewater geochemistry data, the hydroponic study, experiments. Although Equation 4-1 does not provide a mechanistic explanation for sulfide levels, Equation 4-1 shows that sulfate contributes positively to sulfide in sediments (although only slightly) and that iron significantly reduces sulfide in sediments.

This analysis that the NOEC observed in the sulfide hydroponic experiments (90 µM sulfide) is extremely unlikely to occur in natural systems. As noted above, 90 µM was exceeded only four times in the edited data set. At the maximum observed sulfate concentration in the dataset (838 mg/L), and at low observed iron concentrations (5^{th} percentile), the estimated sulfide concentration (27.56 µM) is substantially below the NOEC level.

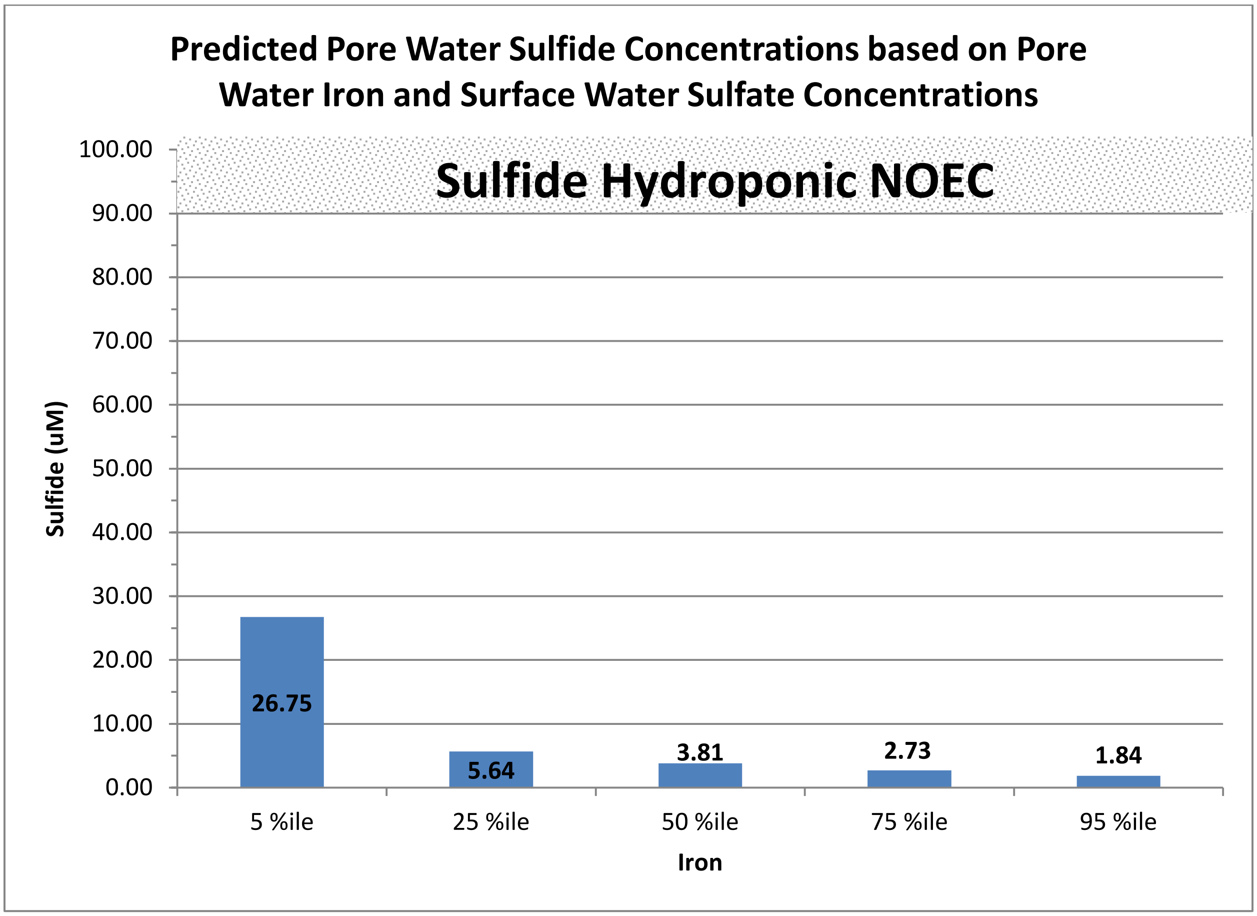

Figure 4-12 **Results of predicted sediment pore water sulfide using the observed 25th percentile surface water sulfate and the 5th, 25th, 50th, 75th and 95th percentiles of porewater iron distributions (for the edited data set from the field survey) as predictor variable**

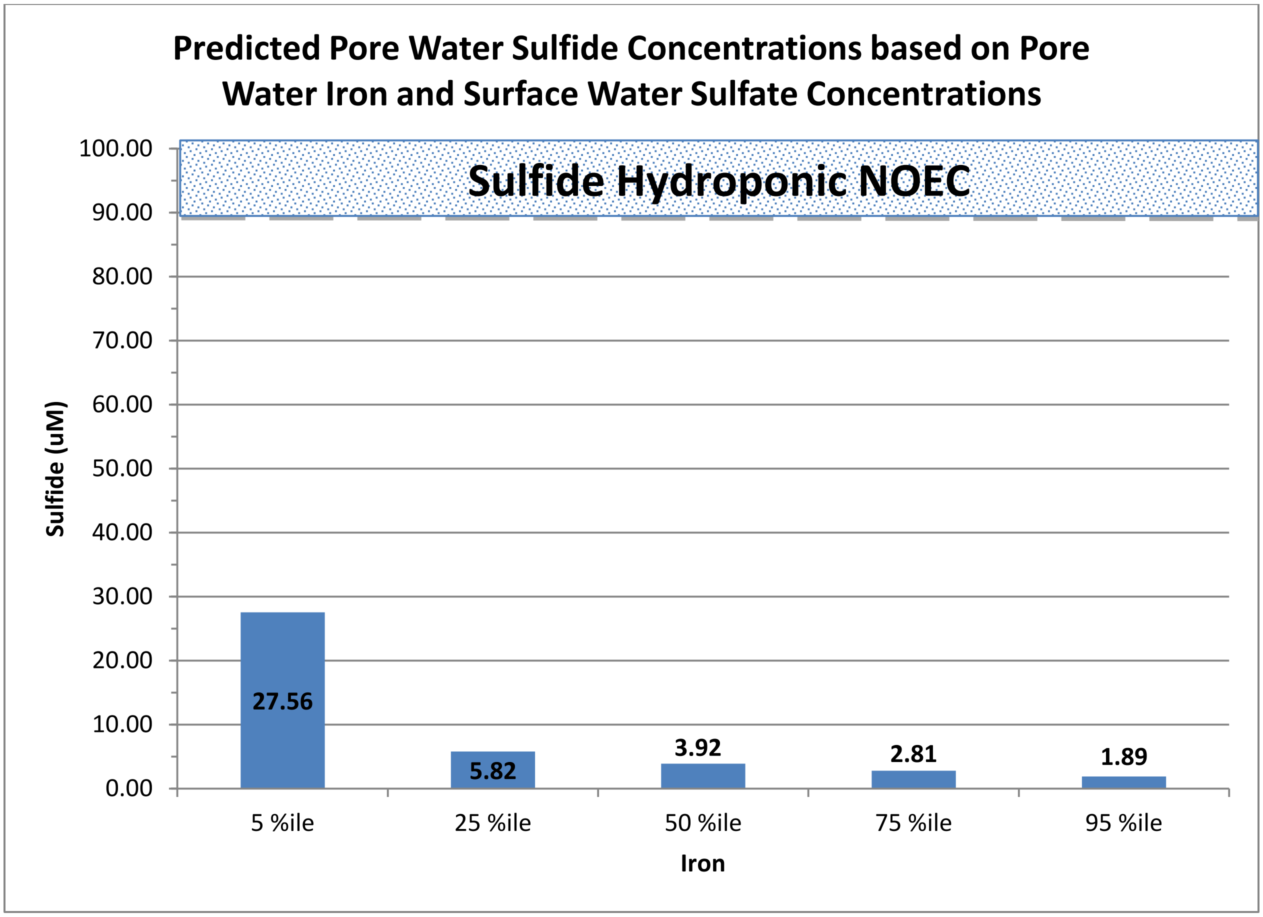

Figure 4-13 **Results of prediction of sediment pore water sulfide using the 838 mg/L surface water sulfate (maximum observed in field survey) and the 5th, 25th, 50th, 75th and 95th percentiles of porewater iron distributions (for the edited data set from the field survey)**

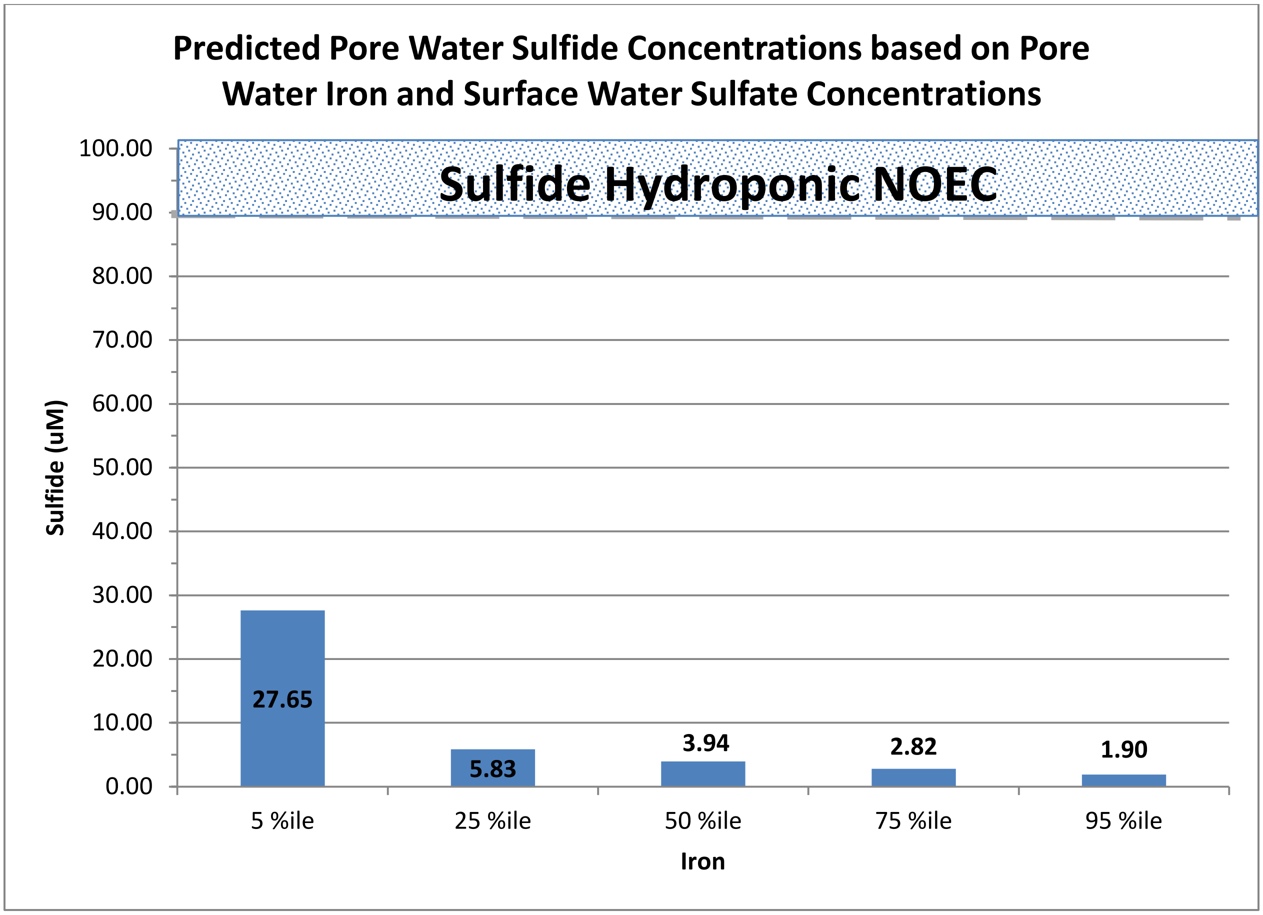

Figure 4-14 **Results of prediction of sediment pore water sulfide using the 1,600 mg/L sulfate (NOEC for sulfate from the sulfate hydroponics experiments) and the 5th, 25th, 50th, 75th and 95th percentiles of iron distributions (for the edited data set from the field survey)**

Thus, even at the highest observed surface water sulfate concentration at the lowest (5th percentile) porewater iron concentration, the sulfide concentration is well below the NOEC observed in the sulfide hydroponic study. The few (4) lakes observed with the highest sulfide are located in or on the Prairie Ecological Province, underlain by Des Moines lobe soils derived from high gypsum, low iron Cretaceous shale, in the semi-arid portion of the state. These conditions, coupled with the fact that most of these lakes are extremely shallow, have low dissolved oxygen and high nutrient levels, leading to enhanced anoxic conditions help explain why these lakes have relatively high sediment sulfide concentrations and no wild rice.

4.3 Integration of Data – Period when “rice may be susceptible to high sulfate”

4.3.1 Overview

The current standard for protection of wild rice applies only “during periods when the rice may be susceptible to damage by high sulfate levels” (MN Rules 7050.0224, Subp. 2). However this phrase is not defined in rule or statute. The MPCA has issued only two permits which specify this period: the permit for Minnesota Power Cohasset plant, originally issued in 1975 and the Mesabi Nugget permit issued in 2012. The 2011 Legislation requires the MPCA to conduct a rulemaking to "designate the specific times of year during which the standard applies" (2011 Special Sessions Laws, 1st Special Session, Ch. 2, Art. 4, Sec. 31)

The Chamber integrated the results of the studies, particularly the sulfide hydroponic study, the root zone geochemistry study, and the sediment incubation experiments to provide a basis for the MPCA to make the designations required by the legislation and define the "periods when the rice may be susceptible to damage by high sulfate levels."

4.3.2 Life cycle of wild rice plants

Dr. Pastor provides a succinct overview of the life cycle of wild rice and the nutrient uptake during that cycle in his hydroponics experiments:

> *Wild rice is an annual plant. It grows in both lakes and rivers in water between 0.3 and 0.67 m depth where there is some water flow. Native stands of wild rice grow in waters that are circum-neutral pH, of low conductivity and hardness, and generally low in nutrient concentrations. In lakes, the most common sediment is an organic-rich silt, but the sediment types range widely (Day and Lee 1990). Sediment in the riverine habitat also ranges widely and may be higher in mineral sediment in the main channels than in backwaters (Meeker 1996).*
>
> *Seeds germinate in the spring and first develop a mesocotyl, or primordial shoot, and a radical, or primordial root. The mesocotyl then grows above the sediment surface, where it develops into a green shoot with a primordial leaf in late spring and early summer. The plant is now at the seedling stage. When the shoot of the seedling reaches the water surface, the plant generates a long narrow leaf which floats atop the water surface; this stage is therefore called the floating leaf stage. Photosynthesis by the floating leaf is used to expand the root system and the beginnings of an aerial shoot which emerges from the leaf axil of the floating leaf and the stem below the water surface.*
>
> *Once the aerial stem and the first aerial leaf emerge, the floating leaf dies and the plant grows taller, putting out additional aerial leaves until late July or early August. Nutrient uptake is very rapid during this stage, and approximately 60-70% of the plant's annual requirement for nitrogen, the most limiting nutrient to both vegetative growth and seed production in most environments, is taken up then (Grava and Raisanen 1978, Sims et al. 2012a,b). In late July or early August, vegetative growth slows and the plant begins to produce a flowering shoot containing male (pollen producing) flowers above female (seed producing) flowers below. Wild rice does not self-pollinate well; instead, as for most graminoids, pollination is largely by wind although bees and flies occasionally visit the male flowers to gather pollen (J. Pastor, personal observations).*
>
> *During the seed production and ripening stage, there is another burst of nutrient uptake from the sediment and the lower vegetative leaves begin to senesce as the nutrients they contain are translocated to the ripening seeds (Grava and Raisanen 1978, Sims et al. 2012a, b). Seeds ripen in late August and through September, although the first two weeks of September are commonly the*

period of peak ripening. The seeds contain a long awn, which helps stabilize them vertically when they are dispersed into the water and thereby allow them to drill into the sediment (Ferren and Good 1977, J. Pastor personal observations). After seed dispersal, the plant dies and its stem, leaf, and root litter are returned to the sediment. Delays in the release of nitrogen from these litters in subsequent years may be responsible for the population oscillations of 3-5 year periods often seen in wild populations (Pastor and Walker 2006, Walker et al. 201 0, Hildebrandt et al. 2012).[82]

A graphical depiction of Pastor's description is shown in Figure 4-15.

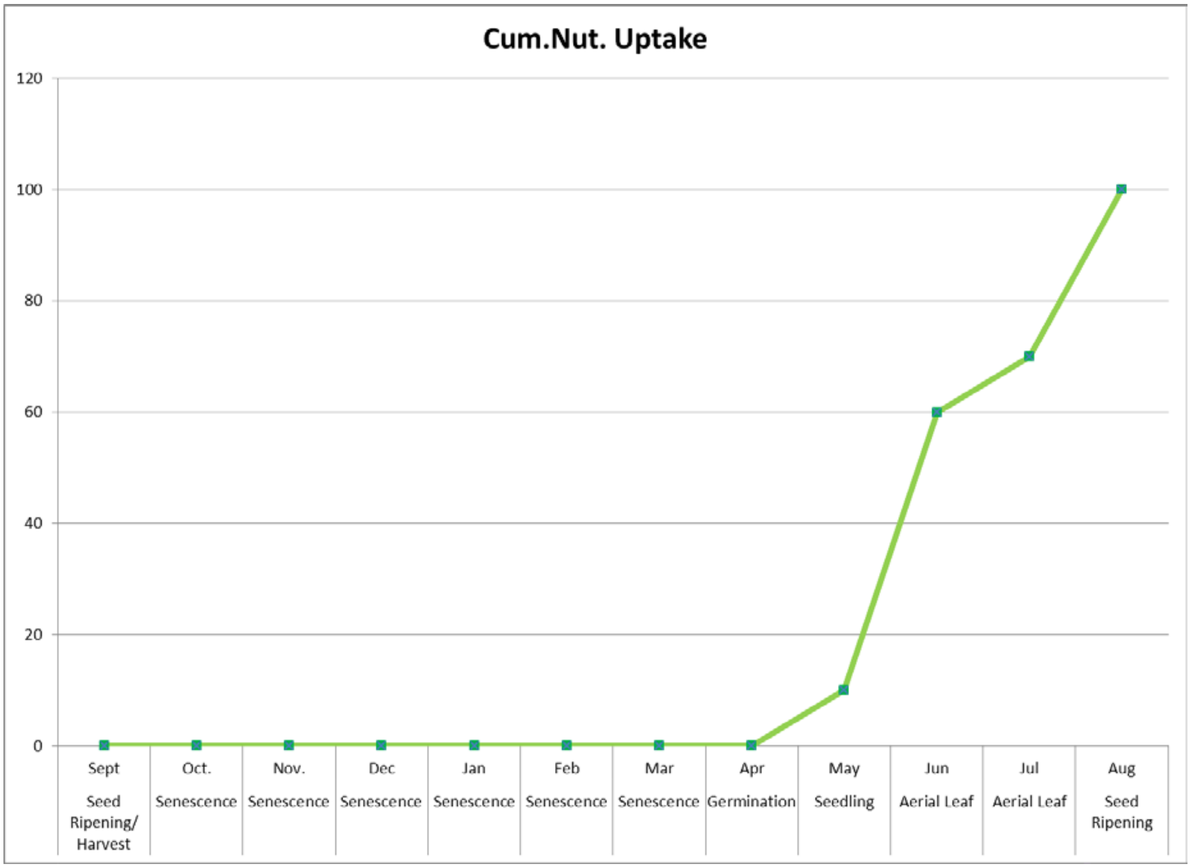

Figure 4-15 Cumulative Nutrient Uptake by Wild Rice

For much of the year, after seed maturation and harvest (or seed drop), there is no nutrient uptake by the wild rice plant, because it has senesced. During germination and early mesocotyl growth, the energy comes from within the seed itself, and there is little uptake or interaction with the environment. During the remainder of the plant's life cycle, nutrient uptake increases with plant growth, until seeds have been set and there is no further need for nutrients.

[82] Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor, University of Minnesota Duluth, December 2013

Sulfur is one of the six macronutrients for plant growth, and low availability of sulfur may therefore limit primary production[83]. Therefore, concentrations of sulfate and sulfide in the environment would have only minimal impact on a plant that is not taking up nutrients (e.g. the senescing plant or the dormant seed).

4.3.3 Integration of Sulfide Hydroponics Study

Exposures up to 90 µM sulfide in a hydroponic experiment did not affect wild rice seed germination or mesocotyl length. . Dr. Pastor concludes, and the data show that:

> *Enhanced sulfide under anaerobic conditions did not affect germination of seeds, mesocotyl weights, or mesocotyl lengths in a rangefinder test at nominal exposure concentrations of 0, 3, 10, 30 and 90 µM sulfides. The rangefinder test was repeated, and because the same results were observed, we did not proceed with any further tests.*[84]

Based on these results, the presence of sulfate or sulfide in the porewater will not impact wild rice plants from roughly September through April. (Germination of course will be determined by the onset of spring, and will vary from year to year.)

4.3.4 Integration of Root Zone Geochemistry Experiments

With regard to the field locations, Dr. Johnson concluded:

> *Sulfide concentrations in sediment pore fluids were almost always less than 10 µM (compared with 3,800 – 7,800 µg/cm^2 sulfate in the overlying water), and were often below the method reporting limit of 0.7 µM. The steep gradients of sulfate, sustained throughout the summer, and a lack of buildup in porewater sulfide indicate a consistent removal mechanism for sulfur in sediments. Iron concentrations at field sites were frequently in excess of 500 – 1,000 µM and precipitation of sulfide as iron sulfides provides a likely explanation for the low dissolved sulfide concentrations.*[85]

Based upon this data and conclusions, as well as the data observed in the field surveys, concentrations of sulfide in porewater will not interfere with wild rice seed germination or mesocotyl growth.

4.3.5 Integration of Temperature Dependent Diffusion Rate Studies

The sediment incubation experiments "investigated the diffusion of sulfate (SO_4^{-2}) into and out of the anoxic regions of two contrasting freshwater aquatic sediments under warm and cold temperatures."[86]

---

[83] *Mineral Nutrition of Higher Plants,* London: Academic Press. Marschner, P.(1995)., and Sulfate transport and assimilation in plants. *PlantPhysiol.* 120, 637–644. Leustek,T. and Saito,K.(1999).

[84] Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor University of Minnesota Duluth, December 2013

[85] Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms, Nathan W. Johnson, University of Minnesota Duluth December 2013

[86] Temperature Dependent Diffusion Rates of Sulfate in Aquatic Sediments, Nathan W. Johnson, Will DeRocher, University of Minnesota Duluth, December 2013

This research addresses whether there are periods when high surface water sulfate levels may occur without damaging or interfering with the growth of wild rice.

Dr. Johnson loaded the two freshwater sediments with 270-280 mg/L sulfate at the beginning of the loading phase (following an equilibrium phase). However, sulfate concentrations in the surface water rose to 350-365 mg/L in one sediment and as high as 650 mg/L in the other, likely due to "bioturbuation by naturally occurring organisms within the sediment, oxidizing the available iron sulfide."[87] By normalizing the sulfate concentrations in the surface water, Dr. Johnson found that "changes in porewater concentration between the 6th week and 9th week were minimal in both North Bay and Partridge River sediments, indicating steady state concentrations had been reached."

In other words, by six (6) to nine (9) weeks following the start of loading of high levels of sulfate in the overlying water, concentrations of sulfate in pore water reached equilibrium within 1 ½ to 2 months. However, during this same phase, sulfide concentrations increased much less:

> *Within twelve weeks of the sulfate spike to overlying water, sulfide concentrations had slightly increased in the porewaters of North Bay cold microcosms (2 µM at 4-6 cm below the sediment water interface) and Partridge River warm microcosms (1.3 µM at 3 cm below the surface, however this is near the 0.7 µM reporting limit of the Hach method used for analysis).*[88]

In the next phase – the recovery phase – lower concentrations of sulfate were introduced to the overlying water. Sulfate diffused back out of the porewaters and into the overlying water. This occurred quite rapidly.

> *Sulfate flux out of the sediment occurred rapidly over the first week of the surface water being replaced with fresh site water during Phase III, the recovery phase. After two overlying water replacements within one week, sulfate levels remained steady at 30-40 mg/L in all of the microcosms except the warm Partridge River trials…"*[89]

Porewater sulfide concentrations at the end of Phase II and throughout Phase III were at or near detection limits in the Partridge River Sediment, and less than 2 µM in the North Bay sediment at the end of Phase II and throughout Phase III. Again, sulfide concentrations are much lower than were observed in the outdoor containers at all treatments.

4.4 Summary

Wild rice is not susceptible to any concentration of sulfate in overlying water between senescence (early September) through seed germination and early stages of seedling growth (mid-April). There should be no sulfate water quality standards applicable during those times.

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[87] Id.
[88] Id.
[89] Id.

If the MPCA decides there needs to be a surface water standard, the standard should be 1,600 mg sulfate/L where wild rice is present. The surface water standard should apply only during the growing season of wild rice (mid-April through early September).

5.0 Recommendations for Revisions of the Water Quality Standard

5.1 Does the scientific evidence indicate that the standard should go up or down and, if so, generally by how much?

Given the high concentration of sulfate needed to have an effect on the growth of wild rice (1,600 to 2,500 mg/L in the hydroponic experiments), and the MPCA's field survey showed no relationship between wild rice coverage and surface water sulfate concentrations, a surface water sulfate standard is unnecessary.

However, if the MPCA decides there needs to be a surface water standard, the standard should be 1,600 mg/L sulfate where wild rice is present. Two sulfate hydroponics experiments were conducted producing results support such an increase[90]:

- 1,600 mg/L (Sulfate did not affect either seed germination or seedling growth other than a slight depression of root lengths at extremely high concentrations (1,600 mg SO4 · L-1). – not statistically significant)[91]
- 2,500 mg/L(The no observed effects concentration (NOEC) for three of the ten Study Day 10 NOEC values were 2,500 mg/L sulfate or lower, and seven of ten SD 10 NOEC values were 5,000 mg/L sulfate. For Study Day 21, eight of ten concentration endpoints exhibited NOEC values of 5,000 mg/L sulfate, indicating that sulfate was generally not toxic at the highest concentration that could be tested within the limits of solubility of the salts.[92])

Wild rice has been observed growing at concentrations near the 1,600 mg/L level. Concentrations above 2,500 mg/L may impact wild rice because of the overall salt content, not because of the specific toxicity of sulfate to wild rice. Multiple observations of wild rice have been made in waters with concentrations well above the current 10 mg/L water quality standard, both in the University of Minnesota Field Survey and in private surveys. Wild rice has been observed at concentrations of 838 mg/L (field survey FS303, Second Creek)[93] and 1,040 mg/L (Mesabi Nugget Wild Rice Survey).[94]

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[90] In accordance with EPA guidelines and state rules for establishing water quality regulations, hydroponic testing is required. See Minn. Rules 7050.0218 and "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses," USEPA, Office of Research and Development, Environmental Research Laboratories, Duluth MN; Narragansett, RI, Corvallis, OR, 1985

[91] Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor, University of Minnesota Duluth, December 2013

[92] Definitive Hydroponics-Based Wild Rice (*Zizania palustris*) Sulfate Toxicity Testing Fort Environmental Laboratories, Inc. December 2013

[93] Wild Rice Sulfate Standard Field Surveys 2011, 2012, 2013: FINAL REPORT, Amy Myrbo, University of Minnesota, December 2013

[94] 2013 Wild Rice Survey and Water Quality Monitoring *Partridge River and Second Creek Prepared for* Mesabi Nugget Delaware, LLC, January 2014

Based on laboratory hydroponic studies, porewater sulfide does not affect wild rice seed germination or mesocotyl growth. Field studies show that porewater sulfide only affects wild rice at very high concentrations, only in lakes in the western and southern portions of the state, and only where porewater iron concentrations are very low.

5.2 Should there be a different standard for lakes/wetlands, or streams, or paddy rice?

No. At an appropriately set standard of 1,600 mg/L, there is no need for a separate stream or lake water quality standard. MPCA has not conducted research on wetlands.

5.3 What more can be said about the "period when the rice may be susceptible to high sulfate"?

Wild rice is not susceptible to sulfate concentrations in overlying water between senescence (early September) through seed germination and early stages of seedling growth (mid-April). As a result, any sulfate water quality standard should not apply between early September and mid-April. If MPCA determines to impose a 1,600 mg/L sulfate water quality standard at locations where wild rice is present, that standard should apply only during the wild rice growing season (mid-April through early September).

5.4 Summary

A surface water sulfate standard is not necessary given the lack of any impact from surface water sulfate on the growth of wild rice. There is little, if any, correlation between surface water sulfate and wild rice growth or density. If the MPCA decides to set a surface water quality standard, it should be set at or near 1,600 mg/L sulfate. The standard should apply to lakes and streams. There should be no water quality standard for sulfate (where wild rice is present) during the time when wild rice has senesced and the time when juvenile seedling growth is established (early September through mid-April). The 1,600 mg/L sulfate water quality standard (where wild rice is present) should apply only during the growing season for wild rice (mid-April through early September).

6.0 Acknowledgements

The Chamber's comments were prepared by a team of scientists and policy experts that consisted of several individuals holding post-graduate degrees (M.S. and Ph.D.) and decades of applied experience in aquatic toxicity assessment, water resources, soil science, rice nutrient dynamics, forest resources and genetics, chemical engineering, statistics, salinity effects on plants (laboratory and field, a professor emeritus in soil science and statistics, and federal and state environmental permitting and rulemaking.

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Mike Bok, PhD. Senior Manager – environmental statistician

Barr Engineering Co.

Rachel Walker, PhD, Senior Environmental Scientist – wild rice biology and nutrient dynamics, wild rice surveys, project management
Mike Hansel, Senior Chemical Engineer and Vice President – water quality rulemaking, environmental permitting
John Borovsky, Senior Environmental Scientist and Vice President – soils and groundwater interaction, environmental permitting
Lindsey Tuominen, Biostatistician – statistical analyses

University of Minnesota

David Grigal, PhD, Professor Emeritus Soil Water and Climate

University of California Davis

Steve Grattan, PhD. Department of Soil Science, plant-water specialist

Fort Environmental Labs

Douglas Fort, PhD. President, aquatic and terrestrial toxicology, developmental toxicology, toxicology/pharmacology

ALLETE/Minnesota Power

Kurt Andersen, Environmental Audit Manager, aquatic toxicity assessment

Appendix A

Private Wild Rice Surveys

Company	Year	Title
Arcelor Mittal	2011	2011 Wild Rice Field Survey for ArcelorMittal
	2010	NPDES Wild Rice and Water Quality Monitoring Report – SD030 - NPDES Wild Rice and Water Quality Monitoring Report – SD030 - SD030 to SD012 (Wyman Creek)
Cliffs - Dunka	2011	Wild Rice Literature Review and 2011 Field Survey for the Dunka Mining Area Technical Memo
Cliffs - Northshore	2013	Wild Rice Literature Review and 2011 Field Survey for the Dunka Mining Area Technical Memo
Essar Steel	2010	2010 Water Quality and Wild Rice Monitoring Report
HibbTac	2011	2011 Wild Rice Survey for Hibbing Taconite Company Technical Memo
Keetac	2009	2009 Water Quality, Hydrology, and Wild Rice Monitoring - Swan Lake, Hay Lake, Moose Lake, Hay Creek, and Hart Creek
	2010	2010 Water Quality, Hydrology, and Wild Rice Monitoring Year End Report - Swan Lake, Hay Lake, Moose Lake, Hay Creek, and Swan River - Keetac Expansion Project
	2011	2011 Water Quality, Hydrology, and Wild Rice Monitoring Year End Report - Swan Lake, Hay Lake, Moose Lake, Hay Creek, and Swan River
Mesabi Nugget	2009	2009 Wild Rice Survey and Sulfate Monitoring
	2010	Lower Partridge River and St. Louis River, October 2009
	2012	2010 Wild Rice Survey and Sulfate Monitoring
	2013	St. Louis River and Second Creek March 2011
Minntac	2013	2013 Wild Rice and Water Quality Sampling Report Dark River and Dark Lake
PolyMet	2009	2009 Wild Rice and Sulfate Monitoring - Spring Mine Creek, Embarrass River, Partridge River, Pike River, and Lower St. Louis River
	2011	2011 Wild Rice and Water Quality Monitoring - Second Creek, Spring Mine Creek, Trimble Creek, Unnamed Creek (PM 11), Wyman Creek, Embarrass River, Partridge River, and Pike River
	2012	2012 Wild Rice and Water Quality Monitoring Summary
	2013	2013 Wild Rice and Water Quality Monitoring Summary
Utac	2011	Wild Rice Field Survey for United Taconite LLC Technical Memo

Appendix B

Development of Power Relationship

The relationship in Equation 4-1 and Equation 4-2 were developed as discussed below. . Minitab Statistical Software was used for the statistical analyses presented here.

Step 1 – Eligible Data

The data from the f*ield survey* were explored with respect to sulfate, sulfide, and iron. A total of 267 samples were included in the f*ield survey* database.[95] Because of the interest in natural systems, data from paddies (12 samples) were not used. Only the stream and lakes data were explored in this analysis (initial data set of 255 samples from multiple stream and lake locations, some locations having multiple samples).

Upon examination of the data, several data use issues were identified. First, several field samples (46 samples) were missing surface water sulfate, sediment pore water iron and/or sediment pore water sulfide observations. These samples were removed from the data set. Second, duplicate samples (class = "5 Survey Duplicate") were also not included in the statistical analysis. Third, it was noted that for 26 samples the reported surface water sulfate was < 0.5 mg/L.

There are numerous possible methods for handling left-censored data (i.e., values reported as below the method detection limit), the simplest methods being: 1) analyze these data as 0.5 mg/L (i.e., set the values as equal to the detection limit), or 2) delete all samples with reported less than values.

Unilaterally deleting 26 samples (> 10% of the database) from a preliminary analysis is ill advised, so the Chamber used method 1) above to address left-censored data. While other more sophisticated methods of handling left censored data are available (e.g. using a random number generator to generate random numbers between 0 and the detection limit, using ½ the detection limit, or using regression imputation), time constraints for performing an initial preliminary analysis prohibited exploration of such methods. There were also a few samples with iron (4 samples) and sulfide (3 samples) that were reported as below the detection limit. The iron and sulfide values for these samples were treated the same as sulfate (i.e., the values were set equal to the detection limit).

As identified in Section 2.3.2.3, two samples in the field study had extremely high porewater sulfide concentrations: Bean Lake in Becker County (sulfide concentration 16 mg/L) and Lady Slipper Lake in Lyon County (one measurement at 14.8 mg/L, other measurements below 10 mg/L). These samples were identified as outliers and were not included in the data analysis. Resolution of the above data issues ultimately resulted in 198 samples in the edited data set (i.e., n = 198).

Step 2 – Simple Regression

The most appealing and useful relationship for regulatory purposes is an estimation of sulfide (the suspected toxic constituent) as a function of sulfate (the constituent whose regulation is of interest) and iron (the constituent known to mitigate the toxicity of sulfide). Using the edited lakes and streams data set (n = 198), the following linear regression was developed:

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[95] LacCore_dataexport_updated_Jan_29_2014.xlsx

Equation B-1

$$S = 0.354 + 0.00032\ SO_4 - 0.000012\ Fe$$

where S is sediment pore water total sulfide in mg S/L (0 to 10 cm depth), sulfate (SO_4) is surface water sulfate in mg sulfate/L, and iron (Fe) is sediment pore water iron in µg iron/L. The relationship had an adjusted multiple $r^2 = 0.070$ and was statistically significant (*prob. < 0.001*). Although the r^2 value is low, the regression coefficient for iron was nonetheless significant (*prob. < 0.001*), whereas the regression coefficient for sulfate was not significant (*prob. = 0.440)*. The low explanatory power of the relationship is due to the large range of values. The relationship does indicate that sulfate is not a statistically significant contributor to sediment sulfide, and iron is a strong factor in reducing sediment sulfide concentrations.

Step 3 Conduct simple regressions on log-transformed values

Because the range of concentrations spanned several orders of magnitude, the variables were transformed to logarithms, and the following linearized relationship was developed:

Equation B-2

$$\text{Ln } S = 0.839 + 0.161 \ln SO_4 - 0.407 \ln Fe$$

Equation B-2 had an adjusted multiple r^2 of 0.460, and was significant (*prob.* < 0.001). The regression coefficients for iron and sulfate were both significant (*prob. < 0.001*) indicating that both sulfate and iron are contributors to sediment sulfide concentration.

The higher explanatory power of the relationship occurs because the logarithms of the high concentrations do not contribute markedly to the error sum of squares, and because the underlying data distributions for porewater sulfide and surface water sulfate are lognormal. The data distribution for [porewater iron is neither normal nor lognormal.

Step 4 Estimate Power Law Equation

The edited lakes + streams data were then fitted to a power function to explore the relationship between sulfate (in surface water), sulfide (in sediment pore water) and iron (in sediment pore water). We used the power function because it passes through the origin (i.e., if sulfate = 0, sulfide should = 0), and because it can approach and ultimately assume a linear shape (if the exponents =1). The result was Equation B-3.

Equation B-3

$$S = 6.42 * SO4^{0.00427} * Fe^{-0.445}$$

This relationship has an r^2 between observed and predicted = 0.595 and was significant (*prob. <0.001)*, see Equation B-3.

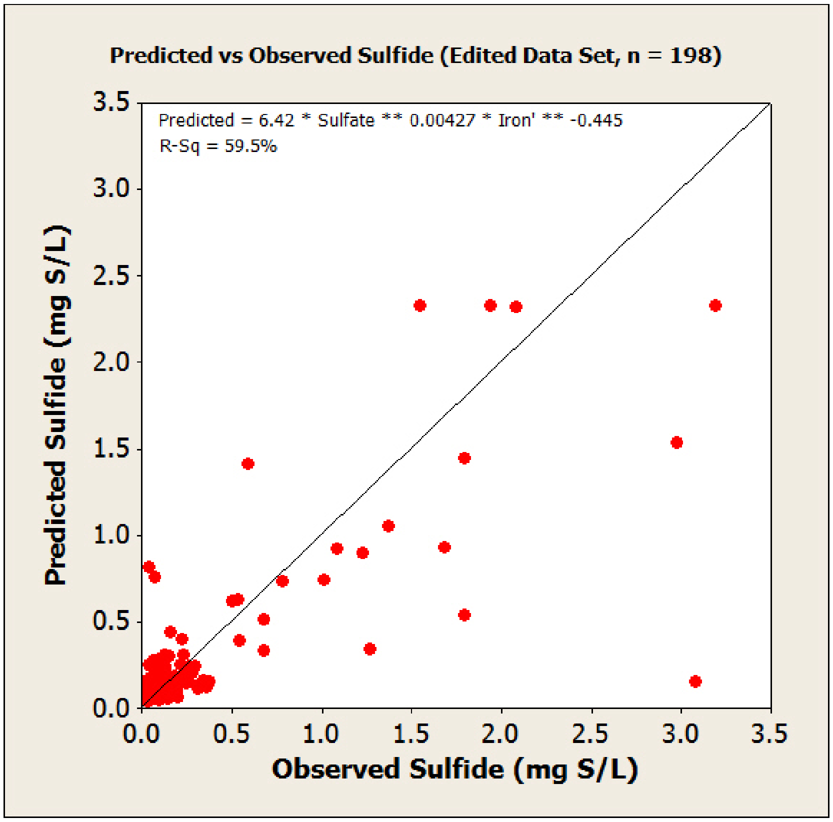

Figure B-1 **Relationship between observed sediment pore water sulfide in lakes and stream sediments and that predicted by a non-linear relationship with surface water sulfate and sediment pore water iron as predictor variables, n = 198 (1:1 line shown)**

Step 7 Explore an alternative method to address less than detect values

As an alternative to treating sulfate values < 0.5 mg/L as 0.5 mg/L (e.g., set the value equal to the detection limit), we repeated the analysis that resulted in Equation B-3 using a censored data set where all sulfate samples with a reported concentration of <0.5 mg/L sulfate (26 samples) were deleted from the analysis (n = 172). (Note: the iron and sulfide values that were included as equal to the detection limit were not eliminated from the data set.)

The resulting power law relationship (n = 172) was:

Equation B-4

$$S = 6.68 * SO4^{-0.00772} * Fe^{-0.439}$$

This relationship had an r^2 between observed and predicted = 0.589 and was significant (*prob. <0.001)*.

Deleting the less than values for sulfate had little effect on the explanatory power (r^2) of the non-linear relationship as compared to that associated with Equation B-4. This analysis might be taken as a lower bound on any other methods of addressing less than detect values. Since including all values as equal to the detection limit and eliminating all less than detect values resulted in nearly identical relationships and with nearly identical r^2 and significance, exploring other more sophisticated techniques for addressing left-censored data were not considered. Figure B-2.

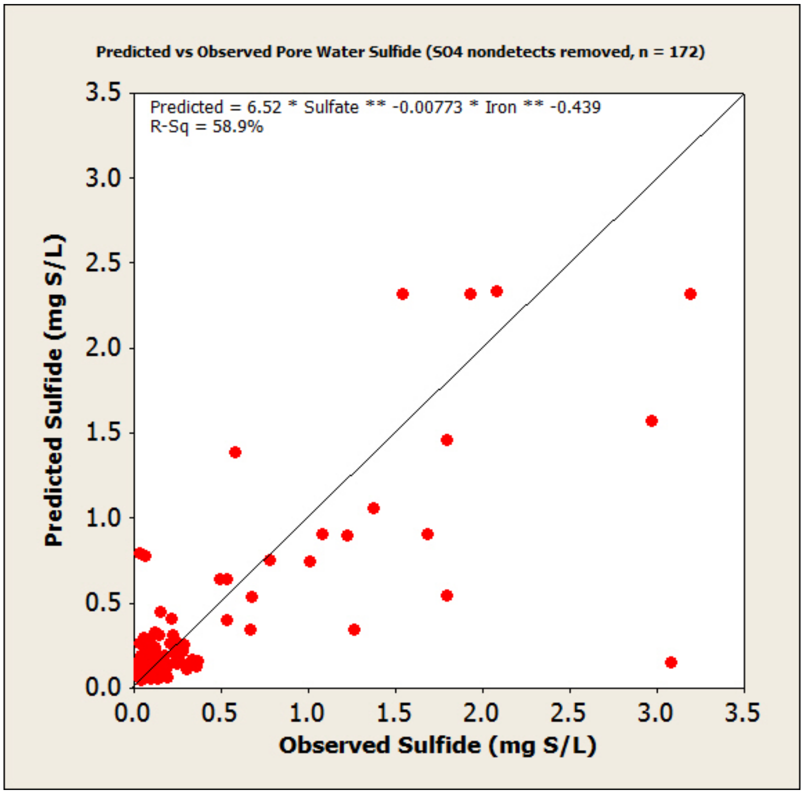

Figure B-2 **Relationship between observed sediment pore water sulfide in lakes and stream sediments and that predicted by a non-linear relationship with surface water sulfate and sediment pore water iron as predictor variables, sulfate values less than detect removed,**

However, noting the very small influence of surface water sulfate on the prediction of porewater sulfide, a test was run to determine whether a relationship between porewater sulfide and porewater iron only would have similar explanatory power. Using the data set that was used for Equation B-3 (n=198) resulted in Equation B-5 (note, this is the same as Equation 4-2):

Equation B-5

$$S\ (\text{mg S/L}) = 6.51 * (\text{Fe}\ \mu\text{g/L})^{-0.446}$$

The relationship had an r^2 between observed and predicted = 0. 595 and was significant (prob. < 0.001).

The relationships described in Equation B-5 show that sulfide concentration in sediment pore water is dictated by the coincident iron concentration in sediment pore water almost regardless of the sulfate concentration in the surface water (Figure B-3).

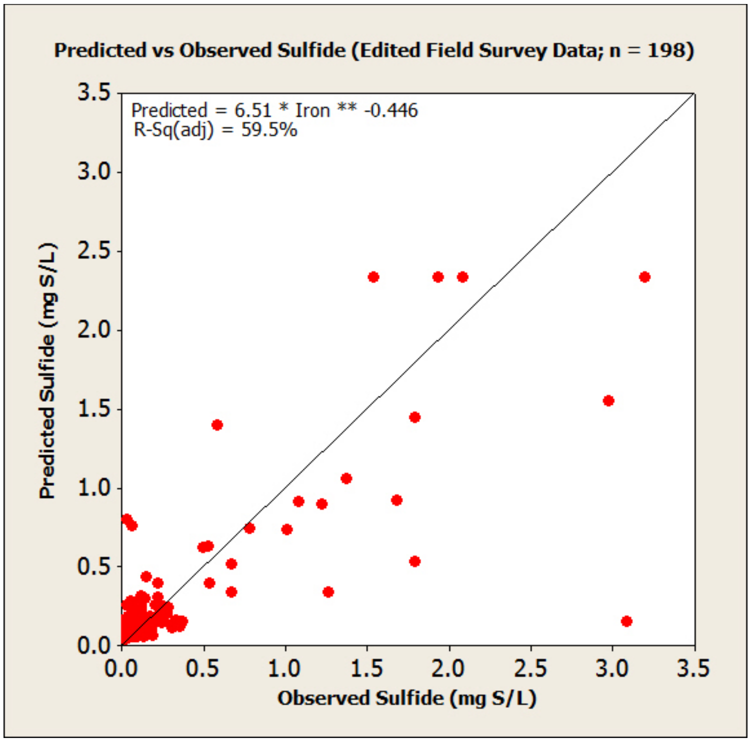

Figure B-3 **Relationship between observed sediment pore water sulfide in lakes and stream sediments and that predicted by a non-linear relationship with only sediment pore water iron as a predictor variable, n = 198 (1:1 line shown)**