

Final Technical Support Document: Refinements to Minnesota's Sulfate Water Quality Standard to Protect Wild Rice



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Availability of manuscripts based on MPCA-sponsored research that are cited in this document

From 2011-2013 MPCA sponsored extensive research into the effect of elevated sulfate and sulfide on wild rice and potential wild rice habitat. Much of the research was carried out by researchers at the University of Minnesota (at both the Duluth campus and Twin Cities campus) and the St. Croix Watershed Research Station of the Science Museum of Minnesota. Four manuscripts were prepared for publication in peer-reviewed scientific journals. A paper by Pastor et al. (see below) was published in January 2017. A group of three papers that refer to each other were submitted to the Journal of Geophysical Research. Because they refer to each other, they cannot be published until all three are accepted for publication. As of August, 2017, two of the three papers have been accepted, and one is still in review.

The MPCA is not posting the manuscripts on the MPCA website, because it is unethical to publish a scientific paper more than once, and most journals now regard the posting of a paper on a website as a publication. However, sharing a copy of a manuscript is not considered to be a publication. Therefore, copies of the four manuscripts are available from the MPCA, and may be requested but must not be posted on a website.

Published:

(Pastor et al., 2017) Pastor, J., B. Dewey, N. W. Johnson, E. B. Swain, P. Monson, E. B. Peters, and A. Myrbo. 2017. Effects of sulfate and sulfide on the life cycle of *Zizania palustris* in hydroponic and mesocosm experiments, *Ecological Applications*, 27, 321-336. This paper is available from the MPCA.

Accepted for publication:

(Myrbo et al., in press-1) Myrbo, A., E.B. Swain, D.R. Engstrom, J. Coleman Wasik, J. Brenner, M. Dykhuizen Shore, E.B. Peters, and G. Blaha. In press-1. Sulfide generated by sulfate reduction is a primary controller of the occurrence of wild rice (*Zizania palustris*) in shallow aquatic ecosystems. This manuscript is available from the MPCA.

(Pollman et al., in press) Pollman, C.D., E.B. Swain, D. Bael, A. Myrbo, P. Monson, and M. Dykhuizen Shore. In press. The evolution of sulfide in shallow aquatic ecosystem sediments – an analysis of the roles of sulfate, organic carbon, iron, and feedback constraints using structural equation modeling. This manuscript is available from the MPCA.

Submitted, but not yet accepted:

(Myrbo et al., submitted-2) Myrbo, A., E.B. Swain, N.W. Johnson, D.R. Engstrom, J. Pastor, B. Dewey, P. Monson, J. Brenner, M. Dykhuizen Shore, and E.B. Peters. Submitted-2. Increase in nutrients, mercury, and methylmercury as a consequence of elevated sulfate reduction to sulfide in experimental wetland mesocosms. This manuscript is available from the MPCA.

Contents

Availability of manuscripts based on MPCA-sponsored research that are cited in this document.....	iii
Contents.....	iv
Figures and Tables	v
Introduction	1
Chapter 1. Numeric wild rice sulfate standard.....	3
A. Confirmation that porewater sulfide is a primary controller of wild rice occurrence.....	3
B. That other factors affect wild rice does not negate the need to protect wild rice from excess sulfide....	23
C. Identification of 120 µg/L as the protective sulfide concentration	31
D. Relationship between surface water sulfate and porewater sulfide.....	40
E. Development of an equation to calculate a numeric sulfate standard for each wild rice water.....	45
F. Comparison of an equation-based standard to fixed standards: error rates and concerns	48
Chapter 2. Evidence that a different standard is needed for some wild rice waters	67
Alternate numeric sulfate standard for false positive wild rice waters	67
Evidence that site-specific standards may be needed for false positive wild rice waters	76
Chapter 3. Implementation of the wild rice sulfate standard	84
Sediment Sampling and Analysis.....	84
Conforming with the Sulfate Standard.....	91
Duration (Averaging Time)	91
Frequency (how often the magnitude may be exceeded).....	95
Appendix 1. Other potential consequences of increasing sulfate concentrations.....	98
Appendix 2. Wild Rice Seeds and Food Value	101
Appendix 3. Results for the 2015 sediment pilot study	105
Appendix 4. Statistical characterization of wild rice density in 10 wild rice waters over 12 years	112
Appendix 5. EC10 values from MPCA-sponsored hydroponic data	113
Appendix 6. EC10 estimates from experimental mesocosms	116
Appendix 7. Protective sulfide estimates from MPCA-sponsored field survey	118
Appendix 8. Empirical demonstration that a MBLR probability of 0.5 is most accurate.....	123
Appendix 9. Examination of a proposed protective sulfide of 300 µg/L.....	127
Appendix 10. Educational credentials and qualifications of TSD and SONAR authors.....	132
References.....	134

Figures and Tables

Figures

Figure 1-1. Average temperatures across the United States in winter (top) and in summer (bottom).	10
Figure 1-2. Estimates of protective sulfide concentrations for biological endpoints from hydroponic, mesocosm, and field data	34
Figure 1-3. Logistic fit of wild rice seedling weight gain against time-weighted arithmetic average sulfide concentrations	35
Figure 1-4. Binary logistic regression of the presence and absence of wild rice for field survey sites with water transparency greater than 30 cm (Class B data, N=96).	36
Figure 1-5. Empirical examination of the average proportion of sites with wild rice above a given porewater sulfide concentration (sites excluded with transparency < 30 cm).	37
Figure 1-6. Relationship between sulfate in surface water and sulfide in porewater.	41
Figure 1-7. Conceptual model of the primary variables affecting the relationship between surface water sulfate and porewater sulfide.	42
Figure 1-8. The misclassification rate compared across a range of potential sulfate standards.	49
Figure 1-9. The proportion of false positive and false negative prediction errors calculated for potential protective sulfide concentrations ranging from 50 to 400 $\mu\text{g/L}$ (in increments of 10 $\mu\text{g/L}$).	50
Figure 1-10. Probability of wild rice presence as a function of porewater sulfide (binary logistic regression; $p=0.001$; $N=108$).	51
Figure 1-11. Probability of wild rice present at densities (A) greater than 25 stems/ m^2 (binary logistic regression; $p=0.013$) and (B) 40 stems/ m^2 (binary logistic regression; $p=0.076$). (Based on sites with wild rice in the Class B dataset, $N=67$.)	51
Figure 1-12. Probability of wild rice being present at a density of less than 10 stems/ m^2 (binary logistic regression; $p=0.0003$). (Based on sites with wild rice in the Class B dataset, $N=67$.)	52
Figure 1-13. Probability of wild rice presence and the probability that the wild rice has a density of less than 10 stems/ m^2 , plotted against porewater sulfide concentrations.	52
Figure 1-14. Two different ways (as described by Fielding and Bell, 1997) of interpreting the false negatives and false positives associated with a spectrum of potential sulfate criteria (potential standards) and their classification accuracy associated with a protective sulfide concentration of 120 $\mu\text{g/L}$	55
Figure 1-15. Observed accuracy observed when the MPCA survey data are assessed against (A) standards calculated with the proposed equation, and (B) a fixed sulfate standard of 10 mg/L .	59
Figure 2-1. Prediction accuracy of the proposed equation for 222 site visits to 115 different waterbodies (data set Class G), plus density of wild rice occurrence, at two levels of density.	68
Figure 2-2. Trend in the efficiency of conversion of sulfate in surface water to sulfide in porewater (71
Figure 2-3. Prediction accuracy of the proposed equation for dataset Class B (a subset of 108 different waterbodies in Class G), plus density of wild rice occurrence, at two levels of density.	77
Figure 2-4. Location of the watershed that includes Monongalia Lake	79
Figure 3-1. Example of variable growth patterns of wild rice biomass across years. Reprinted with permission: Wild Rice Monitoring Handbook by Tonya Kjerland (2015)	84

Figure 3-2. Graph of confidence interval widths compared to sample size for sediment TOC for each of the waterbodies sampled. See Table 3-1 for an explanation of the waterbody acronyms.	89
Figure 3-3. Graph of confidence interval widths compared to sample size for sediment iron for each of the waterbodies sampled. See Table 3-1 for an explanation of the waterbody acronyms.	90
Figure 3-4. Relationship between average sulfate concentration from 2013 and porewater sulfide measured at the end of the 2013 growing season.	95
Figure A5-1. Logistic regressions using initial sulfide hydroponic concentration to predict plant growth. A) weight change. B) weight gain.	113
Figure A5-2. Logistic regressions using arithmetic time-weighted mean (TWM) sulfide hydroponic concentrations to predict plant growth. A) weight change. B) weight gain.	114
Figure A5-3. Logistic regressions using geometric time-weighted mean (TWM) sulfide hydroponic concentrations to predict plant growth. A) weight change. B) weight gain.	115
Figure A6-1. Cook's distances for regression of percent filled seeds against porewater sulfide.	116
Figure A6-2. Regressions of percent filled seeds against porewater sulfide in wild rice mesocosms sampled August 2013	117
Figure A6-3. Regressions of number of plants emerged against porewater sulfide in wild rice mesocosms sampled August 2013 (Pastor et al., 2017).	117
Figure A7-1. Comparison of the use of untransformed data in logistic regression (a-c) to the use of log-10 transformed data (d-f).	119
Figure A7-2. a. Logistic regression of all data in Class B, showing the 10% effect horizontal line. b. Logistic regression of all data with transparency > 30 cm.	120
Figure A7-3. Percent of sites above the sulfide concentration that contain wild rice. A. Class B sites where water transparency was greater than 30 cm (N=96). B. All sites in Class B (N=108).	122

Tables

Table 1-1. Purpose, strengths, and limitations of the components of the MPCA-sponsored wild rice research.	5
Table 1-2. Summary statistics for sulfate concentrations in various datasets.	8
Table 1-3. Correlations of field variables with wild rice and porewater sulfide.	16
Table 1-4. Spearman correlation coefficients for selected environmental variables measured at the field sites.	19
Table 1-5. The 12 field variables that are significantly correlated with the presence/absence of wild rice, as determined through binary logistic regression	20
Table 1-6. Reported environmental requirements for suitable wild rice habitat.	24
Table 1-7. Reported threats to existing natural stands of wild rice.	29
Table 1-8. Estimates of protective sulfide concentrations for wild rice from hydroponic, mesocosm, and field data, based on change-point analysis, EC10 estimates, and visual identification of a decrease in a graph of the proportion of field sites with wild rice present.	33
Table 1-9. Calculated protective surface water sulfate concentrations for three wild rice lakes.	47
Table 1-10. Calculated false classification percentages for potential protective sulfide concentrations from 50 to 400 µg/L	56
Table 1-11. Calculated false classification percentages and diagnosis error rates for potential sulfate standard concentrations from 0.5 to 40 mg/L (Class B dataset; N=108).	57
Table 1-12. Examples of false negatives relative to a fixed numeric standard of 10 mg/L: sites with sulfate less than 10 mg/L, but with greater than the calculated protective sulfate concentration, as calculated by the multiple binary logistic regression (MBLR120).	58
Table 1-13. Examples of false positives relative to a numeric sulfate standard of 10 mg/L: sites with sulfate greater than 10 mg/L, but with sulfate concentrations lower than the calculated protective sulfate concentration.	61
Table 1-14. Phosphorus standards developed by the state of Vermont (Vermont DEC, 2014) and associated misclassification rates.	63
Table 1-15. Effect on median protective sulfate concentrations (for waters with wild rice) of developing equations with different datasets, and different protective sulfide concentrations.	65
Table 1-16. Comparison of wild rice occurrence and density between two groups: waterbodies with porewater less than 120 µg/L and waterbodies with sulfide between 120 µg/L 300 µg/L.	66
Table 2-1. All waterbodies in the field survey that exhibited at least one false positive (Class G dataset)	72
Table 2-2. All waterbodies in the field survey that exhibited at least one true positive (dataset Class G)	80
Table 3-1. Summary results from 2015 pilot study, consisting of 25 sediment cores collected and analyzed from each of six wild rice waters.	86
Table 3-2. Lowest calculated sulfate value of composite samples compared to sulfate values at various percentiles calculated from the 25 individual samples analyzed from each waterbody of the pilot study.	88
Table A2-1. Wild rice seed weight estimates found in the literature.	101
Table A2-2. Literature ranges of number of seeds per head.	102
Table A2-3. Seeds and stem values to support daily energy needs of a dabbling duck.	102

Table A3-1. Results for sediments collected from Bowstring River.	106
Table A3-2. Results for sediments collected from Clearwater River.	107
Table A3-3. Results for sediments collected from Hesitation Wildlife Management Area.	108
Table A3-4. Results for sediments collected from Mission Creek.	109
Table A3-5. Results for sediments collected from Monongalia Lake.	110
Table A3-6. Results for sediments collected from Mississippi River.	111
Table A4-1. Average wild rice density in ten wild rice waters, as monitored by the 1854 Treaty Authority	112
Table A4-2. Statistical characterization of wild rice density in 10 wild rice waters over 12 years	112
Table A5-1. EC10, EC20, and EC50 values for initial mean sulfide hydroponic concentration.	114
Table A5-2. EC10, EC20, and EC50 values for arithmetic TWM sulfide hydroponic exposures.	115
Table A5-3. EC10, EC20, and EC50 values for geometric TWM sulfide hydroponic exposures.	115
Table A7-1. Calculated sulfide EC10 values, based on wild rice presence/absence.	121
Table A8-1. Rates of wild rice presence and density above and below 120 µg sulfide/L. (Class B dataset)	124
Table A8-2. Effect that different probability levels (that sulfide would exceed 120 µg/L) have on the number of waterbodies in four levels of wild rice density	125
Table A8-3. Effect that different probability levels (that sulfide would exceed 120 µg/L) have on the number of waterbodies with and without wild rice	126
Table A9-1. Number of Class B waters in three different groups, separated by porewater sulfide concentration.	127
Table A9-2. Comparison of the odds of wild rice presence between any two sulfide concentration groups.	128
Table A9-3. Number of Class B sites of different density in three different sulfide concentration groups.	129
Table A9-4. Results of a two-sample proportion test to find the probability of groups having the same proportion in each group.	130

Introduction

The federal Clean Water Act requires states to designate beneficial uses for all water bodies (i.e. “waters”) and develop water quality standards to protect each use. Water quality standards include several components:

- Beneficial uses — identification of how people, aquatic communities, and wildlife use waters.
- Numeric standards — typically the allowable concentrations of specific chemicals in a waterbody, established to protect beneficial uses. Can also include measures of biological health.
- Narrative standards — statements of unacceptable conditions in and on the water.
- Antidegradation protections — extra protection for high-quality or unique waters and existing uses.

Minnesota Rules Chapter 7050 assigns a series of beneficial use classifications to all waters of the state. These use classifications set out the beneficial uses that apply to Minnesota waters. Water use classifications, and their accompanying narrative and numeric standards and antidegradation provisions, make up the state’s set of water quality standards. Aquatic life and recreation, industrial uses, agriculture and wildlife, and domestic consumption are some of the beneficial uses that these standards protect. Although there is a lot of commonality among the beneficial uses established by states – for example, every state designates and protects drinking water as a beneficial use – states may also set beneficial uses that reflect the unique nature of their waters and aquatic resources.

Minnesota’s Class 4 water quality standards protect the waters of the state so that they are suitable for “the agriculture and wildlife designated uses.” One subclass of Class 4 is Class 4A waters (Minn. R. 7050.0224, Subp 2), which must be clean enough “to permit their use for irrigation without significant damage or adverse effects upon any crops or vegetation usually grown in the waters or area.” In 1973, Minnesota established a unique beneficial use by establishing a subset of the Class 4A use class called “water used for production of wild rice” and setting a numeric standard to protect the production of the wild rice grain. Wild rice is an important plant species in Minnesota. It provides food for waterfowl, is economically important to those who harvest and market it, and is also an important cultural resource to many Minnesotans.

The specific pollutant from which the “water used for production of wild rice” is protected is sulfate. Sulfate is a natural chemical commonly found in surface and groundwater. It can also be found at varying concentrations in discharges from permitted facilities such as mining operations, municipal wastewater treatment plants, and industrial facilities. The observed relationship between the presence of wild rice in waters with lower sulfate levels, and its absence in waters with elevated sulfate, led to the adoption of the wild rice sulfate standard in 1973.

Minnesota’s wild rice sulfate standard came under scrutiny in the 2000s. Of particular interest was better understanding the effects of sulfate on wild rice in order to understand the appropriateness of the standard and its implementation. The need to clarify which waters support the wild rice beneficial use was also identified.

In 2011 the Minnesota Legislature provided \$1.5 million in funding for the MPCA to conduct a Wild Rice Sulfate Standard Study to gather additional information about the effects of sulfate and other substances on the growth of wild rice. The legislation also required the MPCA to undertake rulemaking to identify wild rice waters and to make any other needed changes to the sulfate standard following completion of the study.

Following the completion of the Wild Rice Sulfate Standard Study in December 2013, MPCA reviewed the results and developed a preliminary analysis of the research, which it then shared with stakeholders

in March 2014 (MPCA, 2014). MPCA staff met with many partners and stakeholders, and continued to refine the analysis of the research based on comments received, review of additional literature and additional statistical analyses. The result of this effort was completion of the [Analysis of the Wild Rice Sulfate Standard Study — Draft for Scientific Peer Review](#) in June 2014 (MPCA, 2014).

MPCA then contracted with Eastern Research Group, Inc. (ERG) to convene and facilitate a scientific peer review of the study and analysis. Full details, background documents and the final report from ERG on the scientific peer review process can be found on the [MPCA's webpage about the wild rice sulfate standard study](#).

MPCA refined its analysis based on the peer review and tribal and Advisory Committee feedback, and in March 2015 MPCA released a Draft Proposal for Protecting Wild Rice from Excess Sulfate (MPCA, 2015).

The Draft Proposal included:

- A proposed draft approach to the wild rice water quality standard;
- A draft list of waters where the standard would apply; and
- Draft criteria for adding waters to the list over time as new or additional information becomes available.

The Draft Proposal was shared broadly, including through a Request for Comments (RFC) asking the public for comments and information about wild rice sulfate standard rulemaking.

As a result of comments and questions received following release of the Draft Proposal, MPCA has re-analyzed data from the studies using different statistical approaches. This reanalysis included review of the following:

- Field survey data used to relate wild rice presence to sulfide in the sediment;
- Field survey data that relate sulfate to sulfide;
- Basic assumptions relating sulfate to wild rice;
- Choice of which data set of sites from 2011-2013 field work would be most appropriate to use in analyses; and
- Variables controlling conversion of sulfate to sulfide.

The MPCA then published a draft Technical Support Document (draft TSD; MPCA, 2016) in 2016 as the next step in the ongoing effort to better understand the effects of sulfate on wild rice to inform an evaluation and, as needed, a revision to the standard.

The Draft TSD was shared broadly and the MPCA received partner and stakeholder input. This Final TSD provides the main scientific support for the MPCA's proposed changes to the wild rice sulfate standard. It revises and updates the draft TSD, providing additional analyses and explanations. Some information provided in the Draft TSD has been moved into the Statement of Need and Reasonableness (SONAR, MPCA 2017). Together, the SONAR and this TSD, along with their exhibits and reference materials, support the MPCA's proposed changes to the wild rice sulfate standard.

Chapter 1. Numeric wild rice sulfate standard

This chapter of the TSD focuses on the mechanism by which sulfate impacts wild rice, and the numeric standard for protecting wild rice from excess sulfate. This chapter is organized as follows:

Part A introduces the primary hypothesis that guided MPCA's technical investigation (namely: if sulfate is harmful to wild rice, sulfate acts by being converted to hydrogen sulfide (sulfide) in the sediment in which wild rice grows), and presents evidence that sulfide exerts significant control over the presence and absence of wild rice in Minnesota's shallow aquatic systems.

Part B refutes the argument that it is not necessary to protect wild rice from elevated sulfide, given that there are multiple other environmental stressors affecting the occurrence of wild rice in water bodies aside from sulfide, such as changes in water levels, impacts of watershed development, and the presence of invasive or competitive species.

Part C presents the evidence used to identify 120 micrograms per liter ($\mu\text{g/L}$) in sediment porewater as the sulfide concentration that is protective of wild rice populations in natural water bodies. Identification of a protective sulfide concentration is a necessary step in the development of a sulfate standard. The next step is to develop a quantitative relationship between sulfate and sulfide.

Part D shows that the relationship between surface water sulfate and porewater sulfide differs among water bodies, and is dependent on sediment concentrations of organic matter and iron.

Part E presents an equation that allows the calculation of a numeric sulfate standard for each wild rice water, as an alternative to maintaining the existing 10 mg/L standard or establishing a different fixed number as the sulfate standard for all wild rice waters.

Part F shows that the equation-based sulfate standard is more accurate than any fixed standard at protecting wild rice from elevated sulfide. Any fixed standard has a higher error rate (being too high or too low than necessary to be protective) than the equation.

A. Confirmation that porewater sulfide is a primary controller of wild rice occurrence

The MPCA began its investigation of the effect of sulfate on wild rice in 2010 by reviewing the scientific literature. After this initial evaluation, MPCA determined that additional studies were needed to better understand the effects of sulfate on the growth of wild rice. In early 2011, MPCA staff scientists prepared a draft research protocol that was designed to further investigate the effects of sulfate on wild rice. On May 9, 2011, MPCA sponsored a discussion of the draft research protocol that included 36 scientists with pertinent expertise (13 from the University of Minnesota, seven from Federal agencies, six from Minnesota tribes, five from the MDNR, and five scientists with other affiliations). The scientists discussed the draft research protocol, which hypothesized that if sulfate is important in controlling the occurrence of wild rice, the active agent would be a result of bacterial conversion of the sulfate to hydrogen sulfide (H_2S) in the sediment where wild rice seeds germinate and grow. In water-saturated sediment, which are usually anoxic, some bacteria that degrade organic matter in the sediment "respire" sulfate, rather than oxygen, producing hydrogen sulfide. The chemical form of hydrogen sulfide varies with pH; below pH 7 H_2S dominates, and above pH 7 the bisulfide ion (HS^-) dominates. For simplicity in this document the sum of the two chemical species is referred to as sulfide.

The 2011 Legislature provide funding to research the effects of sulfate and other substances on wild rice. The research protocol was revised in response to the expert discussion, and finalized on November 8, 2011 (MPCA, 2011). Following a preliminary data collection effort in 2011, in 2012 the MPCA issued a Request for Proposals and ultimately contracted with groups of scientists at the University of Minnesota Duluth and Twin Cities campuses to undertake a study to better understand the effects of sulfate and other substances on wild rice. The MPCA study focused on collecting data on the relationship between sulfate, sulfide, and wild rice through three major parallel study components.

The components each had a specific purpose and associated strengths and limitations (Table 1-1). The study was designed so that the individual components together provided a better understanding of the effects of sulfate on wild rice. The three major study components were:

- Field surveys of wild rice habitats to investigate physical and chemical conditions correlated with the presence or absence of wild rice, including sulfate in surface water and sulfide in the sediment porewater of the rooting zone.
- Controlled laboratory hydroponic experiments to determine the effect of elevated sulfate and sulfide on early stages of wild rice growth and development.
- Outdoor container (mesocosm) experiments using natural sediments to determine the multi-year response of wild rice and other variables to a range of sulfate concentrations in the surface water.

Scientists also conducted additional research via a laboratory experiment to examine the effect of temperature on the movement of sulfate between water and sediment.

Table 1-1. Purpose, strengths, and limitations of the components of the MPCA-sponsored wild rice research.

	Field survey	Laboratory hydroponic experiments		Outdoor container experiment	Sediment incubation laboratory experiment
		Sulfate	Sulfide		
Main purpose	Expand understanding of environmental conditions correlated with the presence & absence of wild rice	Evaluate effects of sulfate on wild rice seed germination and growth of seedlings	Evaluate effects of sulfide on wild rice seed germination and growth of seedlings	Evaluate effects of sulfate loading on sulfide and wild rice life cycle, over multiple years	Evaluate effect of temperature on movement of sulfate into and out of underlying sediment
Endpoints	Concentrations of chemicals in surface water, porewater, & sediment (e.g., sulfate & sulfide) vs. wild rice occurrence	Growth of wild rice sprouts (biomass, root and shoot elongation); germination rate of seeds	Growth of wild rice sprouts (biomass, root and shoot elongation); germination rate of seeds.	Growth of wild rice (biomass, plus number and weight of seeds); sulfide concentrations in rooting zone	Sulfate concentrations in overlying water over time; sulfate, iron, sulfide, and anion tracers in sediment porewater; simple model
Key strengths	Most reflective of actual environmental conditions; multiple wild rice stands and breadth of characteristics sampled	Controlled dose-response experiment; controlled exposure to known concentrations of SO ₄	Controlled dose-response experiment; controlled exposure to known concentrations of sulfide	Controlled dose-response experiment. Includes natural sediment matrix as rooting environment; involves entire growth cycle, multiple years	Controlled experiment with natural sediment and water
Key limitations	Least controlled; annual visit for most sites, 3x/year for a subset; not definitive on cause and effect	Only evaluates early growth stages; leading hypothesis is that sulfate is converted to sulfide, which is directly toxic	Only evaluates early growth stages; unable to simultaneously keep roots anaerobic and shoots aerobic	Eventual steady states with various sulfate loads may not mimic the environment, since there is no loading of other key constituents, such as iron, from groundwater or the watershed.	Provides preliminary assessment of sediment from two sites that may inform, but is not fully transferrable to other sites; no groundwater movement; no wild rice plants grown
Reference	Myrbo et al. (in press-1.)	Pastor et al. (2017)		Pastor et al. (2017); Myrbo et al. (submitted-2)	DeRocher & Johnson (2013) Report to the MPCA.

Impact of porewater sulfide on plants and animals

In 2010, when the MPCA began its investigation on the impact of elevated sulfate and sulfide on wild rice, MPCA could find no scientific information specific to sulfide impacts on wild rice. However, elevated sulfide is a well-documented concern for other aquatic plants that root in sediment (reviewed by Lamers et al., 2013).

EPA has provided guidance on surface water criteria for sulfide, setting a level of 2 micrograms per liter ($\mu\text{g/L}$) as the maximum that can be present in a surface water before sulfide is likely to harm aquatic life. Though EPA produced this value 30 years ago (EPA, 1986), remarkably little attention has been given since then to the potential toxicity of sulfide to benthic animals and aquatic plants that root in sediment. In a major review, Bagarinao (1992) concluded that sulfide had been "...largely overlooked as an environmental factor for aquatic organisms." In a discussion of sediment toxicity testing, Wang and Chapman (1999) also observed that the biological implications of sulfide in sediments are poorly understood and "all too often ignored."

Little information has been developed about how sulfide controls the occurrence of plants and animals in water-saturated sediments and soils. A recent review (Kinsman et al., 2015) concluded that the potential toxicity of porewater sulfide is likely shaping the plant and animal communities of aquatic ecosystems, yet little data has been collected. In an exception to the paucity of data, Simkin et al. (2013) showed that porewater sulfide controlled the distribution of wetland plants more than nutrients. In their introduction, they wrote "...it is puzzling that there has not been more work to investigate the possible role of sulfide as a master variable controlling plant community composition within inland wetland ecosystems." Lamers et al. (2013), in a review of sulfide toxicity to aquatic plants, pointed out that traditional toxicity testing generally neglects the chemistry of the rooting zone.

Aquatic plants that root in marine sediment have evolved in a high-sulfate, high-sulfide environment. Ocean water averages 2,800 mg/L sulfate, so anoxic bacteria in marine sediment can potentially produce high concentrations of sulfide as bacteria degrade sedimentary organic matter. Recently, elevated sulfide has been implicated as the causative agent in a worrying global decline of marine seagrasses, which in some ways are a marine analog to wild rice. Seagrasses, which are perennial, and wild rice, which is an annual plant, are distantly related monocots that both inhabit shallow waters, although seagrasses are often totally submerged. Seagrasses colonize shallow coastal areas worldwide, providing habitat for animals and numerous ecological services. Seagrasses successfully live in the presence of high sediment sulfide by producing high amounts of oxygen through photosynthesis and transporting that oxygen to the roots, which detoxifies the sulfide by converting it back to sulfate. As a result, seagrasses require some of the highest light levels of any plant group (Orth et al., 2006). The primary hypothesis to explain the global decline of seagrasses is that sulfide toxicity is occurring more often as a result of a variety of human activities, including those that reduce underwater light or oxygen levels. In freshwater systems, elevated sulfide could be a result of sulfate pollution, so it makes sense to regulate sulfate in freshwaters. But because sulfate is uniformly high in ocean water, factors other than sulfate have been implicated in increased sulfide production or toxicity. Seagrasses are particularly vulnerable to any processes that reduce light availability, such as eutrophication or dredging of sediment (Orth et al., 2006). In addition, factors that enhance porewater sulfide concentrations have been implicated in the decline of seagrasses, including increased temperature (Koch and Erskine, 2001), increased sediment organic matter (Govers et al., 2014), and iron-poor sediment (Marbà et al., 2008). Sulfide concentrations are greater in iron-poor sediment because iron can remove sulfide from solution. It is thought that only dissolved sulfide is potentially toxic to plants and animals.

Evidence that elevated sulfide can exclude wild rice from otherwise suitable habitat

The MPCA-sponsored field survey of potential wild rice habitat, conducted 2012-2013 (Myrbo et al., in press-1), involved sampling 108 different sites and quantifying 65 field variables (Table 1-2). Some waterbodies were sampled on more than one date. For statistical purposes, a sub-set of the data (called “Class B”) was identified where each waterbody is represented by the sample closest to August 11 (the median sample date), in an effort to control for any seasonal variability. Myrbo et al. used the Class B dataset to evaluate the relationship between wild rice presence (or absence) and environmental variables through binary logistic regression (Table 1-3). In addition, Myrbo et al. correlated wild rice density at a site to the environmental variables through nonparametric Spearman tests (Table 1-3).

Field data used for statistical tests of variables that may control wild rice

The Class B data set was used for statistical tests that correlate the presence or density of wild rice against environmental variables that may control wild rice. Class B was used because this data set is the best available approximation of a random sample of potential wild rice waters that includes porewater sulfide and other variables pertinent to wild rice hypotheses. The primary hypothesis prior to conducting the MPCA field survey was that sulfide, rather than sulfate, controlled wild rice presence and absence (MPCA, 2011). It was essential to sample sites that did not have wild rice, in addition to sites with wild rice, so that variables that control the absence of wild rice could be assessed (see analysis below for further discussion of this point). Therefore, sampling only known wild rice sites would not have allowed the assessment of potentially important variables such as sulfate and sulfide. Furthermore, there was no existing data on porewater sulfide in wild rice habitat, and incomplete data on sulfate in surface waters, although the general trend of sulfate concentrations across Minnesota was known. Since it was likely that porewater sulfide was a function of sulfate in surface water, the field survey was conducted to sample a range of sulfate in waterbodies that could potentially host wild rice. Since it had been noted by naturalists and scientists that both white and yellow waterlilies co-occur with wild rice (Pillsbury and McGuire, 2007), the presence of waterlilies was used to identify potential wild rice habitat when wild rice plants could not be found in a waterbody (for further discussion, see below). Therefore, in an effort to determine the effect of elevated sulfate on wild rice, known waters with wild rice (which tended to be low in sulfate) were sampled, plus potential wild rice habitat where elevated sulfate was suspected based on geography or local land use, resulting in the Class B data set. The representativeness of the Class B data can be assessed by comparing quantile sulfate concentrations against other data sets (Table 1-2). Class B includes data from both lakes and streams. The distribution of sulfate concentrations in the Class B data set is appreciably higher than wild rice lakes, but closely approximates the randomized survey of Minnesota lakes conducted by the EPA in 2012 (Table 1-2).

In general, Minnesota streams and rivers have higher concentrations of sulfate than lakes; randomized EPA surveys found that the median, or typical, stream sulfate concentration is 17.0 mg/L, compared to 3.0 mg/L in lakes. The median concentration in Class B streams of 10.0 mg/L is appreciably higher than the median of Class B lakes, 4.1 mg/L, but not as high as the EPA’s random survey. It is unclear why the Class B stream data did not better follow the distribution of sulfate across Minnesota, but it may be because a smaller number of streams were sampled than lakes (27 compared to 81) and because the field crews were mainly sent to sites known to host wild rice (80% of stream sites had wild rice, compared to 55% of lakes).

Overall, the Class B data set is the best available approximation of a random sample of potential wild rice waters. Extrapolation of the data is mainly performed later in this TSD to assess the accuracy of models that relate sulfate in surface water to sulfide in porewater.

Table 1-2. Summary statistics for sulfate concentrations in various datasets.

Data Set	Quantile Sulfate Concentration (mg/L)			Number of sites
	25%	50%	75%	
Lakes with reported wild rice (Available sulfate data for lakes listed in MDNR, 2008)	1.0	1.8	3.6	520
All Minnesota Lakes (2012 EPA National Lakes Assessment)	0.3	3.0	13.1	50
Class B Lakes	1.0	4.1	14.6	81
Class B Streams	1.6	10.0	16.6	27
All Minnesota Streams and Rivers (2008 EPA National Rivers and Streams Assessment)	2.0	17.0	47.3	52
All Class B waterbodies (Lakes and Streams)	1.2	5.9	15.6	108

When the field crews could not find wild rice in a waterbody, they sampled the water and sediment at a location where wild rice would most likely be growing if it were to grow in that waterbody. These “non-wild rice” sampling locations were usually identified by the presence of either white or yellow waterlilies. The presence of waterlilies is taken to indicate that the habitat is similar to the habitat required by wild rice, because waterlilies and wild rice frequently co-occur (Pillsbury and McGuire, 2009). In addition, in an analysis of 1,753 MDNR aquatic plant surveys from shallow Minnesota lakes, the odds of finding wild rice where there are water lilies are 27 times the odds of finding wild rice where there are no water lilies, with a 95% confidence interval of 20-36 times. This high odds ratio is strong evidence that wild rice and waterlilies share many habitat requirements, although it appears that waterlilies may have a higher tolerance to elevated sulfide concentrations.

Statistical evaluation of variables that may control wild rice

Binary logistic regression (BLR) is the classic method for scientists to identify environmental variables that control the suitability of habitat for a particular species of interest (Hosmer and Lemeshow, 1989; Peeters and Gardeniers, 1998; van der Heide et al., 2009). BLR is “binary” in the sense that it classifies field sites as having, or not having, the species of interest—in this approach, the density of the species is irrelevant to the classification. Conservation biologists use binary information (presence/absence) in the analysis of habitat suitability; density is rarely used because representative density data are difficult to obtain and density can be a function of factors unrelated to the long-term suitability of the habitat. For example, even in excellent wild rice habitat the density of wild rice in a given year can be reduced by a hydrologic event or by animals that use wild rice for food or for nesting material. The entire wild rice plant provides food during the summer for herbivores such as Canada geese, trumpeter swans, muskrats, beaver, white-tailed deer, and moose, and the stems are harvested for nesting material by loons, red-necked grebes, and muskrats (MDNR, 2008, p. 9).

The field survey quantified the wild rice density (stems/m²) in four 1-m diameter circles around the boat where the sediment samples were collected; this does not represent the entire waterbody. The field crew did not attempt to sample a site that was “typical” of the wild rice bed, which would have been

difficult to determine. Rather, it was considered adequate to take the canoe or small boat anywhere into the wild rice bed for sampling of the water and sediment. There was no reason to expect that the wild rice density at the sampling site would be well correlated with any single environmental parameter, because wild rice density fluctuates significantly from year to year for many reasons, such as temporary nitrogen immobilization in plant litter, hydrologic fluctuation, herbivory, and other disturbances (Tables 1-6 and 1-7).

Using BLR, Myrbo et al. (in press-1) identified 12 variables that may be important in controlling the presence or absence of wild rice, as they were all significant at the 0.05 probability level or better (Tables 1-2 and 1-3). To examine the relationships among all the field variables, a Spearman correlation matrix was calculated (Table 1-4), which revealed that many of the 12 variables are correlated with each other. Because Spearman correlations are not designed for binary data, the correlation matrix included the wild rice density (stems/m²), despite the fact that the density variable just represents wild rice density around the boat at the sediment sampling site and did not characterize the wild rice bed as a whole. Somewhat surprisingly, the Spearman correlations between wild rice stem density and environmental variables generally agreed the BLR results, although sometimes at lower significance levels (Table 1-4).

Identification of three causative variables in wild rice presence/absence

Myrbo et al. (in press-1) concluded that, out of the 12 identified potentially causative variables, there are just three factors that have direct effects on wild rice — and that the other potentially causative variables are actually correlated with the truly causative factors. The three causative factors are porewater sulfide, surface water transparency, and surface water temperature. These can be understood as having effects on wild rice that, although independent of each other, also interact, especially in reinforcing correlations with other variables such as a negative correlation with total nitrogen in the surface water (see Table 1-5). Surface water nitrogen is an example of a field variable that does not act directly on wild rice, but nevertheless is significantly correlated with the absence of wild rice because it has mechanistic relationships to more than one of the three directly causative variables (Table 1-5).

The available evidence, coupled with the established scientific understanding of the biogeochemical processes of shallow aquatic ecosystems, suggest that the three causative variables act as follows: Elevated porewater sulfide reduces the growth of wild rice, either by direct toxicity or indirectly by impairing nutrient uptake (Pastor et al., 2017); water transparency below 30 cm essentially excludes wild rice from a waterbody due to light limitation (Myrbo et al., in press-1); and elevated temperatures limit the geographic range of this species of wild rice, *Zizania palustris* (Myrbo et al., in press-1).

Regarding the temperature effect, although the measured temperature variable was summer surface water temperature, the actual mechanism is more likely that the sites with higher summer temperatures also are the sites where winters fail to be sufficiently cold. The seeds of *Zizania palustris* are said to need at least three months of submersion in near-freezing water in order to break dormancy (Cardwell et al., 1978), but the needed winter and spring temperatures to break dormancy are incompletely understood (Atkins et al., 1987; Kovach and Bradford, 1992). Atkins et al. (1987) performed an experiment that incubated wild rice seed at 5 C for 5, 6, and 7.5 months, and found the highest germination after 7.5 months, but did not investigate other incubation temperatures. They also found that germination rates were greater in diurnally fluctuating temperatures rather than in constant temperatures.

Cultivated varieties of Minnesota’s wild rice are grown successfully in the Central Valley of California, which has a warmer summer than Minnesota (Fig. 1-1), indicating that high summer temperatures are likely not the limitation of wild rice range. But the winters of the Central Valley of California are too warm to break the seed dormancy, so Central Valley wild rice farmers store the seed for the next growing season over the winter under water in refrigerated facilities (Marcum, 2007). Therefore, a reasonable hypothesis generated by the observed negative correlation of wild rice occurrence with temperature is that the southern border of the wild rice range may be limited by too-warm winter temperatures, in addition to a progressively greater loss in potential shallow-water habitat due to geographic patterns in both geology and land use. MDNR (2008, p. 30) suggested that climate change-caused increases in winter temperatures could threaten the occurrence of wild rice at the southern portion of its range, due to inadequate seed exposure to cold temperatures. The strongest temperature effect of climate change in Minnesota is warming winters (Seeley, 2006, p. 84).

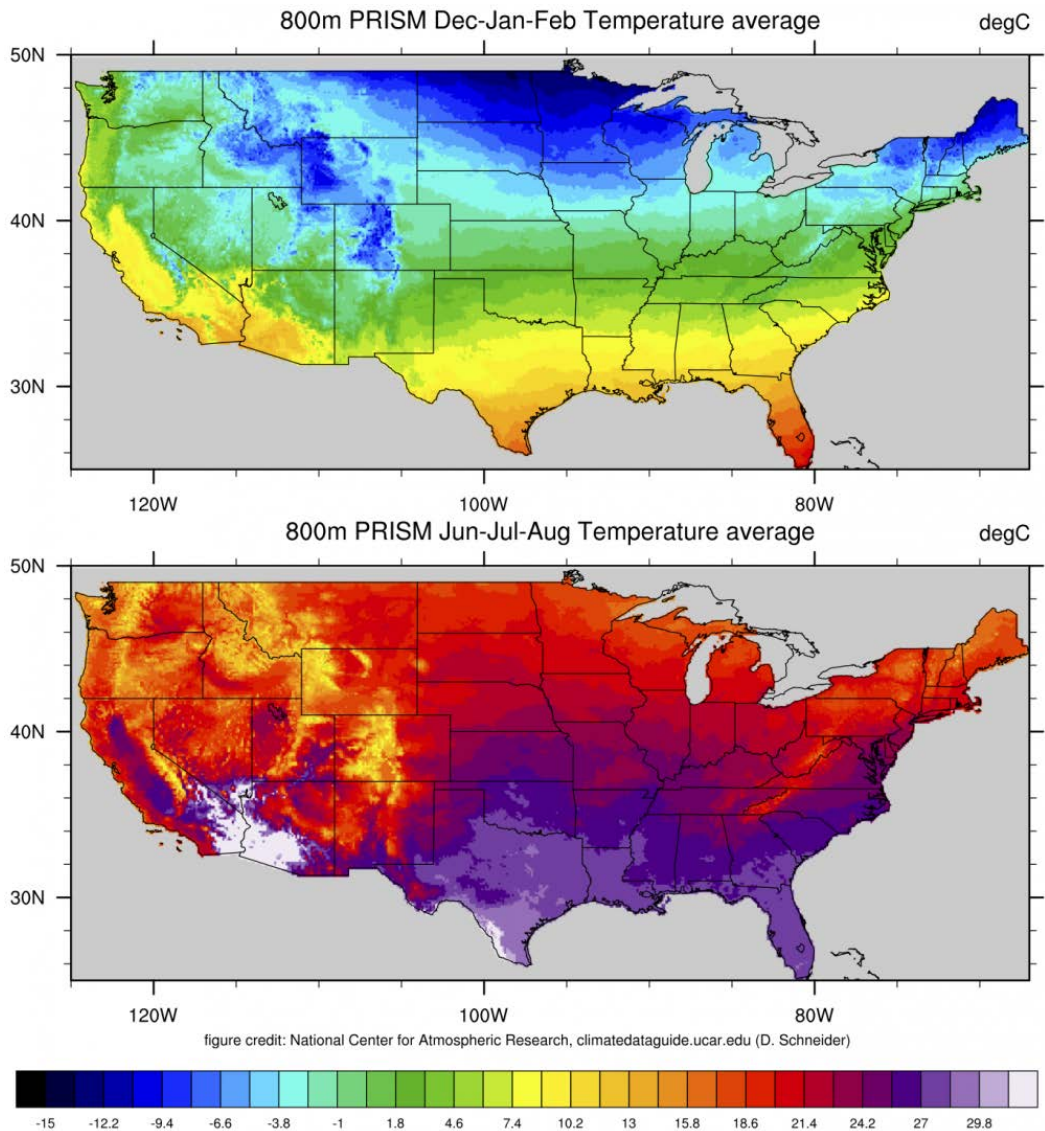


Figure 1-1. Average temperatures across the United States in winter (top) and in summer (bottom). From <https://climatedataguide.ucar.edu>.

Interaction of the three causative variables with other environmental variables

Even though the three causative variables may act independently of each other, there are likely interactions among the three variables, plus secondary effects that are correlated with wild rice presence/absence but are not causative (Myrbo et al., in press-1). For instance, additions of sulfate to waterbodies increases sulfide production, which interacts with iron to release phosphate that had been associated with iron. Furthermore, sulfate-stimulated microbes decompose sedimentary organic matter that would have not otherwise decomposed, which releases the nutrients phosphorus and nitrogen to the surface water, allowing increased phytoplankton growth. Therefore, sulfide production is correlated with increased phytoplankton, which reduces water transparency, inhibiting wild rice growth. Elevated phosphorus and nitrogen in surface water are significantly correlated with the absence of wild rice in waterbodies. These correlations most likely occur because a) the nutrients were released as a result of sulfate-enhanced decomposition of organic matter, producing toxic levels of sulfide (Myrbo et al., submitted-2), and b) their increased concentrations in surface water produced lower water transparency, which limits wild rice growth (Myrbo et al., in press-1; Table 1-5).

The production of sulfide, while negative for wild rice growth at higher porewater concentrations, also affects other variables, causing other observed correlations with wild rice (Tables 1-2, 1-3, and 1-4). These sulfide-related correlations with wild rice can be either negative, such as between wild rice and porewater potassium (K), or positive, such as the positive correlation of wild rice with porewater iron. The latter is the easiest to understand, because dissolved sulfide and dissolved iron react with each other to form a solid precipitate of iron sulfide. When porewater iron is high, sulfide is low, resulting in a positive correlation between porewater iron and wild rice, which is weaker ($p < 0.01$) than the negative correlation between porewater sulfide and wild rice ($p < 0.001$).

By performing multiple binary logistic regression (MBLR) of variables against the presence/ absence of wild rice, it is possible to determine if a variable acts independently of the three causative variables, or is simply correlated with one or more of the causative variables. In MBLR, porewater iron does not improve a model with just porewater sulfide as the predictor, and therefore the positive correlation of porewater iron with wild rice is likely just caused by the effect of sulfide on wild rice. Similarly, the significant correlation of wild rice with the concentration of total sulfur in the sediment ($p=0.048$) is probably a consequence of the strong correlation between total sulfur and porewater sulfide ($p<0.001$). The addition of the variable total sulfur to a regression does not improve the explanatory power of a regression just based on porewater sulfide, indicating that total sulfur is negatively correlated with wild rice because it is correlated with porewater sulfide, the actual causative factor (Myrbo et al., in press-1).

The negative correlations of porewater potassium (K) and surface water total nitrogen (TN) with wild rice are likely the result of their positive correlations with porewater sulfide (Table 1-5). These correlations are most easily understood in terms of the role that sulfide production plays in shallow aquatic systems: when sulfate-respiring bacteria dominate microbial activity, sulfide production is proportional to the decomposition of sedimentary organic matter, which releases the nutrients contained in the decaying plants, including potassium and nitrogen (Myrbo et al., in press-1; Myrbo et al., submitted-2; Lamers et al., 1998). In the field survey data, porewater sulfide is also positively correlated with surface water potassium and porewater concentrations of total nitrogen, ammonia, and silica (Table 1-4), all of which are released as plants decompose. The controlled sulfate-addition outdoor mesocosm experiment provides evidence that sulfide production also mobilizes phosphorus from the sediment into the overlying water, an effect supported by a positive correlation between sediment acid-volatile sulfide (AVS) and surface water phosphorus in the field data (Table 1-4). (Note that when sulfide is produced, it can either stay in the porewater or precipitate with iron; AVS is the sum of the two forms).

The strong correlation between porewater potassium with the absence of wild rice (Tables 1-4, 1-5) is remarkable, as potassium is an essential plant nutrient and therefore it is unlikely that the association is based on toxicity to wild rice. Rather, it is likely that the association is a result of the simultaneous mobilization of potassium with the production of sulfide as plant matter is decomposed simultaneously with the conversion of sulfate converted to sulfide by bacteria. Potassium does not bond covalently with organic compounds and is readily leached out of dead organic matter (Troeh and Thompson, 2005). Silica phytoliths dissolve as plant matter decomposes, allowing additional potassium that had been trapped within the phytoliths to be released into sediment porewater (Nguyen et al., 2015). Wild rice and other wetland macrophytes develop abundant phytoliths that release dissolved silica upon the decomposition of the plant tissue (Struyf and Conley, 2009). Porewater silica, potassium, and sulfide are all significantly correlated with each other (Table 1-4). The negative correlation of porewater potassium with wild rice may be magnified by its additional positive correlation with elevated water temperature (Table 1-5), which plausibly not only accelerates decomposition, but also the dissolution of silica phytoliths in the decomposing organic matter (Kamatani, 1982; Gudasz et al., 2010).

The release of potassium as sulfide is produced during the decomposition of plants is the likely explanation for an observed negative correlation between elevated potassium in surface water and wild rice growth and abundance metrics (Walker and Tuominen, 2014). Walker and Tuominen surveyed wild rice density and sampled surface water lakes and streams in northeastern Minnesota, but did not sample porewater sulfide.

Even though nutrients that limit plant growth (phosphorus and nitrogen) are not toxic to wild rice, the release of plant nutrients associated with the conversion of sulfate to sulfide can increase phytoplankton growth, reducing the light available to wild rice. Water transparency is negatively correlated both with the occurrence of wild rice and wild rice density (Table 1-3). In the field survey data, water transparency is negatively correlated with surface water phosphorus and nitrogen, but not with porewater sulfide. The lack of correlation with porewater sulfide implies that lowered transparency is negative for wild rice regardless of the porewater sulfide concentration, a conclusion confirmed in MBLR analysis (Myrbo et al., in press-1). Aside from phytoplankton abundance, transparency is also controlled by water color, with which it is highly correlated (Spearman's $\rho = -0.68$). Water color is also negatively correlated with the stem density of wild rice, but is not correlated with porewater sulfide (Table 1-4).

The temperature of the surface water measured when each site was sampled is negatively correlated with the presence of wild rice, but temperature is not correlated with porewater sulfide or transparency (Tables 1-2, 1-3, and 1-4), suggesting that temperature limits the range of wild rice independently of porewater sulfide and transparency. In MBLR, water temperature improves a model based on porewater sulfide, suggesting that temperature acts independently of sulfide. Higher temperatures may increase sulfide production by enhancing the activity of the microbial community, but that effect is already accounted for in the concentration of sulfide, so temperature must have an independent negative effect on wild rice (Myrbo et al., in press-1). Consistent with the overall climatic patterns across Minnesota, the surface water temperature variable is negatively correlated with both latitude and longitude (Table 1-4). Although wild rice occurrence is correlated with the measured surface water temperature during the summer field surveys, as discussed above the actual mechanism acting on wild rice habitat may be winters that fail to be sufficiently cold to break seed dormancy (sites with warmer summer temperatures would be the same sites with warmer winter temperatures).

Minnesota varieties of wild rice grow well in the warmer summer temperatures of the California Central Valley, but the Central Valley winter is too warm to break the dormancy of wild rice seeds. Therefore,

the correlation in the field survey between higher summer temperatures and lower probability of wild rice occurrence is more likely due to overly warm winter temperatures than overly warm summers. In the field survey data, temperature is not correlated with other variables that are known to control wild rice occurrence (sulfide and water transparency), and therefore elevated temperatures seem to have an independent negative effect on wild rice occurrence.

How access to oxygen may allow wild rice to detoxify sulfide

Wetland plants, including wild rice, have adaptations to survive long-term rooting in anoxic sediment, a condition that is fatal to virtually all terrestrial plants. Wetland ecologists recognize the production of sulfide in anoxic sediment as one of the major challenges facing plants that root in water-saturated soils (Ponnamperuma, 1972; Kirk, 2004; van der Valk, 2012). To deal with elevated sulfide, wetland plants have adaptations that allow them to decrease the toxicity of sulfide, increasing their chances of successful growth and reproduction. Wetland plants can detoxify sulfide by two broad routes involving oxygen: 1) releasing oxygen from their roots to oxidize porewater sulfide, thereby decreasing sulfide concentrations and associated toxicity, and/or 2) transporting oxygen to their roots to allow internal detoxification of sulfide that has penetrated the root tissue.

Each of these detoxification mechanisms require oxygen to be transported to the roots of the plants. Oxygen is moved to the roots of wetland plants through specialized tissue, aerenchyma, that forms a conduit from the leaves to the roots. Plants adapted to growth in water-saturated soil (or sediment), such as wild rice, transport oxygen to tissues under water and to the roots because there is no oxygen in the sediment. On a volumetric basis, the maximum oxygen content of water is at least 27 times lower than in the atmosphere (Caraco et al., 2006).

Wild rice could obtain oxygen to send to the roots from either the atmosphere (if it has grown enough to reach the water surface) or photosynthetically-produced oxygen, or both. Experiments with another grass genus, *Phragmites*, have shown that release of oxygen from roots is much greater if the plant has access to the atmosphere, rather than being completely submerged (Armstrong et al., 1999). There is no evidence that wild rice would not also transport more oxygen to the roots when emergent from water compared to completely submerged. In the *Phragmites* experiments, oxygen transport to the roots by submerged plants was always at least 40% less than in plants that emerged into the atmosphere. Submerged plants released more oxygen from roots when illuminated, due to photosynthesis, but the roots of emergent plants released more oxygen even in the dark night than the roots of illuminated submerged plants during the day.

Access to the atmosphere could help explain the large difference between the apparent toxicity of sulfide to wild rice as measured in a hydroponic test in which wild rice seedlings were completely submerged (Pastor et al., 2017) and a hydroponic test in which wild rice seedlings were allowed to emerge into the atmosphere (Fort et al., 2017). The lesser toxicity of sulfide in the latter experiment would be explicable if the wild rice seedlings were able to use the elevated oxygen concentrations from the atmosphere to enhance internal detoxification. However, under natural conditions, the seedlings (a maximum of 21-days old) would not have access to the atmosphere because the stems would not yet have elongated sufficiently to reach the water surface. Perhaps neither hydroponic test perfectly mimicked the natural environment, given that it is not definitively known to what sediment depth wild rice seedlings begin development. If seeds germinate at depth in the anoxic sediment, the elongating stem has the potential to be exposed to elevated sulfide; it should not be assumed that only the roots are exposed elevated sulfide. When seeds ripen in the fall, plants drop their seed into the water. The

individual seeds sink into the water, oriented by a rudder-like awn, and work their way into soft sediment near the parent plant (Aiken et al., 1988). Viable wild rice seeds can stay buried for multiple years in the sediment. Little is known about the environmental cues that causes a seed to germinate, after which it utilizes the energy stored in its starch to elongate its stem upward out of the sediment and through the overlying water to the surface of the waterbody. Oelke et al., (1982) observed that seeds may germinate and emerge successfully from sediment while buried in up to “3 inches in flooded soil” (7.6 cm). Meeker (2000) performed experiments where he examined the ability of seeds to successfully germinate and emerge after burial at sediment depths of 0 cm (the control), 4 cm, or 8 cm. Seeds buried 4 cm emerged from the sediment at a similar rate to the control, but the 8 cm treatment emergent rate was significantly lower. Meeker was not studying exposure to sulfide, so it is unknown what the porewater sulfide concentration was in his experiments.

Pastor et al. (2017) began their experiments with 1- to 2-cm long germinated seeds and exposed the whole seedling to the sulfide treatment. Fort et al. (2017) sprouted wild rice seed at a depth of 1 cm in the hydroponic solution and provided a trellis so that the developing seedling could emerge into the atmosphere, which allowed access to the atmosphere much earlier in development than would occur in nature. In contrast, the hydroponic exposure in the Pastor et al. experiment may actually mimic the exposure of seeds that germinate while buried in up to “3 inches in flooded soil” (Oelke et al., 1982) (7.6 cm). In the three Pastor et al. hydroponic experiments, the controls (zero sulfide) grew to 11 to 14 cm (Pastor, 2013), but the seedlings in the highest sulfide treatments only grew to a maximum of 5.3 to 7.6 cm, a similar distance that germinated wild rice might elongate through anoxic sediment with elevated sulfide concentrations. Thus, the Pastor et al. experiment may mimic the sulfide exposure of seeds that germinate while buried under 8 cm of sediment.

In an outdoor mesocosm experiment, sulfide was significantly lower in sediments with wild rice, indicating that adult wild rice releases oxygen from its roots, oxidizing sulfide (Myrbo et al. mesocosm paper, submitted-2). However, wild rice plants may not need to oxidize the entire pool of elevated sulfide in the porewater to reduce sulfide toxicity if the plant can increase the supply of oxygen to roots and submerged tissues, detoxifying sulfide through the second route. Given the *Phragmites* finding, the ability of wild rice to transport oxygen to the roots and detoxify sulfide would likely be enhanced once the growing seedling reaches the water surface, where it not only can access much higher oxygen concentrations, but where it can photosynthesize at higher rates, producing more oxygen. Therefore, germination in shallow water might allow wild rice to detoxify porewater sulfide more efficiently, both internally and externally. Internal detoxification of sulfide has not been looked for in wild rice, but has been demonstrated in plant tissues from other wetland plants (Lee, 2003; Lamers et al., 2013).

Two mechanisms of internal detoxification of sulfide have recently been described in plants: (1) the conversion of sulfide to the amino acid cysteine by the enzyme OAS-TL C (Alvarez et al., 2012), and (2) the oxidation of sulfide by sulfur dioxygenase (SDO), which produces thiosulfate (Krüssel et al., 2014). The first mechanism does not depend on oxygen availability. The second mechanism, the enzyme SDO, not only requires oxygen, but the detoxification of sulfide can be quantified by measuring the consumption of oxygen (Krüssel et al., 2014; Birke et al., 2015).

In summary, it likely that wild rice is better able to detoxify sulfide after a seedling has grown long enough to reach the atmosphere at the water surface, at which time it grows a floating leaf. The floating leaf can be the source of oxygen that is sent to the roots, where SDO can detoxify sulfide by combining sulfide with oxygen. Wild rice is unusual among grasses in that the stem develops before the root, probably because the seedling may have to grow between 50 and 100 cm before reaching the water

surface, at which time floating leaves supply oxygen and energy for root development (Aiken, 1986; Pastor et al., 2017).

Wild rice may be able to tolerate higher levels of porewater sulfide when the seedlings can reach the water surface faster, which would be aided by shallower water and more nitrogen availability. This may be a reason for the lack of an observed negative sulfide effect on cultivated wild rice, since cultivated wild rice is fertilized with nitrogen and water levels are managed. In addition, there is evidence that wetland plants fertilized with nitrogen can better oxidize sulfide around the roots, reducing the potential toxicity (Howes et al., 1986). Five of seven cultivated paddies sampled during the MPCA-sponsored field study exhibited dense wild rice stands where porewater sulfide exceeded 120 µg/L, the concentration identified as protective for natural stands of wild rice. The sulfate standard to protect wild rice needs to maintain porewater sulfide at levels low enough to allow growth and reproduction in natural waters of varying depths and nutrient contents.

Table 1-3. Correlations of field variables with wild rice and porewater sulfide. For sites where multiple samples were collected, the site is represented by the closest sample to August 11, in either 2012-2013 (termed the “Class B” data set; N=108). The variables are ordered by the significance of the variable’s correlation with the presence or absence of wild rice, as measured by binary logistic regression (Myrbo et al., in press-1). (PW=porewater; SW=surface water; Sed=Sediment; *=p <0.05; **=p <0.01; ***=p <0.001).

Field Variable	Spearman Correlation with Field Variable				Binary Logistic Regression for the presence/absence of wild rice	
	Porewater sulfide Correlation (rho)	PW sulfide Correlation significance	Wild rice density Correlation (rho)	Wild Rice Density correlation significance	Regression P value	Regression significance
PW K	0.46	***	-0.36	***	0.0008	***
PW sulfide	1.00		-0.35	***	0.0012	**
Water Depth (m)	0.11	not sig	-0.24	*	0.0028	**
Transparency (cm)	-0.07	not sig	0.24	*	0.0031	**
SW TN	0.22	*	-0.23	*	0.0054	**
Sed Se % dry	0.08	not sig	-0.22	*	0.0059	**
SW Temp	0.17	not sig	-0.17	not sig	0.0077	**
PW Fe	-0.58	***	0.21	*	0.0109	*
SW pH	0.28	**	-0.24	*	0.0200	*
SW TP	0.05	not sig	-0.11	not sig	0.0353	*
Latitude	-0.06	not sig	0.19	*	0.0376	*
Sed TS % dry	0.40	***	-0.21	*	0.0483	*
PW Na	0.33	***	-0.25	**	0.0670	not sig
PW Zn	-0.08	not sig	-0.09	not sig	0.0746	not sig
SW Cl	0.29	**	-0.18	not sig	0.0783	not sig
SW K	0.29	**	-0.08	not sig	0.0922	not sig
Sed Cu % dry	0.00	not sig	-0.14	not sig	0.0940	not sig
Sed Al % dry	-0.05	not sig	-0.11	not sig	0.1109	not sig
Sed AVS % dry	0.29	**	-0.10	not sig	0.1317	not sig
SW sulfate	0.44	***	-0.17	not sig	0.1475	not sig
Sed NAl P % dry	-0.06	not sig	-0.04	not sig	0.1958	not sig
Longitude	-0.15	not sig	-0.16	not sig	0.2141	not sig
SW Ca	-0.06	not sig	0.22	*	0.2489	not sig
PW As	-0.43	***	0.15	not sig	0.2642	not sig
Sed TP % dry	0.07	not sig	-0.10	not sig	0.2697	not sig
SW Alkalinity	0.22	*	0.24	*	0.2786	not sig

(continued)

Field Variable	Spearman Correlation with Field Variable				Binary Logistic Regression for the presence/absence of wild rice	
	Porewater sulfide Correlation (rho)	PW sulfide Correlation significance	Wild rice density Correlation (rho)	Wild Rice Density correlation significance	Regression P value	Regression significance
PW TN	0.31	**	-0.23	*	0.2963	not sig
Sed % Coarse of ORG	-0.06	not sig	0.05	not sig	0.3360	not sig
Sed % Fine of ORG	0.05	not sig	-0.05	not sig	0.3575	not sig
Sed Zn % dry	-0.06	not sig	-0.03	not sig	0.3825	not sig
PW Ca	-0.01	not sig	0.22	*	0.4443	not sig
PW NH ₄	0.33	***	-0.22	*	0.4505	not sig
Sed TEF _e % dry	-0.35	***	-0.01	not sig	0.4795	not sig
PW DOC	-0.05	not sig	-0.01	not sig	0.4865	not sig
Sed Org P % dry	0.07	not sig	-0.08	not sig	0.5468	not sig
PW Si	0.33	***	-0.02	not sig	0.5548	not sig
PW Cu	-0.09	not sig	-0.07	not sig	0.5704	not sig
SW Na	0.26	**	-0.05	not sig	0.5859	not sig
SW Conductance	0.35	***	0.12	not sig	0.6028	not sig
SW Color	-0.11	not sig	-0.20	*	0.6122	not sig
PW TP	0.12	not sig	-0.06	not sig	0.6341	not sig
Sed TIC % dry	0.20	*	0.05	not sig	0.6519	not sig
Sed Inorg LOI	-0.16	not sig	-0.04	not sig	0.6668	not sig
Sed coarse org % dry	0.07	not sig	0.08	not sig	0.6737	not sig
Sed TN % dry	0.14	not sig	-0.08	not sig	0.6807	not sig
SW Fe	-0.33	***	0.02	not sig	0.6827	not sig
Sed fine org % dry	0.09	not sig	-0.07	not sig	0.6971	not sig
SW Mg	0.40	***	0.10	not sig	0.7151	not sig
Sed coarse inorg % dry	-0.15	not sig	0.00	not sig	0.7194	not sig
Sed fine inorg % dry	0.11	not sig	0.07	not sig	0.7267	not sig
Sed Water content	0.15	not sig	-0.07	not sig	0.7274	not sig
Sed Exchangeable P % dry	0.17	not sig	-0.03	not sig	0.7350	not sig
Sed % Coarse INORG	-0.15	not sig	-0.05	not sig	0.7489	not sig
PW Mn	-0.30	**	0.10	not sig	0.7608	not sig
Sec Ca % dry	0.21	*	0.08	not sig	0.7614	not sig

(continued)

Field Variable	Spearman Correlation with Field Variable				Binary Logistic Regression for the presence/absence of wild rice	
	Porewater sulfide Correlation (rho)	PW sulfide Correlation significance	Wild rice density Correlation (rho)	Wild Rice Density correlation significance	Regression P value	Regression significance
Sed % Fine of INORG	0.15	not sig	0.06	not sig	0.7661	not sig
Sed TOC % dry	0.10	not sig	-0.06	not sig	0.7854	not sig
Sed Mg % dry	0.23	*	0.09	not sig	0.8195	not sig
Sed Apatite P % dry	0.08	not sig	0.01	not sig	0.8495	not sig
PW pH	0.03	not sig	-0.04	not sig	0.8976	not sig
Sed org LOI	0.08	not sig	-0.06	not sig	0.9263	not sig
Sed CO ₃ LOI	0.25	**	0.06	not sig	0.9677	not sig
PW Mg	0.33	***	0.11	not sig	0.9843	not sig
Sed As % dry	-0.13	not sig	0.17	not sig	0.9913	not sig
Sed Mn % dry	-0.06	not sig	0.13	not sig	0.9915	not sig

Table 1-5. The 12 field variables that are significantly correlated with the presence/absence of wild rice, as determined through binary logistic regression (Myrbo et al., in press-1). Below the name of each field variable is the nature of the correlation with wild rice presence (positive or negative) and the average value at the sites with wild rice present and at sites with wild rice absent. The Spearman correlation coefficient is termed “rho.” (*=p<0.01; **=p<0.01; ***=p<0.001)

Field Variable (positive or negative correlation with wild rice presence) Site averages: Present, Absent	Significance of binary logistic regression with wild rice presence/absence (p value)	Likely reason for correlation with wild rice presence/absence	Spearman Correlations					
			Porewater Sulfide		Water Transparency		Water Temperature	
			rho	Significant?	rho	Significant?	rho	Significant?
Porewater sulfide (negative) 165, 795 µg/L	0.0012	Elevated porewater sulfide is correlated with the absence of wild rice most likely because elevated sulfide reduces the growth of wild rice.	1.00		-0.07	not sig	0.17	not sig
Surface water temperature (negative) 22.1, 24.4 °C	0.0077	Elevated surface water temperature is negatively correlated with wild rice occurrence, independent of water transparency. Temperature is weakly correlated with porewater sulfide (rho= 0.17); warmer summer and winter temperatures likely have a negative effect on wild rice that is independent of sulfide.	0.17	not sig	-0.08	not sig	1.00	
Latitude (positive) 46.6, 46.1 degrees	0.0376	Minnesota has strong latitudinal gradients in many environmental factors, but latitude’s strongest correlation in this data set is with water temperature (transparency, sulfate, and sulfide are not significantly correlated). It is most likely that northern latitude sites are correlated with the presence of wild rice presence because they are colder.	-0.06	not sig	0.13	not sig	-0.51	***
Water transparency (positive) 84, 66 cm	0.0031	Reduced transparency is correlated with the absence of wild rice, independent of porewater sulfide and surface water temperature. Wild rice is rarely observed at transparencies below 30 cm.	-0.07	not sig	1.00		-0.08	not sig
Porewater potassium (negative) 3.5, 6.1 mg/L	0.0008	There is no reason to expect that elevated porewater potassium (K) is harmful to wild rice, as it is a plant nutrient. Rather, it is likely that porewater K is simply correlated with porewater sulfide because sulfide production is associated with enhanced decomposition of organic matter, which releases the plant nutrients K, N, and P. The negative correlation of porewater K with wild rice is magnified by its additional correlation with temperature, which could be driving non-sulfide related organic decomposition, which would also release K to porewater.	0.46	***	-0.10	not sig	0.33	***

(continued)

Field Variable (positive or negative correlation with wild rice presence) Site averages: Present, Absent	Significance of binary logistic regression with wild rice presence/ absence (p value)	Likely reason for correlation with wild rice presence/absence	Spearman Correlations					
			Porewater Sulfide		Water Transparency		Water Temperature	
			rho	Significant?	rho	Significant?	rho	Significant?
Surface water total nitrogen (negative) 0.9, 1.2 mg/L	0.0054	There is no reason to expect that elevated nitrogen is directly harmful to wild rice, although elevated N likely encourages growth of algae or macrophytes that shade or compete with wild rice. Total nitrogen is correlated with all 3 causative factors that are negative for wild rice occurrence, in that it is not only associated with reduced transparency, but it is also correlated with sulfide and temperature, which are both associated with enhanced decomposition of sediment organic matter.	0.22	*	-0.61	***	0.23	*
Surface water total phosphorus (negative) 41, 62 µg/L	0.0353	There is no reason to expect that elevated phosphorus is directly harmful to wild rice, although elevated P likely encourages growth of algae or macrophytes that shade or compete with wild rice. Total phosphorus is not only correlated with reduced transparency, but it also correlated with water temperature, which is associated with enhanced decomposition of organic matter.	0.05	not sig	-0.58	***	0.27	**
Porewater Fe (positive) 11.0, 7.0 mg/L	0.0109	Porewater iron is negatively correlated with porewater sulfide, which is most likely the causative factor for the correlation with the occurrence of wild rice.	-0.58	***	0.04	not sig	-0.09	not sig
Sediment total sulfur (negative) 3.9, 6.9 mg/g	0.0483	Sediment total sulfur is positively correlated with porewater sulfide, which is most likely the causative factor.	0.40	***	0.03	not sig	-0.08	not sig
Sediment total selenium (negative) 1.0, 1.3 µg/g	0.0059	The slightly higher selenium at sites without wild rice is most likely caused by the co-precipitation of selenium and sulfur by sulfate-reducing bacteria, as shown by Hockin and Gadd (2003). Selenium is correlated with sediment total sulfur (rho=0.35, p < 0.001).	0.08	not sig	-0.21	*	0.13	not sig

(continued)

Field Variable (positive or negative correlation with wild rice presence) Site averages: Present, Absent	Significance of binary logistic regression with wild rice presence/ absence (p value)	Likely reason for correlation with wild rice presence/absence	Spearman Correlations					
			Porewater Sulfide rho Significant?		Water Transparency rho Significant?		Water Temperature rho Significant?	
Surface water pH (negative) 7.9, 8.3 pH units	0.0200	It is unlikely that elevated pH (8.3 ± 0.9 at sites without wild rice compared to 7.9 ± 0.8 at sites with wild rice) is harming wild rice. Rather, elevated surface water pH is reflecting elevated water temperature, which reduces the solubility of CO ₂ , raising the pH. Synergistically, pH is also correlated with porewater sulfide, which is stoichiometrically related to the generation of alkalinity, raising the pH.	0.28	**	-0.05	not sig	0.35	***
Water Depth (negative) 52 , 67 cm	0.0028	Although water depth could control wild rice presence, this metric does not characterize the waterbody, but rather where the field crews took the sample. When wild rice was not present, field crews usually sampled at water lilies, which tended to grow in slightly deeper water than wild rice. The correlation with temperature likely is driven by the tendency of warmer sites to not host wild rice. The significant positive correlation with transparency does not drive the negative correlation with wild rice occurrence, as it is the wrong sign, and therefore inexplicable.	0.11	not sig	0.22	*	0.19	*

B. That other factors affect wild rice does not negate the need to protect wild rice from excess sulfide

Multiple stressors affect wild rice in nature.

Some comments received in regards to the March 2015 Draft Proposal (MPCA, 2015) focused on regulating sulfate. Others suggested that a) it is inappropriate to regulate sulfate without also addressing the many other factors, aside from sulfide, that likely control the presence of wild rice, and b) factors other than sulfate (and sulfide) are more important in controlling the suitability of wild rice habitat. It was further suggested that it is not appropriate to use field data to identify a sulfide concentration that is protective of wild rice both because field data are inherently variable and in light of the multiple stressors that were not studied in the MPCA-sponsored research, especially a) changes in water levels from year to year, b) impacts of development, and c) presence of invasive or competitive species.

It is true that there is more “noise” in field data than in a controlled experiment. Because of this noise, or data variability, it is more challenging to detect a statistically significant impact of a particular stressor in field data; there is more statistical power in controlled laboratory experiments (Chapman, 2002). It is important to conduct controlled laboratory experiments to determine that a particular stressor (such as sulfide) has the potential to negatively affect a species, but the ecological significance of that effect is ambiguous until mesocosm or field data are collected (Chapman, 2002). If, despite environmental variability, a statistically significant relationship is demonstrated in the field that reinforces the laboratory finding, then there is little question that the chemical is important in controlling the occurrence of that species in the environment.

Despite the challenge of documenting a statistically significant relationship in field data, the binary logistic regression (BLR) analysis found a statistically significant negative correlation between the concentration of sulfide in the sediment porewater and the occurrence of wild rice ($p=0.001$, Table 1-5). Performing *multiple* BLR with more than one variable demonstrated that porewater sulfide is one of three primary independent variables correlated with wild rice occurrence (Myrbo et al., in press-1): porewater sulfide, water transparency, and water temperature. The statistical analysis strongly supports the conclusion that sulfide independently affects wild rice presence and absence ($p=0.001$; Table 1-3), which implies that limiting sulfate availability has the potential to protect wild rice from elevated sulfide. Analysis of the MPCA field data shows that porewater sulfide is simultaneously controlled by surface water sulfate and sediment concentrations of total organic carbon (TOC) and total extractable iron (TEFe) (Pollman et al., in press; discussed in Part D of this chapter). Interestingly, sulfate, TOC, and TEF_e do not have any statistically significant effect on wild rice occurrence when considered individually ($p=0.15$, 0.79 , and 0.48 , respectively; Table 1-3; Myrbo et al., in press-1). These three environmental variables only have a relationship to the occurrence of wild rice when they are considered simultaneously, given that particular combinations of the three can produce excessive concentrations of porewater sulfide (Part E of this Chapter).

Factors that act independently of porewater sulfide may also affect wild rice growth, such as hydrological changes and exotic species (Tables 1-6 and 1-7), but unless a factor has an effect on the relationship between sulfate and sulfide, consideration of such a factor is irrelevant to the mission of protecting wild rice from excess sulfide. The only factors that have been identified that have an effect on porewater sulfide are sulfate, sediment TOC, and sediment iron (Pollman et al., in press). However, one exception may be sites with upwelling groundwater; it has been reported that such sites may be favorable habitat for wild rice (Table 1-6). Consistent upward groundwater flow would break the usual relationship between sulfate in surface water and sulfide in porewater, because sulfate would be less likely to move downwards into the sediment when groundwater is moving upwards. Therefore, at some

sites the sulfate concentration of the groundwater may be more important than the surface water in controlling the production of porewater sulfide, but statistical analysis shows that at most sites porewater sulfide is a function of surface water sulfate (Pollman et al., in press). Even if this were not the case, the possibility that groundwater, rather than surface water, controls porewater sulfide in a specific wild rice bed does not negate the validity of the empirically observed, statistically significant, relationship between surface water sulfate, sediment iron, sediment TOC, and porewater sulfide as a general matter (Part D of this chapter, below; Pollman et al., in press).

Table 1-6. Reported environmental requirements for suitable wild rice habitat. Most reports comment on factors affecting the relative growth or density of wild rice rather than factors that control the presence and absence of wild rice, which is how conservation biologists identify factors that are critical to favorable habitat for a species.

Environmental requirement for suitable wild rice habitat (source)	Relevant findings from MPCA-sponsored research
Wild rice grows best when surface water sulfate is less than 10 mg/L	
<p>“No large stands of rice occur in waters having a SO₄ content greater than 10 ppm, and rice generally is absent from water with more than 50 ppm.” (Moyle, 1944)</p> <p>“Wild rice has marked preference for the quality of water in which it grows and is not found in prairie waters which have appreciable amounts of sulfate or “alkali” salts. In Minnesota the range is mostly limited to waters with concentration of sulfate or “alkali” salts lower than 10 parts per million of sulfate ion. Plantings of wild rice seed in prairie waters with higher concentrations of sulfates have generally failed. (Moyle and Krueger, 1964)</p>	<p>The effect of sulfate depends on the background sediment chemistry of the particular waterbody. Elevated sulfate sometimes allows excessive porewater sulfide to develop (Myrbo et al., in press-1; Pollman et al., in press).</p>
Wild rice grows best when surface water alkalinity greater than 40 mg/L	
<p>“Best growth is made in carbonate waters having total alkalinity greater than 40 ppm.” (Moyle, 1944)</p> <p>“Lakes that have had wild rice for many years usually have the following characteristics: (4) they usually are fairly limy and have a total alkalinity exceeding 40 parts per million (there are stands, however, in softer water);” (Moyle and Krueger, 1964)</p>	<p>There is no statistically significant effect of alkalinity on wild rice presence (Table 1-3; Myrbo et al., in press-1).</p>
High phosphorus can have adverse effects on wild rice	
<p>“Wild rice grows within a wide range of chemical parameters (i.e. alkalinity, salinity, pH, and iron; Meeker 2000). However, productivity is highest in water with a pH of 6.0 to 8.0 and alkalinity greater than 40 ppm. While researchers have observed that natural wild rice stands are relatively nutrient rich, excess levels of some nutrients, especially phosphorus, can have significant adverse effects on productivity (Persell and Swan, 1986).” (MDNR, 2008, p. 14)</p>	<p>Wild rice absence is correlated with elevated surface water pH and phosphorus, which are both associated with high phytoplankton levels, reducing transparency (Table 1-3; Myrbo et al., in press-1).</p>

(continued)

Environmental requirement for suitable wild rice habitat (source)	Relevant findings from MPCA-sponsored research
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Nitrogen and phosphorus are limiting nutrients for wild rice

“Nitrogen and phosphorus are limiting nutrients for wild rice (Carson 2002).” (MDNR, 2008, p. 14). Five years of experimental data as well as a model of N cycling in wild rice ecosystems (Pastor and Walker 2006) suggest that delays in nutrient availability the year following deposition of large amounts of immobilizing litter cause biomass and population oscillations over a cycle potentially four years in length (Walker et al., 2010).

Rooted aquatic plants obtain their nutrients from the sediment, rather than from the surface water (Barko and Smart, 1986). There is no statistically significant relationship between sediment nitrogen or phosphorus and wild rice presence (Table 1-3; Myrbo et al., in press-1). Greater sedimentary N and P may increase growth of individual wild rice plants, but not control presence/absence of wild rice.

Wild rice grows best when carp populations are low

Judging from lake names, it once grew farther south in central Minnesota where it has probably been exterminated by carp.” (Moyle and Krueger, 1964)

No data were collected on carp presence.

“Common carp feed primarily on invertebrates in bottom soils. Their feeding action dislodges plants and suspends fine particles into the water column. The increased turbidity, caused both by disturbed sediments and by algae stimulated by the phosphorus released from disturbed sediments, shades out aquatic plants. Turbidity then increases as non-vegetated lake bottoms are disturbed by wind. The reduction in aquatic vegetation also allows for increased boat traffic and wave action that can further dislodge plants such as wild rice (Pillsbury and Bergey, 2000).” (MDNR 2008, p. 27)

Low water transparency can likely cause the absence of wild rice (Myrbo et al., in press-1).

Wild rice grows best in habitat with moving water

“The crop grows best in lakes having some water moving through and often is lacking from stagnant lakes and pools. It is frequent along streams and at lake inlets and outlets.” (Moyle, 1944)

No data were collected on water movement.

“Lakes that have had wild rice for many years usually have the following characteristics: (2) they are wide enough to have heavy wave action in spring or have a flow of water through them;” (Moyle and Krueger, 1964)

“Natural wild rice generally requires some moving water, with rivers, flowages, and lakes with inlets and outlets being optimal areas for growth. Seasonal water depth is critical, however. Water levels that are relatively stable or decline gradually during the growing season are preferred. In particular, abrupt increases during the early growing season can uproot plants. Wild rice grows well at depths of 0.5 to 3 feet of water, although some plants may be found in deeper waters (M. McDowell, J. Persell personal communication).” (MDNR, 2008, p. 14)

(continued)

Environmental requirement for suitable wild rice habitat (source)	Relevant findings from MPCA-sponsored research
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Groundwater inflow areas can be favorable habitat for wild rice

Natural wild rice generally requires moving water, with rivers, flowages, and lakes or wetlands with inlets and outlets being optimal areas for growth. In some areas groundwater flows are apparently adequate to meet the need for flowing water (Norrsgard, 2014).

Wild rice is in a group of emergent plant species that had a mild statistical association with groundwater inflow areas of lakes (Nichols & Shaw, 2002).

No information was collected on groundwater movement at the field sites. Upward flow would break the usual relationship between surface water sulfate and sulfide, because sulfate would be less likely to move downwards into the sediment when groundwater is moving upwards.

Surface water with high transparency

Often lacking from bog lakes with dark brown water. (Moyle, 1944)

“...clear to moderately colored (stained) water is preferred, as darkly stained water can limit sunlight and may hinder early plant development.” (MDNR, 2008, p. 14)

“Waters in which wild rice grows are often somewhat brownish or tea-colored — but dark brown water is not favorable, for this cuts down penetration of the light that the first submerged leaves must have if the plant is to grow. In such waters, especially over mucky bottoms, the rice seedlings may be crowded by such submerged plants as coon tail, flat-stemmed pondweed and star duckweed. Waterlilies may invade wild rice stands and shade out the rice.” (Moyle and Krueger, 1964)

Lower water transparency is correlated with absence of wild rice (Table 1-3; Myrbo et al., in press-1).

Wild rice occurs in shallow water between 0.5 and 3 feet depth

“Lakes that have had wild rice for many years usually have the following characteristics: (1) they contain much water shallower than four feet; “(Moyle and Krueger, 1964)

“Wild rice grows well at depths of 0.5 to 3 feet of water, although some plants may be found in deeper waters (M. McDowell, J. Persell personal communication).” (MDNR, 2008, p. 14)

Consistent with observations of Myrbo et al. (in press-1).

(continued)

Environmental requirement for suitable wild rice habitat (source)	Relevant findings from MPCA-sponsored research
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Wild rice grows best in waters with organic sediment

“Lakes that have had wild rice for many years usually have the following characteristics: (3) they have an organic bottom a few inches to a few feet thick, overlying a hard bottom;” (Moyle and Krueger, 1964)

“Although wild rice may be found growing in a variety of bottom types, the most consistently productive are lakes with soft, organic sediments (Lee, 1986). The high organic matter content with a rather low carbon/nitrogen ratio is necessary to meet the rather high nitrogen needs of wild rice (Carson, 2002). Nitrogen and phosphorus are major limiting nutrients for wild rice (Carson, 2002). Flocculent sediments with nitrogen and phosphorus concentrations less than one gram per square meter are typically incapable of supporting sustained production (Lee, 1986).” (MDNR, 2008, p. 97)

Myrbo et al. (in press-1) found no significant correlations between wild rice occurrence and sediment concentrations of organic matter, nitrogen, or phosphorus, or flocculent sediments (as measured by the water content). Note that Myrbo et al. examined what factors controlled the occurrence of wild rice, not what factors controlled the quality or density of wild rice.

The best wild rice habitat has some summers with high water to reduce the dominance of perennial plants

“As an annual plant sprouting each year from seed, wild rice can have difficulty competing with aggressive perennial vegetation, particularly where natural hydrologic variation has been reduced. Cattail spp.), particularly hybrid cattail (*Typha x glauca*), yellow water lily (*Nuphar variegata*), and pickerelweed (*Pontederia cordata*) are examples of plants that have been cited as competing with wild rice (Norrgard, David, and Vogt, personal communication)” (MDNR, 2008, p. 91).

No pertinent information was collected on competition with perennial vegetation or the effect of high water on the control of perennial vegetation.

“Lakes that have had wild rice for many years usually have the following characteristics: (6) the drainage area feeding the lakes is usually fairly large and the outlet such that there is high water in some summers (times when high water drowns out cattails and other perennial emergent plants that would otherwise crowd out the rice);” (Moyle and Krueger, 1964)

“Cattails and perennial reeds and rushes will crowd out wild rice if allowed to become established. Such plants should be eradicated in paddies and in wild stands drowned out by occasional flooding. Usually there are years of high water (about one year in four) on wild rice stands that have remained as such for a long period of time.” (Moyle and Krueger, 1964)

Yellow water lily (*Nuphar variegata*) and *Utricularia vulgaris* occurrence patterns show that these plants prefer environmental conditions similar to optimal rice habitat, indicating that these plants are likely competitors. Resource managers often assert that wild rice competes with perennial plants such as cattails spp.), pickerelweed (*Pontederia cordata* L.), and waterlilies (*Nymphaea* and *Nuphar*) as a major factor in the disappearance of wild rice stands, but this hypothesis has rarely been tested (Pillsbury and McGuire, 2009).

(continued)

Environmental requirement for suitable wild rice habitat (source)	Relevant findings from MPCA-sponsored research
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In most summers the best wild rice habitat does not have an abrupt rise in water level of more than 6 inches

“Lakes that have had wild rice for many years usually have the following characteristics: (7) water levels which in years of normal or deficient rainfall do not rise sharply (more than 6 inches) at any time during June or July when the wild rice is in the floating-leaf stage.” (Moyle and Krueger, 1964)

No pertinent information was collected on the effect of an abrupt increase in water level on the occurrence of wild rice.

“Water levels that are relatively stable or decline gradually during the growing season are preferred. Abrupt water level increases during the growing season can uproot plants. Wild rice is particularly sensitive to this disturbance during the floating leaf stage. However, some observers feel that water levels kept stable over the long term (multiple years) tend to favor perennial aquatic vegetation over wild rice (David and Vogt, personal communication).” (MDNR, 2008, p. 90)

“Water levels that are relatively stable or decline gradually during the growing season are preferred. Abrupt water level increases during the growing season can uproot plants. Wild rice is particularly sensitive to this disturbance during the floating leaf stage. However, some observers feel that water levels kept stable over the long term (multiple years) tend to favor perennial aquatic vegetation over wild rice (David and Vogt, personal communication).” (MDNR, 2008, p. 90)

Germination of wild rice seeds requires winters with at least 3 months of nearly freezing water

“As an annual plant, natural wild rice develops each spring from seeds that fell into the water and settled into the sediment during a previous fall. Germination requires a dormancy period of three to four months of cold, nearly freezing water (35 F or colder). Seeds are unlikely to survive prolonged dry conditions.” (MDNR, 2008, pp. 14-15). The seed of *Z. palustris* must experience at least 3 months in water at 1 to 3° C (Cardwell et al., 1978).

Warmer summer water temperature is correlated with absence of wild rice, which may be caused by a correlation with winters that are insufficiently cold or long to break seed dormancy (Myrbo et al., in press-1.).

Residential development in a watershed is negative for habitat; deeper habitat may be more sensitive to stress

Wetlands that have lost most of their rice tended to have an increase in residential development within the watershed, and higher ammonium, pH, and water depth. In general, low density rice wetlands tended to be slightly deeper than other sites. Any additional stress may have caused a decline of rice in deeper wetlands while not affecting the rice in shallower wetlands (Meeker 2000, cited in field survey report by Pillsbury and McGuire, 2009)

Cause and effect not investigated, but if development increases nutrients, reduced transparency would affect deeper habitat more than shallow habitat, and pH is correlated with higher nutrients.

Table 1-7. Reported threats to existing natural stands of wild rice.

Increased external loading of sulfate or phosphorus

An increase in sulfate loading to surface water, causing an exceedance of the existing Minnesota sulfate standard of 10 mg/L (MDNR, 2008, p. 25).

An increase in sulfate loading to a surface water that is particularly efficient at converting sulfate to sulfide, increasing porewater sulfide to toxic levels (this document).

A decrease in transparency due to increased phytoplankton growth caused by a) increased external loading of phosphorus (Myrbo et al., in press-1) or b) increased internal loading of phosphorus caused by increased external sulfate loading (Myrbo et al., submitted-2.).

Shoreline and watershed development

Development may entail threats to wild rice apart from enhanced phosphorus and sulfate loading; increased shoreline development reduces aquatic plant cover (Radomski, 2006), although wild rice was not specifically studied, and mechanisms of loss are not documented. Boat traffic may dislodge wild rice (Pillsbury and Bergey, 2000).

Hydrological threats

Dams that maintain stable water levels can favor perennial vegetation over wild rice. (MDNR 2008, p. 22)

Increases in the frequency of rapid increase in water levels, particularly in early summer (MDNR, 2008, p. 21), "...although wild rice is well-adapted to annual fluctuations in water levels, while other aquatic plants may be less suited to such changes." (MDNR, 2008, p. 24) "The emergent stage begins with the development of one or two floating leaves and continues with the development of several aerial leaves two to three weeks later. The floating leaves are apparent in late May to mid-June in Minnesota, again dependent on water depth, latitude, and weather. It is at this stage of growth that wild rice is most susceptible to uprooting by rapidly changing water levels due to the natural buoyancy of the plant. Rising water levels can significantly stress the plant even if it remains rooted." (MDNR, 2008, p. 88)

Groundwater extraction that dries out wild rice habitat. (MDNR, 2008, p. 25)

Impoundments or beaver activity that raise water level in wild rice beds over 3 feet. (MDNR, 2008, p. 21)

Native and exotic species

Carp feeding action dislodges plants and suspends fine particles into the water column. The increased turbidity, caused both by disturbed sediments and by algae stimulated by the phosphorus released from disturbed sediments, shades out aquatic plants. Turbidity then increases as non-vegetated lake bottoms are disturbed by wind. The reduction in aquatic vegetation also allows for increased boat traffic and wave action that can further dislodge plants such as wild rice (Pillsbury and Bergey, 2000). (MDNR,2008, p. 27)

Grazing by Canada Geese (MDNR 2008, p. 24)

Non-native invasive species may harm wild rice. The common carp dislodges plants and reduces water clarity, both by suspending fine particles and releasing phosphorus that enhances algal growth. Hybrid cattail (*Typha x glauca*), a cross of native and non-native cattail (*Typha latifolia* L. and *Typha angustifolia* L., respectively), competes directly with natural wild rice for shallow-water habitat. These plants aggressively form thick mats of roots that can float as water levels fluctuate. A relatively new threat to natural stands of wild rice is the non-native flowering rush (*Butomus umbellatus* L.). Found in similar habitats as native bulrush (*Scirpus* L. spp.), which it resembles, flowering rush can persist in either emergent or submergent forms. Though its distribution in Minnesota is limited, its range is expanding. Another potential threat to natural wild rice in Minnesota is the non-native form of phragmites, or common reed [*Phragmites australis* (Cav.) Trin.] (MDNR, 2008, pp. 27-29).

(continued)

Climate change

Climate change may harm wild rice:

- By allowing carp to spread north (MDNR, 2008, p. 30).
- By excessive warmth, which decreases the occurrence of cold dormancy in southern portion of range that is required for high germination rates (MDNR, 2008, p. 30, Myrbo et al., in press-1).
- Spread of wild rice diseases, such as brown spot (MDNR, 2008, p. 30).
- Extreme precipitation events that increase water depth abruptly (MDNR, 2008, p. 30-31).

Genetic threat

Because wild rice pollen is airborne, some have expressed concerns about unplanned cross-pollination between cultivated stands and natural stands. At this point in time, however, traditional wild rice breeding programs are not thought to pose a threat to natural stands since the cultivated varieties reflect the selection of genes from within the naturally occurring gene pool (MDNR, 2008, p. 26).

C. Identification of 120 µg/L as the protective sulfide concentration

As part of the MPCA-sponsored wild rice research, data were collected to identify a sulfide concentration that would be protective of wild rice in natural waters. Most published information on sulfide toxicity is about effects on animals. The U.S. Environmental Protection Agency (EPA) established national criteria for sulfide in surface waters of 2.0 µg/L to protect aquatic life (EPA, 1986). It is possible for sulfide to accumulate to this concentration in surface waters that cannot be re-supplied with oxygen from the atmosphere and that have low photosynthetically-produced oxygen, such as the hypolimnion of deeper lakes (Wetzel, 2001) or ice-covered shallow lakes (Scidmore, 1957). However, because exposure to atmospheric oxygen quickly detoxifies sulfide by oxidation to sulfate, surface waters in ice-free shallow lakes that are not thermally stratified are unlikely to exceed this criterion for surface water (2.0 µg/L). The challenge faced here is to identify a porewater sulfide concentration that would be protective of wild rice.

Wild rice habitats are vulnerable to accumulation of sulfide in the sediment porewater in which the plants grow. The vulnerability is the result of the combination of the high oxygen consumption by bacteria in sediment containing decaying plant litter, and the low solubility of oxygen in water (about 10 ppm, compared to 210,000 ppm in the atmosphere). On a volumetric basis, there is 27 times as much oxygen in a liter of air than a liter of water (Caraco et al., 2006). Although the scientific literature has long identified rooted aquatic plants as vulnerable to sulfide toxicity (see review by Lamers et al., 2013), at the start of the MPCA-sponsored research effort in 2011 there was no published information specific to the effect of sulfide on wild rice. There is some information on the toxicity of sulfide to white rice (*Oryza sativa*), which is related to wild rice (*Zizania palustris*) and inhabits similar environments, and therefore faces similar environmental challenges. However, it is unclear how applicable data from white rice are to wild rice. Furthermore, many of the studies identify *toxic* levels of sulfide (e.g., 50% effect levels, or EC50), levels that would result in a significant loss of plants. In contrast, the MPCA needs to identify a *protective* level of sulfide. Lamers et al. (2013) reviewed three publications regarding the toxicity of sulfide to white rice, and reported sulfide toxicity as low as 10 micromoles per liter (320 µg/L, or 0.320 mg/L).

Protective concentrations of a chemical have often been identified by exposing organisms to a range of concentrations, and then calculating the concentration at which a minimal effect is observed, such as a 10% or 20% negative effect on growth relative to a control. Effect concentrations of 10% and 20% are termed EC10 and EC20. In an earlier analysis (MPCA, 2014), MPCA had proposed identifying a protective sulfide concentration based on a 20% negative effect (EC20). However, the independent peer review panel recommended that a more conservative protective concentration, such as EC10 or EC5, would be more appropriate for the protection of wild rice. A more conservative (lower) concentration was recommended because this effort involves identifying a protective concentration of a toxin for a single species, in contrast to an ecological community, which is assumed to have functional redundancy among species.

The MPCA has received comments that the use of EC10 is inappropriately over-protective, and that the EPA recommends that water quality standards be based on EC50 for acute exposure of a chemical, and EC20 or EC25 for chronic exposure (MCC, 2015, pp. 16-19) to protect aquatic life. The commenter is referring to EPA guidelines (EPA, 1985 and EPA, 2010), but those guidelines also anticipate that rote application of the basic procedures may not yield the most appropriate standard. Consequently, the EPA guidance provides flexibility for deviation from the normal procedures. For instance, the guidance (EPA, 1985) states:

“Such data might affect a criterion if the data were obtained with an important species, the test concentrations were measured, and the endpoint was biologically important.”

In this case, the flexibility is needed because of the difference between the MPCA's goal of protecting a singular species, wild rice, and the fact that the EPA guidance is designed to protect 95% of a community's species and to preserve the ecological functioning of the community, and not to protect an individual species. The EPA guidance is meant not only to protect multiple species in an aquatic community, but also to be applied when effect concentration data are available from at least eight different groups of aquatic organisms (EPA, 2010). The data are then graphed as a species sensitive distribution (SSD) and generally the 5th percentile of the distribution (the lowest) is accepted as a matter of policy as the concentration that would maintain the viability of most species. Preference is given to using the lower confidence limit of the 5th percentile (NRC, 2013) as the numeric criteria.

The MPCA is not applying this method to the identification of the protective sulfide concentration for wild rice because: (a) the MPCA is updating an existing standard that is specific to just wild rice, rather than the whole aquatic community; and, (b) even if the goal were to develop a standard to protect the community of organisms that inhabit the sediment of shallow aquatic ecosystems, there are not enough high-quality data on the effect of sulfide to rooted aquatic plants and sediment-dwelling animals to perform such an analysis.

EC10 has been identified as a suitable threshold when the goal is to only allow negligible exposure to a potentially toxic chemical (e.g., Merrington et al., 2014; Hommen et al., 2015). The EPA's Science Advisory Board also notes that EC10 has been used when a protective concentration is calculated for a particular species of concern (SAB, 2008).

MPCA staff calculated EC10 values from the hydroponic, mesocosm, and field data (Fig. 1-2, Table 1-8), which are updated from values presented in earlier MPCA reports, such as the March 2015 Draft Proposal (MPCA, 2015), and the Draft Technical Support Document (MPCA, 2016). The following paragraphs explain those EC10 calculations, which are further detailed in the referenced papers and Appendices 5 – 7.

Table 1-8. Estimates of protective sulfide concentrations for wild rice from hydroponic, mesocosm, and field data, based on change-point analysis, EC10 estimates, and visual identification of a decrease in a graph of the proportion of field sites with wild rice present.

	Data set	Protective Sulfide Concentration (µg/L)	
		Estimate	95% Confidence Interval
Minnesota Chamber-sponsored hydroponic experiment (data from Fort et al., 2017)			
EC10, based on hydroponic experiment (MCC, 2015)*	Hydroponic	963	not given
MPCA-sponsored hydroponic experiment (Appendix 5; data from Pastor et al., 2017)**			
EC10, based on regression of weight gain on average initial sulfide*	Hydroponic	251	<11 - 285
EC10, based on regression of weight gain on time-weighted arithmetic mean of sulfide	Hydroponic	106	<11 - 158
EC10, based on regression of weight gain on time-weighted geometric mean of sulfide*	Hydroponic	39	<11 - 66
MPCA-sponsored mesocosm experiment (Appendix 6; data from Pastor et al., 2017)			
EC10, based on regression of percent of filled seeds	Mesocosm	228	0 - 414
EC10, based on regression of number of plants that germinated	Mesocosm	163	0 - 242
MPCA-sponsored field survey (Appendix 7; data from Myrbo et al., in press-1)			
Visual identification of reduction in proportion of waterbodies with wild rice present (N=108)	All sites	120	not applicable
Change-point analysis, based on wild rice density (N=67)	All sites with wild rice	112	25 - 368
EC10, based on binary logistic regression of wild rice presence (transparent sites, N=96)	Transparency > 30 cm	93	14 - 239
EC10, based on binary logistic regression of wild rice presence (all sites, N=108)*	All sites	58	<11 - 117

*Estimates identified in the text as deserving less weight in the weighing of multiple lines of evidence.

**Data from three experiments were merged for the logistic regressions.

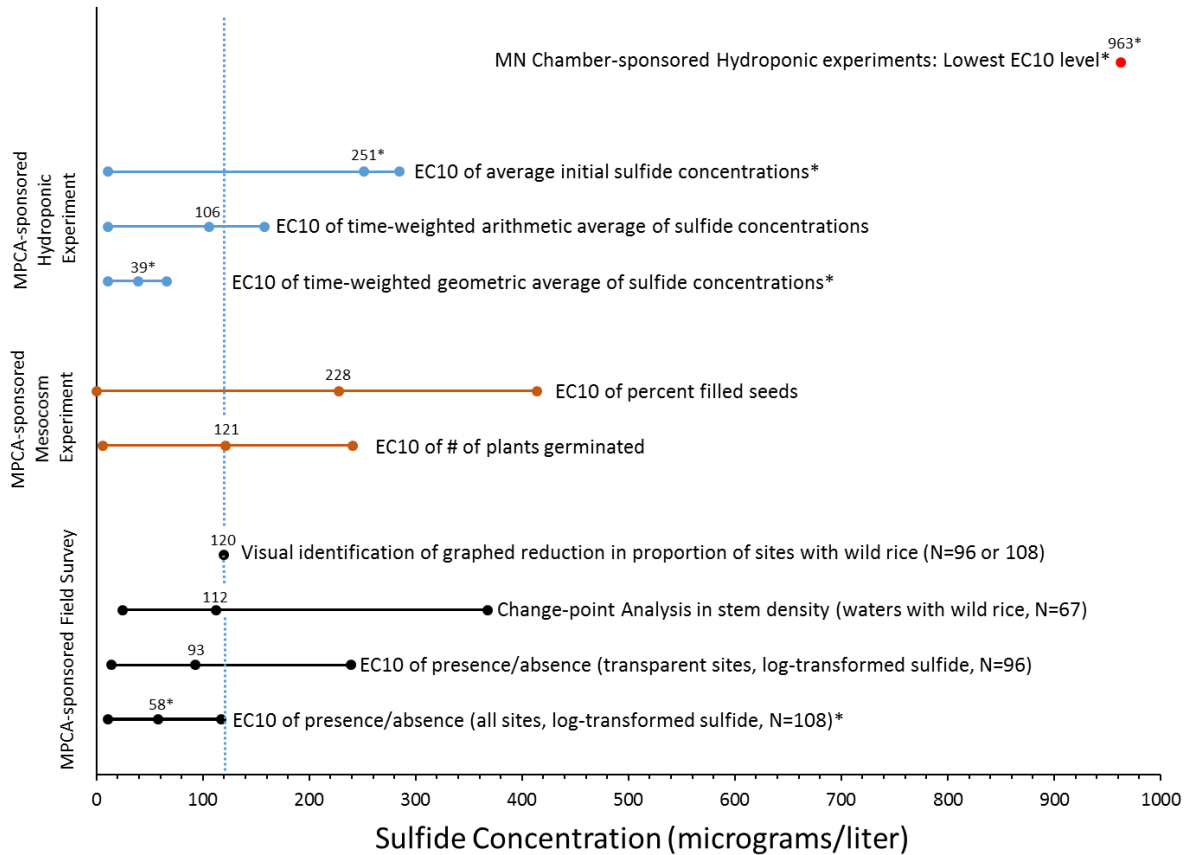


Figure 1-2. Estimates of protective sulfide concentrations for biological endpoints from hydroponic, mesocosm, and field data, based on EC10 estimates, change-point analysis, and visual examination of trends. Estimates marked with an asterisk (*) are identified in the text as deserving less weight in the weighing of multiple lines of evidence.

MPCA-sponsored hydroponic experiments: estimates of protective sulfide concentrations

Three different hydroponic EC10 values were produced by combining growth data from multiple experiments and performing logistic regressions (Ritz et al., 2015). EC10 estimates were made for three different representations of sulfide exposure (initial concentration, arithmetic average, and geometric average) yielding EC10 values of 251, 106, and 39 µg/L, respectively (Appendix 5). The peer review panel (ERG, 2014) concluded that the use of the initial concentration EC10 (251 µg/L) is not warranted, and that it would be more defensible to use either of the time-weighted EC10 values (39 or 106 µg/L). Their reasoning was based on the observation that the sulfide concentrations were measured every two to three days when the hydroponic solution was renewed, and concentrations declined significantly between hydroponic renewals, so that the plants were only exposed to the initial concentration for a short time. The photosynthesizing seedlings produced oxygen that decreased the sulfide concentrations between renewals, especially at low concentrations of sulfide.

The EC10 based on the time-weighted geometric average is lower than the arithmetic average (39 µg/L, compared to 106 µg/L) because a geometric average assumes that the rate of sulfide oxidation was faster at first, and then declined. There is no evidence for a changing oxidation rate, so an EC10 of 106 µg/L is most defensible. Furthermore, it has been argued that calculation of a geometric average is

rarely appropriate when calculating average chemical concentrations for investigations of environmental impact (Parkhurst, 1998). Parkhurst points out that geometric averages are biased low, which accounts for the unusually low EC10 sulfide concentration of 39 $\mu\text{g/L}$. Accordingly, the arithmetic average of 106 $\mu\text{g/L}$ is the most defensible EC10 estimate derived from the hydroponic data. (These EC10 values supersede estimates reported earlier in draft MPCA documents of 299, 160, and 71 $\mu\text{g/L}$, which were calculated with normalized data from each of the three experiments. Further examination of the results indicated that normalizing the data skewed the results, so this updated analysis uses the raw data.)

Of the three approaches to analyzing the hydroponic data, the EC10 estimate that is based on the time-weighted arithmetic average sulfide concentrations (106 $\mu\text{g/L}$) is likely the most reliable estimate (Fig. 1-2). Note that the EC10 is determined by calculating the sulfide concentration associated with a 10% decrease in wild rice growth relative to the growth in the control treatments. The control growth rate is taken as the flat area of “no effect” observed at the lowest sulfide concentrations (for example, the flat area in the left side of the logistic curve in Fig. 1-3).

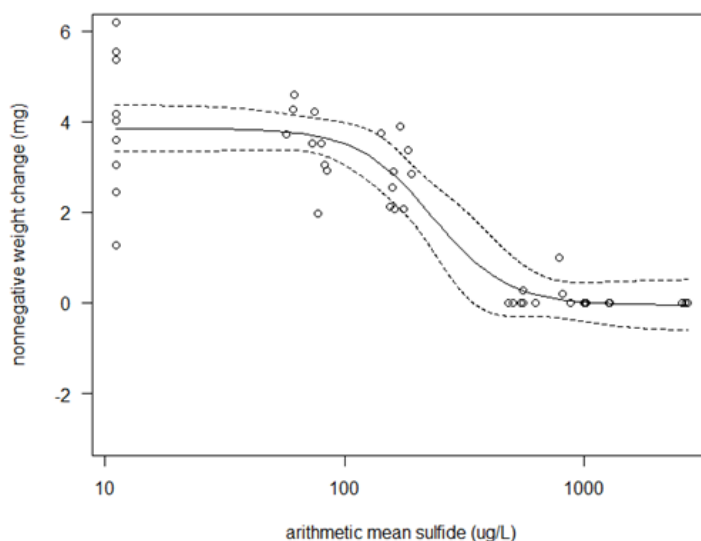


Figure 1-3. Logistic fit of wild rice seedling weight gain against time-weighted arithmetic average sulfide concentrations, using package *drc* in R. Raw data from three experiments are merged together. The EC10 was 106 $\mu\text{g/L}$, with a 95% confidence interval of 11 to 158 $\mu\text{g/L}$.

MPCA-sponsored mesocosm experiments: estimates of protective sulfide concentrations

The MPCA-sponsored mesocosm experiments (described in Pastor et al., 2017) yielded two statistically-significant effects of sulfide on wild rice, (1) percent filled, or viable, seeds and (2) number of plants that emerged in the spring. Calculation of EC10 values from linear regressions (Appendix 6) yields EC10 values of 228 and 121 $\mu\text{g/L}$, respectively, with relatively wide 95% confidence intervals (Fig. 1-2, Table 1-8).

MPCA-sponsored field survey: estimates of protective sulfide concentrations

The field survey of 108 different waterbodies offers several different ways to identify potential protective sulfide concentrations (Appendix 7): binary logistic regression (BLR), change-point analysis, and a visual examination of a graph of the proportion of sites with wild rice present—a non-statistical approach suggested by the 2014 independent peer review panel.

The EC10 derived from a binary logistic regression of wild rice presence/absence of the field data is less precise than expected because when the sulfide data are log-transformed to achieve an appropriate statistical distribution, the curve does not exhibit a flat area of “no effect” at the lowest sulfide concentrations (Fig. 1-4; see Appendix 7 for a comprehensive presentation). Because there is no flat area of no effect, the calculated EC10 value is dependent on selection of the baseline value from which to calculate a 10% effect.

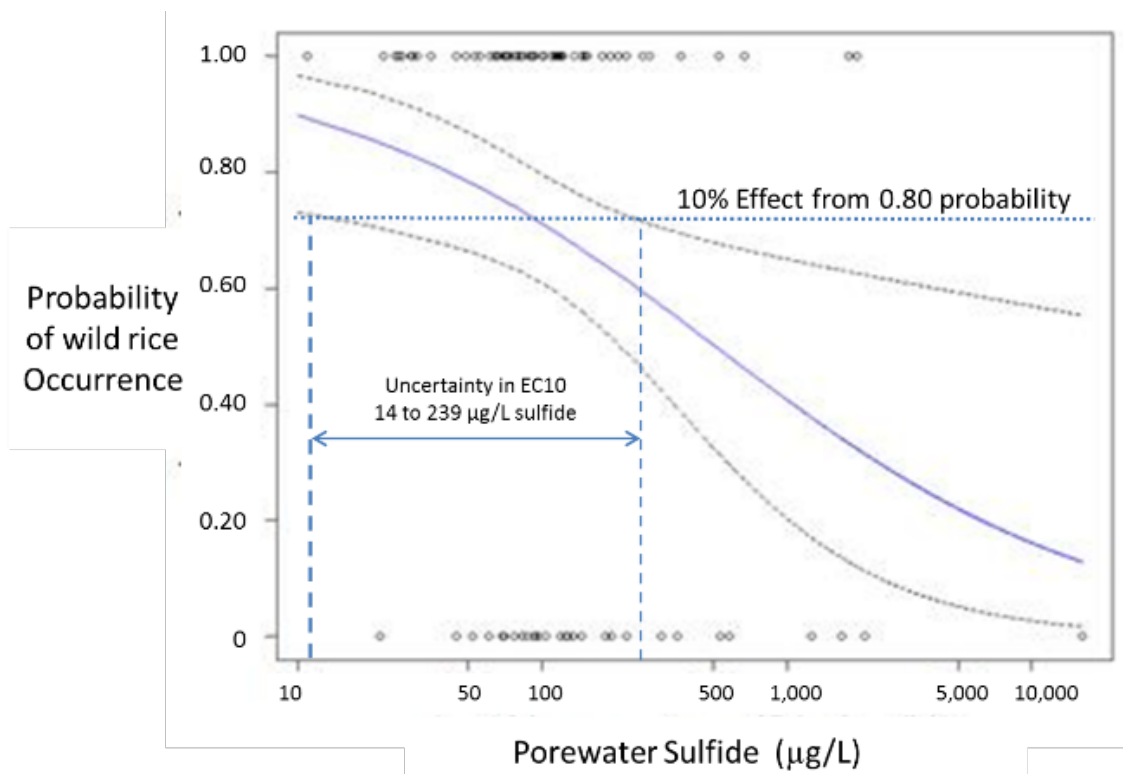


Figure 1-4. Binary logistic regression of the presence and absence of wild rice for field survey sites with water transparency greater than 30 cm (Class B data, N=96). The uncertainty of the EC10 estimate (93 µg/L) was quantified by identifying the range of sulfide concentrations that contain a given EC10 wild rice proportion in their 95% confidence interval based on the binary logistic regression

In the absence of a flat area of the curve, MPCA defined the baseline value as the proportion of sites with wild rice for the 10 sites with the lowest sulfide concentrations (0.80; Appendix 7). Given that definition, binary logistic regression can be used to identify two different protective sulfide concentrations depending on whether low-transparency (< 30 cm water transparency) sites that would not support wild rice are included (Table 1-8). These EC10 values are 58 µg/L for all sites, and 93 µg/L for the 96 sites with sufficient transparency to support wild rice. It is not reasonable to calculate a protective sulfide concentration with data from sites that would not support wild rice no matter how low the sulfide concentration is. Therefore, regression of just sites that would support wild rice, yielding an EC10 of 93 µg/L (95% confidence interval 14 – 239 µg/L), is the most defensible EC10 in this case.

A change-point analysis of wild rice density against sulfide yields an EC10 of 112 µg/L (95% confidence interval 25 – 368 µg/L), which is broadly compatible with the EC10 of 93 µg/L derived from the sites that have suitable transparency to support wild rice.

The third way in which the field data were used to identify a protective sulfide concentration was a non-statistical approach involving direct examination of the data. Visual identification of a reduction in the proportion of sites with wild rice present yields a value of 120 µg/L (Fig. 1-5), which is compatible with both the field survey EC10 based on wild rice presence/absence (93 µg/L, confidence interval of 14 - 239 µg/L) and change-point analysis based on wild rice density (112 µg/L, confidence interval of 25 – 368 µg/L; Table 1-8, Fig. 1-2).

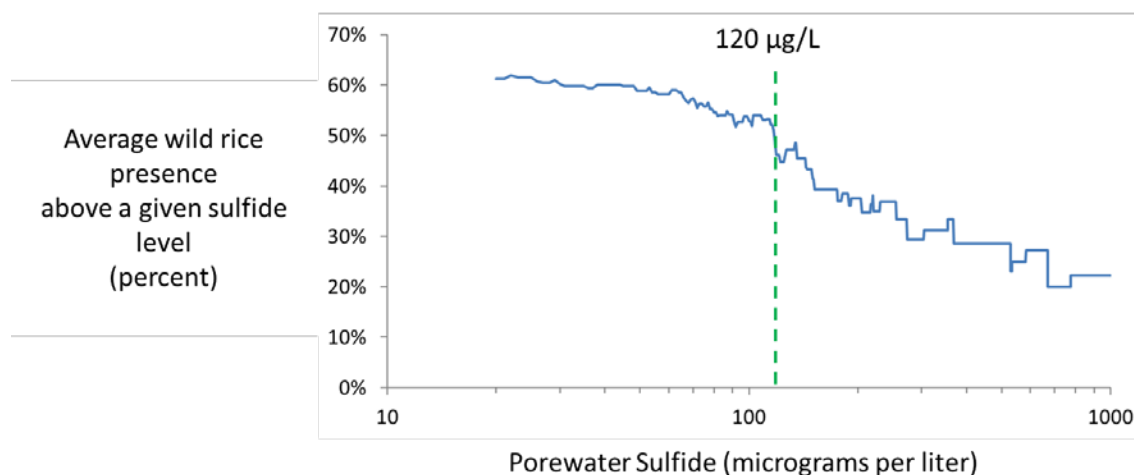


Figure 1-5. Empirical examination of the average proportion of sites with wild rice above a given porewater sulfide concentration (sites excluded with transparency < 30 cm). There is a noticeable decline in the proportion of sites with wild rice when sulfide exceeds 120 micrograms per liter (vertical dashed line).

Minnesota Chamber-sponsored hydroponic experiment: estimate of protective sulfide concentrations

A 21-day hydroponic study was sponsored by the Minnesota Chamber of Commerce (Fort Environmental Laboratory, 2015; Fort et al., 2017) in which wild rice seeds from a Minnesota lake were germinated in solution with a range of sulfide concentrations. Fort et al. (2017) did not calculate effect concentrations, but an EC10 of 963 µg/L was calculated from the Fort study data (MCC, 2015), suggesting that sulfide is less toxic to wild rice than was found in the three MPCA-sponsored studies (hydroponic, outdoor mesocosm, and field survey).

MPCA staff reviewed the design and results of the Fort hydroponic experiments to explore whether there were differences in the experimental approaches that could help account for these differing results. One potential explanation for the difference in the observed toxicity effects lies in the way that the germinated seeds were exposed to sulfide. In the Fort study, seeds were placed on a mesh that was submerged 1 cm in an aquarium open to the atmosphere that initially contained an anaerobic hydroponic solution of a given sulfide concentration; the solution was renewed and monitored daily. During the 21-day experiment, the sprouts were enabled to grow above the surface of the water, into the room air, as the mesocotyl (stem) developed and elongated. As the Fort study report states, "The mesocotyl developed in aerobic conditions under this design. Plastic wire mesh was placed inside the aquaria to provide a trellis to support vegetative growth above the hypoxic culture media." (Fort Environmental Laboratory, 2015, p. 14).

MPCA staff hypothesize that once the wild rice sprouts emerged into the room air, access to oxygen in the room air allowed the sprouts to internally detoxify sulfide by oxidizing it to non-toxic forms of sulfur (see *How access to oxygen may allow wild rice to detoxify sulfide*, in Part A of this chapter). There is

evidence in the scientific literature that aquatic plants can detoxify sulfide through two broad routes that require oxygen. Aquatic plants have special channels in the stem for transporting air, called aerenchyma, for this purpose (Colmer, 2003). Access to the atmosphere is significant because the atmosphere is 21% oxygen (210,000 parts per million, ppm), in contrast to the availability of oxygen in water (a maximum of about 10 ppm). However, as noted in Part A of this chapter, under natural conditions 21-day old wild rice plants would not have access to the atmosphere because the seeds germinate in water that is much deeper than 1 cm, and the stems would not yet have elongated sufficiently to reach the water surface.

Uncertainty surrounding potential sulfide concentrations to protect wild rice

MPCA examined the range of EC10 and change-point estimates from MPCA-sponsored research on hydroponic, mesocosm, and field data (39 to 251 µg/L), plus the EC10 of 963 µg/L estimated from the Fort et al. (2017) hydroponic study (MCC, 2015). The discussion above identifies potential protective concentrations on which there are reasons to place less weight when considering the balance of evidence: two of the three MPCA-sponsored hydroponic EC10 values, the EC10 derived from field survey data that included low-transparency sites that would not support wild rice, and the Minnesota Chamber-sponsored hydroponic experiment. Those estimates with lower weight are identified with asterisks in Fig. 1-2 and Table 1-8.

In a review of plant toxicity endpoints, Clark et al. (2004) suggested that although plant growth is an important metric, successful reproduction is the most important metric in assessing the toxicity of a substance to an annual plant, such as wild rice. In the MPCA-sponsored investigations into the effect of sulfide on wild rice, the best metrics of successful growth and reproduction are (1) the percent of filled seeds (an indicator of seed viability) in the mesocosm experiment, (2) the number of plants that germinated in the mesocosm experiment, (3) the occurrence of wild rice in the field survey, and (4) the density of wild rice in the field survey. The estimates of protective sulfide concentrations from these metrics broadly agree with each other (Fig. 1-2).

The MPCA acknowledges that there is uncertainty in all of the EC10 calculations. The EC10 estimates from the field survey are uncertain due to the lack of a flat curve at low sulfide concentrations (Fig. 1-3). The EC10 derived from the MPCA-sponsored hydroponic experiment is uncertain because (1) sulfide concentrations declined during exposures and (2) the whole seedling was exposed to sulfide, which may not occur in nature except when the plant is germinating from a seed buried several inches in the anoxic sediment. The EC10 values derived from the outdoor mesocosms do not suffer from any obvious flaw, although it should be acknowledged that the mesocosms were not perfect mimics of the environment in that porewater sulfide concentrations were probably not in steady state. Pastor et al. (2017) point out that mesocosms cannot be perfect mimics of natural wild rice waters and be in steady state with controlling variables, because the watershed sources of iron were cut off even as sulfate kept being supplied. As a result, porewater sulfide concentrations increased over time, rather than reaching a steady-state concentration.

In addition, recent publications question whether EC10 can be used as a precise estimate of “no effect,” “negligible effect” or a “protective concentration” (e.g., Hommen et al., 2015; Fox and Landis, 2016). In addition to considering the multiple EC10 values and the change-point estimate, MPCA used a more empirical approach to identify a potential protective sulfide concentration by directly examining the field data for a visual threshold that might be used to identify a protective concentration—an approach explicitly recommended by the peer review panel (ERG 2014, p. 6). The data were examined for a threshold by calculating the average proportion of sites with rice above any given sulfide concentration (Fig. 1-4; Appendix 7), and the pattern simply examined, without any statistical analysis. Such an examination shows that although the percent of sites with wild rice declines as sulfide increases, the decline is relatively slow until the sulfide concentration exceeds 120 µg/L, where there is a notable drop

in the percentage of sites with wild rice present. While a small uptick in the proportion of sites with wild rice occurs between 130-150 µg/L, the percentages never return to the 60% or greater that are observed below 120 µg/L (Fig. 1-5).

Identification of 120 µg/L as the protective sulfide concentration

Based on the analyses described above, MPCA proposes 120 µg/L as the protective concentration of sulfide. Not only is 120 µg/L at a visual break in the proportion of sites with wild rice, but it is within the range of the most defensible estimates of protective sulfide concentrations: 106 µg/L (from hydroponic experiments), 91 µg/L (the field survey EC10 based on wild rice presence), 112 µg/L (the field survey change-point based on wild rice density), 121 µg/L (EC10 based on mesocosm plant germination), and 228 µg/L (EC10 based on mesocosm seed viability) (Fig. 1-2; Table 1-8).

Of the 67 sites sampled in the field survey where wild rice was present, 73% had sulfide concentrations below 120 µg/L. The median sulfide concentration was 85 µg/L, and the average was 165 µg/L; 10% of sites had sulfide concentrations above 235 µg/L. In contrast, the median and average sulfide concentration at sites without wild rice was 126 and 795 µg/L, respectively. It is important to keep in mind that porewater sulfide is not the only environmental variable that affects the presence of wild rice, as discussed above. The analysis of the MPCA field data showed that reduced water transparency and elevated temperature also are associated with the absence of wild rice (Myrbo et al., in press-1), and other factors such as large carp populations and unfavorable hydrology have also been associated with the absence of wild rice (Table 1-6).

D. Relationship between surface water sulfate and porewater sulfide

The relationship between sulfate and sulfide is mediated by biology and chemistry, leading to a situation where superficially there seems to be little relationship between the two.

In the sediment of waterbodies, sulfate in the overlying water can diffuse into the underlying sediment and be converted by bacteria to sulfide. Diffusion of sulfate into sediment and the net retention of sulfur in sediment (as sulfide or as iron sulfide) has been shown in numerous lakes to be a function of the sulfate concentration (Urban et al., 1994). Based on that information, in a survey of many waterbodies one might expect porewater sulfide concentration to be strongly and directly correlated to sulfate concentration in the surface water. However, a plot of porewater sulfide against surface water sulfate (Fig. 1-6) shows only that when sulfate is low, sulfide is also low. But when sulfate is high, sulfide can range anywhere from low to high. This wedge-shaped distribution of data makes sense when one considers that sulfide must be produced from a sulfur-bearing chemical, and that sulfate is relatively mobile and available for the anaerobic bacteria that convert sulfate to sulfide. These bacteria necessarily can produce only limited quantities of sulfide when sulfate concentrations are low.

When sulfate concentrations are higher, there are two possible processes that may serve to limit sulfide concentrations. First, organic matter may be in short supply, limiting the bacteria's active metabolism and subsequent production of sulfide. Second, even if the bacteria are not limited by the availability of organic matter and do produce sulfide, sulfide may be removed from the porewater by precipitation with iron (Pollman et al., in press). As a result, sulfide concentrations associated with waters high in sulfate range from low to high.

Sulfide can also be produced in sediment by the putrefaction of sedimentary sulfur-bearing protein, which has been demonstrated by Dunnette (1989), who studied two eutrophic lakes. Dunnette found that putrefaction accounted for 5% and 57% of the sedimentary sulfide production in the two lakes. However, the overwhelming majority of sulfur retention in oligotrophic lake sediments can be accounted for by the conversion of sulfate to sulfide (Urban et al., 1994). Natural wild rice waters are normally low in nutrients, in contrast to the lakes studied by Dunnette, and so the findings of Dunnette may not be pertinent. Regardless, the following sections demonstrate that a satisfactory model can be developed that predicts porewater sulfide from just sulfate in surface water, sediment organic matter, and sediment iron.

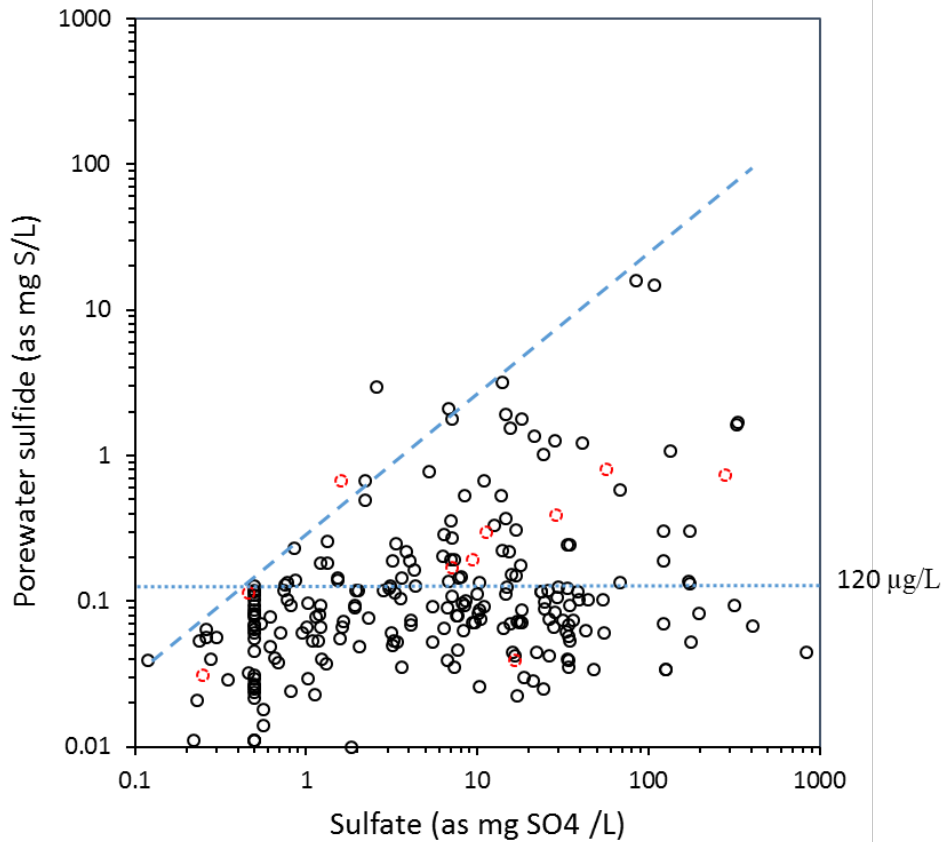


Figure 1-6. Relationship between sulfate in surface water and sulfide in porewater. The dashed line describes the empirically-observed highest net efficiency for the conversion of sulfate to sulfide (Class G data, plus cultivated paddies; N=233). Black symbols = natural waterbodies; Red dashed symbols = cultivated wild rice paddies. The protective sulfide concentration of 120 µg/L is shown.

Use of field data to model the effect of increasing sulfate in surface water

In the development of the research protocol (MPCA, 2011), potentially important environmental variables were identified based on a conceptual model of the processes relating sulfate and sulfide (graphically presented in Fig. 1-7). It has long been known that sulfate, organic matter and iron control porewater sulfide (e.g., Canfield, 1989; Giordani et al., 1996; Eldridge and Morse, 2000). In the interest of also collecting data to evaluate alternative hypotheses, in addition to measuring surface water sulfate, porewater sulfide, dissolved organic carbon (DOC), sediment organic carbon, and dissolved and sediment iron, over 60 other field variables were measured at each field site (Table 1-3).

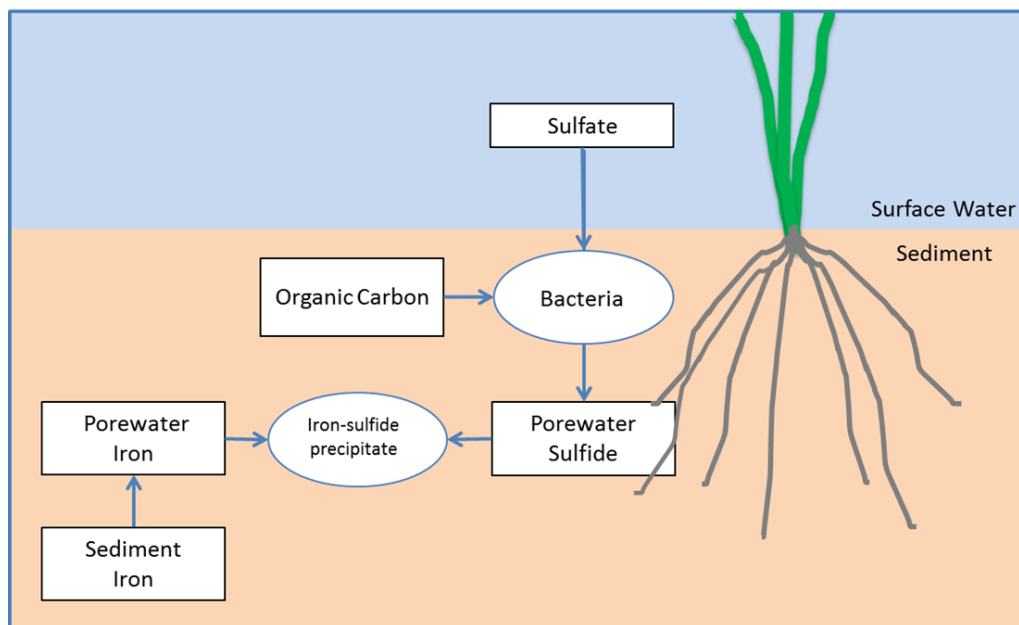


Figure 1-7. Conceptual model of the primary variables affecting the relationship between surface water sulfate and porewater sulfide. As bacteria utilize the energy in organic carbon, they respire sulfate, releasing sulfide. If iron is available, iron-sulfide precipitates form, which detoxifies the sulfide.

MPCA considered two different approaches to modeling the relationship between sulfate in surface water and sulfide in the sediment porewater underlying that surface water: mechanistic and statistical. A mechanistic model uses first principles of chemistry, physics, and biology to quantitatively describe the relationship between variables; to accomplish such a mechanistic model, a relatively complete understanding of the pertinent processes is required. In contrast, a statistical model is developed by fitting field data to mathematical relationships hypothesized by *a priori* understandings of likely chemical, physical, and biological processes operating in the environment (EPA, 2009a).

Mechanistic models, despite the goal of being based on first principles, often are empirically modified with field data to produce more accurate predictions, a process called calibration or parameter estimation (EPA, 2009a). In contrast, a statistical model is fundamentally based on the empirical relationships observed in the field between the variables of interest.

MPCA relied on a model of the statistical relationships of empirically observed data because a statistical model was judged likely to be more reliable than a mechanistic model given the greater data requirements for calibrating a mechanistic model and incomplete knowledge of the processes affecting the net conversion of sulfate to porewater sulfide. The most pertinent attempt to mechanistically model the water-sediment system concerned the potential toxicity of porewater sulfide to seagrass (Eldridge and Morse, 2000), a marine aquatic plant that is analogous to wild rice. A mechanistic model of the chemistry, physics, and biology of a marine system is exactly the same as modeling a freshwater system, except the concentration of sulfate is much higher (about 2,800 mg/L compared to Minnesota's wild rice waters, which range from below 1 to above 500 mg/L). Eldridge and Morse adapted a general model of the decomposition of organic matter by an array of bacteria that respired oxygen, nitrate, iron, and sulfate. The model required input of the concentrations of 13 different chemical species (e.g., organic matter, sulfate, porewater iron, oxygen, alkalinity), and predicted porewater sulfide as one of the seven calculated chemical species, from the simultaneous simulation of 24 different reactions. The model was run to a steady state and the results were compared to environmental data. The modeling was a success in the sense that comparing model results to empirical data reveals how well the processes are

understood. For example, the model fit the measured sulfide better when the model included oxygen release from the roots of the seagrass.

It might have been possible to adapt a mechanistic model like that of Eldridge and Morse (2000) to wild rice, run it to steady state, and compare to the environment. But the model is very complicated and requires a great deal of data to run. The point of developing such a model is to assess how well the processes are understood, and to determine which processes need to be included in the model in order to come close to the observed data. MPCA has a different goal, which is to find a practical model that can relate sulfate to sulfide, to protect wild rice from elevated sulfate concentrations that result in porewater sulfide concentrations that harm wild rice. Therefore, MPCA did not pursue a mechanistic model approach.

Assumption that sulfate, TOC, iron, and sulfide are in a steady state at field sites

Wild rice waters are dynamic ecological systems, with continuous external loading of sulfate and iron, coupled with variable amounts of annual production of wild rice plants, followed by variable decomposition. Yet it is likely that for decades most wild rice waters have experienced relatively constant processes, such as watershed loading of nutrients and sulfate, and soil erosion that carries organic matter and iron. Sulfate concentrations do fluctuate seasonally, but the field data from the MPCA study showed that sulfide concentrations do not fluctuate to a statistically significant degree. It is likely that porewater sulfide is a function of the long-term (e.g., year or more) average sulfate concentration.

It is common, when modeling relatively undisturbed ecosystems, to assume there is not much net change over seasons or years in the concentrations of important variables (e.g., Eldridge and Morse, 2000). In the case of the MPCA field survey, it is reasonable to assume that most of the 108 different sites have experienced no significant recent change in average surface water sulfate or sediment concentrations of TOC, iron, or sulfide. Monthly sampling at 15 different wild rice waters showed no significant change in TOC, iron or porewater sulfide from June through September (Myrbo et al., in press-1). When there is no significant change in concentrations over time, environmental modelers term a dynamic system to be in a “steady state” (Schnoor, 1996, pp. 4-5).

Both mechanistic and statistical modelers often rely on the assumption that a system is in steady state. For instance, in their mechanistic model Eldridge and Morse (2000) assumed that sulfate, organic matter, iron, and sulfide, among other variables, were in steady state. MPCA made the same steady-state assumption to pursue a statistical model that empirically relates the variables that are known to control porewater sulfide (sulfate, sediment organic carbon, and sediment iron). Although the model is based on concentrations of the variables, each concentration reflects the balance between continual input and loss. For instance, the concentration of sediment iron in the model (average concentration in the top 10 cm of the sediment) reflects the balance between new iron arriving to the sediment (inputs), and burial that pushes the layer of recently deposited material successively deeper in the sediment until it is deeper than 10 cm (losses).

Development of a statistical model using structural equation modeling (SEM)

At the suggestion of the peer review panel (ERG, 2014, p. 6), the MPCA employed structural equation modeling (SEM) to test the hypothesized conceptual model (Fig. 1-7). SEM is often referred to as “causal analysis” because it provides a framework for testing hypotheses with empirical data collected in field surveys (Iriando et al., 2003). The structural equation (SE) model that was developed supports the hypothesis that there is a dynamic relationship between production of sulfide from sulfate and precipitation as iron-sulfide solids. A key result from the model is that variations in three external variables (sulfate, sediment TOC, and sediment iron) contribute nearly equally to the observed variations in porewater sulfide (Pollman et al., in press). The model provides strong evidence that

development of a sulfate standard to protect wild rice from elevated sulfide should quantitatively consider the effects of ambient concentrations of sediment iron and organic carbon, in addition to surface water sulfate.

The SE model was validated by conducting a jackknife analysis where the model was refit by withholding a single observation from model estimation, and then using the refit model to predict the log₁₀-transformed value for porewater sulfide. The out-of-sample predictions closely matched the predictions obtained from the fully calibrated model, and also found no problems with unusually influential single observations. In addition, models based on alternative hypotheses and involving additional variables (phosphorus, acid volatile sulfide, dissolved organic carbon) were evaluated and found to offer no advantage over the original hypothesis in the prediction of porewater sulfide (Pollman et al., in press).

In summary, structural equation modeling found that porewater sulfide is controlled equally by the concentrations of surface water sulfate, sediment iron, and sediment organic carbon. Thus, all three variables need to be considered when developing a strategy to ensure sulfide remains at or below a protective level in the sediment of wild rice beds.

E. Development of an equation to calculate a numeric sulfate standard for each wild rice water

The consideration of multiple variables in calculating a numeric concentration for a water quality standard is becoming more common as the scientific understanding of the environment improves. For instance, over time EPA's guidance for developing standards for ammonia has progressed from initially considering a fixed concentration to the present recommendation of adjusting ammonia concentrations for both the pH and temperature of the ambient water (EPA, 2013). Similarly, EPA's 2007 aquatic life freshwater criterion for copper is based on a model termed the Biotic Ligand Model (EPA, 2007 revision). This metal bioavailability model uses receiving waterbody characteristics and monitoring data to develop a numeric copper standard. Input data include temperature, pH, dissolved organic carbon, major cations (Ca, Mg, Na, and K), major anions (SO₄ and Cl), alkalinity, and sulfide.

When the consideration of multiple variables allows the calculation of a significantly more accurate numeric water quality standard, it can make sense to utilize that scientific understanding. The structural equation modeling effort demonstrates that porewater sulfide concentrations can be successfully modeled with three variables: surface water sulfate, sediment TOC, and sediment iron measured as total extractable iron (TEFe). If this scientific understanding can be incorporated into an equation, then the equation can be adopted as the water quality standard, similar to EPA's approach to ammonia and copper standards. A standard that is an equation, rather than a uniform concentration that is applied to all waterbodies, is a reflection of the biogeochemical diversity of the environment. New approaches such as this are needed as environmental regulation progresses from protecting organisms from pollutants that are directly toxic, to protecting them from pollutants whose negative effects are both indirect and a function of environmental conditions that vary from site to site. In the case of sulfate, an equation-based standard would reflect the evidence (discussed below) that an equation will be more accurate than a fixed standard

To identify a protective sulfate concentration for a particular water, it would be logical to employ the relationships revealed by the structural equation model and to work backwards from the protective sulfide concentration (120 micrograms/liter, µg/L) to identify the particular concentration of sulfate that would protect wild rice in that waterbody. A direct way to accomplish this task would be to first arrange the structural equation model into a single equation that expresses porewater sulfide as a function of the variables in the model (sulfate, sediment iron, and sediment TOC). Substituting the protective sulfide concentration for that variable and rearranging the equation would then derive an equation for the protective sulfate concentration as a function of iron and TOC concentrations. Such an equation could be applied to any wild rice water for which TEF_e and TOC are known. However, rearranging the equation in that way results in re-transformation bias, reducing the accuracy of the equation (MPCA, 2015; Pollman et al., in press). Therefore, the MPCA has taken a different approach.

The re-transformation bias seen using the structural equation model can be avoided by predicting the protective sulfate concentration using multiple binary logistic regression (MBLR) with the variables identified from the structural equation model (Pollman et al., in press). By first relying on the structural equation model development to identify the appropriate variables, an MBLR model can be developed using the same field data. MBLR directly predicts the probability of exceeding the protective sulfide concentration threshold as a function of sulfate, TEF_e, and TOC.

MBLR is a predictive analysis; in this case the regression predicts the probability that sulfide is greater than 120 µg/L. The inputs to the regression are the field survey data from 108 different sites for the observed sediment iron, sediment TOC, surface water sulfate and porewater sulfide (the Class B data). All of the Class B is used—including low-transparency waters, waters with wild rice, and waters without wild rice—because the resulting equation is a model of chemical relationships, and does not, and should not, include information on the presence or absence of wild rice. To include only samples where wild

rice was present would certainly bias the data toward lower sulfide concentrations. The model could not be expected to predict higher sulfide concentrations as accurately if a high proportion of high-sulfide sites are excluded (which would happen if only sites with wild rice were included in the regression).

When all 108 samples are used, the MBLR regression is:

$$\text{logit}(\text{sulfide} > 120 \text{ } \mu\text{g/L}) = 9.3176 + 1.8962 * \text{log}_{10} \text{sulfate} - 3.6443 * \text{log}_{10} \text{ iron} + 2.2698 * \text{log}_{10} \text{TOC} \quad (\text{equation 1})$$

An equation to predict a protective concentration of sulfate can be derived if a probability is inserted into the equation. A probability of 0.5 (that sulfide is greater than 120 $\mu\text{g/L}$) produces sulfate values that most accurately predict the sulfide concentrations that were observed during the field survey (see Appendix 8 for a discussion of why 0.5 is the most accurate probability). At a probability of 0.5, the likelihood that sulfide is greater than 120 $\mu\text{g/L}$ is equal to the likelihood that sulfide is less than 120 $\mu\text{g/L}$ —in other words, using a probability of 0.5 to develop the equation produces the sulfate concentration that best matches a porewater sulfide concentration of 120 $\mu\text{g/L}$. Probabilities greater than 0.5 would be under-protective, and probabilities less than 0.5 would be over-protective.

Using a simplified version of the formula, the equation becomes:

$$\text{Logit}(\text{Sulfide} > 120) = \text{constant} + a \text{log}_{10} \text{Sulfate} + b \text{log}_{10} \text{Iron} + c \text{log}_{10} \text{TOC} \quad (\text{equation 2})$$

$$\text{Log}\left(\frac{\text{Probability Sulfide} > 120 \text{ } \mu\text{g/L}}{1 - \text{Probability Sulfide} > 120 \text{ } \mu\text{g/L}}\right) = \text{constant} + a \text{log}_{10} \text{Sulfate} + b \text{log}_{10} \text{Iron} + c \text{log}_{10} \text{TOC} \quad (\text{equation 3})$$

When a probability is chosen, a constant is produced for the left side of the equation (constant2):

$$\text{constant2} = \text{constant1} + a \text{log}_{10} \text{Sulfate} + b \text{log}_{10} \text{Iron} + c \text{log}_{10} \text{TOC} \quad (\text{equation 4})$$

If Sulfate is moved to one side and everything else to the other side, the equation becomes:

$$\text{constant2} - \text{constant1} - b \text{log}_{10} \text{Iron} - c \text{log}_{10} \text{TOC} = a \text{log}_{10} \text{Sulfate} \quad (\text{equation 5})$$

$$\text{log}_{10} \text{Sulfate} = \frac{\text{constant2} - \text{constant1}}{a} - \frac{b}{a} \text{log}_{10} \text{Iron} - \frac{c}{a} \text{log}_{10} \text{TOC} \quad (\text{equation 6})$$

$$\text{log}_{10} \text{Sulfate} = \text{new constant} - \text{new } b \text{log}_{10} \text{Iron} - \text{new } c \text{log}_{10} \text{TOC} \quad (\text{equation 7})$$

$$\text{Sulfate} = 10^{\text{new constant}} \times \text{Iron}^{\text{new } b} \times \text{TOC}^{\text{new } c} \quad (\text{equation 8})$$

Use of a probability of 0.5 produces a $\text{logit}(\text{sulfide} > 120) = 0$, which creates the proposed equation:

$$\text{Calculated Sulfate Standard} = 0.0000121 \times \frac{\text{Iron}^{1.923}}{\text{organic carbon}^{1.197}} \quad (\text{equation 9})$$

Or, alternatively,

$$\text{MBLR}_{120} \text{ Sulfate} = 0.0000121 \times \text{TOC}^{-1.197} \times \text{TEFe}^{1.923} \quad (\text{equation 10})$$

where sulfate is expressed as mg/L , TOC as percent dry weight, and TEF_e as mg/kg .

Operationally, this equation to determine the protective sulfate threshold can be applied to any waterbody for which sediment TOC and sediment TEF_e data are available. Of course, the data need to be produced using methods that are consistent with the procedures used to produce the field data on which the MBLR-based equation was derived.

The effect of different sediments in different wild rice waterbodies is illustrated by calculating the protective sulfate concentrations for three wild rice lakes with contrasting sediment quality. All three lakes had low ambient sulfate concentrations—less than 0.5 mg/L —but because of differences in sediment chemistry, the three have widely different MBLR-calculated protective sulfate concentrations, ranging from 1.2 to 186 mg/L (Table 1-9).

Table 1-9. Calculated protective surface water sulfate concentrations for three wild rice lakes. Note that these examples are for illustrative purposes only.

Study Site	State ID	Sediment Total Organic Carbon (%)	Sediment Iron (mg/ kg)	MBLR120-Calculated Protective Sulfate Concentration (mg/L)
Little Round Lake	03-0302	27.5	3,069	1.2
Elk Lake	15-0010	10.2	8,480	27
Rice Lake	18-0053	35.6	50,389	186

In summary, SEM was used to understand and characterize the relationships between the important parameters in the system that relates sulfate and sulfide, and then MBLR was used to translate the understanding gleaned from SEM into a relationship that can be used to calculate a numeric sulfate standard for each wild rice water.

F. Comparison of an equation-based standard to fixed standards: error rates and concerns

In the development of numeric water quality standards, even when a statistically valid stressor-response relationship is developed there will be a proportion of the waterbodies for which any given standard will be either under-protective or over-protective (EPA, 2009b; McLaughlin, 2012a,b; Vermont DEC, 2014; Smeltzer et al., 2016). To explore this using the MPCA-sponsored field survey data, each waterbody can be compared against a potential sulfate standard to see if the standard would be consistent with its measured porewater sulfide concentration. In other words, does any given sulfate standard that is predicted to be protective of wild rice also result in a porewater sulfide concentration that is protective of wild rice? Conversely, does a sulfate level that is predicted to not be protective of wild rice also result in sulfide levels that are not protective of wild rice? When the sulfate standard is consistent with the measured sulfide, the standard has a correct, or true, classification. A true negative occurs when ambient sulfate is less than the standard and sulfide is less than the protective concentration of 120 µg/L. A true positive occurs when sulfate is greater than the standard and sulfide is greater than the protective concentration of 120 µg/L.

The MPCA field survey data can be examined to determine the proportion of misclassifications (false positive and false negative prediction errors). A false positive error (called a Type 1 error in statistics) occurs when the ambient sulfate concentration exceeds the standard, but porewater sulfide is actually below the protective concentration of 120 µg/L; in such a case, the standard is overly stringent. False positives are a concern because they potentially could cause unneeded investment in sulfate control to keep sulfide at non-toxic levels. A false negative prediction error (a Type 2 error in statistics) occurs when the ambient sulfate concentration is less than the standard, but the porewater sulfide is actually above the protective concentration; in such a case, the standard is insufficiently stringent and adverse impacts may occur where they were not expected. In the development of a water quality standard, while the primary goal is to protect beneficial uses, it is also desirable to minimize the sum of these errors, which is the overall proportion of misclassifications. The total misclassification rate can be calculated for each proposed water quality standard. It has been proposed that it may be optimal to choose a water quality standard that balances misclassifications between false positives and false negatives, so that when an error does occur it is equally likely to be over-protective as under-protective (Vermont DEC, 2014).

The misclassification rate can be calculated for all possible fixed standards using the Class B dataset. At low potential sulfate standards, below 5 mg/L, the misclassification rate is high. For instance at a potential sulfate standard of 1.0 mg/L the misclassification rate is 46% because of a high proportion of false positive errors, indicating that a standard set at 1.0 mg/L would be overly stringent (44% of the sites false positive; 2% false negative; Fig. 1-8). The misclassification rate declines to 32% as the potential sulfate standard rises to 5 mg/L, and then varies between 32% and 37% up to a standard of 40 mg/L. The analysis presented here is limited to potential fixed sulfate standards up to 40 mg/L, above which the proportion of false negatives rises (indicating that fixed standards set above this level would not be reasonable). The lowest misclassification rate, 32% is produced at three potential fixed sulfate standards, 5, 10, and 26 mg/L. At 5 mg/L 24% of the sites would be false positive, and 8% false negative. At 26 mg/L, 4% of the sites would be false positive, and 28% false negative. A fixed sulfate standard of 10 mg/L would be the most balanced between false positives and false negatives, since at 10 mg/L the proportions of the two types of error are equal, at 16%, summing to a total of 32%.

In March 2015 the MPCA proposed to use a SEM-based equation that incorporates sulfate, iron, and organic carbon to calculate sulfate standards for wild rice waters (MPCA, 2015). This approach would result in a misclassification rate of 26% (Fig. 1-8). The refined approach presented in section E of this TSD

of calculating the sulfate standard using multiple binary logistic regression (MBLR) and the same three environmental variables produces a misclassification rate of 16% (9% false positive and 7% false negative). The MBLR equation was validated by applying it to an independent data set (dataset Class V, N=47), which produced a slightly higher misclassification rate of 19% (11% false positive and 8% false negative). The proportion of false negative errors is therefore at least twice as high for a fixed standard of 10 mg/L than for the MBLR-calculated equation-based standard (16%, compared to 8% from Class V, or 7% from Class B) (Fig. 1-8). A major question is whether or not the lower overall error rate of the MBLR equation when compared to a fixed standard (16-19%, compared to 32%) justifies the additional investment in collecting iron and organic carbon data at each wild rice water.

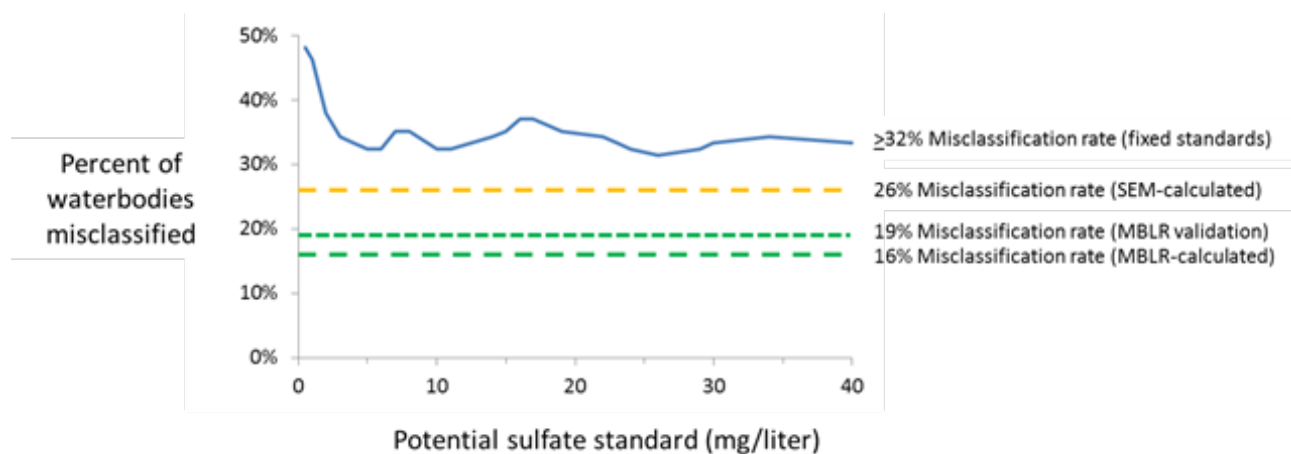


Figure 1-8. The misclassification rate compared across a range of potential sulfate standards. The misclassification rate is the sum of the percentage of false positive and false negative errors at a given potential sulfate standard.

Interplay between protective sulfide concentration and prediction errors

The analysis presented above shows that the MBLR-based equation produces sulfate standards that have fewer false positives and false negatives than any fixed sulfate standard. The equation produces about half the total misclassifications compared to the best fixed sulfate standards. Such comparisons are based on how well matched potential sulfate standards are to sulfide concentrations of 120 µg/L.

But what would the equation-based misclassification rate be if protective sulfide concentrations other than 120 µg/L were chosen? If errors were fewer for a different protective sulfide concentration, would it make sense to base the equation on that sulfide concentration? The interplay between protective sulfide concentrations and prediction errors can be confusing, because it may be tempting to recommend a sulfide concentration as being protective simply because it has relatively few prediction errors. However, it would not be reasonable to promote such a sulfide level unless it were in a range of sulfide concentrations that have a high probability of protecting the beneficial use.

It therefore would be a mistake to designate a sulfide concentration with a low misclassification rate as “protective” without evaluating how well it protects wild rice from that level of sulfide. A critical point to keep in mind is that misclassifications are not correlated with the degree of protection offered by a particular sulfide concentration. If a proposed protective sulfide concentration produces few errors, but the sulfide concentration is not actually protective of wild rice, then the enticement of low errors should be ignored.

Accuracy of calculated sulfate standards across potential protective sulfide concentrations

The potential consequences of designating sulfide concentrations other than 120 µg/L as protective can be investigated through analysis of the Class B dataset. First, the false positives and false negatives can be presented as a percentage of all 108 equation-based predictions across the spectrum of potential protective sulfide concentrations (Fig. 1-9). The sum of false positives and false negatives (the total misclassification rate) dips to 16% between 120 and 130 µg/L, and exceeds 16% at higher sulfide levels until a continuous decline to 9% that begins at 180 µg/L (Fig. 1-9).

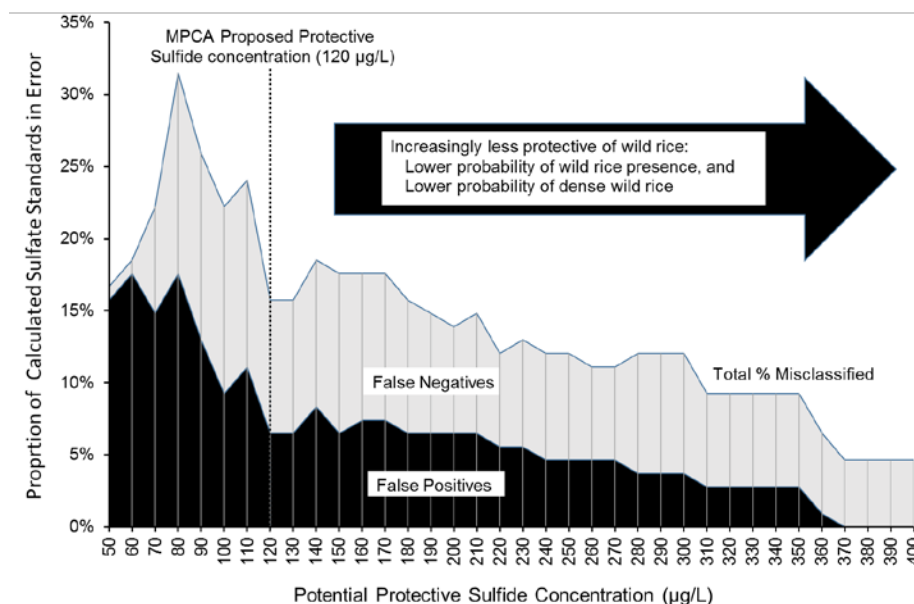


Figure 1-9. The proportion of false positive and false negative prediction errors calculated for potential protective sulfide concentrations ranging from 50 to 400 µg/L (in increments of 10 µg/L). The sum of false negatives and false positives is the total misclassification rate.

Although prediction errors decline when potential protective sulfide concentrations exceed 180 µg/L, such sulfide concentrations would not be as protective of wild rice as 120 µg/L. For instance, the probability that wild rice will be present declines as sulfide concentrations increase (Fig. 1-10), which was one of the lines of evidence included in the identification of 120 µg/L as an appropriate protective sulfide concentration (see Chapter 1, part C, above). In addition, the density of wild rice declines as sulfide concentrations increase; change-point analysis found a statistically significant decrease in density at 112 µg/L, which was another line of evidence examined. The probability that a wild rice water will exhibit dense wild rice (e.g., greater than 25 stem/m² or 40 stems/m²) also declines as sulfide concentrations increase (Fig. 1-11).

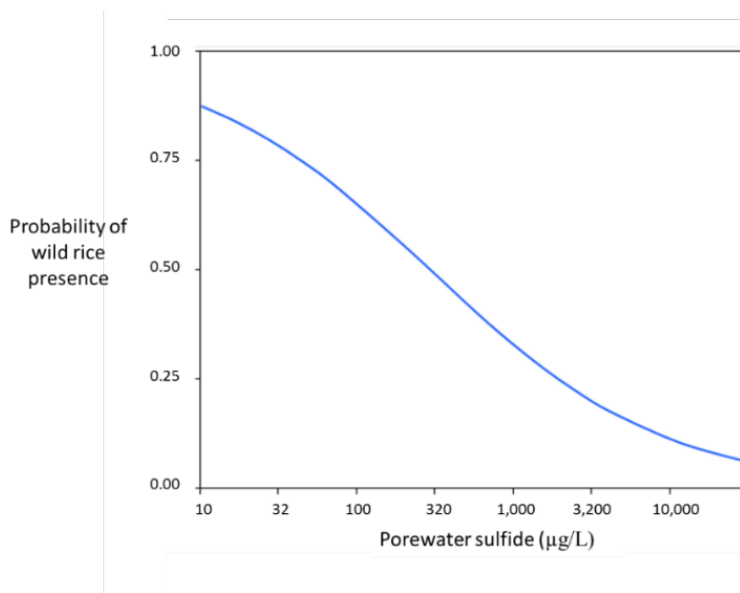


Figure 1-10. Probability of wild rice presence as a function of porewater sulfide (binary logistic regression; $p=0.001$; $N=108$).

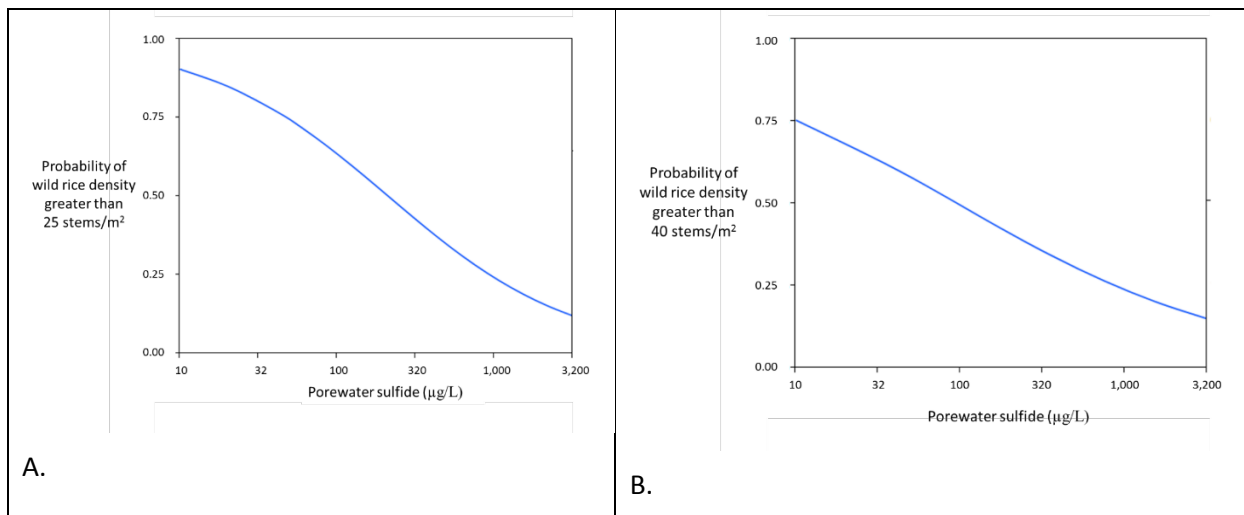


Figure 1-11. Probability of wild rice present at densities (A) greater than 25 stems/m² (binary logistic regression; $p=0.013$) and (B) 40 stems/m² (binary logistic regression; $p=0.076$). (Based on sites with wild rice in the Class B dataset, $N=67$.)

In contrast, the probability that a wild rice water will exhibit sparse wild rice (e.g., less than 10 stems/m²) increases significantly as sulfide concentrations increase (Fig. 1-12). In other words, as sulfide increases and the probability of wild rice even being present declines (Fig. 1-10), it becomes more probable that the wild rice that is present has a low density (Fig. 1-13). For instance, at 120 µg/L, there is a 24% chance that wild rice density is less than 10 stems/m², but at 300 µg/L the probability more than doubles, to 52%. Simultaneously, the probability that wild rice density is greater than 25 stems/m² declines from 60% at a protective level of 120 µg/L to 44% at 300 µg/L.

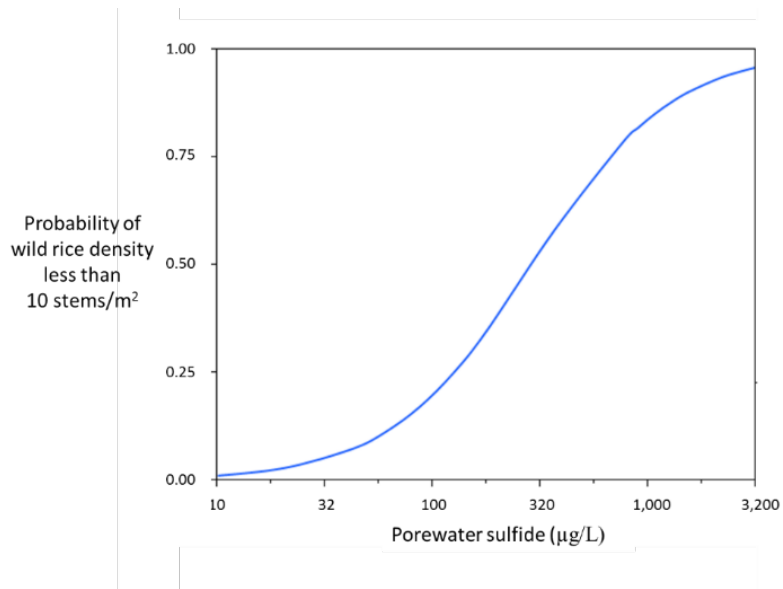


Figure 1-12. Probability of wild rice being present at a density of less than 10 stems/m² (binary logistic regression; p=0.0003). (Based on sites with wild rice in the Class B dataset, N=67.)

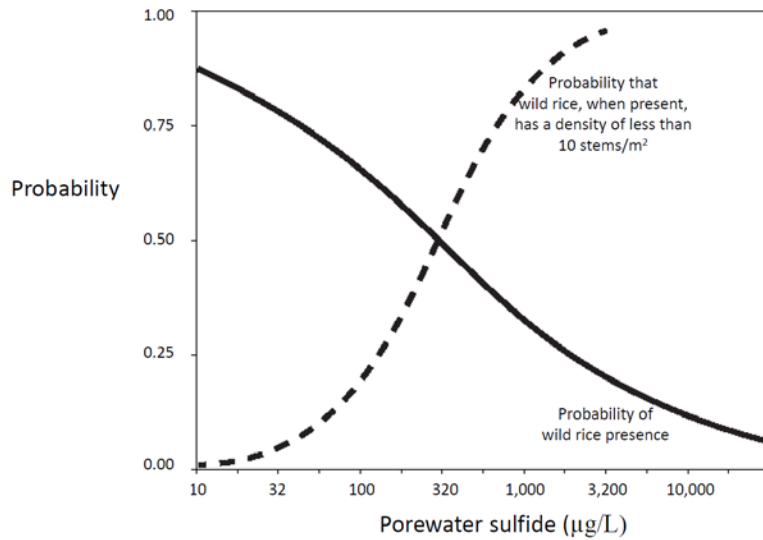


Figure 1-13. Probability of wild rice presence and the probability that the wild rice has a density of less than 10 stems/m², plotted against porewater sulfide concentrations.

Why not make the protective sulfide concentration zero?

It is evident that the highest probability of wild rice presence and a dense population occurs when porewater sulfide is lowest. However, it is unrealistic to have a goal of zero sulfide in the sediment porewater of wild rice waters. Sulfide is a natural chemical that is produced in the environment by naturally occurring bacteria from sulfate, which itself is a common natural chemical. Sulfate concentrations vary naturally across the landscape (Myrbo et al., in press-1), as do the other variables that control the production of sulfide, iron and total organic carbon (Pollman et al., in press). As a result, even under natural conditions there are waterbodies that are not hospitable to sulfide-sensitive species such as wild rice. In addition, there are likely areas within some wild rice waters that have lower iron or higher total organic carbon that naturally produce porewater sulfide that is incompatible with wild rice. For instance, an isolated bay of a wild rice water could plausibly have low sediment iron concentrations because the local watershed is poor in iron or there is no emergent groundwater rich in iron (Maranger et al., 2006). Similarly, a bay of a wild rice water could plausibly have higher sediment organic carbon because it lacks water movement to carry away decaying macrophyte plant material; it has been observed that natural wild rice generally grows best in waters that have some movement (Moyle, 1944; DNR, 2008; Table 1-6).

It is good that sulfate is present at least at minimal concentrations, since sulfate is an essential nutrient for plant growth. Zero sulfide and zero sulfate are not reasonable goals, so the question is, what is a reasonable goal for the protection of wild rice?

The field survey sponsored by the MPCA yields information about the range of sulfide in the porewater of wild rice waters. Of the 108 waterbodies in the Class B dataset, only two were less than the analytical lab's reporting limit of 11 µg/L. Sulfide was therefore likely present in the porewater of sediment as wild rice evolved over the millennia. It is also likely that wild rice, like other wetland plants, has adaptations that allow it to grow and reproduce in the presence of some concentration of sulfide in porewater. The multiple lines of evidence indicate that wild rice populations can thrive if porewater sulfide is less than 120 µg/L.

If it is true that wild rice populations can thrive at sulfide concentrations up to 120 µg/L, why do the graphs of presence and density (Figs. 1-10 and 1-11) imply that wild rice would benefit from every incremental decrease of sulfide below 120 µg/L? The graphs extend down to 10 µg/L, where the probability of wild rice presence is predicted to be 88%, and 75% of wild rice stands are predicted to have density greater than 40 stems/m². If lower sulfide is apparently associated with better wild rice growth, why not make the protective sulfide concentration as low as possible, such as the analytical limit of 11 µg/L?

There are multiple reasons why making the protective sulfide concentration as low as possible is not reasonable, or necessary to protect wild rice:

Firstly, cause and effect is likely backwards at very low concentrations of sulfide. The graphs relating sulfide to wild rice presence and density (Figs. 1-10 and 1-11), support the overall conclusion that wild rice presence and density can be controlled by exposure to sulfide, (Myrbo et al., in press-1; Fort et al., 2017; Pastor et al., 2017). However, when sulfide is low enough for wild rice to grow to dense levels, it is reasonable to assume that oxygen release by the wild rice would decrease sulfide to even lower concentrations, producing the continuous slope observed in the graphs. It is well established that wetland plants can release oxygen from their roots, which is thought to be an adaptation that decreases the toxicity of sulfide by converting it to sulfate (Lamers et al., 2013). Myrbo et al. (submitted-2) found that sulfide concentrations are significantly lower when wild rice plants are present, an observation that provides support for the idea that high densities of wild rice can drive porewater sulfide to very low concentrations. Myrbo et al. (submitted-2) go on to suggest that there may be a tipping point in the exposure of wild rice to sulfide, above which oxygen release is insufficient to detoxify the sulfide and a

wild rice population cannot persist over the long term. In other words, the capacity of wild rice to detoxify sulfide is limited. The shape of the graphs in Figures 1-10 and 1-11 are explicable if sulfide concentrations above 120 µg/L control wild rice presence and density, but that at some concentration below 120 µg/L wild rice starts controlling the sulfide concentration.

Secondly, the accuracy of the equation that would calculate sulfate standards deteriorates for protective sulfide concentrations below 120 µg/L (Fig. 1-9, Table 1-10). The sum of false positives and false negatives is 16% at 120 and 130 µg/L, but from 70 to 110 µg/L ranges between 22% and 32%. Both false positives and false negatives are serious errors. False negative errors could result in ongoing use impairment. False positive errors could lead to inappropriate management interventions using resources that would be better directed elsewhere (Smeltzer et al., 2016). Below 70 µg/L the errors are essentially all false positives (Table 1-10)—where ambient sulfate is greater than the calculated standards, but porewater sulfide is less than the assumed protective sulfide concentration of 50 or 60 µg/L (MPCA did not calculate the misclassification rates below 50 µg/L).

Wild rice exhibits atypical dose-response curves

It was noted earlier (in “MPCA-sponsored field survey: estimates of protective sulfide concentrations,” above) that the logistic curve relating wild rice presence to sulfide does not exhibit a flat area of “no effect” at low sulfide concentrations (Figs. 1-4 and 1-10; Appendix 7). Similarly, the probability of observing high wild rice density does not exhibit a flat area of no effect at low sulfide levels (Fig. 1-11). MPCA staff were initially surprised at the shape of these dose-response curves, since environmental toxicologists typically observe, and expect, a sigmoid-shaped relationship between the growth of an organism and the dose, or concentration, of a chemical (e.g., Landis and Yu, 2003, p. 32). The expectation of a sigmoid-shaped curve is based on the assumption that a chemical has no effect at very low concentrations, but increasingly greater effect as concentrations of the chemical increase. However, environmental toxicologists generally have no expectation that organisms have the ability to decrease the environmental concentration of the toxic chemical, an ability that dense populations of wild rice appear to have when growing in low concentrations of sulfide (through oxidation as described above). The expectation that chemicals affect organisms, and not the other way around, can be explained by the focus of environmental toxicologists on synthetic chemicals that are not natural in the environment, and the assumption that sensitive species do not possess adaptations to reduce the environmental concentration of a toxic chemical. In fact, in their book *Introduction to Environmental Toxicology*, Landis and Yu characterize the sigmoid shape as the typical response of organisms to a “xenobiotic”—xenobiotic being their term for a toxic chemical. The EPA defines a xenobiotic as a chemical “...that does not occur naturally in the environment. Xenobiotics occur as a result of anthropogenic activities such as the application of pesticides and the discharge of industrial chemicals to air, land, or water.” (EPA, 1992, p. 38). Environmental toxicologists have generally not evaluated the effect of a natural toxin such as sulfide on organisms, although there is increasing interest in the effect of sulfide on plants (Lamers et al., 2013). Because high densities of wild rice can further decrease low concentrations of porewater sulfide, as seen by Myrbo et al. (submitted-2), atypical dose-response curves are produced.

Alternative metrics for evaluation of false positives and false negatives

There are multiple metrics of the accuracy of predictions that can be calculated when false positives (FP), false negatives (FN), true positives (TP), and true negatives (TN) are known (Fielding and Bell, 1997). In the discussion above, the false predictions are calculated as the proportion of the total

predictions, which Fielding and Bell call the misclassification rate ($\text{misclassification rate} = (\text{FP} + \text{FN}) / (\text{FP} + \text{FN} + \text{TP} + \text{TN})$). The overall misclassification rate for potential fixed sulfate standards is presented in Fig. 1-8, and the components (false positives and false negatives) are presented in Fig. 1-14a.

A different way to examine the accuracy of predictions is to treat predictions as though they were medical diagnoses, and then to calculate the rate that the diagnosis is incorrect. For instance, a false negative is actually a positive; the “false negative rate” (FNR, as defined by Fielding and Bell) is the proportion of all positives that are false negatives: $\text{FNR} = \text{FN} / (\text{FN} + \text{TP})$. From a medical point of view, the FNR is the rate at which people with a disease are incorrectly diagnosed as not having the disease. Similarly, the false positive rate (FPR) is the rate at which healthy people are incorrectly diagnosed as having a disease. In the world of sulfate water quality standards, the false positive rate is the rate at which the ambient sulfate concentrations in waterbodies exceed the standard, but porewater sulfide is actually below the protective concentration of 120 µg/L.

The State of Vermont identified phosphorus standards to protect against eutrophication by finding the phosphorus concentration where FPR and FNR are equal, so that the standard was equally likely to be over-protective and under-protective (Smeltzer et al., 2016). It is possible to perform this type of analysis for fixed sulfate standards (Fig. 1-14b), but not for equation-based standards, where the error rates are not functions of potential fixed numeric sulfate standards. (Although it is possible to calculate the misclassification rate for the identified protective sulfide concentration of 120 µg/L.)

For potential fixed sulfate standards, the two accuracy metrics (misclassification rate and error rate) produce similarly shaped curves characterizing false positives and false negatives (Fig. 1-14 a,b). In addition, the curves cross at similar sulfate concentrations (10 mg/L and 7 mg/L, respectively), the concentrations where over-protection and under-protection would be balanced.

Early in this section (F) of the TSD it is noted that the overall misclassification rate for the best fixed sulfate standard (32%) is much greater than the misclassification rate for the proposed equation (16% for the dataset it was developed on, and 19% for an independent dataset).

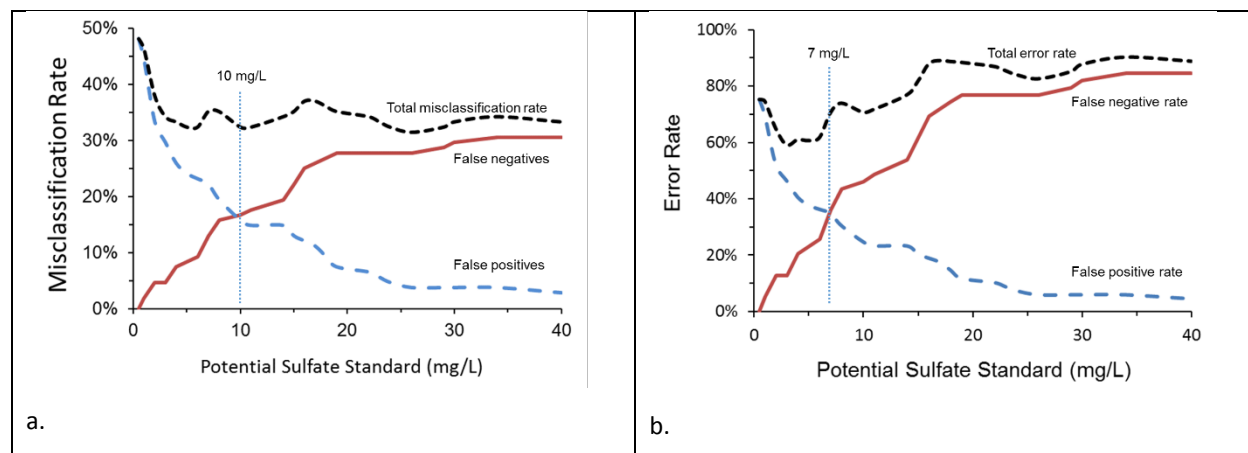


Figure 1-14. Two different ways (as described by Fielding and Bell, 1997) of interpreting the false negatives and false positives associated with a spectrum of potential sulfate criteria (potential standards) and their classification accuracy associated with a protective sulfide concentration of 120 µg/L: a) The total misclassification rate, showing that false negatives and false positives percentages are equal at 10 mg/L, summing to 32%. The total also sums to 32% at 5 mg/L and 26 mg/L, but the former is dominated by false positives (24%) and the latter is dominated by false negatives (28%). b) The total error rate, showing that the false negative rate and false positive rate are equal at 7 mg/L.

Table 1-10. Calculated false classification percentages for potential protective sulfide concentrations from 50 to 400 µg/L (Class B dataset, which approximates a probabilistic sample of waterbodies that could potentially host wild rice; N=108).

Potential Protective Sulfide (µg/L)	True positives	False positives	False negatives	True negatives	False Predictions		
					Percent false positive	Percent false negative	Total percent misclassified
50	89	17	1	1	15.7%	0.9%	16.7%
60	85	19	1	3	17.6%	0.9%	18.5%
70	68	16	8	16	14.8%	7.4%	22.2%
80	51	19	15	23	17.6%	13.9%	31.5%
90	47	14	14	33	13.0%	13.0%	25.9%
100	40	10	14	44	9.3%	13.0%	22.2%
110	35	12	14	47	11.1%	13.0%	24.1%
120	29	7	10	62	6.5%	9.3%	15.7%
130	26	7	10	65	6.5%	9.3%	15.7%
140	22	9	11	66	8.3%	10.2%	18.5%
150	17	7	12	72	6.5%	11.1%	17.6%
160	17	8	11	72	7.4%	10.2%	17.6%
170	17	8	11	72	7.4%	10.2%	17.6%
180	17	7	10	74	6.5%	9.3%	15.7%
190	16	7	9	76	6.5%	8.3%	14.8%
200	16	7	8	77	6.5%	7.4%	13.9%
210	14	7	9	78	6.5%	8.3%	14.8%
220	14	6	7	81	5.6%	6.5%	12.0%
230	12	6	8	82	5.6%	7.4%	13.0%
240	11	5	8	84	4.6%	7.4%	12.0%
250	11	5	8	84	4.6%	7.4%	12.0%
260	11	5	7	85	4.6%	6.5%	11.1%
270	11	5	7	85	4.6%	6.5%	11.1%
280	8	4	9	87	3.7%	8.3%	12.0%
290	8	4	9	87	3.7%	8.3%	12.0%
300	8	4	9	87	3.7%	8.3%	12.0%
310	9	3	7	89	2.8%	6.5%	9.3%
320	9	3	7	89	2.8%	6.5%	9.3%
330	9	3	7	89	2.8%	6.5%	9.3%
340	9	3	7	89	2.8%	6.5%	9.3%
350	9	3	7	89	2.8%	6.5%	9.3%
360	9	1	6	92	0.9%	5.6%	6.5%
370	9	0	5	94	0.0%	4.6%	4.6%
380	9	0	5	94	0.0%	4.6%	4.6%
390	9	0	5	94	0.0%	4.6%	4.6%
400	9	0	5	94	0.0%	4.6%	4.6%

Table 1-11. Calculated false classification percentages and diagnosis error rates for potential sulfate standard concentrations from 0.5 to 40 mg/L (Class B dataset; N=108).

Potential Sulfate Standard (mg/L)	True positives	False positives	False negatives	True negatives	False predictions			Diagnosis Error Rates	
					Percent false positive	Percent false negative	Misclassification rate	False Positive Rate	False Negative Rate
0.5	39	52	0	17	48%	0%	48.1%	75%	0%
1	37	48	2	21	44%	2%	46.3%	70%	5%
2	34	36	5	33	33%	5%	38.0%	52%	13%
3	34	32	5	37	30%	5%	34.3%	46%	13%
4	31	28	8	41	26%	7%	33.3%	41%	21%
5	30	26	9	43	24%	8%	32.4%	38%	23%
6	29	25	10	44	23%	9%	32.4%	36%	26%
7	25	24	14	45	22%	13%	35.2%	35%	36%
8	22	21	17	48	19%	16%	35.2%	30%	44%
10	21	17	18	52	16%	17%	32.4%	25%	46%
11	20	16	19	53	15%	18%	32.4%	23%	49%
14	18	16	21	53	15%	19%	34.3%	23%	54%
15	15	14	24	55	13%	22%	35.2%	20%	62%
16	12	13	27	56	12%	25%	37.0%	19%	69%
17	11	12	28	57	11%	26%	37.0%	17%	72%
18	10	10	29	59	9%	27%	36.1%	14%	74%
19	9	8	30	61	7%	28%	35.2%	12%	77%
22	9	7	30	62	6%	28%	34.3%	10%	77%
23	9	6	30	63	6%	28%	33.3%	9%	77%
24	9	5	30	64	5%	28%	32.4%	7%	77%
26	9	4	30	65	4%	28%	31.5%	6%	77%
29	8	4	31	65	4%	29%	32.4%	6%	79%
30	7	4	32	65	4%	30%	33.3%	6%	82%
34	6	4	33	65	4%	31%	34.3%	6%	85%
40	6	3	33	66	3%	31%	33.3%	4%	85%

False negative sites under the current 10 mg/L standard (but correctly classified by the MBLR-based equation)

Some sites have sedimentary concentrations of iron and organic carbon that allow relatively efficient conversion of sulfate to porewater sulfide. If surface water carried sulfate into the sediment, 100% conversion of only 0.4 mg/L would exceed a sulfide concentration of 120 µg/L, an indication of why regulation of sulfate concentrations less than 10 mg/L would be appropriate when iron availability is low. Highly efficient conversion to sulfide was observed in some sites in the MPCA field survey data set. Among the sites in the MPCA field survey, there are nine sites where a fixed standard of 10 mg/L would not be protective, but the MBLR equation would be protective (Table 1-12). The ambient sulfate concentrations at these sites range from 1.3 to 7.8 mg/L, and observed porewater sulfide concentrations range from 145 to 2,525 µg/L, above the protective concentration of 120 µg/L sulfide. At these sites, wild rice density is generally low (zero to 10.4 stems/m²) with an exception of 69.7 stems/m² observed in the single visit to Bowstring River.

Table 1-12. Examples of false negatives relative to a fixed numeric standard of 10 mg/L: sites with sulfate less than 10 mg/L, but with greater than the calculated protective sulfate concentration, as calculated by the multiple binary logistic regression (MBLR120). As predicted by the MBLR equation, sulfide concentrations are greater than 120 µg/L, the protective sulfide concentration. (Values are average when the site was sampled more than once.)

Site	State ID	Ambient sulfate (mg/L)	MBLR120 (mg/L)	Wild Rice density stems/m ²	Porewater Sulfide (µg/L)	Trans- parency (cm)	Number of Field Samples
Anka Lake	21-0353-00-201	4.3	0.7	10.4	565	89	3
Big Sucker Lake	31-0124-00-203	7.8	2.1	3.8	145	101	1
Bowstring River	S007-219	1.3	0.6	69.7	256	101	1
Gilchrist Lake	86-0064-00-201	7.0	1.7	0.0	355	101	1
Rice Lake	02-0008-00-206	3.6	2.6	0.0	145	31	1
Rice Lake	66-0048-00-203	5.2	2.4	0.0	777	20	1
Rice Lake	73-0196-00-216	4.7	0.9	0.0	2,525	101	2
Rice Lake	74-0001-00-201	3.8	3.2	0.0	217	3	1
Westport Lake	61-0029-00-204	6.7	3.1	3.3	998	86	2

It is useful to examine the implications of the data from the Bowstring River in some detail, because the calculated protective sulfate concentration was extremely low. The Bowstring River was sampled at only one location during the field survey, so it was uncertain how representative the single analyses of TOC and TEF_e were. The following discussion should therefore not be taken as a thorough description of the waterbody, but rather as an opportunity to discuss the effect of sulfide on the probability of wild rice occurrence in a waterbody. This site on the Bowstring River was later sampled in detail as part of an implementation pilot project, described in Chapter 3, *Implementation of the Wild Rice Standard*.

Based on the single sample from the field survey, Bowstring River would have an extremely low calculated sulfate standard, 0.6 mg/L sulfate, based on the MBLR equation (TOC is high, and TEF_e is low). The ambient sulfate concentration was just 1.3 mg/L (1.0 mg/L when sampled in the implementation pilot project 6/23/2015), but produced a porewater sulfide concentration of 256 µg/L, appreciably higher than the protection goal of 120 µg/L sulfide. Despite exceeding the protective porewater sulfide level of 120 µg/L, Bowstring River supported a dense population of wild rice, which is a reminder that 120 µg/L is not a stark threshold below which wild rice can exist and above which wild rice dies. Rather, above 120 µg/L the *probability* of observing wild rice declines progressively as sulfide concentrations increase. According to the logistic regression, the probability of observing wild rice is 69% at 120 µg/L, and declines to a probability of 59% at 256 µg/L (Fig. 1-4). The probability curve suggests that the wild rice population on the Bowstring River is at risk if even slight increases in sulfate occur above the measured ambient concentration of 1.3 mg/L, given that the MBLR-calculated protective sulfate concentration was 0.7 mg/L. If ambient sulfate were allowed to approach 10 mg/L, it is likely that porewater sulfide would become much higher and that the wild rice population would decline in this waterbody. If sulfide reached 500 µg/L, the probability of wild rice occurrence would decline to 50%. As the ambient sulfate concentration increases above the calculated protective concentration for that waterbody, the likelihood of elevated porewater sulfide increases dramatically (Fig. 1-15 a).

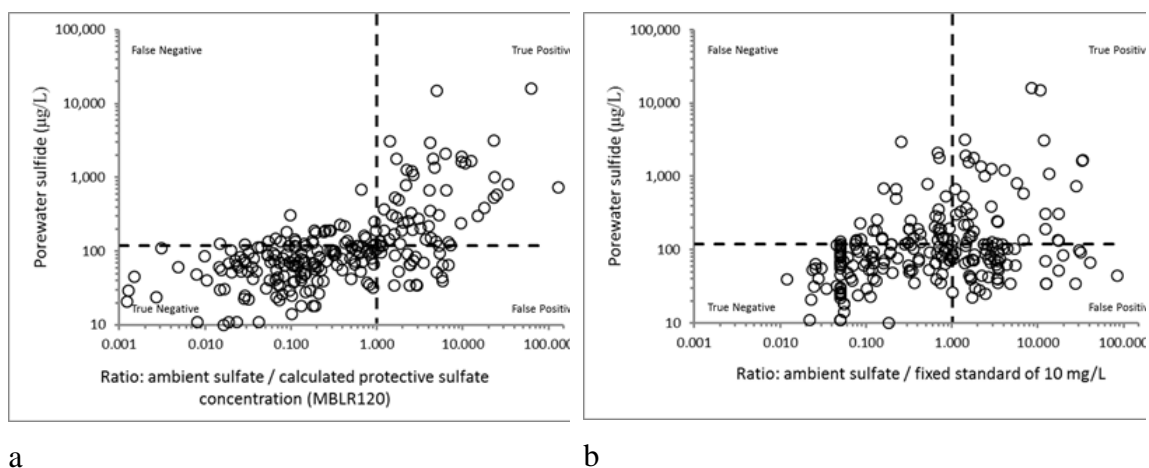


Figure 1-15. Observed accuracy observed when the MPCA survey data are assessed against (A) standards calculated with the proposed equation, and (B) a fixed sulfate standard of 10 mg/L. Sites to the right of the vertical dashed line exceed the potential sulfate standard, the goal of which is to keep porewater sulfide below the protective concentration of 120 µg/L. Sites above the horizontal dashed line actually had porewater sulfide greater than 120 µg/L. The sites in the upper right quadrants (above and to the right of the dashed lines) are correctly classified as exceeding the standard (true positives), whereas the sites in the lower right quadrant are false positives. Sites in the upper left quadrant are false negatives—sites where the ambient sulfate concentration was falsely identified as protective of wild rice, but the porewater sulfide was greater than 120 µg/L. (All survey data are graphed, so that some waterbodies are represented by multiple points; N=222.)

Note that four of the nine sensitive sites noted above that are negatively affected by ambient concentrations of sulfate below 10 mg/L are called “Rice Lake” (Table 1-12), which some assume means that the early settlers had observed obvious wild rice populations. No wild rice plants were observed in these lakes during the field survey. These sites are not notably enriched with sulfate – ambient sulfate ranges from 3.6 to 5.2 mg/L – yet porewater sulfide is elevated above the protective sulfide concentration of 120 µg/L.

If wild rice used to grow in these four lakes, porewater sulfide may have been below the protective sulfide concentration, which implies that these lakes experienced an increase in sulfate or organic matter, or, less likely, the loading rate of iron has decreased. These four lakes lie in areas where there is significant anthropogenic land use, including agriculture. Agricultural activity could increase sulfate loading, either through the use of groundwater for irrigation (since groundwater often has higher sulfate concentrations than surface water) or by the application of sulfur-containing fertilizers. Tile drainage in a watershed may also increase the export of sulfate to receiving waters, because of increased aerobic oxidation of sulfide minerals in the soil (Lamers et al., 2013).

In addition, anthropogenic activity almost always increases loading of phosphorus to surface waters, which likely would decrease transparency. As discussed in section A of this TSD, reduced transparency reduces the probability of wild occurrence independent of the porewater sulfide concentration in that waterbody. Wild rice can be excluded from a site if the water transparency is less than 30 cm (Part B of Chapter 1, above). Three of the four Rice Lakes had low observed transparency (3, 20, and 31 cm), in addition to exceeding the protective sulfide concentration.

False positive sites under the current 10 mg/L standard (but correctly classified by the MBLR-based equation)

Some sites have sediment concentrations of iron and organic carbon that do not result in relatively efficient conversion of sulfate to porewater sulfide. Among the wild rice sites in the MPCA field survey there are six sites where the observed sulfate is greater than 10 mg/L but sulfide concentrations are less than 120 µg/L—sites that under a 10 mg/L fixed sulfate standard would falsely be classified as areas where wild rice is not sufficiently protected. The MBLR equation correctly predicted that these sites would have sulfide below 120 µg/L (Table 1-13), thereby protecting wild rice.

At these six sites, average wild rice densities range from 31 to 141 stems/m². Ambient sulfate concentrations range from 18 to 32 mg/L, well below the calculated protective sulfate concentrations, which range from 93 to 546 mg/L. Observed sulfide concentrations range from 53 to 112 µg/L.

Table 1-13. Examples of false positives relative to a numeric sulfate standard of 10 mg/L: sites with sulfate greater than 10 mg/L, but with sulfate concentrations lower than the calculated protective sulfate concentration. As predicted by the MBLR equation, sulfide concentrations do not exceed 120 µg/L, the protective sulfide concentration. (Values are average when the site was sampled more than once.)

Site	State ID	Ambient sulfate (mg/L)	MBLR120 (mg/L)	Wild Rice density stems/m ²	Porewater Sulfide (µg/L)	Trans- parency (cm)	Number of Field Samples
Hay Lake	31-0037-00-202	26.9	218	141	59	86	2
Mississippi Pool 4 / Robinson Lake	79-0005-02-201	29.6	262.6	46.5	67	90	3
Mississippi Pool 5 / Spring Lake	S007-660	32.5	546.1	39	53	88	5
Mississippi Pool 8 at Genoa	S007-222	31.2	93	31.2	112	86	4
Mississippi Pool 8 at Reno Bottoms	S007-556	18.1	187.6	72.3	71	57	1
Partridge River	S007-443	24.1	302	42.5	80	79	6

Practical implications of false positive and false negative prediction errors

If the ambient sulfate concentration is greater than the standard, the state of Minnesota is required to take action, pursuing either a) completion of a Total Maximum Daily Load (TMDL) study to determine how to reduce sulfate concentrations below the standard, or b) an EPA-approved site-specific standard for the site. The latter would be pursued if there is evidence that the exceedance of the sulfate standard is a *false positive error*. The false positive rate is greater for fixed standards, so a fixed standard would require the pursuit of more TMDLs or site-specific standards when, in fact, neither are needed to protect wild rice.

Because any fixed standard would be less accurate than an equation-based standard, under a fixed standard there would be more cases in which a wild rice water has a rice population judged to be healthy, but where the ambient sulfate concentration exceeds the standard. In such situations, it would be necessary to develop a site-specific standard to protect the beneficial use, which involves significant staff time and resources. This effort would occur less often under the implementation of an equation-based sulfate standard.

When a false positive is calculated for a sulfate discharge, the determination may result in unneeded investment in water treatment—which is why the false positive rate should be minimized.

False negative errors—where it is thought that sulfide will not accumulate to levels that harm the wild rice population when, in fact, it does—will not be recognized for a number of years, because it takes multiple years for sulfide to accumulate in the sediment and harm the rice (Pastor et al., 2017). False negative errors, therefore, potentially cause environmental harm—harm that may be difficult to

reverse, because there is little experience for intentionally oxidizing and detoxifying sulfide once it accumulates in sediment (Ning et al., 2011).

The prediction error rate, the need for site-specific standards, and the occurrence of harm due to false-negative classification can all be reduced by adopting the MBLR equation as the sulfate standard to protect wild rice.

The Mississippi River below the Twin Cities is a good example of the ramifications of retaining a fixed sulfate standard. The MPCA field survey sampled four pools of the Mississippi River below the Minneapolis-Saint Paul Metropolitan Area that have had large populations of wild rice for many years. Ambient sulfate concentrations (18 to 32 mg/L) were well above 10 mg/L. The observed sulfate concentrations are well below the protective sulfate values calculated from the MBLR equation (93 to 546 mg/L). If the wild rice sections of the Mississippi River listed in Table 1-13 were evaluated against a fixed sulfate standard of anything less than 18 mg/L, either a site-specific standard would need to be developed or, under section 303(d) of the federal Clean Water Act, Minnesota would be required to develop a TMDL plan to reduce sulfate concentrations to below the standard. A TMDL plan would entail the calculation of the maximum amount of sulfate that could be discharged to the Mississippi and its tributaries, which would lead to the allocation of the necessary reductions to achieve compliance with the sulfate standard. Development and implementation of such a TMDL would be costly in terms of staffing resources and the potential for additional treatment requirements, and would not appreciably benefit the wild rice populations in these pools of the Mississippi since porewater sulfide concentrations are already less than the protective sulfide concentration of 120 µg/L. However, if the wild rice sulfate water quality standard were based on calculated values generated using the MBLR model, then a TMDL would not have to be developed, Minnesota would not have to determine how the sulfate concentration in the Mississippi could be reduced by as much as 70%, and wild rice would remain sufficiently protected from sulfate impacts.

Comparison of error rates to Vermont's phosphorus standards

The state of Vermont recently adopted, and EPA approved, fixed phosphorus standards to protect aesthetic use in lakes and aquatic biology in streams. Numeric standards were derived in a way to minimize false positive and false negative rates (Vermont DEC, 2014; Smeltzer et al., 2016). Eleven different phosphorus standards were developed, depending on the applicable tiered water use objective. The misclassification rates varied from 17 to 40% (Table 1-14), with a median of 35% - about the same as the best misclassification rate for possible fixed sulfate standards for wild rice (32%).

Table 1-14. Phosphorus standards developed by the state of Vermont (Vermont DEC, 2014) and associated misclassification rates.

Use Objective	Tier*	Phosphorus Criterion (µg/L)	Misclassification rate
Lake Aesthetics	Excellent aesthetic value	17	24%
	Good aesthetic value	18	17%
<hr/>			
Aquatic Life (Small, high-gradient streams)	1	10	39%
	2	10	35%
	3	12	36%
<hr/>			
Aquatic Life (Medium high-gradient streams)	1	9	40%
	2	9	39%
	3	15	22%
<hr/>			
Aquatic Life (Warm-water, moderate gradient streams)	1	18	32%
	2	21	31%
	3	27	39%

*Tier Level of Aquatic Life Use Support

Analysis of Suggested Alternatives to the Protective Sulfide Level and Equation

During the process of developing the proposed revisions, the MPCA received a great deal of comment and advice from stakeholders and interested parties, many of which contained suggested alternate proposals for the sulfate standard. The MPCA considered a number of possible alternatives, including possible fixed standards and that a higher protective sulfide level (and related changes to the equation) might be appropriate.

With the release of the Draft TSD in July 2016 and in discussions of subsequent analyses, primarily with the Wild Rice Advisory Committee, MPCA staff frequently discussed the error rates of the equation. Commenters suggested that there were alternatives to the MPCA’s identified protective sulfide level and equation that would result in a lower error rate (4% compared to 16%) but a similar level of wild rice protection.

Accordingly, MPCA staff have carefully reviewed the suggested changes in the derivation of an equation to calculate protective levels of sulfate. Ramboll (2017) suggested developing an equation with two changes to the work undertaken by the MPCA. The first change is adjusting the dataset used to perform the multiple binary logistic regression (MBLR), using only the 67 waterbodies where wild rice was observed. The second change is to use a protective porewater sulfide concentration of 300 µg/L, and developing the equation to relate sulfide to sulfate from there. Both of these changes would affect the resulting equation to calculate a numeric sulfate standard for a wild rice water.

Dataset used to perform the MBLR

Ramboll (2017) asserted that the chemical relationship between sulfate (in surface water) and sulfide (in sediment porewater) should be developed using regression analysis on a dataset that includes only waterbodies that have wild rice, because such a dataset “is most relevant to the receptor of concern

which is a longstanding approach used by EPA in determining criteria.” In support of this position, Ramboll (2017) asserted:

This is similar to the longstanding EPA policy and practice of data use in development of criteria (USEPA 1994; Stephan et al., 1985; USEPA 2010). For example, when developing aquatic life criteria, EPA uses toxicity data from freshwater species to derive freshwater criteria and saltwater species are used to derive saltwater criteria. Likewise, criteria for warmwater fisheries are derived without toxicity data for coldwater species. [p. 3-1]

and

In keeping with EPA policy and practice, only sites on the proposed wild rice list and with wild rice present should be included. [p. 4-1]

MPCA staff inspected the EPA documents cited by Ramboll (2017) and found no guidance directly pertinent to the question of what datasets should be analyzed when establishing a protective sulfide level and translating a protective sulfide level to a numeric standard to protect wild rice. The primary EPA guidance for the development of a water quality standard is that a state needs to demonstrate that its development procedure is fully protective of designated uses—and that EPA will review proposed standards by looking for a sound scientific rationale (EPA, 1994. p. 3-21, Water Quality Standards Handbook).

As described throughout this TSD, MPCA staff used the field survey data for two purposes: (1) to identify a porewater sulfide concentration that would be protective of wild rice growth and reproduction, and (2) to develop an equation that calculates a protective sulfate concentration that corresponds to the protective sulfide concentration identified in (1). Different subsets of the field data were used in support of each of the two purposes:

(1) Identification of a protective sulfide concentration: As MPCA staff reviewed the multiple lines of evidence for the identification a sulfide concentration to protect wild rice, different subsets of the field dataset were used, depending on the question being asked. For instance, when asking how porewater sulfide affects the probability that wild rice (of any density) will be observed in a waterbody, it was necessary to include waterbodies where no wild rice was observed. When addressing the probability of wild rice being present, two different datasets were analyzed, all 108 waterbodies (yielding an EC10 of 58 µg/L), and a subset that consisted only of sites with sufficient transparency to support wild rice (yielding an EC10 of 91 µg/L). MPCA staff regard the latter estimate as more defensible, since elevated sulfide is not responsible for the lack of wild rice when transparency is inadequate to support wild rice. In contrast, when the question was how sulfide affects the density of wild rice, only waterbodies with wild rice were included in the change-point analysis, which revealed a statistically significant decline in wild rice density at 112 µg/L. (Including waterbodies with no wild rice in this analysis would not have clearly addressed the question of how sulfide affects wild rice density.)

(2) Development of a protective sulfate concentration: MPCA staff developed a mathematical relationship that characterizes the chemical relationship between sulfate and the protective level of sulfide (120 µg/L) by including all 108 waterbodies. MPCA used all available data because the goal was to statistically describe a chemical relationship in the environment, not the effect of sulfide on wild rice.

Using only the 67 wild rice waterbodies has the effect of calculating higher sulfate levels than if all 108 waterbodies are used. This is perhaps because excluding waterbodies without wild rice also excludes many waterbodies with high sulfide, skewing the data used to calibrate the equation.

One way to see the effect of calibrating the equation with different waterbody datasets (and protective sulfide concentrations) is to compare the median potential sulfate standard that each equation produces when applied to the 67 waterbodies within the Class B dataset that had wild rice. The MPCA proposal (protective sulfide of 120 µg/L, equation developed with all Class B data) yields a median

sulfate concentration 14 mg/L (Table 1-15). When an equation is developed only with data from waterbodies with wild rice, the median potential sulfate standard increases to 61 mg/L, which would allow much more porewater sulfide to develop. The effect is magnified if an equation is developed with a protective sulfide concentration of 300 µg/L. If developed with all 108 sites, the median potential sulfate standard would be 20 mg/L. But, if the equation is developed with only with data from sites with wild rice, the median potential sulfate standard would be 209 mg/L (Table 1-15). Calculated sulfate standards are clearly influenced by the dataset used to develop the equation.

MPCA staff concludes that it is most defensible to perform the regression that relates sulfate and sulfide with the entire 108-waterbody data set, rather than the data set that only includes sites with wild rice. First, the point of the regression is to develop a mathematical description of the chemical relationship between the three variables that have been demonstrated to control porewater sulfide: sulfate, TOC, and sediment iron (Pollman et al., in press). Second, since it has been shown that elevated sulfide is one of the primary controllers of wild rice presence in a waterbody (Myrbo et al., in press-1), it is evident that excluding waterbodies without wild rice would likely also exclude waterbodies that have high sulfide, which could skew the results of the regression. In fact, excluding sites without wild rice excludes 77% of the highest sulfide concentrations (10 of the 13 waterbodies with the highest sulfide). It is essential to the goal of the analysis to perform a robust regression that accurately predicts elevated sulfide, and not including data with elevated sulfide is counterproductive to that goal.

Table 1-15. Effect on median protective sulfate concentrations (for waters with wild rice) of developing equations with different datasets, and different protective sulfide concentrations.

Protective sulfide concentration used to develop equation	Median calculated protective sulfate concentration in waters with wild rice (N=67)	
	Equation developed with all Class B Waterbodies (N=108)	Equation developed with only Class B Waterbodies with wild rice (N=67)
120 µg/L	14 mg/L*	61 mg/L
300 µg/L	20 mg/L	209 mg/L**

*MPCA proposal

**Ramboll (2017) proposal

Protective level of sulfide

Ramboll (2017) asserted that a protective sulfide concentration of 300 µg/L provides a similar level of protection for the wild rice beneficial use as 120 µg/L. The evidence given for this assertion is that there is no statistical difference in average wild rice stem densities below the 120 µg/L and below 300 µg/L (55 and 52 stems/m², respectively, p. 3-3) (MPCA’s calculations are slightly different, 57 and 53 stems/m², a difference that is not important in this discussion).

The appropriate way to determine if 300 µg/L provides a similar level of protection as 120 µg/L is different than the test performed by Ramboll (2017). Rather than compare overlapping ranges of porewater sulfide, which violates the fundamental statistical principle that requires independence between two compared groups, it is more appropriate to compare the under 120 µg/L group to the 120-300 µg/L group. If 300 µg/L is similarly protective, there would not be a significant difference between these two groups.

As shown in Table 1-16, the average and median stem densities of the less-than-120 µg/L group are greater than the 120-300 µg/L group (average density of 57 vs 38, and median density of 47 vs 21 stems/m²). A nonparametric statistical test (Wilcoxon-Mann-Whitney) finds that the two groups are significantly different at the p= 0.06 level. A more in-depth analysis shows that waterbodies with porewater sulfide less than 120 µg/L are more likely to have dense (> 40 stems/m²) wild rice than stands with sulfide between 120 µg/L and 300 µg/L (Appendix 9). Wild rice waters with sulfide less than 120 µg/L are 5.6 times as likely as sites with sulfide between 120 and 300 µg/L to have dense wild rice (> 40 stems/m²) than sparse wild rice (< 10 stems/m²) (p<0.5). This is consistent with the change-point analysis, which found that wild rice density is significantly lower at sulfide concentrations greater than 112 µg/L (Appendix 7).

Table 1-16. Comparison of wild rice occurrence and density between two groups: waterbodies with porewater less than 120 µg/L and waterbodies with sulfide between 120 µg/L 300 µg/L.

Group	Number if waterbodies	Number of waterbodies with wild rice	Proportion with wild rice	Average density of wild rice (stems/m ²)	Median density of wild rice (stems/m ²)
less than 120 µg/L	69	49	71%	57	47
120 to 300 µg/L	22	13	59%	38	21

Therefore, wild rice density is significantly less robust between 120 and 300 µg/L than below 120 µg/L. Because density of wild rice in a waterbody is likely related to persistence of the population and to maintaining the beneficial use of wild rice, MPCA concludes that a porewater sulfide concentration of 300 µg/L is not protective of the wild rice beneficial use.

The MPCA did not make any changes to the equation based on the information provided. The proposal does have a lower error rate than the MPCA-proposed approach; however, it is important to remember that while it is desirable to minimize error rates as much as possible, doing so is a secondary consideration. The primary goal and requirement of the standard is to protect the wild rice beneficial use from the impacts of elevated sulfide. The MPCA’s review of the proposal shows that the changes would result in a standard that is not sufficiently protective.

Chapter 2. Evidence that a different standard is needed for some wild rice waters

The basis of the proposed equation to calculate a numeric sulfate standard for wild rice waters is founded upon interrelationships of sulfate, organic carbon, and iron that produces sulfide, which is the toxic agent. With this understanding, there are two probability-based relationships involved in the determination of a sulfate concentration that will protect wild rice from elevated sulfide in the sediment: (1) the choice of a particular sulfide concentration that would be protective of wild rice, and (2) the translation of that sulfide concentration to the corresponding sulfate concentration for that particular waterbody (based on the iron and organic carbon in that waterbody's sediment). As a result, there will be false predictions associated with any sulfate standard, but there will be fewer false predictions when sulfate standards are equation-based, compared to the number of false predictions associated with a fixed standard (as described in part F of Chapter 1).

The first part of this Chapter 2 addresses wild rice waters with false positives and the need for an alternate sulfate standard when sulfide is predicted to be above 120 µg/L but is actually below 120 µg/L. The second part of this Chapter 2 addresses wild rice waters with true positives—sulfide is predicted to be above 120 µg/L and sulfide is actually above that level but the beneficial use is still protected.

Alternate numeric sulfate standard for false positive wild rice waters

A small proportion of wild rice waters in the MPCA-sponsored field study consistently exhibit a porewater sulfide concentration less than 120 µg/L when their ambient sulfate concentrations are greater than their calculated sulfate standards—they are false positives. Such waterbodies are not conforming to the conceptual model on which the equation-based sulfate standard is based, and therefore an appropriate sulfate standard must be determined through an alternative method. Application of an alternate standard could be based on empirical observations.

In the MPCA-sponsored surveys (2011-2013), the dataset that includes all samples in which false negatives and false positives can be identified consists of 222 samples from 115 different natural waterbodies (the dataset termed Class G). In this dataset, at least one false positive was observed in 14 different waterbodies (Fig. 2-1). Thirteen of the 14 waterbodies were sampled more than once, allowing an examination of the consistency of the sulfide predictions. False positives were consistently observed in four of the waterbodies. These four waterbodies consistently had porewater sulfide below 120 µg/L, despite predicted sulfide concentrations above that threshold (Table 2-1). Wild rice was growing in all four of the waterbodies. The most reasonable explanation for unexpectedly low porewater sulfide in these waterbodies is that surface water sulfate was not penetrating downward into the sediment because of upwelling groundwater.

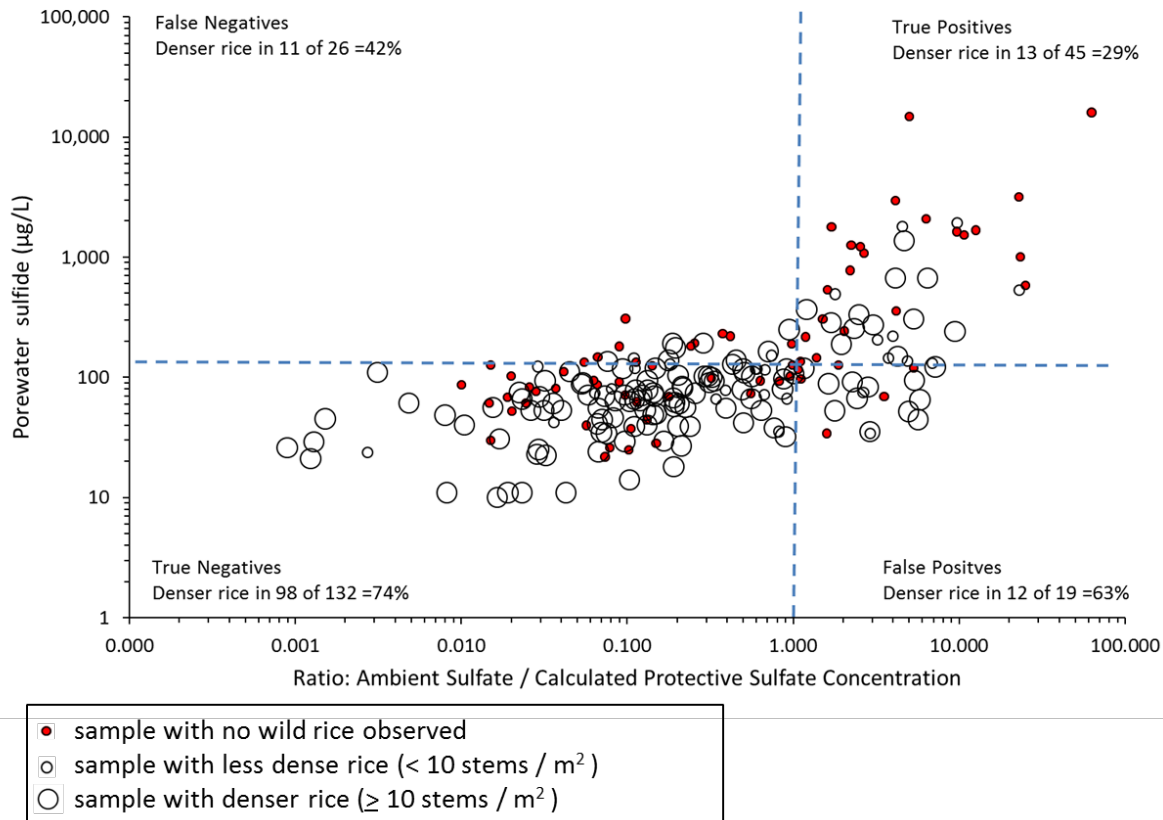


Figure 2-1. Prediction accuracy of the proposed equation for 222 site visits to 115 different waterbodies (data set Class G), plus density of wild rice occurrence, at two levels of density. Of the 222 samples, 19 false positives were found from 14 different waterbodies, of which 12 had wild rice. In 46 site visits to these 14 different waterbodies there were 19 false positives, 10 true negatives, 5 false negatives, and 12 true positives Table 2-1).

False positives (where elevated sulfate does not elevate sulfide) are a concern because they potentially could cause unneeded investment in sulfate control. False positives may merely be the result of random error, especially when ambient sulfate is close to the calculated numeric sulfate standard. Random error is likely the cause of a false positive observed in Second Lake. Second Lake was sampled once in 2011 and once in 2012, and wild rice density was at about the median density of the Class B sites (41 stems per square meter) in both years (37 and 48 stems/m², respectively). In 2012, a false positive was observed when the ambient sulfate concentration in Second Lake was 0.7 mg/L, which was slightly greater than the calculated protective level of 0.6 mg/L, and the measured porewater sulfide, 119 µg/L, was slightly lower than the protective threshold of 120 µg/L (Table 2-1). In 2011, a false negative determination for Second Lake was perhaps an example of random error in the other direction. The ambient sulfate concentration was 0.9 mg/L, lower than the calculated protective level of 1.9 mg/L, and the measured porewater sulfide of 139 µg/L was greater than the protective threshold of 120 µg/L (Table 2-1). Four of the ten sites likely exhibit false positives as a result of random error associated with ambient sulfate levels similar to the calculated protective sulfate concentration (Table 2-1).

More importantly, false positives may also be the result of the failure of a waterbody to conform to the conceptual model upon which the equation is based. As described in Chapter 1 of this TSD, the

conceptual model is supported by significant statistical relationships (Pollman et al., in press), so that most of the waterbodies in the field survey act as described by the model. The model is based on the assumption that porewater sulfide is produced by bacteria in the sediment that are utilizing sulfate transported from the surface water downwards into the sediment. However, there may be wild rice waters where groundwater actively moves upward through the sediment, in which case sulfate in surface water would not play a major role in the production of sulfide. In such cases, ambient sulfate in surface water in comparison to the calculated sulfate standard can produce false positives, depending on the sediment concentrations of organic carbon and extractable iron. Wild rice waters with upwelling groundwater might be most often encountered in gaining streams, which receive water from groundwater, and some lakes that receive groundwater. The interaction of groundwater and surface waters is complicated, and is a function of multiple variables such as the texture and depth of soils, topography, and even seasonal growth of plants that transpire large amounts of groundwater, such as willows (Fetter, 2001).

In a study of 46 Wisconsin lakes, Nichols and Shaw (2002) found that wild rice is statistically associated with shallow areas where groundwater was emerging into the lake. The researchers were not sure why there would be such an association, but it is possible that they happened to sample waterbodies where upwelling groundwater allowed porewater sulfide concentrations to be low enough for wild rice to grow. Nichols and Shaw did not discuss the issue of sulfate and sulfide.

Of the 14 waterbodies in the MPCA study with at least one false positive, upwelling groundwater at four waterbodies seems the likely explanation for unexpectedly low porewater sulfide concentrations (Table 2-1). Three of the four waterbodies are small streams that could be receiving base groundwater flows that keep sulfate in the surface water from moving into the sediment.

For instance, Second Creek (not related to Second Lake, discussed above), was sampled five times and porewater sulfide was less than 120 µg/L in each case despite relatively high sulfate concentrations (303 to 838 mg/L; sulfate was not measured for one of the samplings). Only two of the samples were false positives, because calculated protective concentrations are also relatively high (148 to 947 mg/L) as a result of low sediment TOC and high extractable iron (Table 2-1). Because of interest in this site that combined high sulfate, low sulfide, and robust wild rice density, in 2015 researchers from the University of Minnesota conducted an investigation that measured and modeled groundwater and geochemistry at the site (Yourd, 2017). Yourd found that the model of the geochemical relationships corroborated the findings of Pollman et al. (in press) that sulfide accumulation in porewater depends on the levels of iron and organic carbon—but that hydrologic flux can also play a significant role in the geochemistry of porewater. Yourd concluded that porewater sulfide concentrations in an iron-rich environment like Second Creek may only become elevated when high concentrations of sulfate are able to move into the sediment. Yourd only observed sulfate movement into the sediment when stream water levels were unusually high—which occurred because of a temporary obstruction directly downstream of the study area.

Implementation of an alternate sulfate standard to protect wild rice

When porewater sulfide concentrations are consistently below the protective concentration of 120 µg/L, but ambient sulfate concentrations exceed the equation-based standard (false positives), it is apparent that ambient sulfate concentrations are not being efficiently converted into sulfide. The alternate sulfate standard proposal is based on the idea that when porewater sulfide concentrations are protective of wild rice, the ambient sulfate concentration must also be at a protective concentration.

However, even given these satisfactory conditions, it is not immediately obvious what the applicable sulfate standard should be for a wild rice water that consistently exhibits false positives. An obvious

candidate numerical sulfate standard would be the average ambient sulfate concentration that had been observed for a few years, since that concentration had not caused an exceedance of the protective sulfide concentration of 120 µg/L. But the problem with adopting the average ambient sulfate concentration as the standard is that it is likely, given hydrologic variability, that annual average sulfate concentrations will sometimes be higher in coming years (unless the few years of data were unusual and resulted in the highest possible sulfate concentrations).

Therefore, a reasonable alternate sulfate standard would be higher than the average sulfate concentration observed over just two years of monitoring. But how much higher? One approach is to relate the measured porewater sulfide concentration to 120 µg/L, and to calculate how much higher sulfate could be to maintain porewater sulfide at or below 120 µg/L. Such an approach would need to assume (1) that some surface water sulfate might make its way into the sediment and be converted to sulfide, and (2) that waterbodies have differing empirical efficiencies of converting sulfate to sulfide (the molar ratio of sulfide to sulfate, expressed as a percentage). In the MPCA-sponsored field survey, only 17 of the 115 different natural waterbodies had a sample with efficiency exceeding 50%. The median conversion efficiency of the natural waterbodies was 7.7%. The sulfate-addition experiment of Pastor et al. (2017) offers an opportunity to calculate the efficiency of conversion with different sulfate concentrations interacting with a given sediment (and consistent concentrations of TOC and extractable iron). As the sulfate concentrations increased, the efficiency of conversion declined significantly from a maximum of 4% at the lowest sulfate concentration to a maximum of about 2% (Fig. 2-2).

Therefore, it is likely that the maximum increase in porewater sulfide concentrations as a result of increased sulfate would be proportional to the increase in sulfate. The experimental sulfate additions of Pastor et al. (2017), showing a declining efficiency, suggest that the sulfide increase would be less than proportional. With this understanding, a conservative alternate standard would be an increase in the observed ambient sulfate that is proportional to the degree that 120 µg/L is greater than the observed maximum porewater sulfide concentration. For instance, if the observed porewater sulfide were 80 µg/L and observed ambient sulfate were 110 mg/L, a conservative sulfate standard would be 165 mg/L sulfate ($120/80 * 110$ mg/L).

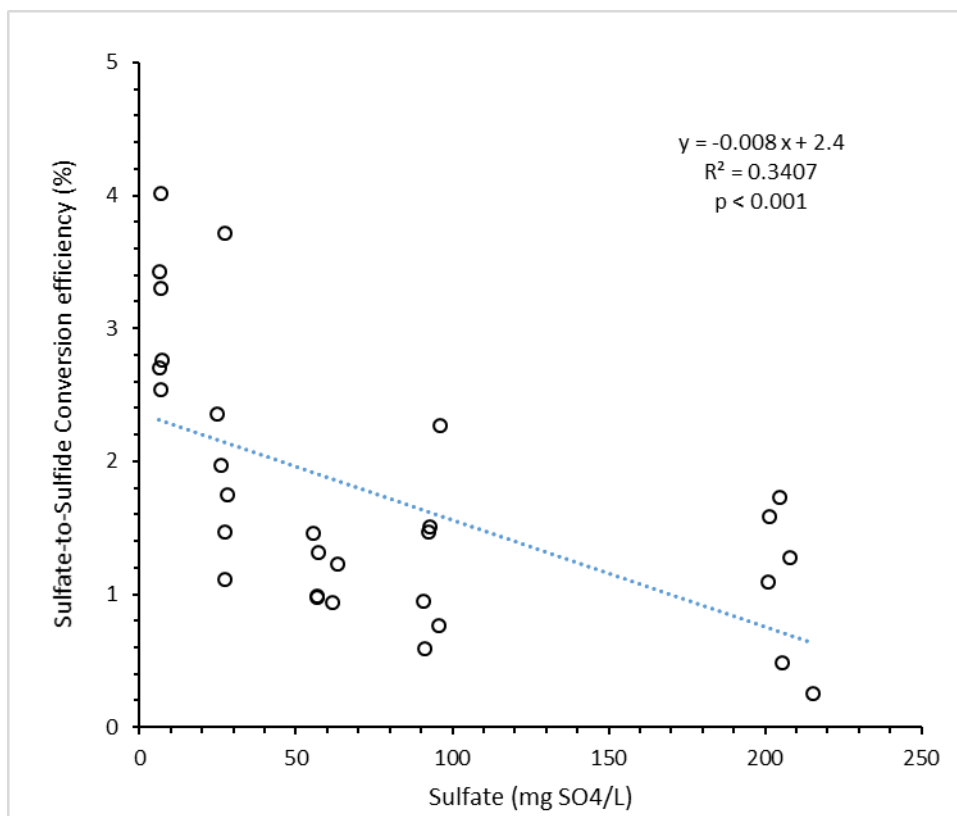


Figure 2-2. Trend in the efficiency of conversion of sulfate in surface water to sulfide in porewater (molar ratio of sulfide to sulfate, as a percentage), after three growing seasons of sulfate additions. The experiment is described in Pastor et al. (2017).

Summary: An alternate sulfate standard to protect wild rice

Implementation of an alternate sulfate standard in a wild rice water would be appropriate when a) ambient sulfate concentrations exceed the equation-based standard and b) porewater sulfide concentrations are demonstrably below the protective concentration of 120 µg/L. The most likely explanation for such observations is the upwelling of groundwater that is lower in sulfate than the surface water. However, sulfate in the surface water may contribute to the production of porewater sulfide if, for instance, groundwater reverses direction seasonally. It might be problematic to set the sulfate standard at the ambient concentration observed over just a few years of monitoring, since natural hydrologic fluctuation may produce an exceedance of the standard. A protective approach to calculating an alternate sulfate standard would be to adjust the observed ambient sulfate concentration by the factor that the protective sulfide concentration of 120 µg/L exceeds the observed ambient porewater sulfide concentration.

Table 2-1. All waterbodies in the field survey that exhibited at least one false positive (Class G dataset) Waterbodies are clustered into three categories in an effort to understand why false positives were produced: 1) Four waterbodies for which the likely explanation is that groundwater was upwelling through the sediment, so that the sites were not accurately modeled by the proposed equation; 2) Four waterbodies for which the likely explanation is random error because sulfate level is only slightly greater than the calculated protective concentration; and 3) Six waterbodies, each of which were sampled at least three times, that exhibited inconsistent behavior, which might be resolved with more extensive sampling. (CPSC120 = Calculated Protective Sulfate Concentration associated with a protective sulfide concentration of 120 µg/L).

Porewater sulfide relative to 120 µg/L	Accuracy Class-ification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)
Waterbodies for which the likely explanation for false positive(s) is upwelling groundwater. Even when sulfate is high relative to CPSC, porewater sulfide is low.												
↓	TN	0.78	0.8	N/A	Second Creek	FS-303	5/30/2013	303	99	13,086	2.20	388.6
↓	TN	0.33	0.8	57.6	S007-220	FS-310	6/14/2013	316	93	31,190	4.22	946.8
↓	FP	2.43	0.6	76.4		FS-323	7/11/2013	405	67	10,036	2.91	166.9
↓	FP	5.66	0.4	66.8		FS-351	8/15/2013	838	45	7,088	1.84	148.0
	N/A	N/A	0.9	27.7		FS-384	9/19/2013	N/A	104	22,634	3.42	657.3
↓	FP	2.64	0.6	0.6	Ox Hide Creek	FS-198	9/7/2012	26.4	75	8,743	24.51	10.0
↓	TN	0.50	0.4	10.5	31-0106-00-203	FS-132	9/7/2012	26.4	42	14,936	14.43	52.7
↓	FP	5.30	1.0	0.0		FS-350	8/14/2013	25.9	119	3,889	12.12	4.9
↓	FP	2.83	0.7	121.0	Turtle River, ND S007-662	FS-358	8/19/2013	198	83	4,262	1.52	70.0
↓	FP	1.78	0.4	56.3	Big Swan Lake	FS-205	8/10/2012	5.47	53	1,719	4.81	3.1
↓	FP	2.27	0.8	133.7	77-0023-00-207	FS-204	8/10/2012	5.49	91	1,731	5.94	2.4

(continued)

Porewater sulfide relative to 120 µg/L	Accuracy Class-ification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)
	↑ FN	0.45	1.2	37.3	Second Lake	P-24	9/7/2011	0.87	139	3,813	25.67	1.92
↓	FP	1.16	1.0	48.4	15-0091-00	FS-105	6/27/2012	0.74	119	2,527	33.3	0.64
↓	TN	0.64	0.8	0.0	Snowball Lake	FS-197	9/4/2012	8.4	94	4,213	6.00	13.2
↓	FP	1.11	0.8	0.0	31-0108-00-202	FS-347	8/12/2013	8.2	97	1,136	1.19	7.4
↓	FP	1.07	1.0	0.0	Trout Lake	FS-219	9/13/2012	38.6	117	12,535	15.00	35.9
↓	TN	0.96	0.9	0.0	31-0216-00-212	FS-356	8/14/2013	39.1	103	11,992	12.59	40.7
	↑ FN	0.19	1.5	41.4	Mississippi R.	FS-208	8/14/2012	18.0	176	2,178	0.41	92.3
↓	FP	1.01	0.9	12.7	Pool 8 at Genoa	FS-311	6/20/2013	29.3	107	1,544	0.62	29.0
↓	TN	0.56	0.9	52.8	S007-222	FS-334	7/29/2013	44.2	102	1,969	0.40	78.3
↓	TN	0.19	0.5	17.8		FS-370	9/9/2013	33.3	62	6,558	1.43	172.4

(continued)

Porewater sulfide relative to 120 µg/L	Accuracy Classification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)
The behavior of the six waterbodies below is inconsistent												
	↑ FN	0.03	1.03	3.8	Sandy Lake	FS-251	9/21/2012	3.1	123	35,905	33.08	105.5
↓	TN	0.09	0.8	0.0	69-0730-00	FS-306	6/11/2013	11.0	91.8	35,357	28.53	122.3
	↑ FN	0.98	1.6	0.0		FS-321	7/9/2013	122	189	36,502	29.51	124.9
	↑ TP	1.11	1.1	0.0		FS-382	9/17/2013	67.9	135	26,645	32.28	61.2
	↑ TP	2.68	9.0	0.0		FS-305	6/11/2013	135	1080	19,094	22.23	50.4
	↑ TP	1.51	2.5	0.0		FS-348	8/13/2013	123	305	13,216	8.23	81.6
↓	FP	2.91	0.3	0.6		FS-380	9/17/2013	126	34	17,868	22.7	43.3
↓	FP	3.53	0.6	0.0		FS-349	8/13/2013	122	70	14,897	20.46	34.6
↓	FP	5.81	0.5	74.4	Unnamed Lake	P-57	9/23/2011	6.42	65	1,946	13.80	1.1
	↑ TP	1.69	2.4	74.4	34-0611-00-201	P-57	9/23/2011	6.42	286	2,311	6.48	3.8
	↑ TP	4.25	1.3	64.9		FS-183	7/30/2012	16.8	150	2,157	5.61	4.0
	↑ TP	4.67	11.4	121.3	Monongalia Lake	FS-77	7/26/2012	21.7	1,370	4,953	18.66	4.6
↓	FP	5.38	0.8	50.0		FS-313	6/23/2013	34.7	94	6,028	19.44	6.4
	↑ TP	7.17	1.0	87.9	34-0158-01	FS-340	7/31/2013	33.6	122	5,530	22.10	4.7
	↑ TP	9.45	2.0	154.4		FS-379	9/13/2013	34.6	242	5,436	26.42	3.7
↓	TN	0.86	0.7	31.6	Stella Lake	P-30	9/14/2011	7.59	80	2,159	2.88	8.8
	↑ TP	4.54	14.9	0.3	47-0068-00	FS-188	8/27/2012	18.1	1,790	1,257	2.34	4.0
↓	FP	1.63	0.7	57.6		FS-341	8/1/2013	24.7	88	1,786	1.35	15.1

(continued)

Porewater sulfide relative to 120 µg/L	Accuracy Classification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)
↑	TP	6.86	1.1	3.2	Dark Lake	FS-322	7/10/2013	175	131	2,480	1.48	25.5
↑	TP	4.89	1.1	2.9	69-0790-00-202	FS-352	8/15/2013	173	136	5,120	3.61	35.3
↑	TP	5.31	2.5	11.1		FS-368	9/5/2013	175	305	3,354	1.94	33.0
↓	FP	4.97	0.4	11.8		FS-369	9/5/2013	176	52	2,037	0.82	35.4
↓	TN	0.15	0.4	25.9	Little Birch Lake	P-47	9/21/2011	3.2	50	4,503	4.46	21.4
↑	FN	0.19	1.6	25.9	77-0089-00	P-47	9/21/2011	3.2	191	2,236	1.75	17.1
↓	FP	2.90	0.3	70.0		FS-54	8/3/2012	7.4	35	1,794	6.02	2.6

Evidence that site-specific standards may be needed for false positive wild rice waters

Some waterbodies will have wild rice when sulfide is greater than 120 µg/L

In the MPCA-sponsored field study, 29 waterbodies out of 115 surveyed (Class G dataset, N=222) had at least one true positive (at least one site visit found sulfide to be above 120 µg/L, consistent with the prediction of the proposed equation). Of the 29, 14 waterbodies had at least some wild rice present (48%), compared to 76% (62 out of 82) of the true negative samples (true negatives are samples conforming with predicted sulfide below 120 µg/L).

In some waterbodies where sulfide exceeds 120 µg/L the wild rice is dense and appears to be thriving. However, when sulfide exceeds 120 µg/L fewer samples have denser rice (for instance, greater than 10 stems/m²). In the Class G dataset (N=222), only 34% of all samples from natural waterbodies have dense wild rice when sulfide exceeds 120 µg/L, whereas 73% of samples have dense wild rice when sulfide is less than 120 µg/L (calculated from Fig. 2-1). In dataset Class B (N=108), which approximates a probabilistic data set of natural waterbodies (Myrbo et al., in press-1), the proportion of samples with denser wild rice is significantly greater when sulfide is less than 120 µg/L (Chi square test; $p < 0.02$). Only 23% of Class B sites have dense wild rice when sulfide exceeds 120 µg/L, whereas 62% of sites have dense wild rice when sulfide is less than 120 µg/L (Fig. 2-3).

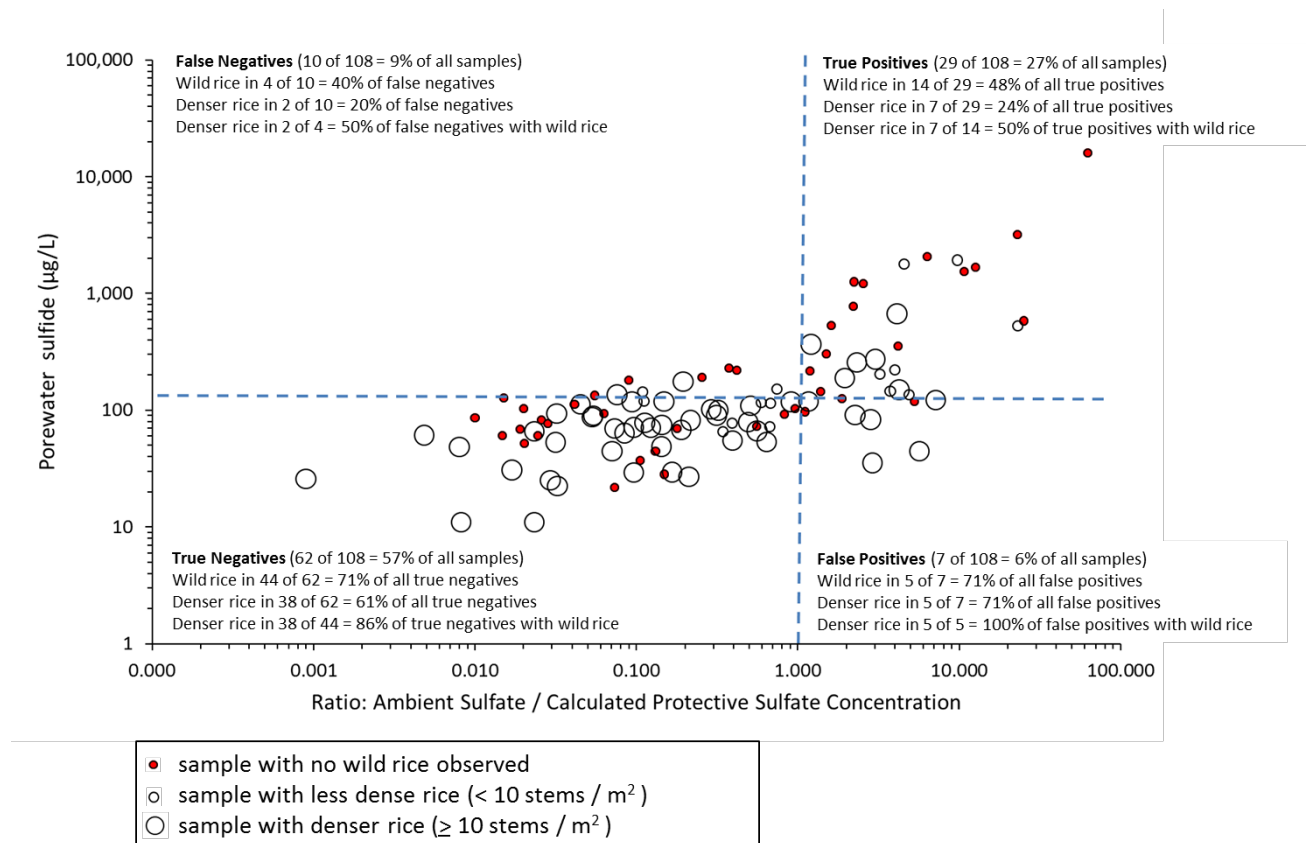


Figure 2-3. Prediction accuracy of the proposed equation for dataset Class B (a subset of 108 different waterbodies in Class G), plus density of wild rice occurrence, at two levels of density.

Possible explanations for wild rice in waterbodies with elevated porewater sulfide

There are several different possible explanations for the observation of wild rice in waterbodies with porewater sulfide in excess of 120 µg/L.

First, 120 µg/L was chosen to be a protective concentration along a gradient of declining probability of wild rice density and occurrence. There is no bright line below which sulfide is not toxic to wild rice and above which sulfide is toxic. As a result, wild rice is sometimes found in waters above the protective concentration, and sometimes in high density (above 40 stems per square meter); however, this occurs much less frequently than at levels of sulfide below the protective concentration. Because no water quality standard is perfect, it is inevitable that there will be some waterbodies where a standard is exceeded even while the beneficial use that the standard is designed to protect is maintained.

Second, it may be that the MPCA survey took too few samples to accurately characterize the specific waterbody. Six of the 14 true positive waterbodies with wild rice are represented by a single sampling (Table 2-2). Additional sampling might reveal that sulfide actually averaged below 120 µg/L. For instance, Stella Lake was sampled three times, and only in one of the three samplings did sulfide exceed 120 µg/L (Table 2-2).

A third possible explanation is that the sampling caught the wild rice population in the process of being extirpated from a waterbody that had sulfide in excess of what could be tolerated by wild rice. Four waterbodies had low densities of wild rice at the time of sampling (Christina, Big Sucker, Dark, and

Sandy, all below 4 stems/m²; Table 2-2). Pastor et al. (2017) concluded that elevated porewater sulfide concentrations cause wild rice populations to decline by adversely affecting the seed production. Perhaps these sites had reproducing wild rice populations in the past that built up a store of seeds in the sediment that can remain viable for multiple years—a so-called seed bank. Not all viable wild rice seeds germinate in a given year. If sulfate has increased in these sites, increasing porewater sulfide, a possible explanation is that the few plants observed are the remnants of the seed bank produced in lower-sulfide years, and that the waterbody's wild rice population is likely to disappear in coming years.

A fourth possible explanation is that other variables that can control wild rice growth and reproduction, such as water depth, transparency, and nutrients such as nitrogen, are sufficiently optimal so as to offset the negative effects of elevated sulfide. For instance, seven cultivated wild rice paddies were sampled during the MPCA-wild rice study, and sulfide exceeded 120 µg/L at five of the seven sites. Wild rice density is very high in these cultivated operations, and growers have learned to optimize variables such as water depth, transparency, and nitrogen so that wild rice grows and produces seed successfully. Growers keep water depth relatively shallow during early seedling growth, which allows wild rice seedlings to quickly reach the water surface, simultaneously affording the plant more light for photosynthesis and access to the atmosphere. High rates of photosynthesis and emergence from the water into the atmosphere both allow more oxygen to be sent to the developing roots, potentially detoxifying sulfide (as discussed in *How access to oxygen may allow wild rice to detoxify sulfide* in Chapter 1, Part A). Wild rice growers also work to maintain high water transparency for seedlings, sometimes treating the water with copper sulfate to reduce the density of suspended algae. The productivity of wild rice is primarily limited by nitrogen; increased nitrogen availability increases the mean seed weight and number of seeds produced per stem (Pastor et al., 2017). Wild rice growers fertilize the paddies with nitrogen (as urea), which also allows wild rice to grow quickly, accelerating stem length and leaf development, and thereby increasing access to oxygen. There is evidence that wetland plants fertilized with nitrogen can better oxidize sulfide around the roots, reducing the potential toxicity (Howes et al., 1986).

Monongalia Lake is an outlier: dense wild rice despite elevated porewater sulfide

Among the 14 true positive waterbodies with at least some wild rice, Monongalia Lake stands out as having multiple samples that document dense wild rice that occurs simultaneously with elevated porewater sulfide (Table 2-2). Three of four samples were true positives, with porewater sulfide of 122, 242, and an unusually high 1,370 µg/L. Wild rice density was very high in all three samples (88, 154, and 121 stems/m², respectively, compared to a Class B median of 41 stems/m²). Observed surface water sulfate concentrations of 22 to 35 mg/L were much higher than the calculated protective sulfate concentrations of 3.7 to 6.4 mg/L. This large (2,255 acres) but shallow (maximum depth 14 feet) lake lies in the Middle Fork Crow River watershed (Fig. 2-4), which is 46% agricultural, 26% wetland, and 10% developed/urban land use upstream of Monongalia Lake (calculated from MFCRWD, 2007). The Middle Fork Crow River flows through Monongalia Lake. Aside from the occurrence of dense wild rice in the presence of elevated porewater sulfide, the only field study parameters that are slightly atypical, compared to the Class B data set, are that in three of four sediment samples TOC, total sulfur, and total nitrogen are all in the upper quartile of Class B sites. Wild rice may be able to grow and reproduce in Monongalia Lake because environmental variables other than sulfide are sufficiently optimal so as to offset the negative effects of elevated sulfide (the fourth explanation, above), although extensive additional study would be required to evaluate that hypothesis. Monongalia Lake stands out as the least-well understood waterbody in the MPCA-sponsored field study in regards to factors that control wild rice growth and reproduction. The unique nature of this lake points towards the need for site-specific research and standard development.

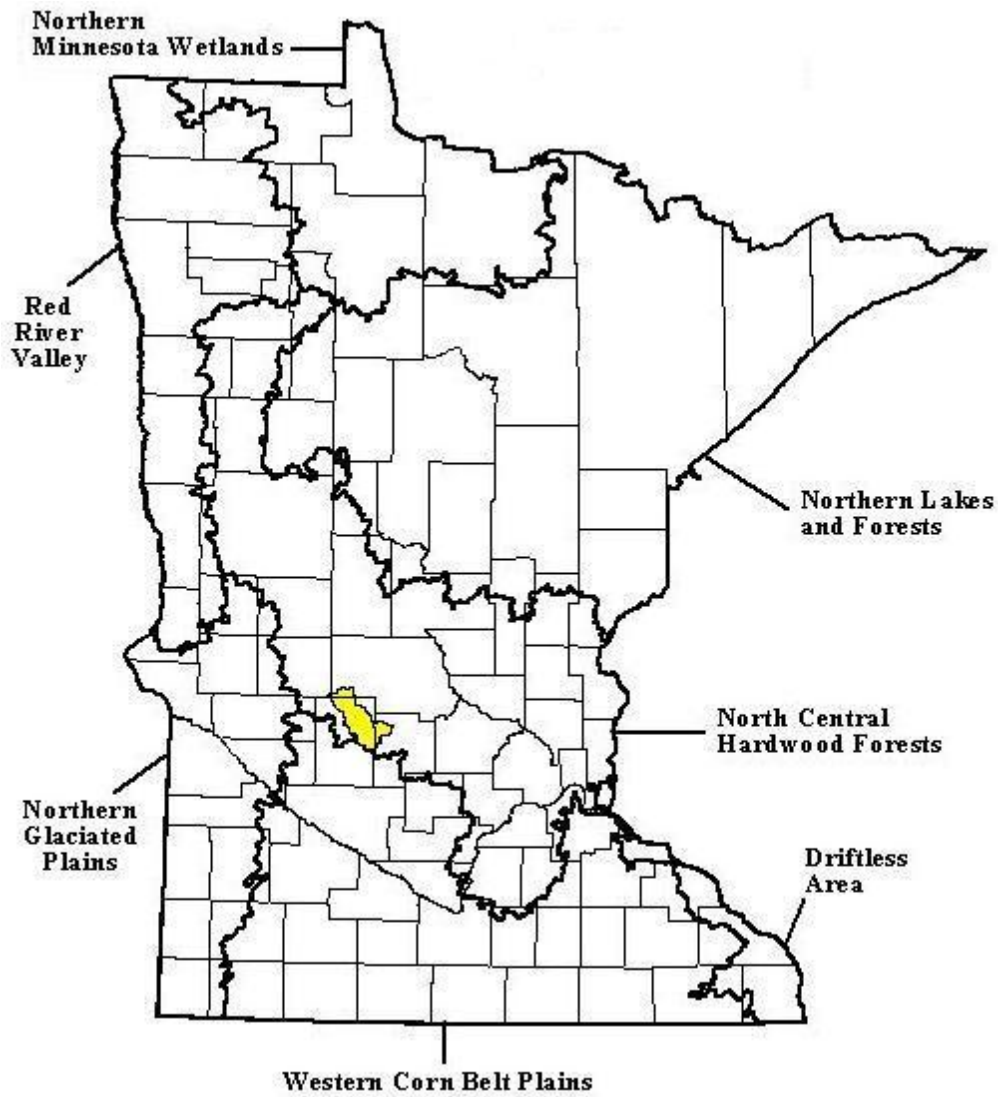


Figure 2-4. Location of the watershed that includes Monongalia Lake (yellow shaded area) (from MFCRWD, 2007).

Table 2-2. All waterbodies in the field survey that exhibited at least one true positive (dataset Class G)

Porewater sulfide relative to 120 µg/L	Accuracy Classification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)	
14 waterbodies with at least one true positive sampling that had wild rice present in at least one survey.													
↑	TP	1.2	3.1	114.9	Pine Lake 15-0149-00-205	FS-190	8/28/2012	14.7	368	4,477	7.08	12.2	
↑	TP	1.8	4.1	3.0	Anka lake	P-35	9/16/2011	2.23	493	2,170	14.84	1.2	
↑	TP	6.4	5.6	25.9	21-0353-00	P-34	9/16/2011	2.23	671	1,485	23.57	0.3	
↑	TP	23.1	4.4	2.3		FS-192	8/29/2012	8.44	530	1,498	22.85	0.4	
↑	TP	3.0	2.3	30.2	Ina Lake 21-0355-00-202	FS-191	8/29/2012	7.08	274	2,216	9.09	2.3	
↑	TP	9.8	16.1	0.6	Christina Lake 21-0375-00-315	FS-339	7/31/2013	14.6	1,930	1,741	8.96	1.5	
↑	TP	2.5	2.8	12.4	Swan Lake (W Bay)	FS-61	8/30/2012	12.5	332	5,827	22.71	5.0	
↑	TP	4.0	1.8	3.8	31-0067-01	FS-62	8/30/2012	14	221	4,821	22.53	3.5	
↑	TP	3.7	1.2	3.8	Big Sucker L. 31-0124-00-203	FS-216	9/12/2012	7.78	145	3,559	21.45	2.1	
↓	↑	TP	4.7	11.4	121.3	Monongalia Lake	FS-77	7/26/2012	21.7	1,370	4,953	18.66	4.6
		FP	5.4	0.8	50.0		FS-313	6/23/2013	34.7	94	6,028	19.44	6.4
	↑	TP	7.2	1.0	87.9	34-0158-02	FS-340	7/31/2013	33.6	122	5,530	22.10	4.7
	↑	TP	9.4	2.0	154.4		FS-379	9/13/2013	34.6	242	5,436	26.42	3.7
↓		FP	5.8	0.5	74.4	Unnamed Lake	P-57	9/23/2011	6.42	65	1,946	13.80	1.1
	↑	TP	1.7	2.4	74.4	34-0611-00-201	P-57	9/23/2011	6.42	286	2,311	6.48	3.8
	↑	TP	4.2	1.3	64.9		FS-183	7/30/2012	16.8	150	2,157	5.61	4.0

(continued)

Porewater sulfide relative to 120 µg/L	Accuracy Classification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)	
↓	↑	TN	0.9	0.7	31.6	Stella Lake	P-30	9/14/2011	7.59	80	2,159	2.88	8.8
		TP	4.5	14.9	0.3	47-0068-00	FS-188	8/27/2012	18.1	1,790	1,257	2.34	4.0
↓		FP	1.6	0.7	57.6		FS-341	8/1/2013	24.7	88	1,786	1.35	15.1
	↑	TP	2.0	1.6	144.8	West Battle L.	FS-228	8/15/2012	4.03	189	3,108	17.37	2.1
					56-0239-00-204								
	↑	TP	4.1	5.6	39.8	Bee Lake	FS-87	8/23/2012	11	670	3,054	13.62	2.7
					60-0192-00-202								
	↑	TP	6.9	1.1	3.2	Dark Lake	FS-322	7/10/2013	175	131	2,480	1.48	25.5
	↑	TP	4.9	1.1	2.9	69-0790-00-202	FS-352	8/15/2013	173	136	5,120	3.61	35.3
	↑	TP	5.3	2.5	11.1		FS-368	9/5/2013	175	305	3,354	1.94	33.0
↓		FP	5.0	0.4	11.8		FS-369	9/5/2013	176	52	2,037	0.82	35.4
	↑	FN	0.0	1.0	3.8	Sandy Lake	FS-251	9/21/2012	3.05	123	35,905	33.08	105.5
↓		TN	0.1	0.8	0.0	69-0730-00	FS-306	6/11/2013	11	92	35,357	28.53	122.3
	↑	TP	2.7	9.0	0.0		FS-305	6/11/2013	135	1,080	19,094	22.23	50.4
	↑	FN	1.0	1.6	0.0		FS-321	7/9/2013	122	189	36,502	29.51	124.9
↓		FP	3.5	0.6	0.0		FS-349	8/13/2013	122	70	14,897	20.46	34.6
	↑	TP	1.5	2.5	0.0		FS-348	8/13/2013	123	305	13,216	8.23	81.6
	↑	TP	1.1	1.1	0.0		FS-382	9/17/2013	67.9	135	26,645	32.28	61.2
↓		FP	1.6	0.3	0.0		FS-381	9/17/2013	126	34	16,172	11.67	79.2
	↑	TP	2.3	2.1	69.7	Bowstring River	FS-214	9/11/2012	1.34	256	1,974	24.34	0.6
					S007-219								

(continued)

Porewater sulfide relative to 120 µg/L	Accuracy Classification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)	
15 waterbodies with at least one true positive sampling for which wild rice was not observed in any site visit.													
↑	TP	1.4	1.2	0.0	Rice Lake 02-0008-00-206	FS-231	8/17/2012	3.6	145	2,159	7.98	2.6	
↑	TP	62.8	133.3	0.0	Bean Lake 03-0411-00-201	FS-85	8/21/2012	85	16,000	1,967	11.85	1.4	
↑	TP	2.5	10.2	0.0	Cromwell Lake 14-0103-00-201	FS-128	8/22/2012	41.2	1,220	2,948	2.85	16.2	
↑	TP	10.7	12.8	0.0	North Geneva L. 24-0015-00-209	FS-176	7/24/2012	15.6	1,540	2,212	13.45	1.5	
↑	TP	22.9	26.6	0.0	South Geneva L. 24-0015-02-208	FS-177	7/24/2012	14.1	3,190	1,618	16.71	0.6	
↓	↑	TP	1.9	1.1	0.0	Upper Panasa L.	FS-59	8/29/2012	29.6	126	895	0.43	15.8
		TN	0.1	0.3	0.0	31-0111-00-202	FS-383	9/18/2013	33.6	40	19,148	2.86	590.3
↑	TP	2.0	2.0	0.0	Lower Panasa L.	FS-60	8/29/2012	33.6	243	8,048	14.12	16.5	
↑	TP	2.2	10.5	0.0	31-0112-00	FS-357	8/15/2013	28.5	1,260	2,347	2.42	12.7	
↑	TP	1.6	4.5	0.0	Little Sucker L. 31-0126-00-202	FS-223	9/14/2012	13.7	534	6,297	16.56	8.5	
↑	TP	23.4	8.4	0.0	Holman L.	FS-218	9/13/2012	24.2	1,010	3,035	29.74	1.0	
↑	TP	25.1	4.9	0.0	31-0227-00-202	FS-353	8/12/2013	68	583	5,094	30.60	2.7	
↑	TP	5.0	123.7	0.0	Lady Slipper L.	P-55	9/22/2011	107.71	14,840	2,814	2.09	21.5	
↑	TP	9.7	13.6	0.0	42-0020-00	FS-79	7/27/2012	330	1,630	3,314	1.85	34.1	
↑	TP	12.6	14.0	0.0		FS-78	7/27/2012	335	1,680	2,719	1.66	26.5	

(continued)

Porewater sulfide relative to 120 µg/L	Accuracy Classification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)
↑	TP	1.7	14.9	0.0	Westport 61-0029-00-204	FS-186	8/1/2012	7.11	1,790	4,917	20.15	4.2
↑	TP	2.2	6.5	0.0	Rice Lake 66-0048-00-203	FS-181	7/27/2012	5.22	777	3,829	21.67	2.4
↑	TP	4.1	24.8	0.0	Rice Lake	FS-184	7/30/2012	2.58	2,970	1,523	15.03	0.6
↑	TP	6.3	17.3	0.0	73-0196-00-216	FS-345	8/7/2013	6.85	2,080	2,012	14.83	1.1
↑	TP	1.2	1.8	0.0	Rice Lake 74-0001-00-201	FS-179	7/25/2012	3.84	217	4,152	19.07	3.2
↑	TP	4.2	3.0	0.0	Gilchrist L. 86-0064-00-201	FS-194	8/31/2012	6.98	355	3,117	20.81	1.7
↑	TP	3.2	1.7	6.7	Westport L. 61-0029-00-205	FS-346	8/8/2013	6.3	205	3,262	19.66	2.0

Chapter 3. Implementation of the wild rice sulfate standard

Sediment Sampling and Analysis

Sediment sampling is conducted to provide the data needed to calculate the numeric sulfate standard for a wild rice water. Sediment total extractable iron (TEFe) and sediment total organic carbon (TOC) concentrations are the two measured variables used in the proposed equation to calculate the numeric sulfate standard. When MPCA developed the draft proposed approach, released in March 2015 (MPCA, 2015), both MPCA and commenters noted that there was spatial variability in the TOC and TEF_e measured in the sediment of a wild rice bed. These two parameters are measured in homogenized 10-cm long sediment cores, which represent many years of sediment accumulation. It is not expected that TOC and TEF_e change significantly over the near term unless unusual hydrologic events occur. No statistically significant seasonal trend was observed in the field data (Myrbo et al., in press-1). To produce data that are pertinent to the protection of wild rice, sediment sampling must be conducted in the places where wild rice grows. Growth patterns of wild rice vary annually as exemplified in Fig. 3-1. It is important to establish how and where sediment samples will be collected in efforts to be representative of the wild rice water.

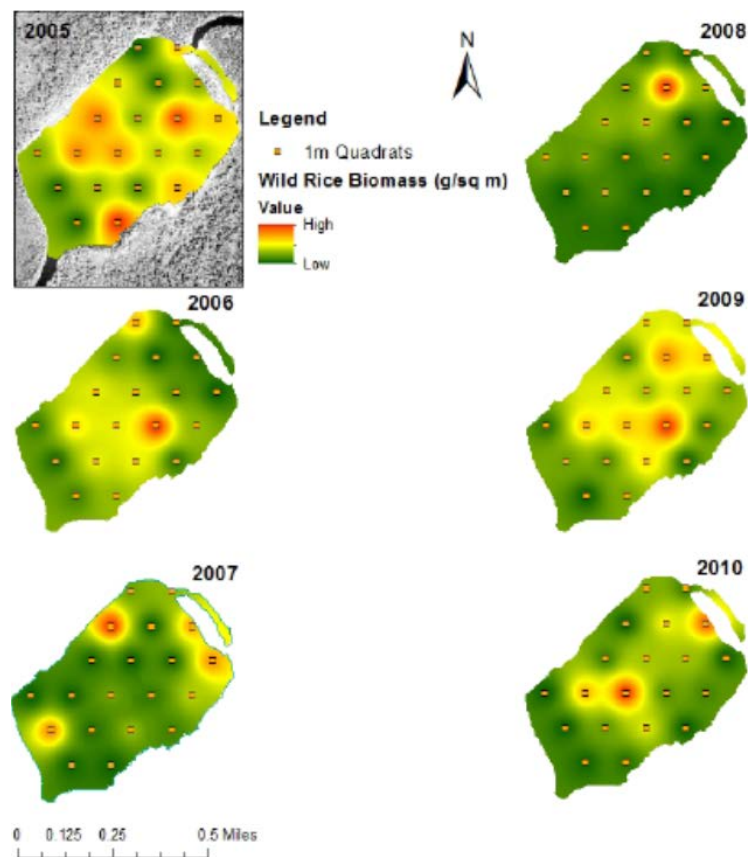


Figure 3-1. Example of variable growth patterns of wild rice biomass across years. Reprinted with permission: Wild Rice Monitoring Handbook by Tonya Kjerland (2015), published by the University of Minnesota Sea Grant College Program; from MN Sea Grant.

To effectively implement an equation-based standard, MPCA must specify how many samples are needed to characterize the sediment of a wild rice water, and how to consider those samples in the calculation of the numeric standard. To inform this approach, in June 2015 a pilot study was conducted to examine spatial variability in sediment TOC and TEF_e found in six wild rice waters. In addition, collecting and analyzing a large sample size from different waterbodies is useful for informing decisions about an appropriate sample size for implementation of the water quality standard. The study sampled six different wild rice waters (four rivers and two lakes) identified on the MPCA draft list of wild rice waters, at areas that were sampled during 2011-2013 as part of the MPCA-sponsored field sampling. Within each wild rice water, 25 individual sediment samples (sediment cores) were collected and analyzed for TOC and TEF_e.

The 25 sediment sampling locations at each waterbody were selected using the following guidelines:

- The coordinates for each wild rice bed were those used for the wild rice field study. The coordinates all corresponded to an access point adjacent to the waterbody. From that point, a representative bed of wild rice was located and a location was identified to begin sampling. This location was at least fifty meters away from the access point. On streams, the starting point was always upstream of the road crossing, if present. From that point, a transect perpendicular to the shoreline (and to water flow) was followed for collecting samples.
- The first sample was retrieved along the transect line where rice was found growing nearest the shoreline.
- The distance between each sample point was approximately 2 meters.
- Sampling stopped at the point along the transect where wild rice growth was not observed or water depth was too great (approximately 4 feet of water; Kjerland, 2015). In cases where wild rice growth was observed all the way to the opposite shore (e.g., a shallow lake or stream), professional judgment was used to determine a reasonable transect length.
- Additional transects were laid out in the same manner as described, parallel to the first transect, as needed to complete collection of the 25 samples.

All sediment samples were collected in the following manner:

- Sediment was collected using 70 mm diameter polycarbonate core tubes.
- The top ten centimeters of the substrate sampled in each core was collected and placed into a plastic bag. Samples were kept on ice in the field.
- In the lab, each bagged sample was gently mixed by hand. A subsample from each bagged sample was placed into a jar for analysis of TOC and TEF_e. These samples were refrigerated until analysis.

Methods for TOC analysis followed EPA method 9060 (EPA, 2004) and analysis of TEF_e followed Balogh et al. (2009) as modified by the Minnesota Department of Health (MDH, 2016).

Table 3-1. Summary results from 2015 pilot study, consisting of 25 sediment cores collected and analyzed from each of six wild rice waters. Total organic carbon (TOC) and total extractable iron (TEFe) were quantified in each core. All data is reported in Appendix 3.

Waterbody (abbreviation)	Mean TOC (%)	SD	CV(%)	Minimum	Maximum
Bowstring River (BRT)	16.0	1.9	12	12.6	19.7
Clearwater River (CLRT)	20.3	2.5	12	16.2	25.4
Hesitation Wildlife Management Area (HWMAT)	21.8	2.8	13	16.1	25.9
Mission Creek (MCT)	3.8	0.9	24	1.5	5.3
Monongalia Lake (MLT)	16.9	7.1	42	2.4	27.2
Mississippi River (MRT)	15.2	4.0	26	6.1	22.0

Waterbody (abbreviation)	Mean TEFe (mg/kg)	SD	CV(%)	Minimum	Maximum
Bowstring River	3,827	640	17	2,169	4,680
Clearwater River	13,439	2,652	20	8,370	19,800
Hesitation Wildlife Management Area	38,088	13,850	36	24,300	74,700
Mission Creek	15,707	3,882	25	7,470	22,500
Monongalia Lake	6,041	1,792	30	2,610	9,000
Mississippi River	5,432	436	8	4,680	6,300

As expected, the measured sediment TOC and TEF_e concentrations were variable (Table 3-1). Paired values of TOC and TEF_e reported from each sediment core analysis were used to calculate a sulfate concentration using the equation being proposed for the water quality standard for sulfate. Analysis of Variance performed on this data set showed significant differences ($p < 0.05$) between wild rice waters, which infers that the variability of sulfate values calculated within a waterbody varied less than the variability between wild rice waters. Given this information, it is important to know how sample size affects variability.

One way to examine this is to compare the variability of the data around the mean to increasing sample size. Graphing this for both TOC and TEF_e shows that as sample size increases, the variability decreases (Figs. 3-2 and 3-3). Variability is displayed as the width of the confidence interval along the y-axis. The rate of narrowing of the confidence interval levels off at a sample size of about 20 to 25. This suggests that a large amount of the sample variability has been accounted for with a sample size of 25 and that further samples would not greatly improve the estimate of either sediment TEF_e or TOC.

Based on this information, the MPCA examined whether this same conclusion could be drawn using composite samples. A composite sample is collected by using the same sampling equipment as described above (core samples), but instead of collecting and analyzing individual samples, a set of samples collected from an area are pooled into a single sample for analysis. MPCA proposes that instead of collecting and analyzing 25 separate core samples from each wild rice water, composites of samples be collected and analyzed from five separate areas within the wild rice water. These five composite samples, each comprised of five individual core samples, would then be analyzed for TOC and TEF_e.

An important question to answer is whether the variability of sulfate values calculated from composite samples is similar to the variability seen in values calculated from single core samples. To investigate this, calculated sulfate values from theoretical composite samples were compared to calculated concentrations from individual cores using data from the 2015 pilot study. Cores from the 2015 pilot study were placed into groups of five and average TOC and TEF_e values were calculated to simulate a composite sample. The groups were determined by the order that the samples were retrieved from the sample area (i.e. first five samples in first composite group, second five samples in second composite group, etc.) to simulate composite sampling in the field. Using the proposed equation, the protective sulfate value was calculated for each TOC and TEF_e pair from individual and composite samples (Appendix 3). Percentile ranks were determined using the full set of individual calculated sulfate values; 10th to 90th percentiles are shown in Table 3-2. The percentiles of each composite calculated sulfate value range from 11% to 93% among the wild rice waters (Appendix 3), showing that the composite samples capture the majority of the variability of the single samples.

To effectively implement an equation-based approach to determining the sulfate standard, a single numeric sulfate standard needs to be determined for each wild rice water. The purpose of sampling sediments in the wild rice bed is to capture the variability of the sediment concentrations of TEF_e and TOC to ensure that the single sulfate standard selected from the group of five representative sulfate values calculated is protective of the wild rice beneficial use throughout the wild rice water. The MPCA compared the lowest composite value from each site to the percentile ranks (Table 3-2; Appendix 3), and observed that they all fall within the 10th and 30th percentiles for the six sites. Selecting the lowest value as the sulfate standard for the wild rice water addresses the need to protect for sensitive conditions where sulfide may accumulate, protecting the wild rice.

There are two reasons that it is not reasonable to use the average calculated sulfate concentration rather than the lowest calculated sulfate concentration. First, the goal of developing a sulfate standard is to allow wild rice to grow throughout the suitable locations in a wild rice water, not just in a subset. Use of an average would protect only a portion of the wild rice, given that use of an average implies that about half of the wild rice would need a lower numeric sulfate standard to avoid high porewater sulfide concentrations. Second, while “average” might sound like it would protect half of the wild rice, in fact, protection might be far less than half. The reason that “average” does not necessarily protect half of the wild rice is that calculation of averages is vulnerable to extreme values. For example, if one of the five calculated potential sulfate standards were extremely high, the average could actually be higher than four of the five values. In such a case, the use of an average as the numeric sulfate standard could conceivably protect only a very small proportion of the wild rice in a wild rice water. For the above reasons, use of the lowest calculated sulfate concentration is much more defensible and reasonable than use of a calculated average concentration.

Table 3-2. Lowest calculated sulfate value of composite samples compared to sulfate values at various percentiles calculated from the 25 individual samples analyzed from each waterbody of the pilot study.

Waterbody	Lowest calculated sulfate value from composites (mg/L)	Calculated sulfate values at various percentiles calculated from 25 individual samples (mg/L)				
		10th	30th	50th	70th	90th
Bowstring River	2.1	2.0	3.3	3.6	3.9	5.3
Clearwater River	22.3	19.7	23.5	24.4	32.3	50.1
Hesitation WMA	104.3	85.7	112.7	142.2	217.2	469.4
Mission Creek	240.1	203.1	247.6	294	312.8	397.1
Monongalia Lake	6.6	5.1	6.8	8.6	10.7	13.8
Mississippi River	5.6	4.6	6.0	6.9	9.3	12.8

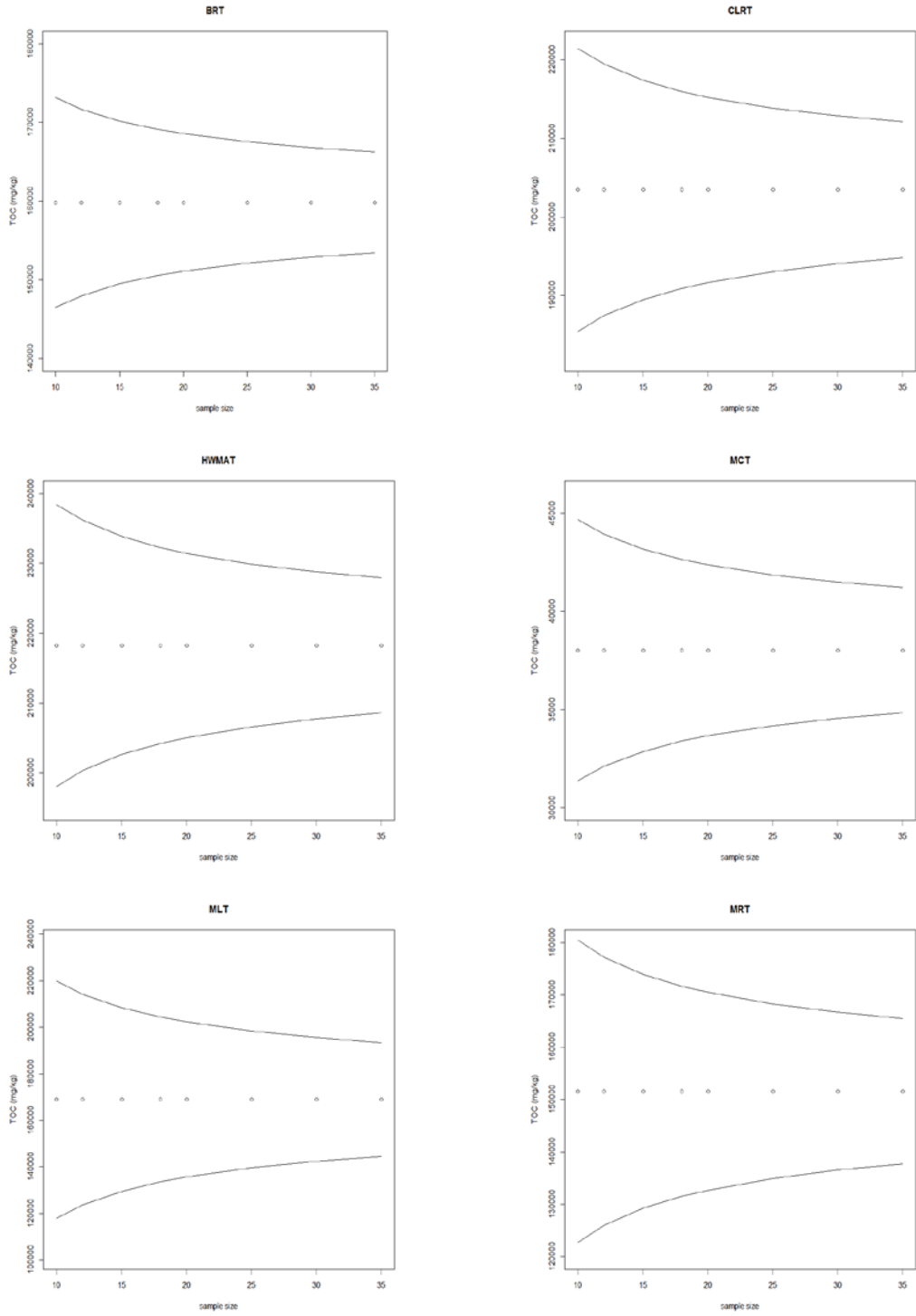


Figure 3-2. Graph of confidence interval widths compared to sample size for sediment TOC for each of the waterbodies sampled. See Table 3-1 for an explanation of the waterbody acronyms.

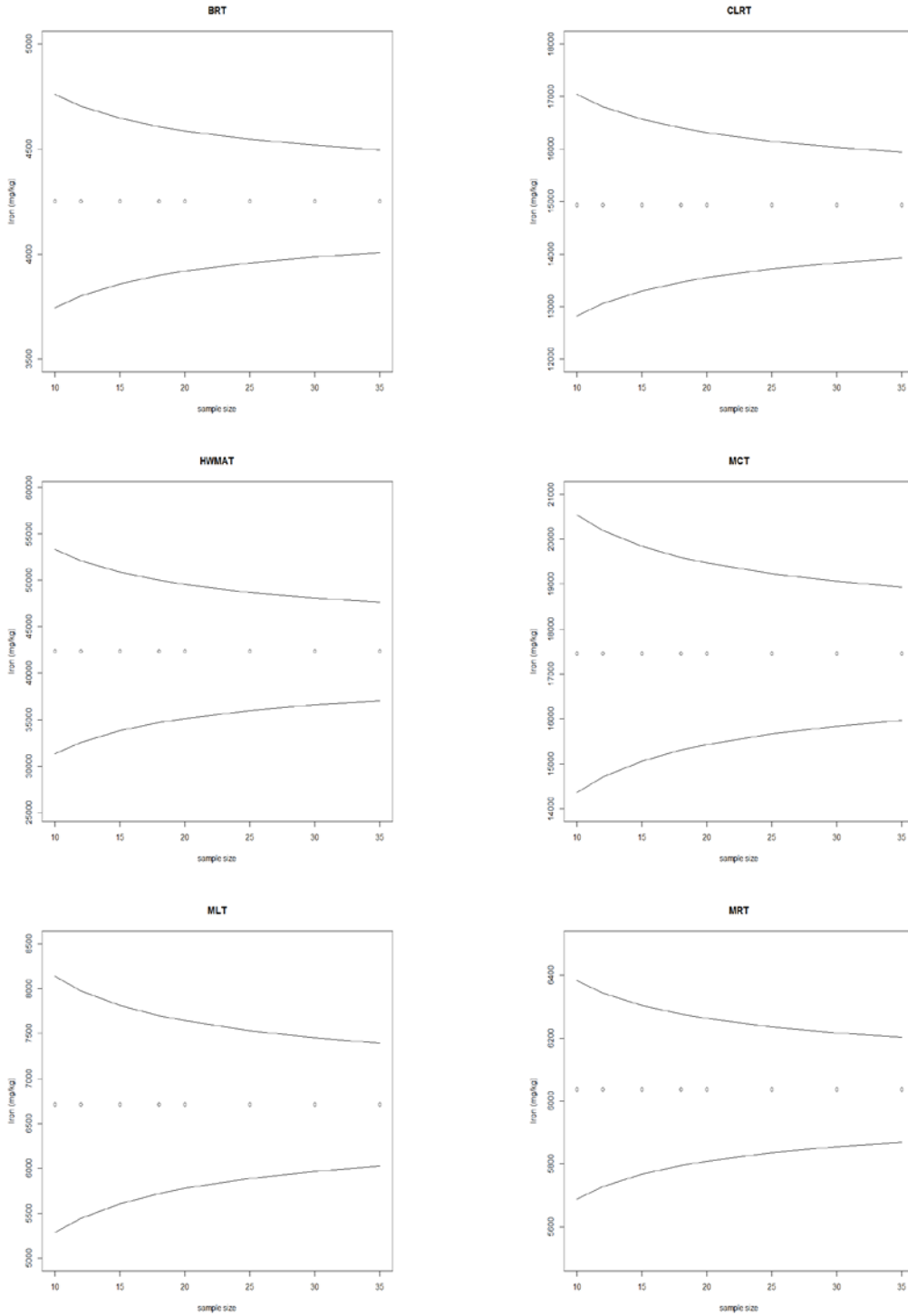


Figure 3-3. Graph of confidence interval widths compared to sample size for sediment iron for each of the waterbodies sampled. See Table 3-1 for an explanation of the waterbody acronyms.

Conforming with the Sulfate Standard

An important part of implementing any water quality standard is determining whether any given waterbody meets the standard. The magnitude, duration, and frequency of a standard not only are the bases for determining how waterbodies are assessed against the standard, but also inform permit requirements.

The magnitude is the level of the standard – in this case the amount of sulfate allowable in the wild rice water to maintain the protective sulfide level. The magnitude will usually be the numeric standard calculated with the equation, using sediment samples collected as described above. It may also be a sulfate concentration derived using the procedures for an alternate standard, or a site-specific standard.

The averaging time of the standard is the duration, and the frequency is how often the magnitude may be exceeded before the standard is considered to be violated. The analysis in Chapter 1 speaks to the magnitude of the standard, while this section discusses the technical information supporting the MPCA's proposed duration and frequency of the standard.

Appropriate duration and frequency are most easily determined for chemicals that are directly toxic to aquatic organisms. Determining duration and frequency for a chemical that has indirect negative effects, such as sulfate, is more challenging.

For this standard, duration is defined as the averaging period for sulfate that was found to be related to observed porewater sulfide concentrations, and frequency is defined as the interval between poor wild rice growth years from which wild rice has the undoubted ability to recover.

Duration (Averaging Time)

Defining duration for a pollutant should reflect the available information about the timeline of impact to the beneficial use. For example, a standard to protect against acutely toxic conditions may be expressed as a “never to exceed” duration, whereas one that protects against impacts over the longer term may be expressed as an annual or even multi-year average.

The MPCA is proposing to apply the standard as an annual average. This means that throughout a year, surface water sulfate concentrations could fluctuate above and below the standard so long as the annual average concentration is below the numeric sulfate standard for that wild rice water.

There are two main factors supporting the use of the annual average. First, sulfate is not directly harmful to wild rice and the conversion of sulfate to sulfide is not instantaneous. Second, the use of an annual average of sulfate concentrations is consistent with the empirical statistical relationships upon which the equation is based.

1. Sulfate is not directly toxic and takes time to convert to sulfide

Expression of the numeric standard as an annual average accounts for the fact that sulfate is not a direct toxicant upon wild rice, but rather that elevated sulfate concentration can lead to elevated sulfide in the sediment porewater, which is the toxicant of concern.

Porewater sulfide is produced biologically under anaerobic conditions throughout the year in the sediment where wild rice grows; accumulation of sulfide in the sediment depends on the sulfate concentration as well as the concentrations of total organic carbon and total extractable iron present in

the sediment. Sulfide can be produced at any time throughout the year (DeRocher and Johnson, 2013). From this understanding, it is reasonable to conclude that the concentration of sulfate in surface water is important throughout the year, not just when wild rice is actively growing.

One possible approach is to implement the standard as a concentration that should never be exceeded. This would be reasonable if sulfate were directly toxic to wild rice. But several studies (Fort et al., 2014; Pastor et al., 2017) demonstrate that sulfate is not directly toxic to wild rice at concentrations encountered in Minnesota. However, over time sulfate can contribute to the buildup of sulfide in the porewater of sediments in which wild rice grows, so it is still important to regulate the concentration of sulfate in surface water.

The effect of elevated sulfate is (a) indirect, and (b) relatively slow. For instance, in a multi-year sulfate addition experiment (treatment sulfate concentrations of 0, 50, 100, 150, and 300-mg/L additions), it was not until the third year of the experiment that wild rice growth and reproduction was significantly affected in the 100 mg/L treatment (Pastor et al., 2017). In this case, the calculated protective sulfate concentration for the sediment used in the experiment was 34 mg/L. Even after five years of sulfate additions the 50 mg/L treatment (which had produced an actual average surface water sulfate concentration of 41 mg/L, less than the target of 50 mg/L because sulfate kept being converted to sulfide in the sediment) had no statistically significant effect on the most sensitive endpoints, seedling survival, seedling germination, and final plant biomass (Pastor et al., 2017). The 41 mg/L average sulfate concentration had not harmed wild rice after five years, which may be because (a) the equation-calculated sulfate concentration of 34 mg/L is sufficiently conservative to be protective of a concentration 20% higher than the calculated standard, or (b) not enough time had passed for the negative impact of elevated sulfate to manifest. It should be noted that the experiment was not a true mimic of likely impacts in the real environment, because the experiment was conducted in plastic tubs that cut off the iron supply from the watershed. Therefore, negative impacts might be observed in the experiment that would not occur in nature, where there is re-supply of iron to the sediment. After five years of sulfate additions, the highest sulfate treatment (300 mg/L) depleted the iron in the experimental tubs, allowing porewater sulfide to increase dramatically (Pastor et al., 2017).

The conversion of sulfate to sulfide is slow because it is a multi-step process. First, sulfate needs to enter the sediment from the overlying water, which in most wild rice sites occurs by diffusion. Diffusion is essentially a consequence of Brownian motion, the vibration of molecules proportional to temperature. While the speed of diffusion is driven by temperature, the direction of diffusion is from areas of high concentrations to areas of low concentration. Diffusion is a slow process, particularly under colder conditions. Second, once sulfate has entered anoxic sediment, the conversion to sulfide is a consequence of the growth of bacteria that respire sulfate instead of oxygen. If the growth of these bacteria is limited by sulfate (they can also be limited by the availability of organic matter), over the long term sulfide production is proportional to the sulfate concentration (Herlihy and Mills, 1985; Urban et al., 1994; Holmer and Storkholm, 2001). Microbial growth is also strongly affected by temperature. Bacteria grow slower under colder conditions.

Not only is the conversion of elevated sulfate to sulfide relatively slow, but the process of sulfate diffusion into the sediment is reversed if there is a decline in the concentration of sulfate in the surface water (DeRocher and Johnson, 2013). After a decline in sulfate concentration, the diffusion gradient is reversed, and unreacted sulfate will diffuse back into the overlying water (until concentrations are equal).

There is limited available information to support the determination of an alternative to a one-year averaging time. A key example is the limited potential to model the effect of varying sulfate

concentrations on porewater sulfide, in order to determine how long sulfate concentrations would need to be elevated to affect sulfide concentrations.

Modeling is one of the most powerful analytical tools available to environmental science. Scientists use models as a way to develop hypotheses, explain complex interrelated processes, and to present their understanding of a particular subject matter. "Modeling" has a broad definition within environmental science; a model can range from a conceptual diagram to a complex computer model involving days of super-computing processing time.

There are no official rules of "modeling" but there are some best practices, including:

- 1) Start simple and add complexity later.
- 2) Add complexity to your model only as necessary.
- 3) A model is only as good as the quality of the data used to develop and implement the model (a phenomenon often communicated as "GIGO", or "Garbage In Garbage Out" – a reminder to not put undue faith in the output of an unvalidated model).

The effect of short-term fluctuations in sulfate concentrations on porewater sulfide concentrations is difficult to model mathematically, and therefore difficult to predict. Among other reasons, modeling is difficult because both diffusion and bacterial growth are affected by temperature, and temperature changes rapidly in the spring and fall. For instance, if sulfate concentrations are temporarily high for a time in the winter, less sulfate will diffuse into the sediment than under warmer conditions, and bacterial conversion to sulfide will be slow because of the cold. If the sulfate concentration then declines, spring comes, and the sediment warms, the sulfate will simultaneously begin to diffuse up into the overlying water and conversion to sulfide will accelerate even as the sulfate concentration declines. The MPCA commissioned a study to examine these interactions (DeRocher and Johnson, 2013) that compared the effect of varying sulfate concentrations at two temperatures. The study confirmed many of the expected relationships between temperature, sulfate, sulfide, and iron, but also produced some unexpected results associated with one of the two sediment sources. The unexpected result (continued release of sulfate from the sediment throughout the experiment) was most likely an artifact of exposing the sediment to oxygen while mixing the sediment during the experimental set up. A major lesson of the study is that the development of a general model that predicts porewater sulfide from varying sulfate concentrations and varying temperature would be a major effort.

No published model tries to address the net effect of fluctuating sulfate concentrations and temperature. Rather, modelling efforts to date either assume constant sulfate concentration (for instance, in the marine environment (e.g., Eldridge and Morse, 2000) or step changes from one concentration to either a higher or lower sulfate concentration (for instance, increases or decreases in the atmospheric deposition of sulfate; Nikolaidis et al., 1989). Moreover, there are no published experimental studies in which sulfate concentrations were purposefully varied and compared to the effect of holding the sulfate concentration constant at the average concentration of the varying system. Thus, there is limited information on the effects of short-term fluctuations in sulfate concentrations in order to inform the MPCA's decision of an averaging time for the standard, beyond the conclusion that sulfate increases act over the longer term—a year or more—rather than days or months.

2. An annual average is consistent with the data and empirical statistical relationships

MPCA developed the equation by using the ambient surface water sulfate concentrations observed in the field survey, which is not significantly different from the annual average concentration (see below). Therefore, the proposed equation is relating the annual average sulfate concentration to porewater sulfide. Myrbo et al. (in press-1) showed that in 14 wild rice waters there is no significant seasonal trend

in porewater sulfide over the wild rice growing season, even though there was a slight increase in surface water sulfate over the summer. If there is an annual cycle in porewater sulfide, it is likely that sulfide is lower in the winter, as studies found (Leonard et al., 1993; Urban et al., 1994), which was attributed to greater winter oxygen penetration, lower sulfate diffusion rates, and decreased bacterial growth rates. The MPCA's equation, which is based on summer porewater sulfide concentrations, is therefore predicting the highest sulfide likely to be encountered in a waterbody.

In addition, Myrbo et al. (submitted-2) found that in experimental mesocosms porewater sulfide was linearly related to annual average sulfate concentration (which varied because the sulfate concentration was readjusted to target concentrations periodically and between adjustments sulfate diffused into the sediment; see Fig. 3-4 in the discussion below).

Implementing the average as an *annual* average is reasonable because of the strong annual temperature and organic matter production cycle in Minnesota, which strongly affect sulfide production. Bacteria only produce sulfide because bacteria are metabolizing decaying plants, which are produced on a strong annual cycle. All wild rice plants die in the fall, producing an abundance of organic matter that drives the production of sulfide, if sulfate is available.

An analysis of repeated samples from 14 different natural wild rice sites showed no significant time trends in sediment total organic carbon or sediment total extractable iron. A slight seasonal increase in sulfate (statistically significant at the $p=0.05$ level; Myrbo et al., in press-1) was observed, which is likely due to temporary dilution after spring snowmelt (Myrbo et al., in press-1). Because it takes many years to accumulate 10 cm of sediment, it makes sense that the iron and total organic carbon measured in the 10-cm long sediment samples show no change over time. Ten-cm long cores represent about 20 years of sediment accumulation (lead-210 dated age at 9-cm depth of cores from eight wild rice lakes average 21 years, with a median of 19 years; unpublished data from A. Myrbo, University of Minnesota).

For the 14 field study sites for which at least 3 samples were taken in one growing season, the sample used in the development of the equation is not significantly different from the average of the samples ($p=0.94$, Wilcoxon signed-rank nonparametric test). Conversely, regression analysis shows that the samples used to develop the equation are good estimates of the average of the samples. Regression analysis of log-transformed data (to approximate a normal distribution) with and without Second Creek yields slopes near 1.0 (0.944 to 0.971) and very high R^2 values (both 0.988). Second Creek had relatively high sulfate concentrations, which averaged 466 mg/L in 2013, in contrast to the 13 other sites, which ranged from 0.74 to 174 mg/L.

The equation therefore relates porewater sulfide to average surface water sulfate concentrations, not to maximum sulfate concentrations. Consequently, it is logical to implement the calculated sulfate standard as an annual average. If the calculated sulfate standard were implemented as a maximum value, the associated porewater sulfide concentration would be lower than the protective value of 120 $\mu\text{g/L}$, which would be over-protective.

Data collected during the sulfate-addition mesocosm experiment of Pastor et al. (2017) provides further evidence that the annual average surface water sulfate concentration is related to the porewater sulfide concentration. In this experiment, sulfate concentrations varied significantly over the year because of conversion to sulfide in the sediment. For instance, in 2013 the highest experimental sulfate treatment averaged 257 mg/L (Fig. 3-4), but ranged from 49 to 308 mg/L. Similarly, the second-highest sulfate treatment averaged 121 mg/L, but ranged from 14 to 151 mg/L. Yet, there is a highly statistically significant relationship between annual average sulfate concentration and porewater sulfide ($p < 0.001$, Fig. 3-4).

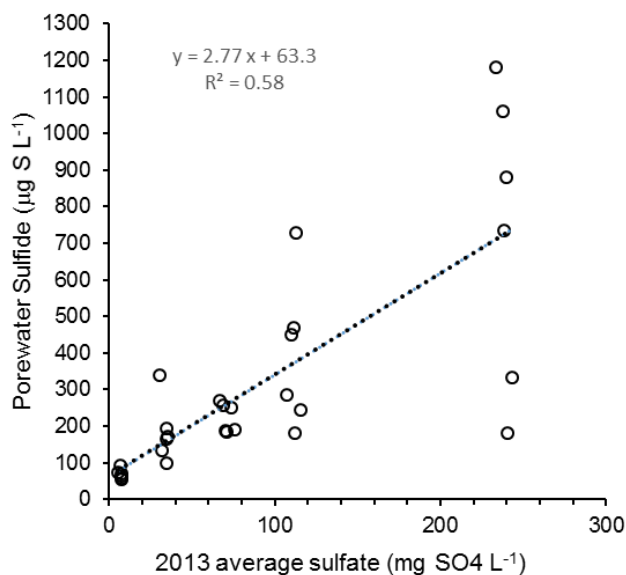


Figure 3-4. Relationship between average sulfate concentration from 2013 (8 measurement dates) and porewater sulfide measured at the end of the 2013 growing season. The regression between average sulfate concentration and porewater sulfide is highly significant ($p < 0.001$).

Frequency (how often the magnitude may be exceeded)

Given natural environmental variability, there is some probability that a water quality standard will be exceeded on occasion, due to factors other than human impacts. For instance, a dry summer can greatly decrease the expected dilution capacity of a receiving water. The question, then, is how frequently the sulfate standard can be exceeded without adversely impacting the beneficial use.

Porewater sulfide concentrations will decrease after an exceedance of a sulfate standard

The level of porewater sulfide is the long-term balance between production and loss of sulfide. If elevated sulfate in one year is followed by a year of lower sulfate, it is expected that porewater sulfide would re-equilibrate to the long-term average. The return to the long-term average sulfide concentration occurs because of a) the un-converted sulfate would diffuse back to the surface water and b) porewater sulfide would be oxidized by oxygen, ferric iron, and other oxidants. The concentration of porewater sulfide in a particular waterbody is, then, the net result of multiple dynamic processes involving sulfide production, sulfide oxidation, and reactions with both ferric (oxidation of sulfide) and ferrous iron (precipitation of sulfide). If sulfate concentrations are temporarily relatively high, producing higher sulfide concentrations, the system will tend to revert to a long-term average porewater sulfide concentration once sulfate is lowered, due to all the processes that affect sulfide—a phenomenon known as “sulfide buffering” (Giordani et al., 2008).

Several studies and reviews have concluded that observed sulfide concentrations are the balance between dynamic sulfide formation and sulfide degradation. Holmer & Storkholm (2001) found that up to 90% of sulfide production is oxidized to sulfate. Leonard et al. (1993) found seasonal variation in sediment sulfide in three lakes in northeastern Minnesota, where there is net loss of sulfide in the

winter. Urban et al. (1994), working in Little Rock Lake in Wisconsin, found that winter oxygen penetration oxidized sulfide in lake sediment, sometimes resulting in sulfate diffusion back into the surface water. The initial spring measurements in the sulfate-addition mesocosms of Pastor et al. (2017) also showed release of sulfate from the sediment into the overlying water (Myrbo et al., submitted-2).

Oxidation of sulfide can occur when sulfide encounters oxygen in the sediment, either through release from plant roots or bioturbation (mixing by benthic animals). Sulfide is also oxidized by the introduction of ferric iron to sediment from the watershed that is mixed downward by bioturbation (sulfide reduces ferric iron, a process that oxidizes the sulfide (Hansel et al., 2015).

As a result of the diffusion of sulfate that never was converted to sulfide back into the surface water, and the multiple processes that oxidize sulfide, temporary high concentrations of sulfate in surface water are not permanently preserved in the sediment as high sulfide. Porewater sulfide concentrations will return to the long-term average after a temporary exceedance of the sulfate standard.

Wild rice populations will recover even if porewater sulfide is temporarily elevated

A waterbody's wild rice population will be able to persist at a high average stem density if the annual average sulfate concentration does not exceed the calculated standard very often. The MPCA had to define what "very often" means in order to define the allowable excursion frequency. Because of the limitations of available environmental knowledge, the severity of an excursion cannot be rigorously related to the impact on a wild rice population. Nevertheless, MPCA expects that a wild rice population will not be significantly harmed by an exceedance that occurs only once in ten years, because that frequency will allow the environmental chemistry and wild rice population to recover between exceedances, thereby providing a high degree of protection.

New findings from the mesocosm experiment described by Pastor et al. (2017) provide some information on potential for wild rice to recover after a decrease in sulfate that had been elevated above the calculated protective concentration. The outdoor experiment grew wild rice in natural sediment at five different levels of sulfate (six replicates of each: control, 50, 100, 150, and 300 mg/L). If a numeric sulfate standard were calculated based on the sediment used in the experiment, it would be 34 mg/L (TOC=8.1%; TEF_e=8,300 µg/g). In the fifth year of treatment at the 300 mg/L level, in 2015, no wild rice plants grew in five of the six replicates, and the sixth replicate had just one plant (the control averaged 22 plants/replicate) (Pastor et al., 2017). As an experiment within the experiment, starting in 2016 no sulfate was added to five of the six replicates. In 2016, two of the mesocosms had three plants germinate and produce abundant seeds. Recovery was more widespread in the spring of 2017 (Pastor, 2017a, b). It is informative that wild rice could begin to recover within two years after four years of sulfate concentrations markedly greater than the calculated protective concentration of 34 mg/L. Through 2013, the 300 mg/L sulfate treatment actually averaged 207 mg/L because of ongoing conversion of sulfate to sulfide (Myrbo et al., submitted-2). These observations support the idea that porewater sulfide and wild rice can recover after occasional one-year exceedances in sulfate concentrations above the standard.

Therefore, it is unlikely that one year of elevated sulfate will have a long-term negative effect on wild rice growth and reproduction, so long as sulfate concentrations do not remain elevated above the allowable annual average for multiple years in a row.

Furthermore, the available scientific evidence supports that even a one-year elevation in sulfide levels in the sediment porewater above 120 µg/L would not have a long-term negative effect on wild rice growth and reproduction, so long as sulfide concentrations do not remain elevated above 120 µg/L for multiple sequential years. Relatively poor reproduction in one year out of five or ten years is extremely unlikely

to have a long-term negative effect on the persistence of a wild rice population, because wild rice populations build up a seed bank in the sediment so that only a portion of dormant seeds germinate in any given year. In fact, wild rice is infamous for oscillating between low and high populations under natural conditions on a 3- to 5-year cycle (Pastor and Walker, 2006). The existence of the seed bank allows wild rice to recolonize a waterbody even if all growing plants are eliminated by an environmental disturbance in a given year (MDNR, 2008). For example, a June 2012 precipitation event completely eliminated wild rice in Kettle Lake (Carlton County), but the following year the density of wild rice was above average (55 stems per square meter, compared to a 10-year average of 41 stems per square meter (Vogt, 2017), (not counting two years of zero density, 2012 and 2016).

Based on the foregoing, MPCA is proposing a one in ten year exceedance frequency as reasonable and protective of the beneficial use.

Appendix 1. Other potential consequences of increasing sulfate concentrations

This TSD largely restricts the discussion of negative effects of increased sulfate concentrations to the accumulation of toxic concentrations of sulfide in the sediment porewater of wild rice beds.

There are two other potential pathways of concern regarding elevated sulfate: potential direct toxicity of sulfate to aquatic organisms (e.g. Wang et al., 2016), and negative consequences of sulfide production even if porewater sulfide remains at low levels. These should be kept in mind even if sulfate concentrations could be increased at a site without harming wild rice due to impacts on porewater sulfide. These considerations, while not directly relevant to the refinement of the wild rice sulfate standard, represent additional insight gained from the MPCA-sponsored study that can be used to inform water quality management decision-making.

The scientific literature includes evidence for multiple hypothesized effects associated with the conversion of sulfate to sulfide in sediment. The outdoor mesocosm experiment conducted by Pastor et al. (2) presented an opportunity to evaluate multiple hypotheses simultaneously. Data from the mesocosms were obtained in August 2013 and 2015, the results of which are reported in Myrbo et al. (submitted-2). In addition, the correlations observed in the MPCA-sponsored field survey are consistent with the science summarized below.

Stoichiometric releases associated with sulfate-enhanced decomposition of organic matter

The shallow-water aquatic ecosystems in which wild rice grows usually accumulate significant concentrations of organic matter; in the MPCA field survey the median concentration of organic matter is 25% on a dry weight basis. The organic matter, which is plant litter that has not fully decomposed, accumulates because decomposition by the microbial community is greatly slowed by limited availability of the principal terminal electron acceptors (TEAs) – oxygen, nitrate, oxidized manganese, oxidized iron, sulfate, and carbon dioxide – which are thermodynamically favored in that order (Froelich et al., 1979). A major reason wetlands accumulate organic matter is that oxygen availability, and therefore decomposition, is significantly reduced in water-saturated sediments; oxygen is consumed by bacteria within a few millimeters into sediment, and is supplied at a very slow rate because of its low solubility in water (10 ppm, compared to 210,000 ppm in the atmosphere). The next thermodynamically-favored TEA, nitrate, is not elevated in most wild rice waters, and therefore is not generally available to support decomposition. Manganese concentrations in sediment are usually minor compared to iron, and will not be discussed here. Ferric iron, the next thermodynamically favored TEA, is mostly present as a solid, and therefore of limited availability to bacteria. Even small increases in sulfate availability can increase bacterial activity, increasing decomposition in rough proportion to sulfate concentrations (Perry et al., 1986; Cook et al., 1986). The production of methane, which occurs when carbon dioxide is utilized as a TEA, is the least thermodynamically favorable and generally occurs when the other TEAs are depleted.

Plants have a relatively constant ratio among the important building blocks of carbon (C), nitrogen (N), and phosphorus (P), which are therefore released proportionally during decomposition of sedimentary organic matter. When sulfate is available, release of C, N, and P can be proportional to sulfide production (Froelich et al., 1979; Weston et al., 2006; Myrbo et al., submitted-2). Other plant components, including potassium (Lamers et al., 1998) and silica (Weston et al., 2006), are also released into solution during decomposition of plants. In the controlled outdoor mesocosm experiment, Myrbo et al. (submitted-2) found that porewater sulfide concentrations are significantly related to increases in products of decomposition in the surface water: total phosphorus, total nitrogen, dissolved organic

carbon, and alkalinity. In addition, enhanced decomposition also increased the concentration of total mercury in the overlying water—mercury that presumably had been associated with the solid organic matter that decomposed. Mercury would have been delivered to the sediment from the atmosphere, either directly in precipitation or dry deposition to the emergent plant material, or indirectly from the watershed (Wiener et al., 2006). In the surface water of the mesocosms, total mercury was highly correlated with dissolved organic carbon, which is known to have a high affinity for mercury (Ravichandran, 2004).

It is not surprising that sulfide production is statistically correlated with predicted changes in the controlled mesocosm experiment, where the only change was the sulfate concentration, and sediment and overlying water were initially the same among treatments. That statistically significant correlations with porewater sulfide are observed in the MPCA-sponsored field survey, given the great heterogeneity in landscapes across Minnesota (Moyle, 1956; Heiskary et al., 1987), reinforces the conclusion that sulfide production is an important process that can control the occurrence of wild rice. Although the importance of sulfide is clear to many wetland scientists, this knowledge is not yet widespread among environmental scientists in general. It is pertinent to repeat a quote from Chapter 1 of this TSD from a study in central New York State of the factors that control plant species distribution in a wetland: "...it is puzzling that there has not been more work to investigate the possible role of sulfide as a master variable controlling plant community composition within inland wetland ecosystems." (Simkin et al., 2013).

Among the 108 field sites sampled in the MPCA survey, sulfide production (measured as acid-volatile sulfide or porewater sulfide) is significantly correlated with porewater total phosphorus, ammonia, and silica, and surface water total nitrogen, total phosphorus, potassium, and alkalinity (Myrbo et al., in press-1). These correlations support the hypothesis that production of sulfide is associated with enhanced decomposition of sedimentary organic matter, releasing the nutrients, alkalinity, and mercury to porewater and surface water.

Sulfate-enhanced release of phosphorus from sediment iron through sulfide production

There are two processes through which increased sulfide production is associated with the release of phosphorus from solid phases in the sediment, thereby increasing concentrations in porewater and surface water: 1) enhanced decomposition of organic matter, releasing phosphorus as discussed above, and 2) the release of phosphorus as a result of the interaction of sulfide and iron in the sediment. The addition of sulfate can produce more sulfide, which is thought to interact with iron in several ways that solubilize phosphorus (Caraco et al., 1989; Smolders and Roelofs, 1993; Maynard et al., 2011). When surface water sulfate and sedimentary sulfide concentrations are low, phosphorus is associated with several different phases of iron (Smolders and Roelofs, 1993; Maynard et al., 2011). When sulfate concentrations increase, sulfide production increases in sediment porewater, which reacts with ferrous iron, precipitating as iron-sulfide compounds, which do not sorb phosphate effectively (Roden and Edmonds, 1997).

Potential for sulfate-enhanced increased production of methylmercury

Increased sulfide production, which can result from an increase in sulfate loading or concentration under certain conditions, has long been known to increase the conversion of inorganic mercury to methylmercury (Gilmour et al., 1992), a phenomenon confirmed in an ecosystem-level sulfate addition experiment in Minnesota (Jeremiason et al., 2006). Methylmercury is the form of mercury that bioaccumulates in fish. Increased production of methylmercury is a significant concern, given that bioaccumulation of methylmercury in fish is a major cause of water quality impairments in Minnesota,

resulting in a state-wide effort to reduce mercury contamination in fish (MPCA, 2007). Increased sulfide production not only has been shown to mobilize inorganic mercury from sediment, but also to increase the proportion of that mercury that is converted to methylmercury (Myrbo et al., submitted-2).

Other changes associated with conversion of sulfate to sulfide

The calculated numeric sulfate standard to protect wild rice should not be taken to mean that it is automatically defensible to increase average ambient sulfate concentrations to that level, even if wild rice would not be harmed. Indeed, even if the calculated sulfate concentration is not thought to be directly harmful to aquatic biota, and even if none of the three classes of chemical changes that are associated with increased sulfide production, described above, there may be other concerns about increasing sulfate concentrations and sulfide production.

The produced sulfide has a number of non-exclusive potential fates. The sulfide could 1) remain in the sediment porewater as free sulfide, 2) diffuse into the surface water, to be oxidized to sulfate, 3) be oxidized in the sediment, 4) volatilize to the atmosphere, or 5) react with metals (usually forming iron-sulfide compounds), forming insoluble precipitates in the sediment. The net concentration of sulfide remaining in the sediment can be quantified as acid-volatile sulfide (AVS), which can be a useful indicator of cumulative sulfide production.

Some of the consequences of sulfide production are not necessarily negative, and some have as yet poorly understood ramifications. For instance, one stoichiometric consequence of the conversion of sulfate to sulfide is the production of alkalinity. While it is not clear that additional alkalinity is negative, it is a change in the aquatic ecosystem, and some organisms seem to have different alkalinity optima (e.g., Moyle, 1945; Vestergaard and Sand-Jensen, 2000).

The production of alkalinity is not necessarily permanent, because the conversion of sulfate to sulfide is reversible. In many of the field survey sites most of the sulfide was precipitated as a solid when it reacted with iron, forming iron-sulfide compounds. If the waterbody dries and the sediment is exposed to oxygen in the atmosphere, the sulfide can be oxidized, and upon rewetting release sulfate as sulfuric acid, negating the alkalinity that had been produced earlier when the sulfide was produced. This has been documented in eastern North America (Kerr et al., 2012) and Australia, where the issue of restoration of wetlands with sulfidic sediment has been addressed (Ning et al., 2011). If the alkalinity had been produced gradually and washed downstream, the production of acid might not be buffered. Thus, even though the production of iron-sulfide solids detoxifies the porewater sulfide by removing it from solution, the accumulation of sulfidic sediment, which can be quantified as AVS, also represents the potential for episodic release of sulfate, which could be acidic.

Appendix 2. Wild Rice Seeds and Food Value

Part of the beneficial use established is the use of wild rice as a food source for wildlife. Information on the amount of wild rice needed to support wildlife, especially waterfowl, may be helpful in considering the beneficial use, and is provided here.

Literature estimates of wild rice seed weights and number of seeds per stem

Ranges for wild rice seed weights found in the scientific literature, other reports, and personal communications are shown in the Table A2-1. Similarly, Table A2-2 shows some literature ranges for the values for number of seeds per seed head in wild rice populations in Minnesota and Wisconsin. Although the ranges are quite large, means and medians of number of seeds per stem in natural wild rice waters are about 50 seeds per stem.

Table A2-1. Wild rice seed weight estimates found in the literature.

Seed weight estimates (dry weight in mg)	Description of paper or research	Reference
17.1 mg-42.3 mg (range of lakes and rivers)	Lacustrine and riverine populations of wild rice in northern Minnesota and Wisconsin, four river and four lake pairs	Eule-Nashoba, 2010; Eule-Nashoba et al., 2012
35.39-37.81 mg (range in lakes)		
20.63-23.77 mg (range in rivers)		
Seeds in lakes were 11.9 to 18.2 mg larger than their paired riverine populations. Mean seed mass in lake populations was 41 percent larger than in river populations.		
2011 24.6 (1.24) 2012 27.8 (0.9) 2013 29.7 (1.1)	Means of samples from six control tanks with standard errors in parentheses	Pastor, 2013
20-30 mg (dry)	Personal communication of literature values from natural stands and weighing of hand-harvested rice	David Schimpf, retired associate professor of biology at University of Minnesota Duluth and technical advisor to MPCA wild rice advisory committee
River rice may average somewhat lower than lake rice		

Table A2-2. Literature ranges of number of seeds per head.

# of grains per head	Description of research, study	Source
Range 19-115 grains per head on 14 stands of wild rice (all are lakes)	Survey of 14 stands of wild rice-Table 1 from 1941	Moyle, 1942, Fisheries Research Investigational Report # 40
Seed scars/panicle Range =23.9-132.8	Lacustrine and riverine populations of wild rice in northern MN and WI, four river and four lake pairs	Eule-Nashoba, 2010; Eule-Nashoba, 2012
12.75-102.35 number of seed scars/panicle Mean of seed scars/panicle 46.39 standard deviation of 29.7	17 wild rice populations in northern Wisconsin	Lu et al., 2005

The MPCA is using an estimate of 25 mg for wild rice seed weight and an estimate of 50 seeds per wild rice stem in the calculations to put into context the food value of wild rice. Table A2-3 shows the number of seeds and stems to support the daily energy needs for a dabbling duck.

Table A2-3. Seeds and stem values to support daily energy needs of a dabbling duck.

Mass of wild rice required to meet the daily energy expenditure of a dabbling duck	Daily Intake of seeds corresponding to 85 grams	Number of stems corresponding to 3,400 seeds (assuming 50 seeds per stem)
85 grams	3,400 seeds	68 stems

The MPCA initially considered criteria for identifying a wild rice water of ¼ acre of wild rice with an average density of 8 stems per square meter, or ½ acre of wild rice with 4 stems per square meter would meet at a minimum the food energy needs of a pair of ducks for two months. Although these criteria were not carried forward into the proposed rule, they support the MPCA’s proposal that a single stem of wild rice (or other small amount) is insufficient to demonstrate the beneficial use and support identifying a water as a wild rice water.

Food value of wild rice

The nutritional value that the wild rice grain affords to waterfowl is equivalent to or exceeds the caloric energy values provided by many other wetland plants and agricultural grains (Sherfy, 1999; Gray et al., 2013). Wildlife researchers use these food energy values in determining species-specific daily energy expenditures for waterfowl supported by a given amount of food over a given management area. These calculations are often expressed as duck-energy days (or duck days) and represent the number of days a given amount of food will support a duck or group of ducks. These types of calculations have been used to build complex models to estimate the carrying capacity of large regions for a variety of species.

In its simplest form, duck-energy days can be determined by the following equation:

$$\text{Duck-energy days} = \frac{\text{Food available (grams dry weight)} \times \text{True Metabolizable Energy (kcal/gram dry weight)}}{\text{Daily Energy Expenditure (kilocalories/day)}}$$

Daily Energy Expenditure (kilocalories/day)

This can be simplified to solve for the amount of wild rice needed to meet the daily energy requirements of a single duck.

$$\frac{\text{Daily Energy Expenditure average dabbling duck (kcal/day)}}{\text{True Metabolizable Energy (kcal/g dry weight)}} = \text{g of wild rice needed by a duck for a day}$$

True Metabolizable Energy (kcal/g dry weight)

The MPCA is using a value of 294.35 kcal/day as the daily energy expenditure for an average dabbling duck (Reinecke and Kaminski, 2006) and a value of 3.47 kcal/g (Sherfy, 1999) for the True Metabolizable Energy of wild rice.

$$294.35 \text{ kcal/day divided by } 3.47 \text{ kcal/g} = 84.82 \text{ g wild rice needed to feed an average dabbling duck for one day.}$$

While it is recognized that ducks do not only eat wild rice, the approximate 85 gram per day can be used to estimate the number of ducks that can theoretically feed on a given amount of wild rice. See below for details and discussion of values used for estimates of daily energy expenditure and true metabolizable energy and calculation of grams of wild rice needed to feed a dabbling duck for a day.

Daily Energy Expenditures

Daily Energy Expenditures (DEE) for waterfowl are calculated based on the strong relationship between body mass and basal metabolic rate (BMR) or resting metabolic rate (RMR) within and among species of birds (King, 1974; Prince, 1979; Miller and Eadie, 2006).

The Mississippi Alluvial Valley (MAV) is a waterfowl management area in the Lower Mississippi that is important to migrating and wintering waterfowl. Resource managers of the Mississippi Alluvial Valley Joint Venture have developed a DEE of 294.35 that is based on: 1) the daily energy requirements of an dabbling ducks during fall and winter and 2) the population goals in the MAV for seven dabbling ducks and the wood duck (Reinecke and Kaminski, 2006). Previously, the daily energy expenditures of the mallard were used as a surrogate for all the species found in the joint venture. This value is a reasonable estimate for daily energy expenditure for ducks eating wild rice as the dabbling ducks found in the Mississippi Alluvial Valley include many of the same species found in Minnesota and include species that consume wild rice such as the mallard and blue-winged teal.

True Metabolizable Energy (energy value of waterfowl foods)

True metabolizable energy (TME) is recognized as a valid expression of dietary quality that can be measured rapidly and reliably (Miller and Reinecke, 1984). The TME of waterfowl foods is an important component for accurate assessments of waterfowl energetics. It can be calculated indirectly using a regression model, or measured experimentally by feeding birds a controlled diet and measuring excretory energy.

There is a lack of TME values for common waterfowl food and species. The species studied most frequently include the Mallard, American Black Duck, Northern Pintail, Blue-Wing Teal, Carolina Wood Duck and Canada Goose. Eadie et al., cite values of true metabolizable energy (TME) of white rice as ranging from 3.34 to 3.76 kcal/gram. (Eadie et al., 2008). One study reports a mean value of 3.47 kilocalories/gram (3.07 to 3.92 range) for the TME of wild rice (*Zizania aquatica*)(Sherfy, 1999) and was based on blue-winged teal. Mallard values were not available for wild rice.

The MPCA is planning to use the mean TME of 3.47 kilocalories per gram for wild rice reported in 1999 by Sherfy as the TME for wild rice.

Calculation of amount of wild rice needed to an average dabbling duck for a day

294.35 kcal/day divided by 3.47 kcal/g = 84.82 g wild rice needed to feed an average dabbling duck for one day.

Although ducks do not only eat wild rice, this value can be used to estimate the number of ducks that can obtain food from a given amount of wild rice. A wild rice water of 0.25 acres of wild rice with a stem density of eight stems per square meter or 0.50 acres with a stem density of four stems per square meter would meet at a minimum the food energy needs of a pair of ducks for two months.

Appendix 3. Results for the 2015 sediment pilot study

Raw sediment analytical data, selected statistics and calculated values of sulfate for sites sampled during the 2015 sediment pilot study. Individual ID = individual sediment core sample; % TOC = percent sediment Total Organic Carbon; TEF_e = sediment Total Extractable Iron; Composite = Average of 5 individual samples composited as one sample; Calculated sulfate = sulfate value calculated using the equation: Sulfate = 0.0000121 x (Iron^{1.923}/Organic Carbon^{1.197}); Organic Carbon = TOC and Iron = TEF_e.

Table A3-1. Results for sediments collected from Bowstring River.

Individual ID	TOC (% Dry)	TEFe (mg/kg)	TOC Composite (% Dry)	TEFe Composite (mg/kg)	Calculated Sulfate Composite (mg/L)	Calculated Sulfate Individual (mg/L)
1-1	16.8	4230				3.9
1-2	16.9	4050				3.5
1-3	19.1	4230				3.3
1-4	17.2	4140				3.6
1-5	16.3	3600	17.3	4050	3.5	3.0
1-6	15.0	2520				1.6
1-7	14.4	3330				3.0
1-8	13.5	4500				5.7
2-10	13.5	3870				4.3
2-11	16.0	4140	14.5	3672	3.5	4.0
2-12	15.1	4590				5.2
2-13	17.0	4320				4.0
2-14	15.7	4320				4.4
2-15	14.0	3510				3.4
2-16	14.8	3690	15.3	4086	4.1	3.5
2-9	15.6	3870				3.6
3-17	14.2	3780				3.8
3-18	12.6	4320				5.7
3-19	15.6	3870				3.6
3-20	15.1	4680	14.6	4104	4.3	5.4
3-21	19.0	3060				1.8
3-22	16.4	3150				2.3
4-23	17.7	3240				2.2
4-24	18.4	2160				1.0
4-25	19.7	4500	18.2	3222	2.1	3.6
Mean=	16.0	3827				3.6
Std. Dev=	1.9	640				1.2
CV (%)=	12%	17%				34%
min=	12.6	2160				1.0
max=	19.7	4680				5.7

Percentiles for individual calculated sulfate values

10th	30th	50th	70th	90th
2.0	3.3	3.6	3.9	5.3

Table A3-2. Results for sediments collected from Clearwater River.

Individual ID	TOC (% Dry)	TEFe (mg/kg)	TOC Composite (% Dry)	TEFe Composite (mg/kg)	Calculated Sulfate Composite (mg/L)	Calculated Sulfate Individual (mg/L)
1-1	19.2	11700				23.4
1-2	21.0	15300				35.3
1-3	16.6	8370				14.6
1-4	21.0	12600				24.3
1-5	16.2	13500	18.8	12294	26.4	37.8
1-6	20.3	18900				55.1
1-7	25.4	13500				22.1
2-10	19.9	14400				33.5
2-11	16.5	10800				24.1
2-12	17.5	11700	19.9	13860	31.1	26.2
2-13	25.3	12600				19.4
2-14	18.9	18000				54.7
2-15	24.5	12600				20.2
2-8	21.2	12600				24.0
2-9	17.7	15300	21.5	14220	29.7	43.3
3-16	21.8	9900				14.6
3-17	21.5	12600				23.6
3-18	21.0	13500				27.7
3-19	22.5	12600				22.3
3-20	18.4	11700	21.0	12060	22.3	24.7
3-21	22.2	13500				25.9
3-22	19.0	19800				65.3
3-23	20.9	12600				24.4
3-24	19.9	11700				22.4
3-25	20.2	16200	20.4	14760	34.0	41.2
Mean=	20.3	13439				30.0
Std. Dev=	2.5	2652				13.0
CV (%)=	12%	20%				43%
min=	16.2	8370				14.6
max=	25.4	19800				65.3

Percentiles for individual calculated sulfate values

10th	30th	50th	70th	90th
19.7	23.5	24.4	32.3	50.1

Table A3-3. Results for sediments collected from Hesitation Wildlife Management Area.

Individual ID	TOC (%) Dry)	TEFe (mg/kg)	TOC Composite (%) Dry)	TEFe Composite (mg/kg)	Calculated Sulfate Composite (mg/L)	Calculated Sulfate Individual (mg/L)
T1	22.7	45000				255.7
T10	25.6	27000				82.9
T11	23.9	36000				156.5
T12	23.4	29700				110.9
T13	24.7	26100	24.1	32760	129.5	81.1
T14	25.9	36000				142.2
T15	23.0	29700				113.2
T16	25.4	31500				112.6
T17	23.6	39600				190.9
T18	22.9	39600	24.2	35280	148.6	197.9
T19	21.4	25200				90.0
T2	19.8	48600				349.2
T20	19.3	37800				222.1
T21	22.1	30600				125.8
T22	22.1	34200	20.9	35280	176.4	155.8
T23	19.0	74700				838.5
T24	20.7	58500				472.9
T25	20.0	56700				464.1
T3	18.3	69300				759.2
T4	16.1	41400	18.8	60120	558.6	328.6
T5	17.4	27000				131.6
T6	17.6	24300				106.0
T7	20.9	26100				99.0
T8	24.7	25200				75.8
T9	25.1	32400	21.1	27000	104.3	120.5
Mean=	21.8	38088				231.3
Std. Dev=	2.8	13850				204.5
CV (%)=	13%	36%				88%
min=	16.1	24300				75.8
max=	25.9	74700				838.5

Percentiles for individual calculated sulfate values

10th	30th	50th	70th	90th
85.7	112.7	142.2	217.2	469.4

Table A3-4. Results for sediments collected from Mission Creek.

Individual ID	TOC (% Dry)	TEFe (mg/kg)	TOC Composite (% Dry)	TEFe Composite (mg/kg)	Calculated Sulfate Composite (mg/L)	Calculated Sulfate Individual (mg/L)
1-1	3.1	11700				207.0
1-2	3.6	15300				294.0
1-3	5.1	16200				213.7
1-4	4.5	18900				333.7
1-5	3.7	11700	4.0	14760	240.1	170.4
1-6	2.8	10800				203.9
1-7	3.3	13500				255.8
1-8	4.0	12600				175.1
2-10	3.3	17100				400.1
2-11	3.6	18000	3.4	14400	277.0	392.7
2-12	4.2	17100				303.2
2-13	1.5	7470				202.6
2-14	3.2	14400				302.8
2-15	2.6	11700				251.9
2-16	4.5	16200	3.2	13374	259.2	250.1
2-9	4.4	14400				205.4
3-17	4.0	17100				314.0
3-18	3.9	18000				359.3
3-19	5.3	19800				298.1
3-20	5.2	19800	4.6	17820	293.0	307.8
3-21	4.6	20700				391.3
3-22	5.0	22500				413.4
3-23	3.8	22500				582.0
3-24	2.4	10800				247.0
3-25	3.6	14400	3.8	18180	375.0	263.4
Mean=	3.8	15707				293.6
Std. Dev=	0.9	3882				93.6
CV (%)=	24%	25%				32%
min=	1.5	7470				170.4
max=	5.3	22500				582.0

Percentiles for individual calculated sulfate values

10th	30th	50th	70th	90th
203.1	247.6	294.0	312.8	397.1

Table A3-5. Results for sediments collected from Monongalia Lake.

Individual ID	TOC Dry (%)	TEFe (mg/kg)	TOC Composite (% Dry)	TEFe Composite (mg/kg)	Calculated Sulfate Composite (mg/L)	Calculated Sulfate Individual (mg/L)
1-1	4.3	3330				12.4
1-2	9.9	5310				11.3
1-3	19.2	6930				8.6
1-4	2.4	2610				15.7
1-5	23.1	8910	11.8	5418	9.6	11.1
2-6	2.5	2610				14.8
2-7	19.1	6750				8.2
2-8	10.6	4950				9.1
3-10	18.7	6210				7.2
3-11	12.5	9000	12.7	5904	10.3	23.6
3-9	17.4	7740				11.9
4-12	14.7	5760				8.3
4-13	20.1	6300				6.7
4-14	24.1	4410				2.7
4-15	24.7	7110	20.2	6264	6.6	6.7
5-16	19.3	7830				10.8
5-17	23.6	8820				10.6
5-18	22.6	6300				5.9
6-19	27.2	6570				5.1
6-20	25.1	4950	23.6	6894	6.6	3.3
7-21	21.6	5760				5.2
7-22	8.5	4050				8.1
8-23	14.8	4950				6.1
8-24	18.3	6750				8.6
8-25	17.9	7110	16.2	5724	7.3	9.8
Mean=	16.9	6041				9.3
Std. Dev=	7.1	1792				4.4
CV (%)=	42%	30%				47%
min=	2.4	2610				2.7
max=	27.2	9000				23.6

Percentiles for individual calculated sulfate values

10th	30th	50th	70th	90th
5.1	6.8	8.6	10.7	13.8

Table A3-6. Results for sediments collected from Mississippi River.

Individual ID	TOC Dry (%)	TEFe (mg/kg)	TOC Composite (% Dry)	TEFe Composite (mg/kg)	Calculated Sulfate Composite (mg/L)	Calculated Sulfate Individual (mg/L)
1-1	19.3	4680				4.0
1-10	22.0	4770				3.5
1-11	15.1	5310				6.8
1-12	12.4	6120				11.4
1-13	16.6	5940	17.1	5364	6.0	7.6
1-14	11.8	5760				10.7
1-15	14.1	6030				9.5
1-16	12.3	5670				9.9
1-17	9.7	5220				11.3
1-18	9.2	5580	11.4	5652	10.8	13.7
1-19	6.1	4860				17.2
1-2	16.0	5130				6.0
1-3	19.4	5130				4.7
1-4	14.0	5400				7.7
1-5	16.9	5220	14.5	5148	6.8	5.8
1-6	20.8	5220				4.5
1-7	17.5	5130				5.4
1-8	17.6	5850				6.9
	16.8	5670				6.8
2-20	18.6	5130	18.3	5400	5.6	5.0
2-21	15.8	5220				6.3
2-22	17.8	5490				6.0
2-23	15.2	5940				8.4
2-24	16.5	6300				8.5
2-25	7.6	5040	14.6	5598	7.9	14.0
Mean=	15.2	5432				8.1
Std. Dev=	4.0	436				3.4
CV (%)=	27%	8%				43%
min=	6.1	4680				3.5
max=	22.0	6300				17.2

Percentiles for individual calculated sulfate values

10th	30th	50th	70th	90th
4.6	6.0	6.9	9.3	12.8

Appendix 4. Statistical characterization of wild rice density in 10 wild rice waters over 12 years

The long-term data collection by the 1854 Treaty Authority (Vogt, 2017) constitutes the best available information on variation over time in average wild rice density among a variety of waterbodies. From 2005 to the present, the 1854 Treaty Authority conducted surveys of wild rice density in ten wild rice waters using a consistent methodology (described in Kjerland, 2015). Surveys were conducted in late August or early September when the rice was standing and reaching maturity. Wild rice density in each waterbody was determined from at least 20 sample plots of an area of 0.5 m² each. The annual average density for each of the ten waters (Table A4-1) ranged from a minimum of zero (which occurred three times) to a maximum of 408 stems/m², with an average of 46 stems/m². The median, or most typical, density was 30 stems/m² (Table A4-2).

Table A4-1. Average wild rice density in ten wild rice waters, as monitored by the 1854 Treaty Authority (Vogt, 2017). Density is average stems per square meter from a minimum of 20 fixed sampling points.

Year	Big Rice Lake	Breda Lake	Cabin Lake	Campers Lake	Cramer Lake	Kettle Lake	Little Rice Lake	Round Island Lake	Stone Lake	Vermilion River
2005	58	80	86	29	80	11	61	408	48	88
2006	13	66	56	46	58	36	16	95	17	201
2007	11	53	82	59	46	76	30	40	42	116
2008	11	69	21	67	28	32	14	11	25	153
2009	4	85	26	75	29	19	6	44	10	90
2010	7	76	99	74	74	65	12	88	38	42
2011	5	42	9	27	28	8	11	154	26	87
2012	7	15	20	0	17	0	12	45	5	75
2013	11	25	14	17	17	55	9	60	5	113
2014	5	61	28	35	29	42	4	37	26	97
2015	4	49	27	40	36	64	24	23	25	99
2016	4	11	20	24	85	0	13	24	11	100

Table A4-2. Statistical characterization of wild rice density in 10 wild rice waters over 12 years (data from Table A4-1).

percentile	stems/m ²
10	7
15	11
25	14
50	30
75	65
85	75
90	88

Appendix 5. EC10 values from MPCA-sponsored hydroponic data

Data used

Dr. John Pastor conducted three experiments where his team added sulfate to hydroponic wild rice at concentrations of 0 to 2,880 $\mu\text{g/L}$, described in Pastor et al. (2017). Pastor's team measured the weight of the plants and the mean sulfide at the beginning of the study and at the end of the study. The variables used for this analysis are:

To measure sulfide concentration:

- Mean initial sulfide concentration ($\mu\text{g/L}$)
- Arithmetic time weighted mean (TWM) sulfide concentration ($\mu\text{g/L}$)
- Geometric TWM sulfide concentration ($\mu\text{g/L}$)

To measure plant growth:

- Weight change (mg)
- Weight gain: the weight change with any weight loss set to 0 (mg)

Curve fitting

Logistic regressions and estimates of EC10, with 95% confidence intervals, were conducted with routine *drc* (R, Ritz et al., 2015).

Predicting weight gain from mean initial sulfide

Fig. A5-1 shows the log logistic curve fits for both predicting the weight change (Fig. A5-1A) and predicting the weight gain (Fig. A5-1B).

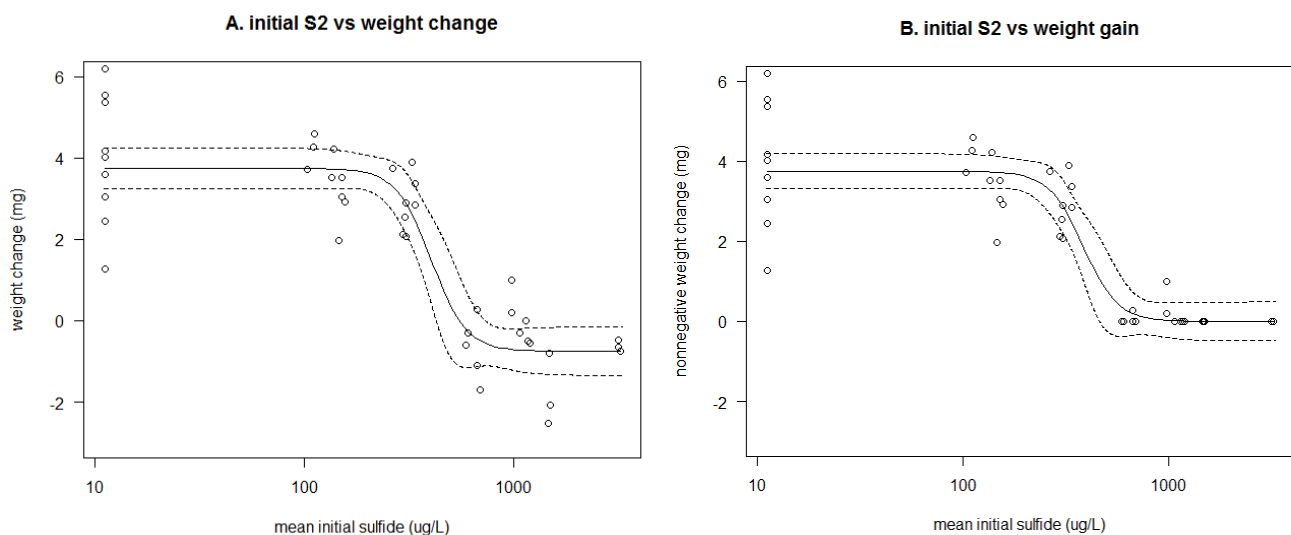


Figure A5-1. Logistic regressions using initial sulfide hydroponic concentration to predict plant growth. A) weight change. B) weight gain.

In both graphs that the weight change stays constant until well over 100 $\mu\text{g/L}$ initial sulfide. The resulting EC10, EC20, and EC50 values are in Table A5-1. While the EC values are lower when using weight gain vs weight change, the numbers are not significantly different. For both, concentrations below 250 $\mu\text{g/L}$ look to be protective.

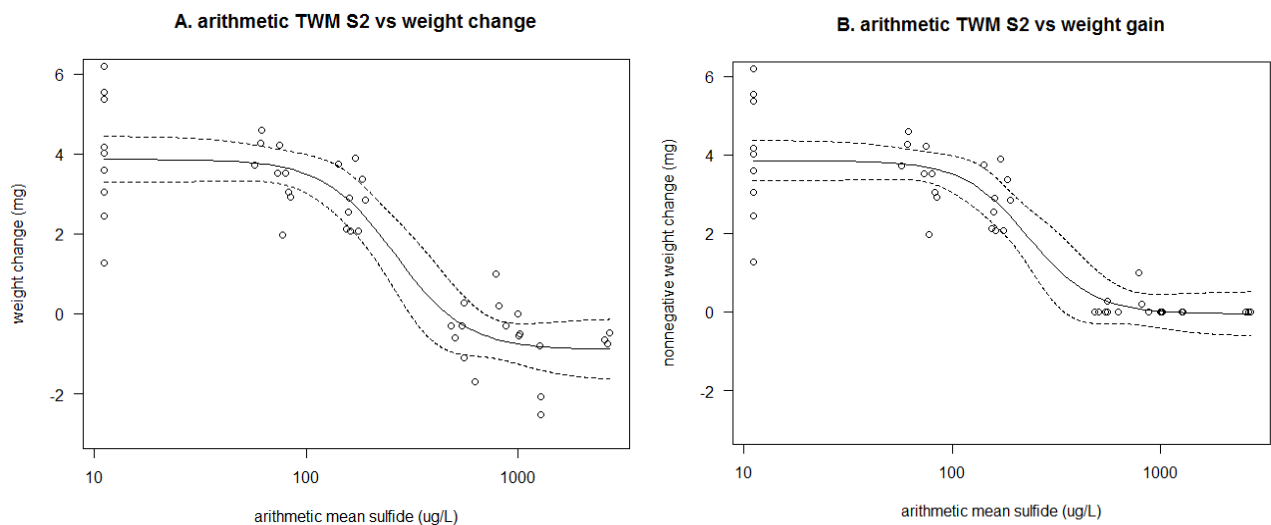
Table A5-1. EC10, EC20, and EC50 values for initial mean sulfide hydroponic concentration.

	Wt change	Estimate	In 95% CI	Wt gain	Estimate	In 95% CI
EC10	3.37 mg	255 $\mu\text{g/L}$	<11 $\mu\text{g/L}$ - 322 $\mu\text{g/L}$	3.465 mg	251 $\mu\text{g/L}$	<11 $\mu\text{g/L}$ - 285 $\mu\text{g/L}$
EC20	2.99 mg	296 $\mu\text{g/L}$	235 $\mu\text{g/L}$ - 363 $\mu\text{g/L}$	3.080 mg	294 $\mu\text{g/L}$	234 $\mu\text{g/L}$ - 353 $\mu\text{g/L}$
EC50	1.87 mg	378 $\mu\text{g/L}$	324 $\mu\text{g/L}$ - 474 $\mu\text{g/L}$	1.925 mg	384 $\mu\text{g/L}$	331 $\mu\text{g/L}$ - 486 $\mu\text{g/L}$

Predicting weight gain from arithmetic time weighted mean sulfide concentration

Fig. A5-2 shows the log logistic curve fits for both predicting the weight change (Fig. A5-2A) and predicting the weight gain (Fig. A5-2B).

Figure A5-2. Logistic regressions using arithmetic time-weighted mean (TWM) sulfide hydroponic concentrations to predict plant growth. A) weight change. B) weight gain.



In both graphs that the weight change starts to decrease around 100 $\mu\text{g/L}$. The resulting EC10, EC20, and EC50 values are in Table A5-2 below. Once again, the EC values for weight gain (i.e. no negative weight change) are lower than the EC values for weight change (which allows for weight loss). However, the numbers are similar, and the confidence intervals are large enough to confirm they are not significantly different. When the arithmetic TWM sulfide concentration is used, the EC10 is between 100 and 110 $\mu\text{g/L}$.

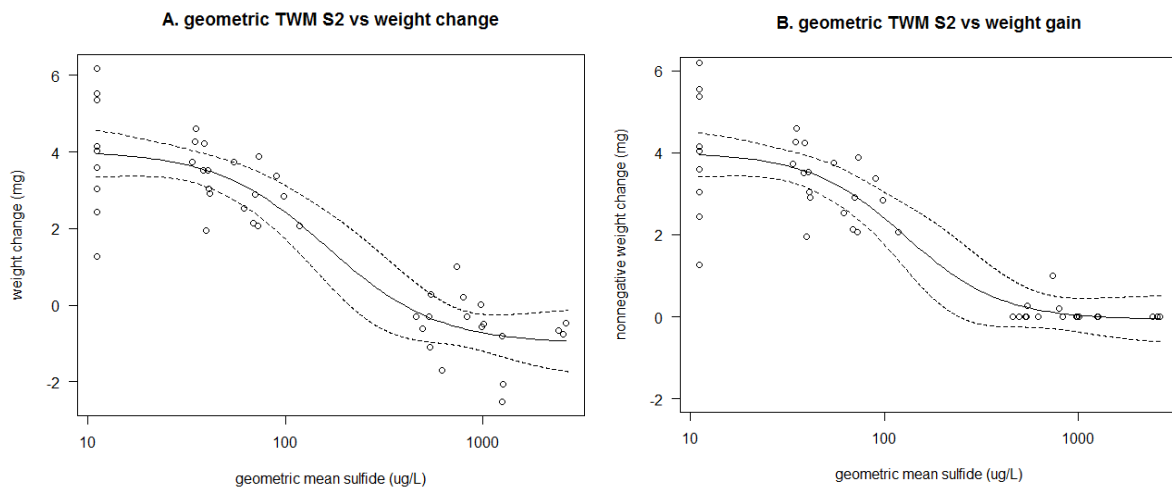
Table A5-2. EC10, EC20, and EC50 values for arithmetic TWM sulfide hydroponic exposures.

	Wt change	Estimate	In 95% CI	Wt gain	Estimate	In 95% CI
EC10	3.474 mg	103 µg/L	<11 µg/L - 162 µg/L	3.465 mg	106 µg/L	<11 µg/L - 158 µg/L
EC20	3.088 mg	140 µg/L	95 µg/L - 195 µg/L	3.080 mg	141 µg/L	98 µg/L - 188 µg/L
EC50	1.930 mg	227 µg/L	174 µg/L - 312 µg/L	1.925 mg	231 µg/L	182 µg/L - 335 µg/L

Predicting weight gain from geometric time weighted mean sulfide concentration

Fig. A5-3 shows the log logistic curve fits for both predicting the weight change (Fig. A5-3A) and predicting the weight gain (Fig. A5-3B).

Figure A5-3. Logistic regressions using geometric time-weighted mean (TWM) sulfide hydroponic concentrations to predict plant growth. A) weight change. B) weight gain.



The geometric time weighted mean results in lower mean estimates of sulfide, and therefore, the curve has almost no “plateau”, but shows an effect on wild rice growth almost immediately. As a result, the EC10 values are very low (around 40 µg/L), and have tighter confidence intervals than the other two estimates of sulfide concentration.

Table A5-3. EC10, EC20, and EC50 values for geometric TWM sulfide hydroponic exposures.

	Wt change	Estimate	In 95% CI	Wt gain	Estimate	In 95% CI
EC10	3.558 mg	38 µg/L	<11 µg/L - 67 µg/L	3.551 mg	39 µg/L	<11 µg/L - 66 µg/L
EC20	3.163 mg	59 µg/L	37 µg/L - 96 µg/L	3.157 mg	60 µg/L	40 µg/L - 90 µg/L
EC50	1.977 mg	128 µg/L	89 µg/L - 216 µg/L	1.973 mg	127 µg/L	91 µg/L - 224 µg/L

Appendix 6. EC10 estimates from experimental mesocosms

Data used

Dr. John Pastor conducted a multi-year experiment where his team added sulfate to outdoor mesocosms in which wild rice grew in natural sediment at five different levels of sulfate (six replicates of each: control, 50, 100, 150, and 300 mg/L). Porewater sulfide was measured in the sediment of each of the 30 mesocosms in August, 2013. The experiment had been initiated in June, 2011.

The variables used for this analysis are:

Porewater sulfide.

To quantify wild rice response:

- Percent of filled (viable) seeds
- Number of plants that emerged from the sediment

Percent filled seeds as a function of porewater sulfide

Using all the data, the EC10 using percent filled seeds and the baseline sulfide of 69.28 $\mu\text{g/L}$ is: 288 $\mu\text{g/L}$ with a 95% confidence interval of (0, 648).

However there was a statistical outlier, mesocosm #29, which had a porewater sulfate of 1180 $\mu\text{g/L}$ but still had 53.5% of the seeds filled. The number of plants that emerged was low. Since this mesocosm had a Cook's distance of 0.6, which was twice as high as the next highest distance (Fig. A6-1), the regression was recalculated without mesocosm #29. Without mesocosm #29, the EC10 is: 228 $\mu\text{g/L}$ with a 95% confidence interval of (0, 414) (Fig. A6-2).

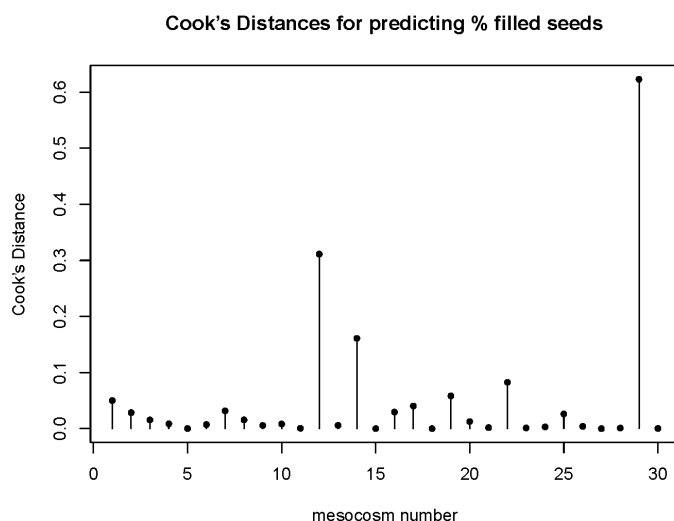


Figure A6-1. Cook's distances for regression of percent filled seeds against porewater sulfide.

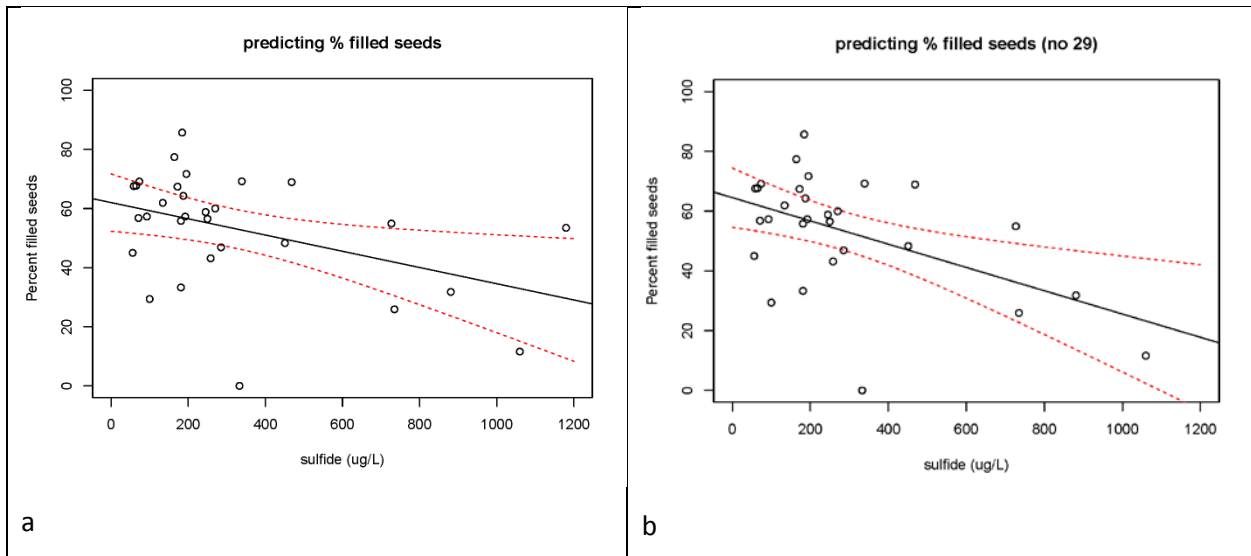


Figure A6-2. Regressions of percent filled seeds against porewater sulfide in wild rice mesocosms sampled August 2013 (Pastor et al., 2017), with all data (a), and without outlier mesocosm #29 (b).

Number of emerged plants as a function of porewater sulfide

The EC10 using number of emerged plants does require a log transformation of sulfide in order to fit a linear model. When this is done, the EC10 is:

121 $\mu\text{g/L}$ with a 95% confidence interval of (6, 241) (Fig. A6-3).

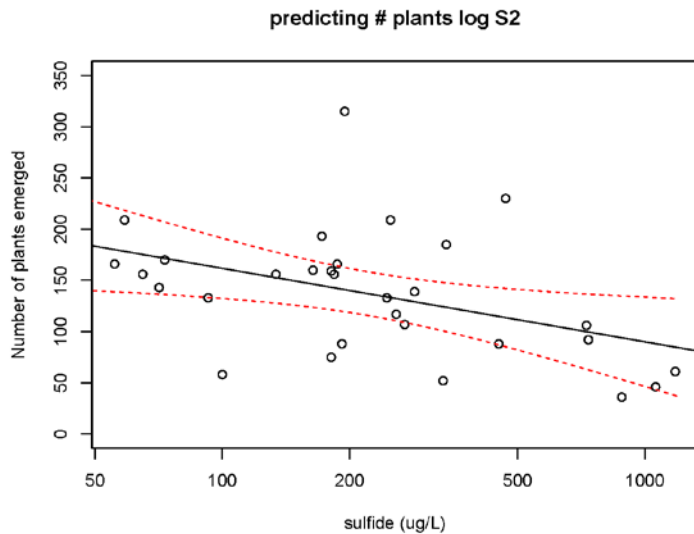


Figure A6-3. Regressions of number of plants emerged against porewater sulfide in wild rice mesocosms sampled August 2013 (Pastor et al., 2017).

Appendix 7. Protective sulfide estimates from MPCA-sponsored field survey

Goal of analysis

Utilizing the large dataset from the MPCA-sponsored wild rice field study, to investigate the relationships between potential protective sulfide levels and error rates, wild rice presence, and wild rice density.

Data used

The Class B data set from the wild rice field study was analyzed to identify potential protective sulfide concentrations. This data set includes 108 different sites measured as close to mid-August as possible. Relevant measurements include:

- Porewater sulfide (in $\mu\text{g/L}$)
- Wild rice density (stems/ m^2)

Potential protective sulfide concentrations

Using the wild rice field survey data, three different methods were used to identify potential protective sulfide concentrations: (1) EC10 estimates from regressions, (2) Visual examination of a graphical representation of the proportion of sites with wild rice present, and (3) Change-point analysis.

1. EC10 estimates from regressions of field data:

The binary logistic regression that relates porewater sulfide to the presence and absence of wild rice can be calculated a number of different ways, producing different EC10 estimates of protective sulfide concentrations. The production of multiple EC10 estimates suggests that the selection of a protective concentration of sulfide should be the outcome of weighing multiple lines of evidence, rather than relying on a single calculation. Each estimate has a range of uncertainty. A reasonable protective sulfide concentration lies within the overlapping uncertainty ranges. An exploration of uncertainty around the estimates was recommended by the independent peer review panel (ERG 2014, p. 6).

Initially, MPCA conducted binary logistic regressions of wild rice presence/absence against linear sulfide concentrations. However, re-examination of the data used in the logistic regression found that porewater sulfide concentrations are skewed (Fig. A7-1a), and that a log-10 transformation would approximate normality (Fig. A7-1b).

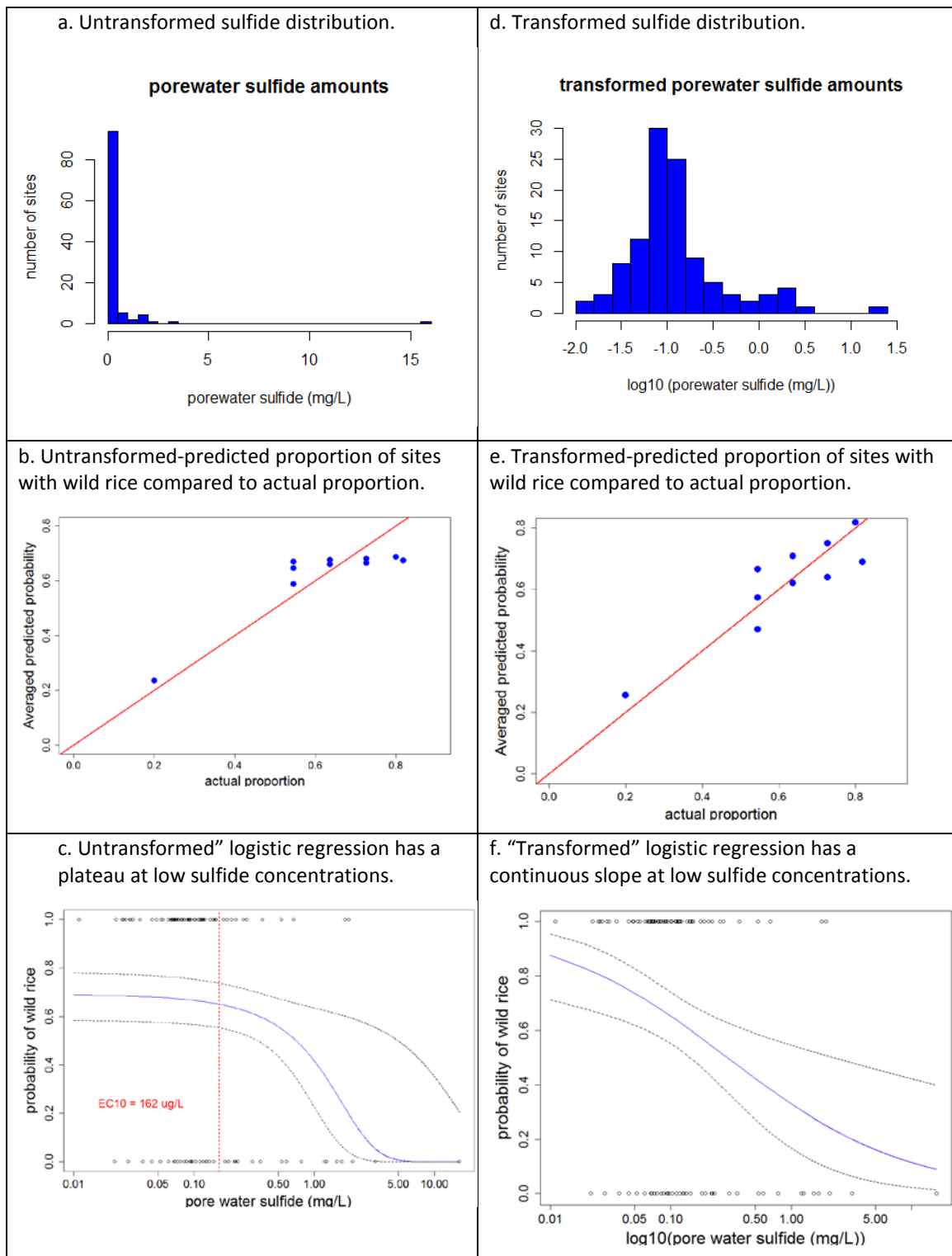


Figure A7-1. Comparison of the use of untransformed data in logistic regression (a-c) to the use of log-10 transformed data (d-f). Sulfide is graphed in both c and f on a log scale, but the modeling used untransformed data in c. In b and e the data are divided into deciles in order to assess the accuracy of the logistic prediction.

It is apparent that transforming the data results in more accurate predictions of the proportion of sites with wild rice (the data follow the 1:1 line better in Fig. A7-1e than in Fig. A7-1b). However, the logistic curve based on transformed sulfide data (Fig. A7-1f) is problematic for the calculation of an EC10

because of the absence of a plateau. Calculation of an EC10 assumes that there is a plateau, or baseline, of “no effect” of a stressor, from which a 10 percent effect adverse can be calculated. But the re-calculated logistic curve (Fig. A7-1 f) exhibits a continuous slope as sulfide declines, all the way down to the analytical reporting limit of 11 $\mu\text{g/L}$, which makes it difficult to identify a “baseline” probability from which to calculate a 10 percent effect. To complete the exercise of calculating an EC10, MPCA assumed a baseline probability based on the proportion of sites with wild rice for the 10 sites with the lowest sulfide concentration (8 out of 10 sites had wild rice, a baseline probability of 0.80). Because low-transparency sites (< 30 cm) generally do not support wild rice regardless of how low sulfide is in the porewater, it is likely more accurate to calculate an EC10 for sulfide from a data set that does not include low-transparency sites that did not support wild rice because of low light (Fig. A7-2b).

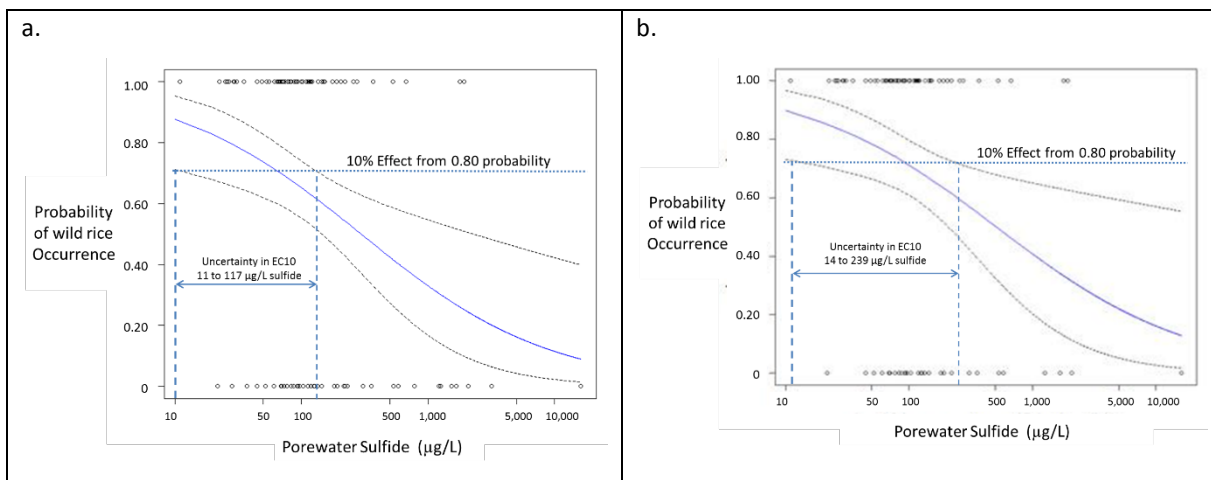


Figure A7-2. a. Logistic regression of all data in Class B, showing the 10% effect horizontal line. b. Logistic regression of all data with transparency > 30 cm. The horizontal 10% effect lines span the distance between the lower and upper 95% confidence intervals (Table A7-1), which is the indication of uncertainty (the confidence intervals shown are calculated for the probability at a given sulfide concentration, not for uncertainty in sulfide for a given effect level).

The calculated EC10 values range from 58 $\mu\text{g/L}$ (all 108 sites) to 93 $\mu\text{g/L}$ (the 96 sites with transparency greater than 30 cm). The uncertainty of the EC10 estimates was quantified by identifying the range of sulfide concentrations that contain a given EC10 wild rice proportion in their 95% confidence interval based on the binary logistic regression (Fig. A7-1; Table A7-1). Note that the uncertainty around the calculated EC10 values is relatively large, ranging from the sulfide reporting limit in the field survey, < 11 $\mu\text{g/L}$, to 239 $\mu\text{g/L}$ (Table A7-1).

Table A7-1. Calculated sulfide EC10 values, based on wild rice presence/absence.

Data Set	EC ₁₀ (µg/L)	Uncertainty of sulfide concentrations around the EC ₁₀ (µg/L)	
		Lowest value within the 95% CI	Highest value within the 95% CI
All Sites (N=108)	58	< 11	117
Sites with transparency > 30 cm (N=96)	93	14	239

The peer review panel concluded that the MPCA field survey provided some of the best data available to investigate the relationship between wild rice and sulfide, and recommended that MPCA conduct a statistical analysis of the probability of wild rice occurrence as a function of the porewater sulfide levels (ERG, 2014). Binary logistic regression (BLR), described in Part B of Chapter 1, is “binary” in the sense that it classifies field sites as having, or not having, a wild rice population – the density of the wild rice is irrelevant to the classification. It is useful to note that Minnesota lakes with wild rice that are monitored by the 1854 Treaty Authority average 46 stems/m² with a median density of 30 stems/m² (Appendix 4), and in the MPCA-sponsored field survey, average wild rice density was 51 stems/m² with a median density of 41 stems/m² (Myrbo et al., in press-1).

MPCA’s use of the binary logistic regression for the calculation of an EC₁₀ was questioned in a comment on the March 2015 Draft Proposal as a non-standard statistical technique (MCC, 2015). MPCA found no objections raised in the scientific literature when this same statistical technique was used to assess the effect of selenium on mallard egg viability and duckling mortality (Adams et al., 2003). The Adams et al. (2003) study was subsequently cited favorably in an EPA guidance document (EPA, 2007); this guidance was subsequently explicitly approved by the EPA Science Advisory Board as an exceptional analysis of toxicity in a field setting: “Toxicity in wildlife from metals exposures is generally poorly understood and is rarely quantified in field settings. A few notable exceptions are those mechanisms described in avian waterfowl exposure to [selenium] (Adams et al., 2003)...” Thus, it appears that MPCA’s statistical analysis of the wild rice field data, although perhaps not traditional, is supported by the scientific literature, EPA guidance, and the Science Advisory Board.

A protective sulfide concentration was identified as a 10% decrease (a 10% effect concentration, or EC₁₀) from control conditions using a logistic regression where the probability of wild rice presence was predicted against log 10 transformed sulfide concentration (Class B data with water transparency > 30 cm data). The EC₁₀ was 93 µg/L (95% confidence interval of 14 -239 µg/L). However, the “control” condition, or baseline, was difficult to define, since there was no range of sulfide concentrations where the probability of wild rice was constant. Therefore, the EC₁₀ of 93 µg/L is misleadingly precise.

2. Visual examination of a graphical representation of the proportion of field sites with wild rice present against sulfide concentrations:

The visual examination identified a protective sulfide concentration of 120 µg/L. Focusing on sulfide between 20 and 1000 µg/L, the proportion of sites with wild rice present above each measured sulfide concentration was graphed for (1) all sites or (2) for all sites with water transparency greater than 30 cm.

(30 cm is identified as a threshold because 11 of 12 sites with lower transparency did not have wild rice; Myrbo et al. (submitted-2) identified water transparency as one of the environmental variables aside from porewater sulfide that controls wild rice presence in Minnesota waterbodies). The proportions above each sulfide concentration were graphed versus the sulfide concentration. In both graphs, a potential protective sulfide concentration was identified as a dip at 120 $\mu\text{g}/\text{L}$ in the proportion of sites with wild rice (Fig. A7-3).

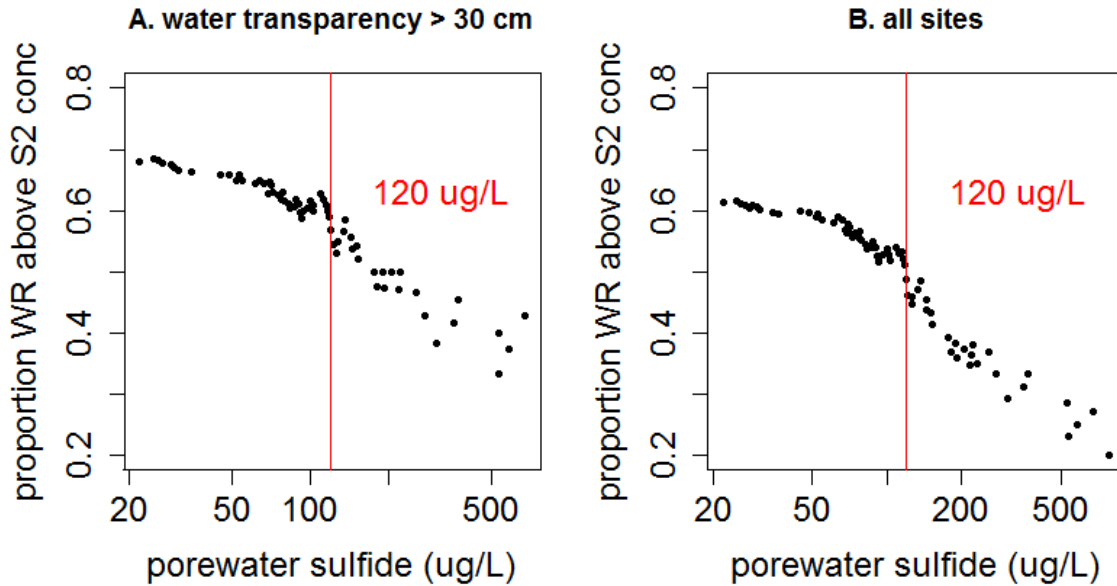


Figure A7-3. Percent of sites above the sulfide concentration that contain wild rice. A. Class B sites where water transparency was greater than 30 cm (N=96). B. All sites in Class B (N=108).

3. Change-point analysis of the field data:

To assess whether the visual identification of 120 $\mu\text{g}/\text{L}$ is supported through a recognized objective procedure, change-point analysis was used to identify sulfide concentrations that are associated with changes in the trend in the density of wild rice in relation to porewater sulfide. A potential protective sulfide concentration was found by ordering the data from lowest sulfide to highest sulfide concentration, then performing change-point analysis on the wild rice density (average number of stems per square meter). The resulting sulfide concentrations was 112 $\mu\text{g}/\text{L}$, although the confidence interval is wide (95% confidence interval 25-368 $\mu\text{g}/\text{L}$).

Appendix 8. Empirical demonstration that a MBLR probability of 0.5 is most accurate

Background

The MPCA used multiple binary logistic regression (MBLR) to develop an equation to calculate the surface-water sulfate concentration corresponding to a porewater sulfide concentration of 120 µg/L (given the TOC and extractable iron in the sediment of a particular wild rice water). MPCA chose to use MBLR to avoid re-transformation bias in solving for the sulfate concentration that is incurred with some other statistical models, following the recommendation in Pollman et al. (in press).

However, many people are unfamiliar with the steps necessary to derive an equation that predicts sulfate from a MBLR model. First, the output of a logistic regression cannot be used to directly predict the sulfate concentration of interest. Rather, the MBLR-based model output is an equation that predicts the probability that sulfide would be greater than 120 µg/L (given the TOC and extractable iron in the sediment of the wild rice water). The model can be rearranged to predict the sulfate concentration of interest, but only if a probability is inserted into the model. This TSD asserts that the appropriate probability is 0.5.

Theoretical basis for choosing 0.5

A probability of 0.5 yields sulfate concentration predictions that have an equal chance of being too high or too low. Choosing probabilities that are higher or lower than 0.5 would bias predictions either generally higher than the best sulfate standards (probabilities greater than 0.5) or lower (probabilities less than 0.5). For instance, if a probability of 0.8 is used, a sulfate standard would be calculated that produces an 80% probability of the of the porewater sulfide being over 120 µg/L, which would be under-protective. Conversely, if an equation is developed with a probability of 0.2, the resulting equation would produce an 80% probability of porewater sulfide being below 120 µg/L, which would be over-protective. A probability of 0.5 is balanced between the possibilities of over- and under-protection, and is most likely to accurately calculate a sulfate standard that is related to 120 µg/L.

Choosing a probability of 0.5 therefore has a result similar to the goal of the more familiar statistics that seek to characterize the most likely prediction, by achieving a “best fit” or describing the “central tendency” through calculations of medians, means, or linear regressions. All of these approaches use a variety of methods to identify a value that is neither too high nor too low. MBLR maximizes the probability of choosing an accurate dependent variable (sulfate, in this case), whereas other approaches maximize accuracy through other methods; for instance linear regression minimizes the distance of the best fit line to the data points of the dependent variable.

Empirical demonstration that 0.5 yields the most accurate prediction of a protective sulfate concentration

The MPCA has identified a porewater sulfide concentration of 120 µg/L as the optimal concentration to serve as a regulatory protective threshold. This concentration of 120 µg/L is optimal to protect wild rice because the MPCA-sponsored survey of potential wild rice waters found that the density and probability of occurrence of wild rice decreases at or above that concentration.

If porewater sulfide could be directly regulated, there would be no need to regulate sulfate. However, porewater sulfide is a function of three variables (sulfate, sediment TOC, and sediment iron), and only sulfate makes sense to regulate, since the sediment characteristics are natural landscape features. Therefore, the goal is to choose a model that can predict sulfate concentrations that most accurately correspond to actual porewater sulfide concentrations.

Even though 120 µg/L is the optimal sulfide concentration to serve as a threshold, it is not perfect. That is, wild rice does sometimes occur in waterbodies where the field survey found porewater sulfide at concentrations greater than 120 µg/L. In addition, although the density of wild rice (measured in stems per square meter, stems/m²) was generally lower when sulfide concentrations were greater than 120 µg/L, some of the waterbodies had dense rice (described here as greater than 40 stems/m²).

Nevertheless, it is clear that wild rice is more likely to be present, and more likely to be dense, if a waterbody has porewater sulfide less than 120 µg/L (Table A8-1).

Table A8-1. Rates of wild rice presence and density above and below 120 µg sulfide/L. (Class B dataset)

Stems/m ²	Sulfide less than 120 µg/L			Sulfide greater than 120 µg/L		
	Number of sites	% of all sites	% of sites with wild rice	Number of sites	% of all sites	% of sites with wild rice
Over 40	28	41%	57%	6	15%	33%
Between 10 & 40	15	22%	31%	3	8%	17%
Less than 10	6	9%	12%	9	23%	50%
No wild rice observed	20	29%	0%	21	54%	
Total	69	100%	100%	39	100%	100%

In waterbodies with porewater sulfide less than 120 µg/L, it is 2.7 times more likely to encounter dense wild rice (over 40 stems/m²) than in higher (>120 µg/L) sulfide waters (41% vs. 15%). In addition, wild rice is less likely to be found in waterbodies with higher sulfide; in 54% of high-sulfide waterbodies wild rice was not found, in contrast to 29% of the low-sulfide waterbodies. Furthermore, when wild rice is present in a high-sulfide waterbody, it is 4.1 times more likely to have low density wild rice (less than 10 stems/m²) (50% vs 12%). A low density of wild rice (less than 10 stems/m²) may indicate that the population is not reproducing or germinating well (Table A8-1).

The goal of choosing an equation that calculates the sulfate concentration corresponding to a sulfide concentration of 120 µg/L is to find an equation that best corresponds to the empirical observations seen in the effect of porewater sulfide on both wild rice density and presence/absence (Table A8-1).

The ability of the MBLR-based equation to reproduce the pattern of empirical observations can be assessed with the data from the MPCA field survey. The proposed equation produces a calculated protective sulfate concentration (CPSC) that corresponds to 120 µg/L, based on the sediment TOC and iron from each waterbody. In principle, sulfate concentrations greater or less than the CPSC should have the same effect on wild rice density and presence/absence as when porewater sulfide is greater or less than 120 µg/L. The question is, then, what probability produces the most accurate equation at reproducing the pattern of measured porewater sulfide?

First, let us examine the effect that using different probability levels to develop sulfate equations have on the number of waterbodies with different levels of wild rice density. If equations are developed with a high probability that sulfide would be greater than 120 µg/L (e.g., 0.9), then CPSCs are very high; only 4 waterbodies would exceed the calculated sulfate standards to protect wild rice, and only one of the four had wild rice, and the density is less than 10 stems/m² (Table A8-2). Using a high probability would therefore produce calculated standards that would be severely under-protective. Conversely, if equations are developed with a low probability that sulfide would be less than 120 µg/L (e.g., 0.1), then CPSCs are very low, and 76% of waterbodies would exceed the calculated sulfate standards (Table A8-2). In reality, only 36% of waterbodies have porewater concentrations greater than 120 µg/L, so using a low probability would be severely over-protective.

In Table A8-2, the number waterbodies predicted by the equations developed with a range of probabilities (0.1 to 0.9) that sulfide would exceed 120 µg/L can be compared to the empirically

observed sulfide levels in the wild rice survey of 108 potential wild rice waterbodies. The predicted numbers that most closely match the empirical observations are printed in bold. Densities that have no bold densities are associated with probabilities of 0.1, 0.2, 0.8, and 0.9. Therefore, equations developed with probabilities 0.3 through 0.7 are most likely to reflect the observed relationship between porewater sulfide and wild rice density.

Table A8-2. Effect that different probability levels (that sulfide would exceed 120 µg/L) have on the number of waterbodies in four levels of wild rice density, compared to the empirically observed relationship of wild rice with porewater sulfide (last line of the table). Calculations are based on the Class B data set, which best approximates a probabilistic data set.

Probability that sulfide >120 µg/L	Sulfate less than CPSC				Sulfate greater than CPSC			
	Density over 40 stems/m ²	Density between 10 & 40 stems/m ²	Density less than 10 stems/m ²	No wild rice observed	Density over 40 stems/m ²	Density between 10 & 40 stems/m ²	Density less than 10 stems/m ²	No wild rice observed
0.9	34	18	14	38	0	0	1	3
0.8	32	18	13	35	2	0	2	6
0.7	29	16	8	33	5	2	7	8
0.6	26	16	8	29	8	2	7	12
0.5	24	16	8	24	10	2	7	17
0.4	24	14	5	22	10	4	10	19
0.3	21	13	3	19	13	5	12	22
0.2	16	11	2	18	18	7	13	23
0.1	9	5	0	12	25	13	15	29
	Observed sulfide less than 120 µg/L				Observed sulfide greater than 120 µg/L			
	28	15	6	20	6	3	9	21

Second, the range of potential probabilities can be further narrowed by comparing the success of the calculated equations in reproducing the pattern that is empirically observed between porewater sulfide and the presence and absence of wild rice, in contrast to comparing against wild rice density (evaluated above). The best matches occur when the probability is set to 0.5 (Table A8-3).

Two logical goals of developing a water quality standard to protect wild rice is to maximize the occurrence of wild rice when a waterbody conforms to the standard, and to minimize the occurrence of wild rice when a waterbody exceeds the standard. While wild rice occurrence is the protection goal, significant occurrence of wild rice at levels above the calculated standards would mean the calculation is inaccurate. These two goals are best met with a probability of 0.5. When sulfate is less than the calculated standard, the maximum proportion with wild rice occurs when the equation is developed with a probability of 0.5 (Table A8-3). Conversely, when sulfate is greater than the calculated standard, the minimum proportion also occurs when the equation is developed with a probability of 0.5 (Table A8-3).

Table A8-3. Effect that different probability levels (that sulfide would exceed 120 µg/L) have on the number of waterbodies with and without wild rice, compared to the empirically observed relationship of wild rice with porewater sulfide (last line of the table). (WR = wild rice) (Class B dataset)

Probability that sulfide >120 µg/L	Sulfate less than CPSC			Sulfate greater than CPSC		
	WR vs no WR	total	% with wild rice	WR vs no WR	Total	% with wild rice
0.7	53 vs 33	86	62%	14 vs 8	22	64%
0.6	50 vs 29	79	63%	17 vs 12	29	59%
0.5	<u>48 vs 24</u>	<u>72</u>	<u>67%</u>	<u>19 vs 17</u>	<u>36</u>	<u>53%</u>
0.4	43 vs 22	65	66%	24 vs 19	43	56%
0.3	37 vs 19	56	66%	30 vs 22	52	58%
	Observed sulfide less than 120 µg/L			Observed sulfide less than 120 µg/L		
	49 vs 20	69	71%	18 vs 21	39	46%

In conclusion, use of a probability of 0.5 produces an equation that produces sulfate concentrations that correspond most accurately to the sulfide threshold of 120 µg/L. Use of probabilities less than 0.5 produce equations that are over-protective; the calculated sulfate standards are lower, and would classify more waterbodies as exceeding standards that have porewater sulfide less than 120 µg/L and dense wild rice populations. Conversely, use of probabilities greater than 0.5 produce equations that are under-protective; the calculated standards are higher, and would classify fewer waterbodies as exceeding standards that actually have porewater sulfide greater than 120 µg/L and less dense wild rice populations. Use of a probability of 0.5 is therefore the best approach for developing an equation that calculates protective sulfate concentrations.

Appendix 9. Examination of a proposed protective sulfide of 300 µg/L

Goal of analysis

Other scientists have presented evidence in favor of 300 µg/L as a potential protective sulfide concentration threshold. The goal here is to examine 300 µg/L versus 120 µg/L as potential protective sulfide thresholds by looking at how each concentration is associated with wild rice occurrence and density.

Data analyzed

The field data from 2012-2013 was analyzed, during which time 108 different waterbodies were sampled. If there was more than one sample from a waterbody in this time, the sample that was closest to August 11th was used to ensure consistency. This dataset, which approximates a probabilistic survey of potential wild rice waters, is referred to as the Class B dataset. Wild rice density (in stems per square meter) and porewater sulfide (in µg/L), among other variables, were measured at each site.

Wild rice presence

In order to examine the pattern of wild rice occurrence and density associated with the two potential sulfide concentrations thresholds, three groups were created: a group with sulfide below the MPCA proposed standard of 120 µg/L, a group with sulfide concentrations between 120 µg/L and the proposed 300 µg/L threshold, and, finally, a group with sulfide concentrations above 300 µg/L. If the 300 µg/L sulfide concentration is protective, then it would be expected to only find a significant difference between the group under 300 and the group over 300, but not between the group under 120 and the group over 120 but less than 300. If the 120 µg/L is the better choice for a protective sulfide threshold, it would be expected that wild rice presence is significantly greater below 120 than above 120 (both the 120-300 group and the above 300 group). However, it would not be expected to see a significant difference between the 120-300 µg/L group and >300 µg/L group. The number of waters for each group are in Table A9-1:

Table A9-1. Number of Class B waters in three different groups, separated by porewater sulfide concentration.

	Under 120 µg/L	between 120 and 300 µg/L	Over 300 µg/L
Wild rice present	49	13	5
Wild rice absent	20	9	12

First, the question is if there are any differences between the three groups. A chi square test for independence was performed to see if any of the three sulfide level groups were different with respect to wild rice presence. With a p value of 0.0063, there is evidence of a significant difference among the three sulfide level groups with regards to presence or absence of wild rice.

To find out where the difference lies, the odds of wild rice presence for any two sulfide concentration groups were compared, and a two sample proportion test with continuity correction was conducted to obtain the p value (Table A9-2).

Table A9-2. Comparison of the odds of wild rice presence between any two sulfide concentration groups. Significant differences are in bold.

Group 1	Group 2	Odds ratio	95% Confidence Interval	P value
Under 120	Between 120 & 300	1.70	(0.63, 4.59)	0.434
Under 120	Over 300	5.88	(1.83, 18.86)	0.0037
Between 120 & 300	Over 300	3.47	(0.90, 13.31)	0.1286
Under 120	Between 120 & 300 plus Over 300	2.86	(1.26, 6.47)	0.0334
Under 120 plus between 120 & 300	Over 300	5.13	(1.65, 15.93)	0.0060

One advantage of expressing statistical results with odds ratios is that they are relatively easy to put into words. For example, looking at the second line of Table A9-2, the odds of having wild rice when the sulfide is under 120 (group 1) is 5.88 times the odds of having wild rice when the sulfide is over 300 (group 2). The 95% confidence interval for this odds ratio of 5.88 ranges from 1.83 to 18.86. Since this interval does not include 1.0 (a value of 1.0 means the odds are the same), there is a significant difference between the under 120 and the over 300 groups. Since the interval is entirely over 1.0, the under 120 µg/L group (group 1) has significantly higher odds of having wild rice than the over 300 group (group 2). The p value for a proportion test based on these numbers is 0.0037.

Based on these odds ratios and p values, there is not a significant difference between the under 120 and the between 120 & 300 groups with regards to wild rice presence (Table A9-2). However, there is not a significant difference between the between 120 & 300 group and the over 300 group, either. Only the under 120 is significantly different from the over 300 group. Since there is not a significant difference between the 120 to 300 group versus the over 300 group, it is not possible to definitively say that 300 is protective. However, this could be largely due to the small number of sites in each group—the odds ratio is a not-statistically-significant 3.47 (p=0.13), which means that the odds of having wild rice when the sulfide is between 120 and 300 µg/L would be almost 3.5 times the odds of having wild rice when the sulfide is above 300 µg/L. Further, there is not a significant difference between under 120 and between 120 & 300 (p=0.43), so even though wild rice is significantly more likely to be present under 120 µg/L sulfide than above 300 µg/L, based on presence/absence, it is not possible to conclude that 120 is better than 300 as a protective sulfide value.

The final two lines of Table A9-2 are calculated in an attempt to merge groups to look at 120 as a cutoff versus 300 in hopes it is possible to derive a more definitive answer. If the two groups are merged together and compared to the third group, there are significant differences between the groups. The odds of having wild rice when sulfide is under 120 µg/L are 2.86 times the odds of having wild rice when the sulfide is over 120 µg/L. Since the confidence interval is entirely above 1.0, the sites with sulfide under 120 µg/L are significantly more likely to have wild rice than those over 120 µg/L sulfide (p=0.03). Moreover, the odds of having wild rice below 300 µg/L sulfide are 5.13 times the odds of finding wild rice above 300 µg/L, and this is also statistically significant. Therefore, based on presence/absence data it is not possible to determine whether the 120 µg/L or 300 µg/L sulfide concentration threshold is more protective.

Wild rice density

The analysis was then expanded to examine the density of the wild rice stands above and below these potential protective sulfide concentrations. Because wild rice density fluctuates from year to year in natural wild rice waters, low density is not necessarily indicative of an unhealthy population in any single waterbody. However, any condition that is statistically associated with lower density among a variety of different waterbodies should be suspected of impairing the reproduction and/or germination of wild rice populations, and therefore decreasing the probability that a population will successfully persist over the long term. Conversely, conditions statistically associated with higher wild rice density can be interpreted as associated with a higher probability that a wild rice population will persist over the long term.

To statistically associate porewater sulfide with low and high wild rice densities, reasonable thresholds for identification of relatively low density and high density were identified. Low and high density thresholds of 10 and 40 stems/m² were assessed against the long-term wild rice surveys conducted by the 1854 Treaty Authority on ten relatively un-impacted wild rice waters over 12 years (Appendix 4). The long-term data have a median, or most typical density, of 30 stems/m² among the ten waterbodies. The “high” density of 40 stems/m² corresponds to the 56th percentile (44% of annual visits to these ten waterbodies had wild rice density greater than 40 stems/m²). The “low” density of 10 stems/m² corresponds to the 14th percentile; in the long-term survey, 86% of the visits to these ten waterbodies were greater than 10 stems/m². For comparison, the sites with wild rice in the Class B dataset have a median density of 41 stems/m². In other words, Class B sites rated as “high density” had greater wild rice density than 50% of the Class B sites with any wild rice, and 56% of the surveys conducted by the 1854 Treaty Authority.

Therefore, the number of sites in the Class B dataset with wild rice density was examined in three categories: over 40 stems/m², below 10 stems/m², and in between those two ranges. Therefore, there are four groups for analysis: the dense group (over 40 stems/m²), the intermediate group (between 10 and 40 stems/m²), the sparse group (under 10 stems/m²), and the group with no wild rice. The information is in Table A9-3, below. There are not only differences in the proportion with wild rice, there are also differences among the density groups.

Table A9-3. Number of Class B sites of different density in three different sulfide concentration groups.

	Under 120 µg/L	between 120 and 300 µg/L	Over 300 µg/L
Over 40 stems/m²	28	5	1
Between 10 and 40 stems/m²	15	2	1
Below 10 stems/m²	6	6	3
Wild rice absent	20	9	12

There are enough groups with a low number of observations per group that a Fisher’s test (nonparametric) is preferred over a Chi square test (parametric) to confirm statistical differences. A Fisher’s test of independence resulted in a p value of 0.002, which indicates that at least two groups differ.

The odds ratios and confidence intervals were then calculated to compare the under 120 to the 120-300 group. In addition, a two-sample proportion test with continuity correction was calculated to find the probability that the groups have the same proportion in each group. The use of the continuity correction on small sample sizes per group results in a slightly different test than the odds ratio, so a confidence interval that does not include 1 (and indicates a significant difference)

may occur when the p value is over the standard cutoff of 0.05. For the sites with sulfide over 300 µg/L, there are not enough lakes per group in the 3 wild rice groups to run odds ratios or proportion tests. Therefore, the odds ratios all compare the under 120 group to the 120-300 group (Table A9-4).

Table A9-4. Results of a two-sample proportion test to find the probability of groups having the same proportion in each group. Comparisons that are significantly different are in bold.

Group 1	Group 2	Stem density	Odds ratio	95% Confidence Interval	P value
Under 120	Between 120 & 300	>40 vs 10-40	0.75	(0.13, 4.32)	1.0
Under 120	Between 120 & 300	>40 vs <10	5.60	(1.28, 24.56)	0.044
Under 120	Between 120 & 300	>40 vs no WR	2.13	(0.73, 7.46)	0.235
Under 120	Between 120 & 300	>40 vs <40	3.47	(0.90, 13.31)	0.375
Under 120	Between 120 & 300	>40 vs <10 & no WR	3.23	(1.03, 10.45)	0.072
Under 120	Between 120 & 300	10-40 vs <10	7.50	(1.17, 48.15)	0.065
Under 120	Between 120 & 300	10-40 vs no WR	3.38	(0.63, 17.97)	0.262
Under 120	Between 120 & 300	10-40 vs <10 & no WR	3.54	(1.28, 9.84)	0.024
Under 120	Between 120 & 300	>10 vs <10	6.14	(1.54, 24.54)	0.018
Under 120	Between 120 & 300	>10 vs no WR	2.76	(0.90, 8.48)	0.127
Under 120	Between 120 & 300	>10 vs <10 & no WR	3.55	(1.28, 9.84)	0.024
Under 120	Between 120 & 300	<10 vs no WR	0.45	(0.11, 1.79)	0.429

The comparisons in Table A9-4 show that the groups with wild rice density at or above 10 stems/m² (whether they are over 40, between 10 and 40, or 10 and up) are significantly different from the groups with wild rice density below 10 stems/m² (whether they are between 0 and 10, or both no wild rice and between 0 and 10). Since the confidence intervals are all over 1.0, there is significantly higher odds of observing dense wild rice if the sulfide concentration is below 120 µg/L than if the sulfide concentration is between 120 and 300 µg/L.

Discussion

Based on wild rice presence versus absence, it is not possible to find a statistically significant difference between those sites with sulfide below 120 µg/L and those with sulfide between 120 µg/L and 300 µg/L. However, when wild rice density is examined, there is significantly higher density for those sites with sulfide below 120 µg/L compared to those with sulfide between 120 µg/L and 300 µg/L. Therefore, while sulfide concentrations between 120 µg/L and 300 µg/L do not produce a significant difference in the proportion of sites with wild rice, sites with porewater sulfide less than 120 µg/L are more likely to have dense wild rice than stands with sulfide between 120 µg/L and 300 µg/L. Wild rice waters with sulfide less than 120 µg/L are 5.6 times as likely as sites with sulfide between 120 and 300 µg/L to have dense (>40 stems/m²) than sparse wild rice (<10 stems/m²).

It should be noted that the statistical tests described in this report were conducted in accordance with a fundamental assumption of statistical analysis, which is that groups being compared are independent of

each other. That is, there was no overlap in the waterbodies between groups; no waterbody was in more than one group in any given statistical test. For instance, it would not be appropriate to statistically compare under 300 µg/L to under 120 µg/L, since 79% of the data in the under 300 µg/L group is the data from the under 120 µg/L group.

Conclusions

Based on a statistical analysis of the MPCA field survey, a protective sulfide concentration of 300 µg/L would not be as protective of wild rice as a concentration of 120 µg/L. The 22 waterbodies in the survey with sulfide concentrations between 120 and 300 µg/L were just as likely to have wild rice as waterbodies with sulfide below 120 µg/L, but were significantly less likely to have dense wild rice (greater than 40 stems/m²). Waterbodies in the <120 µg/L sulfide group are 5.6 times as likely to have dense wild rice as waterbodies in the 120-300 µg/L sulfide group.

Appendix 10. Educational credentials and qualifications of TSD and SONAR authors

The following MPCA staff conducted research, analyzed data, and authored the documents that are the basis for the MPCA proposal for establishing the level of sulfide and sulfate to protect wild rice.

Name	Qualifications
David Bael Economic Policy Analyst	Ph.D. Candidate, Applied Economics (University of Minnesota); Masters of Public Policy (University of Minnesota); B.S. Biology and B.S. Management Science (Massachusetts Institute of Technology); 5 years experience in the analysis of issues related to environmental economics and finance
Baishali Bakshi Economist, Water Quality Standards	Ph.D. Economics (University of California-Irvine); 8 years experience in data analysis and natural resources policy.
Gerald Blaha Research Scientist	University of Minnesota / Century College – coursework in biology / air and water analysis; 40 years experience in water quality standards and water use classifications
William Cole Supervisor	M.Aq. Aquaculture (Auburn University); B.S. Biology (John Brown University); 10 years experience developing and implementing water quality standards
Elise Doucette Policy Specialist	B.S. Biology (University of Minnesota-Duluth); 15 years experience in water quality regulations.
Patricia Engelking Planner Principal	B.A. Chemistry (Washington University); 28 years of experience in water quality
Stephanie Handeland Hydrologist	B.S. Geology (Winona State University); 22 years experience in water quality (NPDES) wastewater permitting; 9 years experience mining permitting
Elizabeth Kaufenberg Research Scientist	M.S. Water Resources Science (University of Minnesota); B.S. Environmental Science (UW-River Falls); 8 years experience in water quality (co-authored 4 papers)
Scott Kyser Wastewater Engineer	M.S. Civil and Environmental Engineering (University of Minnesota-Twin Cities); B.A. Biology (Gustavus Adolphus College); Registered Professional Engineer in Minnesota; 6 years experience in environmental engineering
Shannon Lotthammer Director	M.S. Ecology (University of Minnesota-Twin Cities); B.S. Biology (University of Minnesota-Duluth); 23 years experience in state and local environmental management.
Phillip Monson Research Scientist	M.S. Entomology (University of Maine); B.S. Biology (University of Minnesota-Duluth); 15 years experience developing and implementing water quality standards
Carol Nankivel Planner Principal	B.S. Soil Science (University of Minnesota); 35 years experience in preparation of administrative rules
Catherine Neuschler Manager	Master of Public Affairs (School of Public and Environmental Affairs, Indiana University); B.A. Environmental Studies (Macalester College); 11 years experience at the MPCA.

(continued)

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Michael Schmidt	J.D. (University of Minnesota); 8 years experience in the Clean Water Act and state water law
Marta Shore Data Analyst (now teaching in the biostatistics program at the University of Minnesota)	M.S. Statistics with supporting work in Biostatistics (University of Minnesota); B.A. Biology (University of Chicago); 10 years experience performing statistical analyses
Edward Swain Research Scientist	Ph.D. Ecology (University of Minnesota); B.A. Biology (Carleton College); 33 years experience in aquatic ecology research and publication of peer-reviewed findings (authored or co-authored 30 papers)

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