Findings of Expert Witnesses

(By Expert)

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Kurt Anderson

1. Summary

The MPCA has not provided a single documented example of a decline in natural wild rice stands from the impacts of elevated sulfide or sulfate.

The MPCA has not provided information regarding how sulfide, which they propose to be the toxic agent, might actually be impacting wild rice. They have not provided a mechanism regarding how, or when, sulfide affects wild rice above their proposed "protective" level.

The foundation of the proposed standard – the MPCA's proposed protective level of sulfide for wild rice – contains numerous unresolved contradictions, based on the MPCA's own field research and peer-reviewed, published laboratory studies.

1) Fifty-seven percent of the waterbodies with sulfide levels above the MPCA's proposed "safe" level have wild rice present – including some of the densest stands in the entire state.
2) The densest stand of wild rice in a natural lake had sulfide levels eleven times higher than what the MPCA is proposing as "safe".
3) The densest stand of paddy rice – the densest stand observed anywhere in the state-funded study– had sulfide levels over three times higher than what the MPCA is proposing as "safe". The MPCA has chosen to exclude this commercial paddy data.

The MPCA has chosen to exclude data from their own funded laboratory research showing no impact to wild rice in the rooting zone at levels nearly 30 times higher (no effect in the rooting zone at 3,060 ppb sulfide) than their proposed protective level of 120 ppb sulfide.

The MPCA has chosen to discount peer-reviewed, published research that studied the effects of sulfide on wild rice in the rooting zone – a study which was designed based on the MPCA's own hypothesis, and the MPCA's own peer reviewers' recommendations.

Removing sulfate to reach this unsupported "safe" level of sulfide represent an incredible potential risk to the State's economy. Costs for affected wastewater treatment facilities could be between $20-30 million for a flow of 1 million gallons per day (MGD) wastewater treatment facility. Estimates for larger wastewater treatment facilities could run into the hundreds of millions of dollars. Annual operations and maintenance costs could add millions more. These costs will fundamentally impact the economies and societies of Minnesotans for generations to come, funneling energy and monies away from the real challenges that wild rice faces.

The MPCA has not evaluated whether removing sulfate to these levels might actually result in detrimental impacts to the aquatic community, including ion imbalances and nutrient deficiencies due to removal of essential minerals and salts.

Put simply, I feel the proposed sulfide standard is not based on a complete and robust evaluation of the available data and scientific results. Based on the information in the enclosed comment letter and the other information received during the rulemaking process, I urge you to direct the MPCA to withdraw this flawed proposal, and address the significant contradictions and inconsistences discussed herein.

2. Executive Summary

The MPCA has used both field and laboratory (Pastor et al[i], Fort et al[ii]) data to rightly conclude that sulfate in the water column is not directly toxic to wild rice until levels far exceed the existing 10 parts per million (ppm) water quality standard.

Clearly, sulfate did not impact wild rice health or abundance at levels anywhere near the existing 10 ppm sulfate limit in either the laboratory of the field. The MPCA has rightly determined, based on modern research, to not continue implementation of the existing 10 ppm sulfate standard.

While the MPCA has concluded that this "safe" level of sulfide is 120 parts per billion, this proposed protective value – which is the very foundation of the proposed rule -- is far lower than the data would suggest is needed to protect wild rice. There is an abundance of contradictory information to support this assertion in the MPCA's own laboratory and research data, as well as in independent, peer-reviewed, published research conducted by a nationally-accredited laboratory

Certainly, it would also be unreasonable to set a protective level so far below the toxic threshold that it is meaningless to the resource we are seeking to protect. Yet there is much data to suggest this is exactly approach the MPCA has chosen to take: they have selected specific data, some it from questionable test design, to support a protective sulfide level so far below any toxic threshold it is essentially meaningless from an environmental benefit perspective. At the same time, they have chosen to ignore other contradictory lines of evidence that would support a more logical protective level -- with a significantly reduced error rate[iii] -- that would still set a protective level of sulfide far below toxic thresholds.

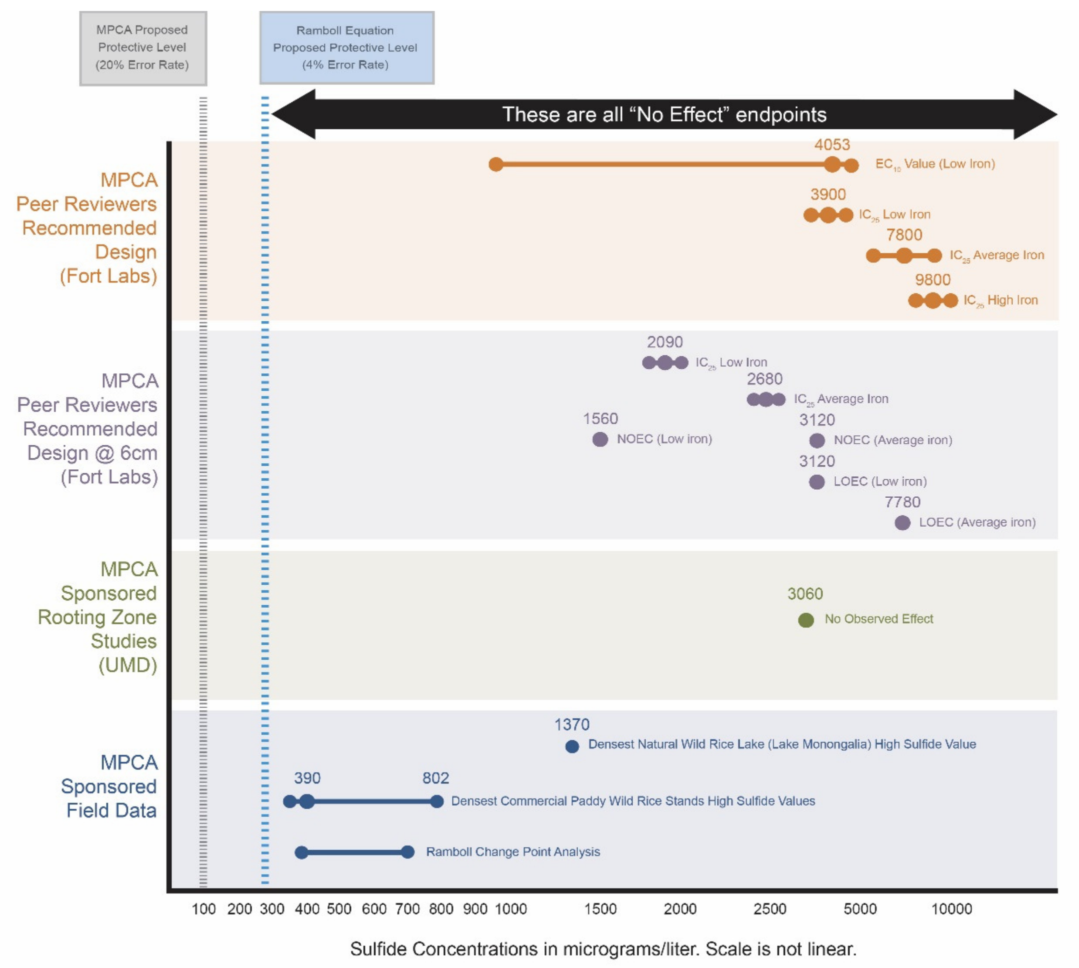
The MPCA's hydroponics testing at the University of Minnesota-Duluth (UMD) found no impact of sulfide at any tested concentration – up to 3,060 ppb – to wild rice in the rooting zone. This high level of sulfide – nearly thirty times higher than the MPCA's proposed protective threshold -- does not represent a toxic threshold. It's a "no impact" level, with wild rice roots in sulfide-enriched water actually growing better than the controls in some cases.[iv]

After these tests failed to show an impact, the MPCA made a policy decision to test the entire wild rice plant, including shoots and leaves. It cannot be overstated how unconventional and contradictory this policy decision was; it violates not only the MPCA theory that sulfide affects wild rice in *the rooting zone*, but also general scientific understanding of where sulfide actually occurs in the natural environment.

Despite numerous and widespread concerns with test design and test performance, the MPCA has chosen to rely on unconventional outdoor container (mesocosm) studies which killed 72-84% of the controls, and the MPCA has failed to acknowledge the serious quality control issues associated with this study. Significantly, the MPCA states the following on page 38 of the TSD: "The EC_{10} 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 (emphasis added)". It must be noted that any experiment that kills the control organisms over seventy percent of the time can reasonably be considered to contain one or more obvious flaws. Also, a design that completely eliminates the mitigating factors of groundwater upflow in the sediment can reasonable be considered to be an obvious flaw that warrants significant scrutiny. This failure to rigorously examine test results and test quality of these outdoor container studies is yet another indication that the MPCA has not taken a reasonable approach in developing multiple lines of evidence to support their proposed protective sulfide level.

The MPCA has taken a highly questionable approach toward evaluating field research data. They have failed to provide any explanation on a central contradiction to their proposed protective sulfide vale: i.e. why is wild rice growing in dense, thick stands at levels far above (up to ten times higher) than the proposed protective level?

The MPCA has chosen to exclude the information from commercial paddy rice in their evaluation, stating the management practices used in commercial paddies as the basis for this exclusion. Their rationale for this is entirely unclear, however, because the MPCA has yet to demonstrate how or when sulfide might be affecting wild rice.



The conservative nature of laboratory study results is also supported quite well by the field data, which shows the densest stands in the entire state have levels far exceeding the MPCA's proposed protective value. Again, these are not sparse, marginal stands; they represent the thickest natural wild rice waterbodies (Lake Monongalia) and the thickest wild rice paddies (FS-326).

They have left these anomalies unaddressed, and have seemingly failed to distinguish between correlation and causation. In fact, when one takes a more straightforward view of the field data, even the correlation between increased sulfide and decreased rice does not hold true.

3. History of Existing Water Quality Standard for Sulfate and Wild Rice in Minnesota

Note that not only is wild rice absent or in present in low densities in the generally higher sulfate/sulfide regions of the Northern Glaciated Plains, Lake Agassiz & Aspen Parklands, and the Red River Valley, but it is also largely absent or present in low quantities in much of the (lower sulfate/sulfide) Northern Superior Uplands and Northern Minnesota and Ontario peatlands. Based on this distribution, ecoregion type appears to be a far more significant factor in wild rice distribution than sulfide levels, yet the MPCA has failed to examine these critical components – and the dangers of confusing correlation with causation -- in their proposed rulemaking.

While Moyle indicated he thought a 10 ppm sulfate level was a potential limiting factor in wild rice abundance, he also noted wild rice growing in waters containing up to 282 ppm sulfate (Moyle 1944).

In 1968, E.R. Brooks noted an upper limit of 60 ppm might be closer to an upper sulfate limit, though he noted that trying to determine a limit for sulfate was too singular of a focus; rather, he felt it was the mix of cations and anions that mattered (Brooks, 1968)

Also in 1968, University of Saskatchewan researchers noted that large amounts of sulfate in the soil – up to 1500 ppm -- did not appear to deter wild rice growth in Jackfish Bay (Vicario and Halstead, 1968). They also noted that sulfate levels above 500 ppm seemed to limit plant weight in laboratory experiments however, optimal wild rice growth occurred between 250-500 ppm sulfate.

Other scientists found no impacts to wild rice in the field at sulfate levels of to 170 ppm (Paulishyn and Stewart, 1970). Others recommended an upper threshold of 200 ppm sulfate for paddy rice development (Roaglsky, Clark, and Stewart).

When the MPCA adopted a 10 mg/L sulfate standard to protect wild rice in their water quality rules. Apparently, this was based off of Moyle's fieldwork that was published in 1944 and 1945, and did not account for the additional research conducted by other researchers in the intervening three decades.

4. From a Water Quality Standard to a Wastewater Permit Limit: A Case-Specific Example

Based off the testimony from various experts, as well as field observations and studies, a 40-60 mg/L sulfate limit was eventually adopted at Boswell instead of the 10 ppm standard, and Boswell continues to discharge industrial effluent under those permit limits to this day. With the exception of one other permit in 2013[v], the standard has not been enforced elsewhere at any time.

Fast-forward forty-two years, to 2017 and the proposed rulemaking by the MPCA. The wild rice stands downstream of Boswell are still present and healthy. In the most recent formal field studies[vi], wild rice in the higher sulfate waters downstream of the Boswell NPDES discharge had higher biomass and abundance than the wild rice upstream of the discharge.

Had the MPCA used their proposed 2017 approach back in 1975, the sulfate limit calculated from the MPCA new equation based formula would have been somewhere between 20-25 mg/L, a number based off sediment data collected downstream of Boswell in 2016 by Barr Engineering[vii].

This is exactly the same situation that could unfold across Minnesota of the proposed sulfide-based rulemaking goes forward as currently designed: Extremely high levels of wastewater treatment investment would be needed, with no guarantee or even reasonable chance that environmental benefit for wild rice will actually occur as a result.

5. **Multiple Lines of Evidence: Research on the Effects of Sulfate - Laboratory (or Hydroponics) Research**

The ecotoxicity, or "hydroponics" experiments, were water-only exposures that subjected wild rice seedlings to different levels of sulfate in the water column. Survival and growth of the seedlings were measured, and compared to the levels of sulfate, to determine the "dose response" of wild rice to sulfate. This test sought to isolate the effects of sulfate on wild rice, without the potential interferences of sediment.

These hydroponics tests, conducted by the University of Minnesota-Duluth (UMD), concluded that sulfate levels up to 1,600 mg/L had no statistically significant impact to wild rice seedling germination or growth[viii]. These results were confirmed by an independent ecotoxicity study conducted by Fort Environmental Laboratories (FEL) under Good Laboratory Practice (GLP) protocol, a study which was funded through the Minnesota Chamber of Commerce (Chamber). The FEL study determined the no observed effect concentration (NOEC) of sulfate to wild rice seedlings was 2,500 mg/L on test day 10 and 5,000 mg/L on test day 21. The FEL study was submitted to the MCPA on January 7, 2014, via the Chamber.

These independent tests show roughly the same effect level: sulfate is not toxic to wild rice seedlings until levels are extremely high; nearly twice as high as any recorded sulfate levels in the state's field survey[1].

6. **Multiple Lines of Evidence: Research on the Effects of Sulfate - Mesocosm Research**

Significant seedling mortality is noted in 2012 and 2013. This includes the control exposures, which only had survival ranging from 16-28%. Wild rice seedlings obviously should not have significant mortality if conditions are suitable for wild rice's health and growth; by comparison, the minimum survival control criteria for most chronic effluent ecotoxicity tests is 80%.

7. **Multiple Lines of Evidence: Research on the Effects of Sulfate - Field Research**

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[1] Second Creek in St. Louis County had the highest recorded sulfate level, at 838 mg/L. It also had high wild rice stem density, at ~80 stems/m^2.

. Note that stem density can vary from year to year, due to natural cycling of wild rice population densities.

This data shows that lakes, streams, and cultivated paddies can support moderate to high wild rice densities above 60 stems/m^2 with sulfate levels ranging from just above 10 ppm (Clay Boswell) all the way to 838 ppm (Second Creek).

This field research is among the most compelling information to suggest the existing 10 ppm sulfate standard is neither needed nor necessary to protect wild rice.

8. Multiple Lines of Evidence: Research on the Effects of Sulfide - Laboratory Experiments

However, the attempt to set a water quality standard based on sediment parameters (sulfide) is extremely unusual, and no other similar approaches have been made by other state agencies that this commenter is aware of.

At the MPCA's direction, the UMD researchers again conducted hydroponics testing, this time to determine the toxicity of sulfide on wild rice. Rangefinder tests are often used to set up an approximate range of test concentrations for the actual, definitive exposures. In this case, the rangefinding tests indicated no significant impact on wild rice seed germination or mesocotyl (see below) growth at levels up to 3,060 µg/L, or 3.06 mg/L.[ix]

Based on the lack of observed toxicity of sulfide, MPCA chose not to conduct definitive testing on the effects of sulfide on seed germination or mesocotyl growth of wild rice. It should be noted that both seeds and mesocotyls can be logically expected to be in the "rooting" zone of wild rice (i.e. the sediment) although mesocotyls could, in some case, also be expected to emerge into the overlaying water.

Surprisingly, these rangefinder tests were conducted by exposing sulfide to the entire seedling, which is in direct contradiction to the MPCA's theory that sulfide was impacting wild rice in the rooting zone, not the rooting zone plus the shoot and leaf zone. Again, sulfide cannot be present in the water column where oxygen is present, yet the MPCA chose to expose the portions of wild rice in the water column to a toxicant that effectively cannot exist outside the sediment.

Only the shoots and leaves were affected; the parts of the plant in the rooting zone – seeds (germination), roots, and mesocotyls -- were unaffected.

Seedlings exposed all sulfide levels had higher growth roots than the average initial root length. The exposure with 3,060 µg/L sulfide (the highest sulfide concentration) had 8% longer root lengths on average than the controls (2.74 cm compared to 2.55 cm). The 3,060 µg/l sulfide exposure was the highest concentration tested.

In short, the only measurements -- and subsequently conclusions – that can be derived from the UMD sulfide study are based upon the most ecologically irrelevant exposure pathways – sulfide affecting the shoots and leaves, a place where the scientific consensus is that sulfide cannot exist.

Results from this well-controlled exposure produced significant amounts of data. Most notably, the lowest observed impact for any exposure was an impact to emergence at levels of 1,560 µg/L sulfide, which occurred at the lowest iron concentration (0.8 mg/L) at test day 10. It is important to note that this impact was only noted at test day 10; no impact was observed at the 1,560 µg/L level on test day 21. On Test day 21, the lowest observed adverse effect concentration was 3,100 µg/L at this very low iron level. However, even the most conservative, low-iron, water-only, and temporary (again, no effect was noted at this concentration after 21-days) ”no effect” level of 1,560 µg/L sulfide is more than ten times higher than the MPCA's current proposed protective value of 120 µg/L.

The research also confirmed the MPCA's hypothesis regarding the role of iron. As iron concentrations increased, levels of the free, toxic form of sulfide decreased. As would be expected, toxicity to wild rice seedlings also subsequently decreased. At levels of 2.8 and 10.8 mg/L iron, toxicity was not observed until sulfide reached 7,800 µg/L.

Indeed, sulfide levels in the rooting zone were maintained at the correct dosages in the Fort test design throughout the test duration. The empirical analytical data plainly and directly conflicts with the MPCA's statement, above. Sulfide was present at constant levels, in the rooting zone, throughout the Fort exposure at the desired levels. It was never detoxified; it simply wasn't nearly as toxic when it couldn't attack the green parts of the wild rice plant.

These paradoxical, unproven statements and positions represent a critical departure in the MPCA's approach toward the rulemaking process. Disregarding rigorously designed, peer reviewed research -- refined from the original design, based on the MPCA's own peer reviewers' recommendations -- for unsupported, unproven reasons is highly concerning.

9. Multiple Lines of Evidence: Research on the Effects of Sulfide - Field Research

Put another way, having sulfate levels above 10 ppm meant there was a higher likelihood of having wild present than if levels were below 10 ppm. Therefore, the MPCA made a logical conclusion that this line of field evidence did not support the existing 10 ppm sulfate standard.

: Eighty-one waterbodies have sulfide levels above 120 ppb, of which a full fifty (62%) have wild rice present.

That last bears repeating - -the average sulfide values in wild rice water is approximately 37% higher than what they are proposing as protective.

This is a major assumption, and again, one would assume the field evidence would show a strong link between lily pad presence and wild rice presence. Yet once again, we would be completely wrong; the MCPA's own field data shows that out of the 263 waterbodies, **this assumption fails 115 times.** One hundred and fifteen times, one of the following is true:

This is a very unorthodox approach toward interpreting field data.

Yet the field data contains an incredible amount of conflicting information that the MPCA has chosen not to evaluate. For example, the densest natural wild rice stand in the entire MPCA filed study, Lake Monongalia, had sulfide levels up to eleven times higher (1,370 ppb) than the proposed protective standard. This kind of discrepancy begs numerous serious questions about the validity of the MPCA's proposed safe level of 120 ppb sulfide, and Lake Monongalia is not the only waterbody where this is observed. Fifty seven percent (57%) of waterbodies with sulfide above 120 ppb have wild rice present, including some of the densest stands in the state.

It is also striking to note that the MPCA has chosen to exclude commercial paddy rice data they collected, despite the fact commercial wild rice stands have the same species of wild rice, and operators and owners specifically design paddies to optimize wild rice production. These commercial paddies, described in more detail later in this document, unsurprisingly have dense wild rice stem counts. They also have high sulfide levels; eight of the twelve are above the MPCA proposed protective level, with the highest sulfide value over 800 ppb. Perhaps the most telling point is this: Of the ten commercial paddies with sufficient data, the MPCA's formula predicts there is too much sulfate present in eight of them. If there were permitted discharges to waterbodies such as these – or to Lake Monongalia, or many other waterbodies which produce some of the densest wild rice stands year after year – the MPCA rulemaking could require those dischargers to remove sulfate, with the cost likely in the millions of dollars

10. An Alternative Approach For Field Data Analysis

First, we must strip away the MPCA's unsupportable assumption that wild rice should be present if lily pads are present. Indeed, we must take this a step further; because there are so many factors affecting whether wild rice can grow in a waterbody, we must remove all waterbodies from the analysis which do not have wild rice present.

This leaves us with 54 streams/rivers and 116 lakes, all with varying densities of wild rice, from 0.3 to 154 stems m^2. Forty-seven (47) of these waterbodies do not have stem densities reported. We also see a wide range of sulfide concentrations in this dataset, from 2,080 ppb all the way down to less than the detection limit of 11 ppb.

Instead, when we do a simple trend-line analysis (the green line in the above graph), it actually shows **an increase in wild rice abundance as sulfide levels increase**.

When one plots this data, we see **that just as in natural lakes and streams, as sulfide increases in commercial paddies, wild rice abundance increases**.

This equation results in "protective" sulfate values ranging from 0.36 to 2.45 mg/L. Actual levels of sulfate in these paddies ranges from 0.25 to 279 mg/L.

This regulation, in turn, could not only not help protect wild rice, it could actually cause harm the wild rice population.

11. Conclusion

All we know is that the Agency has proposed a rulemaking with numerous unaddressed contradictions, and they have failed to act in a reasonable manner when evaluating all lines of evidence, and have ultimately failed to show why this regulation, as proposed, is needed.

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.

The suggestion that sulfide acts independently to affect the presence and absence of wild rice implies there is no interaction between sulfide or any other variable that could potentially influence sulfide presence and bioavailability which is not justified based on historical understanding of sediment sulfide and interactions with other factors described below. I found no attempt to evaluate interactions within the logistic regression. Although this complicates the analyses, it does provide ecological credence in that the ecosystem is not a binary function, it operates in an interactive manner with at least several of the variables considered potentially interacting with each other. Not to at least consider interaction elements in the model, is an over-simplification of the system.

The thought that sulfate, TOC, or Fe didn't have a direct impact on the presence or absence of wild rice is not surprising, as is the thought that their significance in terms of affecting the presence or absence of wild rice is simultaneous. Each of these variables interact directly or indirectly with sulfide to module toxicity. Thus, these statements oversimplify a complicated system in which the toxicity of sulfide which is not in question is modulated by other factors including sulfate, and to a greater extent, TOC and Fe begging the question, why was multiple linear regression not used in the analysis of this data?

The point is, comparison of any means of sulfide toxicity in the environment without consideration of the other confounding variables, Fe and TOC is not justified without further statistical analyses.

Bock

Protective Sulfide level

An examination of the field data shows that there are a great many waterbodies in the MPCA dataset that exhibit porewater sulfide concentrations that exceed the MPCA threshold (>120 µg/l) and also possess healthy stands of wild rice. This finding calls into question the validity of MPCA threshold and suggests problems in how MPCA used the field data to derive a threshold.

Based on my analyses, the conservative sulfide threshold I derived is as protective of wild rice health as the 120 µg/l MPCA standard.

The field data used in MPCA's analysis was collected in 2012 (n=83) and 2013 (n=25). Significant flooding was reported in the Duluth region in 2012. This flooding occurred in June, a critical time for the germination of wild rice. MPCA did not discuss the possible important of this flooding on in the 2012 data and the derivation of the sulfide threshold. In fact, more than 75% of the 25 samples with porewater sulfide between 100 µg/l and 150 µg/l were collected in 2012, the samples expected to have the most influence on the MPCA's 120 µg/l threshold.

MPCA's first derivation of a sulfide threshold is based on the 'breakpoint' analysis of the field data described on page 69 of the SONAR and on pages 37 and 39 of the Final Technical Support Document (TSD; MPCA 2017). This threshold is based entirely on MPCA's visual interpretation of the plot. In my professional judgement there is no visual evidence for a breakpoint at 120 µg/l and this value represents a visual artefact. The use of professional judgment, either MPCA's or my own, can easily lead to unconscious biases and has a high potential for erroneous conclusions.

There are statistical methods that can be used to identify breakpoints, specifically piecewise regression (Seber and Wild, 1989). These methods avoid the biases associated with professional judgment and provide a statistical basis for decision making. When these methods are applied to the MPCA field dataset they indicate (1) if the lake with the highest sulfide is excluded (Bean) the 'breakpoint' is more than twice the value identified by MPCA (2) if all water bodies are included the breakpoint is more than 1000x the MPCA value and suggests no sulfide threshold in the field data.

More simply put, the true threshold could be substantially higher than 300 µg/l.

MPCA conducted another analysis of the data using 'change point' analysis to identify a threshold (SONAR 6E p 69). This method has been described in the peer-reviewed scientific literature (Hawkins 2001, Killick et al. 2016) and does not rely on professional judgement.

The results of these analysis show that the single change point identified by MPCA is not unique and in fact does not represent a change point that can be associated with a change in wild rice density.

Although MPCA limited their change point analysis to stem density, this analysis can also be applied to the presence of wild rice and to the presence of dense stands of wild rice with a stem density of greater than 40 stems per square meter (TSD page 50). The algorithms from Hawkins (2001) were used for this binary change point analysis.

These statistical change points are all substantially higher than the MPCA threshold of 120 µg/l, supporting the conclusion that 120 µg/l is below the true threshold.

Although the methods described above are well founded in statistical theory and provide important information regarding the relationship between sulfide and wild rice health metrics, these methods are not typically used to derive protective thresholds. Typically a dose-response statistical model would be used for this sort of data, such as the relationship shown on pages 119-120 of the TSD for probability of wild rice presence versus pore water sulfide. However, the field data do not fit the requirements of such a model; specifically (1) there no well-defined no-effect level due to high variability at all sulfide concentrations, and (2) sulfate is a nutrient required for plant growth (TSD page 53).

Thus, although MPCA does fit the field data to a dose-response curve, the data do not fit the assumptions of the statistical model and therefore any sulfide threshold derived using this method should not be used.

Binary analysis presents an alternative method for analyzing the relationship between wild rice and sulfide that is not affected by the issues that plague the dose-response modeling of the field data.

Two wild rice health metrics were subjected to binary analysis: (1) the presence of wild rice, and (2) the presence of high density stands of wild rice (>40 stems per square meter). These same metrics were used by MPCA in their analyses (presence/absence of rice, presence absence of high density stands of rice) (SONAR 2 page 69).

Thus, these results indicate that the MPCA threshold of 120 µg/l is too low, and higher thresholds (2-3) are just as protective as the MPCA threshold. Furthermore, there are too few data points in the field data with porewater sulfide values high enough (300 µg/l or higher) to reliably determine a true upper threshold (Table 3).

When I analyzed the field data I found no evidence that increasing the sulfide threshold to values 2-3 times the MPCA value would lead to a discernible decrease in the health of wild rice. There is insufficient data to reliably evaluate higher thresholds.

MPCA unreasonably excludes the alternative threshold of 300 µg/l in TSD Appendix 9.

Based on the weight of evidence, I conclude that the 120 µg/l sulfide threshold proposed by MPCA is overly conservative and the true threshold is at least 2-3 times higher than the MPCA threshold.

Protective sulfate level

I found a significant conflict in the performance of the equation that indicates that the equation does not provide sound predictions of the relationship between sulfide and sulfate and therefore is an unreasonable standard. Specifically, if the sulfide threshold is increased one would expect the sulfate threshold for a given water body to also increase. I found that in a large number of instances, when the sulfide threshold is increased the sulfate threshold decreases. Any equation used to derive a sulfate standard must yield higher sulfate standards in a waterbody when higher sulfide thresholds are used. The fact that this is not true for the MBLR equation indicates that the equation is likely to lead to erroneous conclusions and is potentially simply a statistical anomaly. The use of such an equation presents a fundamental flaw in MPCAs approach and should not be used.

Based on my analysis of the sulfide threshold (Rule 7.20; SONAR part 6E) and sulfate equation (Rule 7.26-8.2; SONAR 6E p75-77), I am recommending the following changes:

1. Reject the sulfide threshold of 120 µg/l.
2. Reject MPCA’s equation to predict a waterbody specific sulfate threshold based on TOC, iron, and the sulfide threshold.
3. Explore a more mechanistic approach or evaluate wild rice health in individual water bodies and address those that 1) have lost wild rice relative to historical values or 2) exhibit the qualities expected to support wild rice but that lack stands of wild rice.

Fort

The primary conflict discussed in the testimony provided is the lack of rationale in dismissing a hydroponic study conducted by Fort Environmental Laboratories and published in a well-respected peer-reviewed journal [2].

Since loss of free sulfide increases with increasing Fe addition, we suspect that a significantly proportion of the sulfide was converted to FeS. Therefore, MPCA’s statement is based only on the assumption that only detoxification by the rice plant itself resulted in lower toxicity of sulfide, whereas it is the physicochemistry of the hydroponic environment also resulted in chemical reduction in free sulfide due to conversion to FeS which is misleading.

Thus, the present study should be considered in the evaluation of criteria selection and is important in evaluating other factors in the environment that modulate and often mitigate sulfide toxicity to wild rice.

November 2017 study: Results from this study indicate that for the most sensitive endpoint (mesocotyl emergence), exposure of developing wild rice to sulfide at concentrations ≥3.12 mg/L sulfide was toxic based on assessment of NOEC and LOEC values in the presence of 0.8 mg/L Fe. However, exposure of developing wild rice to sulfide at concentrations ≥7.8 mg/L was necessary to significantly reduce emergence in the presence of 2.8 mg Fe/L. Mesocotyl emergence was the most sensitive endpoint in the study, while seed activation, seedling survival, and phytotoxicity were the least sensitive endpoints. Based on measured sulfide concentrations, Fe reduced free sulfide concentrations in the 2.8 mg Fe/L treatment relative to the 0.8 mg Fe/L treatment.

Hansel

1. Introduction and Overview

MPCA proposes to delete the current standard of 10 mg/L[2]; this proposal is needed and reasonable and fully supported by the multiple lines of evidence.

The proposed new water quality standard is **unneeded** and **unreasonable** because:

- MPCA, though alerted by their own peer review panel, misconceptualized the hydrogeological conditions under which sulfate is delivered to sediment beds. This flawed conceptual model led to the following issues which pervade their analysis:
    - Unreasonably assuming that chemical diffusion of sulfate from an overlying water column to the sediment porewater is a process favored in these environments; and
    - Unreasonably excluding important controlling variables, such as the concentrations of iron and sulfate in groundwater, from field survey data collection.
- MPCA's model and key hypothesis are incorrect and are not supported out by the multiple lines of evidence;
- In considering the evidence, MPCA improperly weighted the multiple lines of evidence by:
    - Unreasonably excluding or discounting peer-reviewed published science that represents the state of the art in determining toxicity of chemicals to organisms;
    - Unreasonably relying too heavily upon non-peer-reviewed, unpublished science and analyses ;
    - Unreasonably failing to take into account other wild rice stressors, and ascribed all deleterious effects on wild rice to sulfide alone.

The MPCA never states (or proves) that the proposed "protective" porewater sulfide and water column sulfate are needed.

MPCA's initial research followed the state-of-the-art toxicity testing performed on aquatic organisms to determine whether individual substances, such as sulfate, are toxic to those organisms.

2. Sulfate is not toxic to wild rice (at concentrations observed in Minnesota Wild Rice waters)

Two state-of-the-art scientific studies clearly demonstrate that sulfate is not toxic at concentrations observed in Minnesota wild rice waters. These studies were conducted in a laboratory where physical conditions were tightly controlled (e.g. temperature, light levels, periods of darkness). Chemical parameters of all other compounds were also strictly controlled, so that only sulfate concentrations varied. Biological parameters were also tightly controlled, with no competition from other competitive or invasive species and no disease parasites. Negative controls – where the wild rice is exposed to zero (or near zero) sulfate concentrations was grown under the same conditions as the exposed wild rice.

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[2] Id at proposed MN rules 7050.0224, Subp. 2

One of the two, Fort et al[3], also used a positive control – where wild rice was exposed to a known toxicant, to be sure that the wild rice was not resistant to chemical toxicants. The Fort et al study also followed Good Laboratory Practices[4], an internationally recognized standard "to ensure the generation of high quality and reliable test data".

Dr. Pastor et al conducted a state-of-the-art controlled toxicity test, and concludes:

"Sulfate exposure concentrations of 0, 10, 50, 100, 400, and 1600 mg SO4/L did not affect germination success, mesocotyl lengths, or the masses of the stem plus leaf (if any) and roots (P > 0.10 for each test)."[5]

Because Dr. Pastor et al struggled early on to grow wild rice in the laboratory, the Chamber commissioned Fort Environmental Labs to conduct similar hydroponic toxicity tests. Fort et al concluded:

"In summary, sulfate concentrations below 5000 mg/L did not adversely affect early–life stage wild rice during a 21-d [ay] period, and effects at 5000 mg/L sulfate were attributable to conductivity-related stress rather than sulfate toxicity in 2 of 4 end points."

There is excellent agreement between Dr. Pastor et al and Fort Labs et al that sulfate is not toxic to wild rice at concentrations seen in Minnesota waters. Dr. Myrbo found the highest concentration of sulfate in wild rice waters to be well under either the 1,600 mg/L sulfate found by Dr. Pastor and the 5,000 mg/L found by Fort Labs et al.

The toxic sulfate levels determined by both Pastor et al and Fort et al, using standard toxicological testing, are more than 1,000 times the current standard of 10 mg/L. It is also interesting that the toxic sulfate levels determined by both Pastor et al and Fort et al, using standard toxicological testing, are more than 1,000 times the median sulfate concentration in streams (17 mg/L) and lakes (3 mg/L).[6] Those same levels are more than double the highest level measured by Myrbo et al. – 838 mg/L at Second Creek.[7]

3. Sulfide is not toxic to wild rice (at concentrations observed in Minnesota Wild Rice waters)

The Peer Review Panel had serious concerns about Dr. Pastor's hydroponic study, and recommended that "If these experiments can be repeated, the panel recommends the following approach:

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[3] Toxicity Of Sulfate And Chloride To Early Life Stages Of Wild Rice (*Zizania Palustris*), Douglas J. Fort, Michael B. Mathis, Rachel Walker, Lindsey K. Tuominen, Mike Hansel, Scott Hall, Robin Richards, S.R. Grattan, and Kurt Anderson, Environmental Toxicology and Chemistry, Vol. 33, No. 12, pp. 2802–2809, 2014 © 2014 SETAC

[4] See OECD webpage at: http://www.oecd.org/chemicalsafety/testing/goodlaboratorypracticeglp.htm

[5] Effects of sulfate and sulfide on the life cycle of *Zizania palustris* in hydroponic and mesocosm experiments, John Pastor, Brad Dewey, Nathan W. Johnson, Edward B. Swain, Philip Monson, Emily B. Peters, and Amy Myrbo, *Ecological Applications*, 27(1), 2017, pp. 321–336 © 2016 by the Ecological Society of America

[6] MPCA Final Technical Support Document: Refinements to Minnesota's Sulfate Water Quality Standard to Protect Wild Rice, August 2011, Chapter 1.A. page 7.

[7] Raw data from Myrbo et al "Sulfide generated by sulfate reduction is a primary controller of the occurrence of wild rice (*Zizania palustris*) in shallow aquatic ecosystems, In press, *Journal of Geophysical Research: Biogeosciences*.

- Use of **a split design**, in which there is a root compartment separated from the shoot. This allows **anaerobic conditions in the root zone to be maintained** and exposure of the root (but not shoots) to the experimental sulfide concentrations.
- Use of an **experimental period of 14 or 21 days**, which is standard in ecotoxicology for aquatic macrophytes. Response measurements should be collected at regular intervals.
- To the extent possible, **use of the same biological endpoints** in the laboratory study as used in the outdoor container and field studies. Decisions on biological endpoints for all the field and laboratory studies in turn will feed into the modeling approaches that can be used. This should be part of the conceptual framework and design for the overall Study and will allow better integration of the study components.
- **A larger sample size**. A power analysis should be done to determine the number of replicates and treatment levels needed.
- We anticipate that **a minimum of six exposure concentrations** should be used, with several treatment levels bracketing the current water quality standard.
- **Maintaining the exposure concentrations throughout the experimental period**. This will be easier if roots are separated from shoots."[8] (Emphasis added)

The results of the Fort Labs study found that at Day 10, with no additional iron, emergence of seedlings was most affected by sulfide, but the lowest observed effects concentration (LOEC) was 3.2 mg/L sulfide.

"Increasing Fe concentrations reduced the toxic effects of sulfide to wild rice," with day 10 LOEC for emergence of seedlings rising to 7.8 mg/L sulfide.

MPCA's essential rejection of the Fort et al sulfide hydroponic study is not reasonable. First, Fort Labs followed as nearly as possible the recommendations of the Peer Review Panel. Second, the Fort Labs study followed Good Laboratory practices and was certified as such. Third, the Fort Labs study followed US EPA guidance for conduct of toxicity testing for the purpose of developing water quality criterion and standards.

Interestingly, despite the fundamental flaws in Dr. Pastor's sulfide hydroponic studies, he found similar results for those parts of the plant which are in contact with the sediment:

"Sulfide concentrations of 0, 96, 320, 960, and 2880 μg/L did **not** affect **germination success of seeds, mesocotyl masses, or mesocotyl lengths** ($P > 0.10$ for each test)."[9] (Emphasis added)

And

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[8] Summary Report of the Meeting to Peer Review MPCA's *Draft Analysis of the Wild Rice Sulfate Standard Study* Saint Paul, MN August 13-14, 2014

[9] Id at 1.

"**Root lengths were only weakly depressed with increasing sulfide concentration** ($P < 0.10$)."[10] (Emphasis added)

Thus, based on the hydroponic tests conducted by Pastor et al and Fort Labs et al, sulfide is not toxic "in the root zone" or "in the sediment" to those parts of the wild rice plant that lives there, at concentrations of 2,800 µg/L to 3,200 µg/L – hundreds of times more than the "protective" level of sulfide proposed by the MPCA – 120 µg/L. These levels are more than 50 times the median concentration determined during the field surveys conducted by Myrbo as well.

Based on these controlled sulfate hydroponic experiments, there is absolutely no scientific support for the proposed "protective" sulfide standard of 120 µg/L sulfide pore water. Nor is there any scientific support for the notion that sulfide is toxic to wild rice at concentrations observed in Minnesota wild rice waters. Therefore, MPCA has not demonstrated the need for or reasonableness of the proposed "protective" sulfide standard of 120 µg/L sulfide in the porewater.

4. MPCA's Conceptual Model and Key Hypothesis do not correspond to natural conditions

MPCA's analysis has yet to address sulfate in groundwater and its likely control of both the concentrations of sulfide in sediment porewater and sulfate in the surface water column.

In stark contrast, MPCA's conceptual model relies on sulfate delivery to the sediment bed from the overlying water column through chemical diffusion; nowhere does MPCA demonstrate that this mechanism is reasonable. The MPCA also neglects the groundwater contributions of dissolved iron.

So, the MPCA's supposition that a tenuous correlation between water column sulfate and porewater sulfide indicates that the "equation works 80% of the time" is problematic. The tenuous correlation found by MPCA may simply reflect two factors that are controlled by the underlying (and unmeasured) influence of groundwater.

Neither MPCA's conceptual model nor key hypothesis is a reasonable depiction of the natural conditions in wild rice waters. They were specifically called into question by the technical peer review panel, who explicitly identified that the field study "requires addressing the full hydrological system (supply by surface water and groundwater)". The conceptual model used by the MPCA is not borne out in the general understanding of the hydrologic cycle, in decades of research on Minnesota lakes and rivers by USGS and other researchers, or by recent research published by the Minnesota DNR.

5. MPCA's Conceptual Model of wild rice waters as "bathtubs" does not reflect natural conditions

Unfortunately, the MPCA treats their dataset as if wild rice waters are essentially "bathtubs" with no interaction between groundwater and surface water, and no interaction between groundwater and sediment and porewater. Thus, MPCA's model ignores the important role of groundwater in bringing nutrients and sulfate into the sediment and porewater. It is unlikely that sulfate from the surface water is the primary source for the formation of sulfide.

Literature demonstrates that groundwater interacts with sediment and wild rice

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[10] Id

Thus, MPCA's model is fundamentally flawed, because it unreasonably implicates the water column as the source of sulfate. It ignores the important role of groundwater in bringing nutrients and sulfate into the sediment and porewater.

MPCA argues that because certain waters don't fit the model (e.g. exhibit "false positives"), the model is correct and the waters that don't fit the model (because groundwater inflow to the sediment prevents the formation of sulfide at levels which have the potential to harm wild rice) are "outliers". Indeed, MPCA presents only one measurement to demonstrate that this occurs; a measurement of Second Creek (see Yourd, 2017)[11]. Those measurements showed that porewater sulfide was lower than the "protective" level of 120 µg/L, "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).

In other words, groundwater "upwelling" through the sediment has been observed as a critical component in the growth of wild rice. Yet, despite the measurements of multiple lakes in Wisconsin and multiple streams and lakes in Minnesota, MPCA holds that the model is still "valid" because of the MPCA's and Dr. Pollman's statistical analysis.

The fact is that groundwater provides much of the flow into wild rice waters, carries with it many nutrients, including dissolved sulfate and iron, and controls the chemistry of porewater in riparian environments. MPCA cannot ignore either the flow or, as will be seen below, the chemistry that accompanies the flow.

6. MPCA's key Hypothesis is not supported by the multiple lines of evidence

Thus, it is unlikely that sulfate from the water column is the main source for sulfide formation in the porewater. It is also likely that iron and dissolved carbon are migrating to the porewater, not from dissolution of the sediment (MPCA's primary source), but are being transported to the sediment, porewater, and ultimately, to the surface water body, via the groundwater flow into the wild rice water. MPCA presents no evidence that sulfate from the water column is the only source for conversion to sulfide in the porewater. It merely makes a policy decision, in the form of the hypothesis, that such is the fact.

In short, MPCA's hypothesis remains a "supposition" or "proposed explanation" – one that is not supported by a general understanding of what controls the chemistry of porewater.

7. State-of-the-Art Controlled sulfate & sulfide toxicity experiments

However, MPCA unreasonably ignored research commissioned by the Chamber as well as other literature, giving all considerably less weight in its weighting of the multiple lines of evidence.

MPCA effectively dismisses the very studies which represent the state-of-the art in toxicity testing, and the best controlled experiments. Pastor et al and Fort et al state-of-the-art controlled hydroponic studies clearly demonstrated that sulfate is not toxic to wild rice at concentrations observed in Minnesota wild rice waters.

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[11] G.-H. C. Ng , A. R. Yourd, N.W. Johnson, and A. E. Myrbo. "Modeling hydrologic controls on sulfur processes in sulfate-impacted wetland and stream sediments" 2017. Journal of Geophysical Research: Biogeosciences 10.1002/2017JG003822

Therefore, MPCA unreasonably relied upon the Pastor et al controlled sulfide toxicity tests (hydroponic tests) in, for example, Figure 1-2 of the TSD[12], and elsewhere throughout the SONAR and TSD.

8. Outdoor Container studies were seriously flawed and cannot be reasonably relied upon.

The MPCA unreasonably relied upon the data generated by Dr. Pastor et al in his outdoor container study. There are serious flaws in the outdoor container studies, because of which the MPCA should have not relied as heavily as it did in developing protective sulfide and sulfate levels.

The MPCA does not resolve the discrepancies between the results of the Fort et al controlled sulfate and sulfide toxicity testing, the Pastor et al controlled sulfate and (properly interpreted) sulfide toxicity testing and the uncontrolled outdoor container studies. These are significant as will be seen.

It is important to note that control mortality at these levels (85%) represents a stressed population of wild rice, and the impact from any added stressors are likely to be greatly exaggerated compared to a healthy population of wild rice.

Thus, the outdoor container tests conducted by Pastor et al did not follow US EPA guidance, and should be given considerably less weight.

Given the serious flaws in the outdoor container data and corresponding Peer Review Panel criticisms, the MPCA cannot reasonably rely upon the results to corroborate a “protective” sulfide” or “protective sulfate” level.

MPCA does not reconcile the differences between the “protective” sulfide levels determined from the hydroponic studies and the outdoor container studies.

Thus, MPCA unreasonably rejects the Fort et al sulfide hydroponic studies, misinterprets the Pastor et al sulfide hydroponic studies, and does not reconcile the fact that there is nearly a factor of 10 difference between these studies and the other studies on which the MPCA relies.

9. Field Surveys were seriously confounded

Unlike the state-of-the-art controlled hydroponic studies, the field surveys are entirely uncontrolled. The wild rice growing in the wild rice waters (and non-wild rice waters) surveyed were subject to weather and all of the other stressors which can affect the presence and density of wild rice.

MPCA acknowledges that several of these other stressors are “statistically significant”, yet does nothing to separate their effects from the effects of sulfide. Instead, MPCA ascribes all ill effects on wild rice to sulfide and sulfide alone.

MPCA unreasonably used data from non-wild rice waters to determine “protective” levels of sulfide and sulfate

MPCA ignores other stressors of wild rice, several of which the MPCA determined were statistically significant, in determining the sulfide and sulfide alone impacts the growth and density of wild rice

[12] [12] MPCA Final Technical Support Document: Refinements to Minnesota’s Sulfate Water Quality Standard to Protect Wild Rice, August 2011, Chapter 1.C. page 34.

MPCA does not prove its hypothesis, in that there is no causal determination that sulfide in the porewater (e.g. the rooting zone) impacts the presence and density of wild rice

MPCA does not resolve the inconsistencies between the results of the hydroponic studies (where only sulfide or sulfate are stressing the wild rice) and the field surveys, where multiple stressors are operating on the wild rice.

10. MPCA did not adequately consider the costs to comply with the proposed rule

The MPCA in the SONAR admits that the costs to reduce sulfate in discharges from municipalities and industry are "prohibitively expensive".

For cities, annual costs can exceed $1 million/year

For industry, because flows are generally higher and sulfate concentrations are higher than municipal wastewater, costs are even more "prohibitive". For taconite mines and processing plants, there are multiple discharge points and multiple sources of sulfate, including scrubbers, mines and waste rock piles, tailings basin, as well as rainfall over the vast areas which encompass a taconite mine and plant.

11. Summary

There are fundamental problems with both the underlying evidence and with MPCA's policy decisions on weighting the relative value of each line of evidence.

First, MPCA's model is unreasonable in that it paints all wild rice waters as essentially bathtubs, with no interaction between the surface water, sediment and groundwater, when multiple lines of research show that the groundwater may be the source of the very compounds which may influence the formation of sulfide, and its effect upon wild rice.

Second MPCA's model is unreasonable in that it assumes that all sulfate migrates to the sediment from the water column, and that all dissolved iron and organic carbon in the porewater (root zone) comes from the sediment. Dissolved iron and organic carbon could just as easily migrate from the water column to the sediment and porewater, and from the groundwater to the sediment and porewater.

Second MPCA's model is unreasonable in that it assumes that all sulfate migrates to the sediment from the water column, and that all dissolved iron and organic carbon in the porewater (root zone) comes from the sediment. Dissolved iron and organic carbon could just as easily migrate from the water column to the sediment and porewater, and from the groundwater to the sediment and porewater. Research has shown that, in fact, groundwater is the more likely source of all of these compounds. MPCA unreasonably ignores the potential contribution of these migrations, and unreasonably relies upon an overly-simplified model to determine the "protective" level of sulfide and sulfate.

Using the standard, hydroponic toxicity tests per US EPA guidance, sulfate is not toxic to wild rice at concentrations well above the concentrations seen in MN wild rice waters. Therefore, the current standard of 10 mg/L sulfate has no scientific validity. The mode of action of sulfate is also now well understood – it, like other salts, exerts osmotic pressure on the plant, and is no more toxic than any other salt. Therefore, there is no need for a "protective" sulfate standard.

Similarly, based on the effects of sulfide on the rooting zone (and those portions of the plant in the sediment and exposed to the porewater), sulfide is not toxic to wild rice at concentrations seen in most Minnesota wild rice waters. While the mode of action is not well understood at this point, it is clear from these experiments, un-confounded by other wild rice stressors, that sulfide is not toxic to wild rice at concentrations seen in Minnesota wild rice waters. Therefore there is no need for a "protective" sulfide standard.

While MPCA conducts a series of statistical analyses to allegedly show that a "protective" sulfide in porewater standard is needed, both the underlying data and the statistical analysis are fraught with errors, and contradicted by the literature. The result is an inconsistent body of evidence, some of which shows that a sulfide in porewater and sulfate in the water column water quality standard may be necessary, and other showing that such standards are neither needed nor reasonable.

For example, the MPCA has not and cannot provide any studies, literature or other evidence that reducing sulfate in discharges to surface waters will effectively reduce sulfide in the porewater in wild rice waters. Indeed, Berndt et al[13] reach an entirely opposite conclusion.

MPCA has not and cannot provide any studies, literature or other evidence that reducing sulfate in the water column will better protect wild rice. None of the controlled hydroponic studies show any evidence for this, nor do the outdoor container studies nor do the field surveys. Again, Berndt et al [14]shows that sulfate in the surface water has little to do with sulfate reduction in the sediment, while groundwater flow provides the bulk of flow as well as sulfate, organic carbon and iron in the sediment.

MPCA has not and cannot provide any studies, literature or other evidence that reducing sulfate in the water column will reduce sulfide in the porewater. This was simply not tested in any of the studies, nor in any of the literature cited by the MPCA. Yet the proposed rule explicitly says that this is what needs to happen to comply with the rule. In wild rice waters where the porewater sulfide exceeds the protective level, dischargers of sulfate will need to reduce their discharges of sulfate. Yet there is no evidence that reducing sulfate in discharges will result in significant reductions in water column sulfate, or that reducing sulfate in the water column will reduce sulfide in the porewater. Considering that cities and industries may be required to expend billions of dollars to reduce sulfate in their discharges, through the use of membrane filtration treatment, MPCA should be able to solidly demonstrate, in at least one wild rice water, that reduction in sulfate results in reduction in porewater sulfide. MPCA has not done so, and thus the proposed rules are unreasonable.

MPCA has not and cannot provide any studies, literature or other evidence that reducing sulfide in the porewater will better protect wild rice.

Therefore, the "prohibitively expensive" costs to comply with the proposed rule may provide no additional protection for wild rice. MPCA has not and cannot provide any studies, literature or other evidence that these "prohibitively expensive" costs will have any positive impacts on wild rice.

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[13] A comparison of results from a hydrologic transport model (HSPF) with distributions of sulfate and mercury in a mine-impacted watershed in northeastern Minnesota, Michael E. Berndt, Wes Rutelonis, Charles P. Regan. Journal of Environmental Management 181 (2016) 74-79
[14] Id

We respectfully request that the current sulfate standard of 10 mg/L[15] be eliminated, as the weight of evidence clearly shows that sulfate is not toxic to wild rice at that concentration or at any other concentration observed in Minnesota wild rice waters.

We respectfully request that the rule be remanded to the MPCA, to address the errors, uncertainties and inconsistencies noted above, particularly the inconsistency that multiple studies show that concentrations of sulfate and sulfide are not toxic to wild rice at concentrations observed in Minnesota wild rice waters, while other studies show that a "protective" concentration of 120 µg/L, and a "protective" concentration of sulfate, which are orders of magnitude smaller than the controlled, state-of-the-art hydroponic test results.

We respectfully request that the rule be remanded to the MPCA until it does a more complete cost analysis, and can demonstrate that the expenditure of billions of dollars will result in better protection of the use of wild rice for harvest by humans and wild life.

We respectfully suggest that MPCA has not met its obligations under the Administrative Procedures Act to demonstrate the need for and reasonableness of the proposed rule, specifically Proposed MN Rules 7050.0224 Subp. 5. A. (Line 7.17 – 7.12), and Proposed MN Rules 7050.0224 Subp. 5. B.1. (Line 7.25 – 8.17)

Hawkins

The analyses led to the conclusions:

1. **The waterbody-specific sulfate standard proposed by MPCA does not differentiate waterbodies hosting wild rice from water bodies that do not.**

The P value of this test falls far short of statistical significance, confirming the visual impression that the proposed SO4 limit has no connection to the presence or absence of wild rice in the water body.

In other words, 60% of the water bodies – a majority – would be misdiagnosed by the proposed standard.

The performance of the proposed sulfate standard for identifying wild rice sites is akin to throwing a die and declaring the water body good if the die shows a 1 or 2 and as bad if the die shows a 3, 4, 5 or 6.

2. **More generally I have been unable to find any function of SO4, TOC and Fe that can differentiate water bodies hosting wild rice from water bodies that do not.**

Thus neither the overall model, nor any of the terms in it, is statistically significant.

[15] Proposed Permanent Rules Relating to Wild Rice Sulfate Standard and Wild Rice Waters, MPCA per Minnesota Reviser RD4324A, Proposed MN Rules 7050.0224, Subp.2

The conclusion then is that these three predictors are not informative about the presence or absence of wild rice. Any model using them to predict presence or absence of wild rice can be no better than random guessing.

Whether for wild rice presence, or for the abundance of the wild rice, SO4, TOC and Fe do not show any predictive information in the field data.

3. Sulfide is a statistically significant but weak predictor of wild rice presence.

On the other hand, however, sulfide explains only 10.53, or 7%, of the total deviance in wild rice presence, leaving the remaining 132.87, or 93% unexplained.

This means that while porewater sulfide is a statistically significant part of the picture of wild rice presence or absence, it is only a modest part of it. Its contribution pales next to that of other characteristics and variables.

Like the proportion of deviance explained, the AUC paints a picture of sulfide as one fairly small part of the picture: statistically significant but far short of determinative.

Here too, sulfide has a statistically significant separation between water bodies with and without wild rice, but is not particularly effective in differentiating between the two.

4. The MPCA assessment of the proposed sulfate rule's performance is questionable.

Contrary to my conclusion that SO4 has no perceptible connection to wild rice, the MPCA document reports quite favorable performance for the proposed water-body-specific sulfate standard. However this performance is against a surrogate endpoint – sulfide being below 120 µg/L – and not the actual endpoint of interest – the presence or absence of wild rice.

Thus the use of this surrogate endpoint seems questionable, as do the resulting conclusions.

5. All four analytes vary substantially from time to time within the same water body.

At this level, two sulfide readings on the same water body have a 1 in 3 chance of differing by more than 100%, a proportion supported by the actual successive sulfide readings.

In other words, the sulfide level of a water body is an elusive, moving target.

In summary, all four analytes show substantial variability over time within the same water body. A snapshot of the chemistry at a given time may produce substantially different values than another time. The steady state assumption is therefore not validated particularly well.

6. SO4, TOC and Fe are statistically significant but imprecise predictors of sulfide.

As expected, all three terms in the model are highly statistically significant, as is the overall regression. However, while significant, the regression explains less than half the variability (R2 = 0.491), implying that other factors and random variability are responsible for most of the sulfide variability.

There is a statistically significant but not very strong relationship of sulfate, Fe and TOC to sulfide, and

There is a statistically significant but not very strong relationship of sulfide to wild rice.

This chain of relationships falls apart when the intermediate of sulfide is removed and one attempts to predict wild rice directly from sulfate, Fe and TOC. Then the unmodeled random variability in the two relationships overwhelms the modest associations, leading to the lack of significant association between SO4, Fe and TOC and the presence or absence of wild rice.

7. **The proposed sulfide cutoff of 120 µg/L is not well supported and would lead to many false alarms.**

In summary, going from a cutpoint of 120 to 274 µg/L produces many fewer alarms, and those alarms that are produced are much more likely to indicate real problems with the wild rice.

8. **A different approach using sulfide in a linear discriminant analysis incorporates explicit recognition of the implications of false positive and false negatives, and further motivates higher sulfide cutoffs.**

Even the lowest of these numbers is above the 120 µg/L proposed in the MPCA document. These numbers provide further evidence that, if sulfide is used as an indicator of suitability for wild rice, a higher sulfide cutoff should provide a better use of resources for followup.

In summary, the data presented give little reason to believe that changes in the sulfate standard will have any effect on the occurrence or health of wild rice. A standard focused directly on sulfide would incur substantial numbers of false positives (water bodies with high sulfide but abundant wild rice) and false negatives (water bodies with low sulfide but no wild rice). More detailed study of these water bodies would be required diagnose their specific properties and actions needed to enhance wild rice.

Richards

MPCA has not demonstrated the reasonableness of the following:

- The porewater sulfide concentrations impacting wild rice health (SONAR E.2)
- The MBLR sulfate equation (SONAR E.4 and E.5)
- The porewater sulfide analytical method (SONAR E.7)

Very few details were provided in the published paper on the data used, definition of EC0, EC10, or definition of initial conditions. The peer-reviewed article does not contain an EC10 so it should be noted that any EC10 based on these data were not evaluated during the peer-review process for publication. In a meta-analysis performed for MPCA, Pastor calculated an EC10 of 299 µg/L.

The quality of the test design and execution are not considered of the quality typically used for determine chemical toxicity as per Good Laboratory Practices[16]. This is reflected by a variety of things:

[16] EPA Good Laboratory Practices (GLP) available on-line: https://www.epa.gov/compliance/good-laboratory-practices-standard-operating-procedures

- The scatter (huge variability) of the weight change
- The gap in sulfide concentrations between 100 µg/L and 1000 µg/L
- The high variability in the measured sulfide concentrations the implicit lack of control of aqueous sulfide
- The lack of daily sulfide measurements
- Treatment of what is really 3 range-finding tests as definitive tests

It is not clear whether MPCA has generated a different random subset and conducted a sensitivity analyses to determine that this is valid approach; nor is there an evident rationale for not using the entire initial weights. As presented in Attachment 1, an option of using the geomean of the minimally generated sulfide measurements was investigated by Ramboll Environ. It is not appropriate to use a geomean on this type of data, a time-weighted average is more applicable.

The sulfide EC10s for the Pastor data vary by more than a factor of two, ranging from 103 µg/L to 255 µg/L. Given the variability in these EC10s and significant criticisms of the Peer Review Panel (see Section 3.2.2) these the sulfide EC10s, and any other ECs that may be based on the Pastor dataset, should be considered rough estimates and weighted less heavily in the determination of a porewater sulfide protective value then the other lines of evidence.

To reiterate, the MPCA presentation of probability of wild rice presence versus porewater sulfide is flawed as there is not a well-defined no-effect level due to the high variability in porewater sulfide concentrations and the fact that sulfate is a necessary wild rice nutrient (TSD page 53).

The proven and known approach of developing water quality criteria by developing a dose-response curve has not been reasonably demonstrated by MPCA. Their presentation contains errors and these errors undermine the confidence in understanding and defining the relationship between porewater sulfide and wild rice health.

MPCA correctly states that water column sulfate does not have a direct effect on wild rice – there is no dose-response curve for sulfate vs. wild rice survival, growth, or reproduction. MPCA presents sulfate as having an indirect effect of wild rice. MPCA has defined porewater sulfide as a toxicant causing adverse impact to wild rice. However, as discussed previously, there is minimal confidence in the sulfide threshold developed by MPCA and MPCA's presentation of dose-response relationship is flawed.

Without confidence in the dose-response for porewater sulfide, a "toxic amount" is difficult to define for use in assuring that protection of designated use is achieved. If MPCA followed the longstanding EPA approach to water quality criteria development, the wild rice water quality standard would be based on the chemical causing the direct effect, porewater sulfide.

EPA has not attempted to establish water quality criteria based on an indirect cause of the effect. EPA's water quality criteria are based on the direct cause. As discussed earlier, EPA has recommended criteria that are not water-column based i.e., selenium fish tissue, methylmercury fish tissue.

And
OECD Good Laboratory Practices available on-line:
http://www.oecd.org/chemicalsafety/testing/goodlaboratorypracticeglp.htm

MPCA should take a page from EPA and use guidance to implement the porewater sulfide threshold. Certainly MPCA would have far more flexibility to allow implementation of the porewater sulfide threshold concentration into water column sulfate concentrations to exist as guidance, and not regulation. This would also allow MPCA the nimbleness needed to respond to additional data, evolving understanding the geochemistry of wild rice waters, and improved statistical methods.

MPCA neglected to explain the Vermont process and highlight how the process was very different from the MPCA approach for the MBLR sulfate equation. In particular, specific to the implementation of the Vermont nutrient criteria, an integrated approach to implementation is also presented by Vermont[17]. The integrated approach used by Vermont allows for compliance with nutrient criteria to be evaluated by either comparison to nutrient criteria or by comparison to nutrient response variables (e.g., macroinvertebrate community health). This integrated approach is used because of the misclassification rates of 20 to 40%.

An integrated approach that might be considered is the presence and health of the wild rice in the wild rice water body and if the wild rice were present and healthy, then compliance is demonstrated. Given the amount of MPCA MBLR sulfate misclassification rate, an integrated approach is warranted.

MPCA, by adopting into rule the translation of the porewater sulfide to water column sulfate with the development of the MBLR sulfate equation, needs a level of confidence (e.g., far lower level of misclassifications) that is not currently shown. In addition, Vermont, in recognition of their high misclassification rate (similar to MPCA's misclassification rate) for nutrients, is using an integrated approach for implementation while MPCA is not.

USEPA has issued and continues to update guidance on criteria development including the type of data and statistical methods to define the dose-response. The current state of the science is recognition that the direct cause of an aquatic life (or human health) adverse impact (or effect) may not be due to water-column exposure[18]. This is similar to MPCA's finding that water column sulfate has no direct effect on wild rice.

The development of water quality criteria for methylmercury, took 10 years of work (science and review) and for selenium, 19 years. Understanding of cause and effect takes time to allow thoughtful consideration given the importance of protecting humans and fish.

MPCA correctly states that water column sulfate does not have a direct effect on wild rice – there is no dose-response curve for sulfate vs. wild rice survival, growth, or reproduction. MPCA presents sulfate as having an indirect effect of wild rice. MPCA has defined porewater sulfide as a toxicant causing adverse impact to wild rice. However, as discussed previously, there is minimal confidence in the sulfide threshold developed by MPCA and MPCA's presentation of dose-response relationship is flawed.

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[17] Vermont DEC. 2014 rev 2016. Nutrient Criteria for Vermont's Inland Lakes and Wadeable Streams: Technical Support Document. Available on-line: http://dec.vermont.gov/sites/dec/files/wsm/Laws-Regulations-Rules/2016_12_22-Nutrient_criteria_technical_support_document.pdf

[18] USEPA. 2001. Water Quality Criterion for the Protection of Human Health: Methylmercury. EPA-832-R-01-001. Available on-line: https://nepis.epa.gov/Exe/ZyPDF.cgi/20003UU4.PDF?Dockey=20003UU4.PDF
and
USEPA. 2016. Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater. Available on-line:https://www.epa.gov/sites/production/files/2016-07/documents/aquatic_life_awqc_for_selenium_-_freshwater_2016.pdf

Using state-of-the-art methods, EPA has shown more than once that a non-water column criteria can be developed from dose-response (aforementioned fish tissue based criteria) and that the confidence one typically has with laboratory water column data, can be achieved in defining a "toxic amount" in fish tissue. If MPCA followed the longstanding EPA approach to water quality criteria development, the wild rice water quality standard would be based on the chemical causing the direct effect, porewater sulfide.

However, the implementation of these criteria to water column levels, or translation to a water column concentration, is considered a separate activity and is not part of the EPA's national recommended criteria. What this means is that while EPA criteria are suitable (and typically encouraged) to be adopted into state water quality standards programs, translation of criterion to water column concentration is not encouraged by EPA to be part of state regulatory water quality standards. It is recognized that the models developed by EPA to go from fish tissue level to water column level are very site-specific and typically data intensive (e.g., multiple years of data needed).

The implementation guidance for methylmercury (to generate a total mercury water column concentration for a water body) was issued 9 years after the final fish tissue methylmercury criterion was issued[19]. EPA has not yet finalized the implementation (and monitoring) guidance for selenium fish-tissue; work that began in 2004[20]. Point being: the amount of data and information needed takes time to generate, validate, and utilize to be able to develop the sound models and recommendations to translate the direct effect (methylmercury in fish tissue or selenium in fish tissue) to water column concentrations (mercury in water column or selenium in water column).

As presented by MPCA, the MBLR sulfate equation (which is a model) is not aligning with porewater sulfide or wild rice health (MPCA uses the term "misclassification") for an alarming number of waterbodies (TSD, page 48 to 62, 67 to 83; SONAR page 77 to 79) as one considers the regulatory impact on agency decisions and actions.

MPCA should take a page from EPA and use guidance to implement the porewater sulfide threshold. Certainly MPCA would have far more flexibility to allow implementation of the porewater sulfide threshold concentration into water column sulfate concentrations to exist as guidance, and not regulation. This would also allow MPCA the nimbleness needed to respond to additional data, evolving understanding the geochemistry of wild rice waters, and improved statistical methods.

MPCA's identification of porewater sulfide as the direct cause of an adverse impact on wild rice is similar to EPA's fish-tissue based criteria. However, EPA has taken the time to generate robust and valid data and methods to translate the fish-tissue criteria to water column chemical concentrations and the translation is adopted as guidance; not a water quality criterion or rule. MPCA, by adopting into rule the translation of the porewater sulfide to water column sulfate with the development of the MBLR sulfate equation, needs a level of confidence (e.g., far lower level of misclassifications) that is not currently shown. In addition, Vermont, in recognition of their high misclassification rate (similar to MPCA's

[19] EPA. 2010. Guidance for Implementing the January 2001 Methylmercury Water Quality Criteria. Available on-line: https://nepis.epa.gov/Exe/ZyPDF.cgi/P1007BKQ.PDF?Dockey=P1007BKQ.PDF

[20] EPA. 2016. Technical Support for Adopting and Implementing EPA's 2016 Selenium Criterion in Water Quality Standards, Draft. Available on-line: https://www.epa.gov/sites/production/files/2016-10/documents/technical-support-adoption-implementation-selenium.pdf

misclassification rate) for nutrients, is using an integrated approach for implementation while MPCA is not.

MPCA does list acceptable analytical performance but neglects to identify the required MDL. My opinion is given MPCA's use of a porewater sulfide threshold of 120 µg/L, the MDL should be at least 3 to 5 µg/L and the RL 10 to 15 µg/L to have confidence in using the data to derive an enforceable sulfate standard. The accuracy (bias) statement presented by MPCA is different than that included in Standard Methods. Further, no documentation or data on the development of an acceptable recovery of 80 to 100% (versus 97.6% to 104.2%) is provided by MPCA.

Ramboll has reached out to over 10 reputable certified (e.g., NELAC) commercial water testing laboratories and none of them either are set-up to run this method or routinely run this method to be confident in the quality of their results at a RL of 10 to 15 µg/L sulfide[21]. One commercial lab who has been a leader in AVS and sulfide analytical method development, Alpha Analytical, noted that colorimetric methods have a high potential for false positives due to naturally colored water.

MPCA needs to fully share all the laboratory quality control data and MDL studies conducted by the state lab to assure that MPCA, existing, new or expanding dischargers, and stakeholders are informed on the reliability and accuracy of Method 4500-$S2^-$ E Sulfide. As of now, neither MPCA nor other parties, can document the reliability and accuracy of a porewater sulfide result that will be key to deriving the enforceable sulfate standard.

As of today, no certified commercial water testing labs are available to conduct this method to a RL of 10 to 15 µg/L sulfide. As MPCA seems to have the most experience with this analytical method, they should engage in public outreach to share their knowledge with commercial labs on reliably and accurately conducting Method 4500-$S2^-$ E Sulfide.

Tedrow

1. SUMMARY

- Controlling competing vegetation in waters intended for WR production is critical for maintenance of desired WR growth, distribution, abundance, and productivity. Competitive exclusion and potential allelopathic influences from competing vegetation can substantially limit WR health and productivity, with the potential for elimination from the area or water resource.
- Achieving and maintaining an appropriate water depth in WR areas is one of, if not the, more important variables to control for WR plant growth, development, reproduction, and abundance. In the absence of appropriate water depth control, WR plants will be under excessive stress, which may result in decreased health and abundance with subsequent elimination from an area or water resource.
- In general, for prairie potholes, in the absence of water depth control and maintenance of a preferable WR water depth, and the almost ephemeral nature of prairie potholes re: presence /

[21] Ramboll personal phone and email communications in August 2017.

absence of standing water, prairie potholes are unlikely to be acceptable habitat for WR production, regardless of chemical characteristics of overlying water and sediment pore water.

  - Prairie potholes are not generally controlled, or controllable, for WR production. Reference of prairie potholes as poor WR habitat specifically due to chemical characteristics that may be detrimental to (WR) growth is incomplete, and not necessarily defensible if not considering the variable hydrological cycle(s) of any specific pothole.

- Suggesting that water lilies are indicative of acceptable WR habitat is an incomplete statement. In the areas presented here, WR and water lilies do occur in the same general area; however, a distinction between higher density populations of each plant appears evident. Therefore, simply stating that the presence of water lilies is an indicator of acceptable WR habitat is overgeneralized.
  - Additionally, water lilies have been observed to grow in areas of this system with water depths exceeding three feet – a depth not conducive to WR plant growth. In a system with non-controlled water depth, water lilies may be a high proportion of the aquatic plant assemblage in the absence of WR. This could also be due to a lack of viable WR seed in the sediment; a potential result of WR germination in excessive water depths without subsequent reproductive success.
- Based on available data, and consideration of biological, physical, and other environmental influences beyond control specific to microbial H2S synthesis, application and enforcement of a sediment porewater sulfide WR protective level is unlikely to be beneficial to WR distribution in MN.

2. INFLUENCES ON WILD RICE GROWTH, HEALTH, AND ABUNDANCE

Increased focus on specific chemical characteristics of surface waters and associated sediment porewaters of wild rice (WR) areas may currently be non-warranted. Initially, system-wide physical and biological characteristics – specifically, water depth and competing vegetation – of waters containing WR should be the focus, if maintenance or management of that resource for WR production is the overall objective. Multiple examples of each of these influences can be observed occurring independently or, as is sometimes the case, concurrently

Based on historical, and current data and observations during laboratory and field experiments, as well as direct field-scale application via WR restoration activities, controlling competing vegetation and maintaining an appropriate water depth for WR should be the first two objectives for maintaining waters for increased WR growth, health, and abundance.

Initially, system-wide physical and biological characteristics of waters containing WR should be the focus, if maintenance or management of that resource for WR production is the overall objective. Specifically, water depth and competing vegetation.

According to published literature sources water depths of 0.5 – 3.0 feet are more conducive to WR growth and propagation (MN DNR 2008; Vogt 2012), and that water depth is the major factor controlling WR abundance and production (Aiken 1989, Oelke et al. 1997, MN DNR 2008; Vogt 2012).

Water depth directly influences WR phenological development and its ability to compete against other aquatic vegetation better able to cope with increased or increasing water depth.

Influences on WR from water depth increases depend on its phenology at the time of increase. If the increase is sudden during the submerged or floating leaf stage, the less developed roots may not be able to anchor the plant, which may then be uprooted (Thomas and Stewart 1969).

If water depth increase is more gradual and the plant is still in the submerged stage, it will take longer to reach the surface with corresponding losses in yields due mostly to decreased tillering, or complete loss of reproductive success due to mortality.

If the plant has achieved floating leaf stage and is then submerged, the plant is placed under stress since gas exchange with the atmosphere has been interrupted – NOTE: at this stage, the cuticle may have already formed further exacerbating WR plant stress due to decreased gas exchange ability while submerged. Some varieties survive by reducing growth and initiating metabolic processes that enable the plant to tolerate temporarily increased water depth.

… higher nutrient levels ensured more robust plant growth that enabled the plants to survive the water level increases. It may also be possible that the WR variety used had a genetic tolerance to depth increases such as shown by Counts and Lee (1988).

If water depth decreases to an extent that the water in the rice areas freezes to or past the sediment-water interface, the seed may desiccate (essentially the same as 'freezer burn') and lose its ability to germinate. In natural stands of WR, this is commonly known as the 'ring effect' whereby no WR grows along the shallower edges of the water body, but is present in some deeper sections (Aiken et al. 1989).

3. WILD RICE IN MN PRAIRIE POTHOLES

Primary factors limiting the restoration of WR in areas previously dominated by WR have been related to water depth and managing competing aquatic vegetation (see amended attachments – 'MN Conservation Volunteer – Wild Rice Renaissance,' and MPR News re: Fond du Lac Band WR restoration activities).

Moyle suggested that WR was primarily found in waters with a total alkalinity less than 40 mg l-1, pH between 6.8 – 7.0, and a sulfate concentration of less than 10 mg l-1.

The hydroponic solution recommended by Malvich and Percich (1993) uses a sulfate concentration of 48 mg l-1. Using this culture solution, Lee and Hughes (2000) found that early WR development was affected at sulfate concentrations in the range 1200 – 1500 mg l-1. Vicario and Halstead (1968) conducted experiments with rice in culture solutions with sulfate that ranged from 0 to 8800 mg l-1. They observed decreases in weight and height when sulfate in the culture solutions went above 220 mg l-1. More recent laboratory studies exposing WR seeds to various concentrations of sulfate and chloride salts under hydroponic conditions concluded that adverse influences from sulfate in particular occurred at concentrations over 1500 mg l-1 (Fort et al. 2014).

Overall, adverse influences on WR growth and development associated with sulfate and / or chloride are more likely due to a general increase in TDS, which tends to disrupt osmotic balance and ion transfer to / from / within the plant.

Lee (1979) in a survey of WR lakes in Minnesota and Ontario found the majority of lakes supporting WR had soft water with average alkalinities of 40 mg l-1 and pH levels of ~ 6.9.

Pip (1984) examined the distribution of 59 species of aquatic macrophytes, including WR, outside and inside the Precambrian shield of central Canada. She found the more important water chemistry parameters associated with their distribution to be pH, TDS, and total alkalinity.

Chloride, phosphorus, and sulfate concentrations were reported as '…of minor importance in both areas.' Wild rice is generally associated with more oligotrophic waters.

Pilsbury and McGuire (2009) attributed losses of WR in Minnesota and Wisconsin to residential and agricultural developments that increased nutrient levels, which can result in increased competition from other aquatic plants including algae. Ammonia and pH changes were specifically implicated.

Reduction in the range of WR has also been attributed to human disturbance including water contamination, recreational activities (boat turbulence), and importantly water level manipulation (Meeker 1996; Bennet et al. 2000).

Finally, although WR distribution may be influenced by water chemistry or at least correlated to water chemistry, WR also affects the water chemistry in which it lives. Lee and McNaughton (2004) showed that water surrounding WR stands contained lower sulfur (S), and higher conductivity, calcium, and iron concentrations than open water areas.

It is notable that there is a range of values for multiple parameters of one to three orders of magnitude suggesting that WR has a wide tolerance range of these characteristics.

All metals were therefore below levels where any adverse influence on WR should occur.

As typically isolated water bodies surrounded by agricultural activities, potholes may not be considered optimal, or even suitable, WR habitat for multiple reasons – the two primary reasons, as the initially more important reasons, are lack of water depth control and aquatic plant management. Influences from surrounding land use patterns may also contribute to the general non-suitability of potholes as WR habitat (nutrient inputs, pesticide / herbicide exposures, localized groundwater use); however, in the absence of data supporting these claims, discussion will be limited to the primary physical (water depth) and biological (competing aquatic vegetation) influences.

In general, in the absence of water depth control and maintenance of a preferable WR water depth, and the almost ephemeral nature of prairie potholes re: presence / absence of standing water, prairie potholes are unlikely to be acceptable habitat for WR production, regardless of chemical characteristics of overlying water, sediment, and sediment porewater.

Adverse influences from aquatic vegetation competing with WR for scarce resources can be exacerbated in the presence of non-controlled water depth.

4. WATER LILIES AS AN INDICATOR OF ACCEPTABLE WILD RICE HABITAT

Although water lilies can occur in the same general area as WR, and in some cases, co-occur, competing vegetation in general adversely influences the abundance of WR.

Additionally, water lilies have been observed to grow in areas of this system with water depths exceeding three feet – a depth not conducive to WR plant growth. In a system with noncontrolled water depth, water lilies may be a high proportion of the aquatic plant assemblage in the absence of WR.

In this particular area, WR and water lilies do occur in the same general area; however, a distinction between higher density populations of each plant appears evident. Due to this observation and the scenarios detailed above, simply stating that the presence of water lilies is an indicator of acceptable WR habitat is overgeneralized.

5. MIGHT THE 120 µg/ L SULFIDE PROTECTIVE LEVEL IMPROVE WR DISTRIBUTION IN MN?

Although as a general rule sulfide as hydrogen sulfide (H2S) can be problematic to organisms, there is a tolerance range associated with what may be the exposure concentration at which adverse responses are observed – WR in this case is no exception. Based on current MPCA field data and observations, WR can grow to a density of > 100 stems per square meter in the presence of hydrogen sulfide concentrations exceeding 120 µg / L.

Based on available data, and consideration of biological, physical, and other environmental influences beyond control specific to microbial H2S synthesis, application and enforcement of a sediment porewater sulfide WR protective level is unlikely to be beneficial to WR distribution in MN.

Since water depth is a primary controlling factor for WR distribution and abundance, and WR tends to prefer shallower water (0.5-3.0 feet deep), the likelihood of oxygenating the entire water column in areas of preferential WR habitat specifically during disturbance events would likely be high. Therefore, one controlling factor for hydrogen sulfide synthesis in nearer-surface sediments is likely water column oxygenation, both duration and frequency; in addition, sufficiently intense disturbance events could disrupt nearer-surface sediments, resulting in oxygenation of the disturbed sediment area and decreasing H2S synthesis potential.

ROL from WR plants could influence the overall composition of the microbial assemblage, further decreasing the potential for microbially mediated H2S synthesis during periods of growth for WR plants.

6. POTENTIAL INFLUENCES FROM IRON PLAQUE FORMATION ON WILD RICE ROOTS

Reduced iron precipitates have been observed to occur during the plant's life stage in which reproduction is the more dominant activity; during this time the seed is maturing, while the remaining portions of the plant are beginning to senesce. Precipitation of reduced iron species could be expected, since plant senescence during seed maturation involves decreased energy allocation to maintaining the shoot, stems, and leaves, and the likely decreased rate of ROL into the rhizosphere.

Although a lower nitrogen content of WR seeds may suggest a less 'healthy' seed, additional generational research is required to investigate influences on germination of viable WR seeds from plants exposed to increased aqueous sulfate, with generally decreased seed nitrogen. Since this was an observed association under more controlled laboratory conditions, field verification of these observations would be required to allow a more applied perspective to these data.

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[i] Pastor, 2014
[ii] Fort, et al, 2014

[iii] The “Ramboll equation”, detailed in Appendix 9 of the Technical Support Document
[iv] Reference page of the Pastor report
[v] KeeTac NPDEs permit, need reference
[vi] Reference needed
[vii] Independent research conducted by Barr Engineering, November 2016
[viii] Reference Pastor et al, 2013
[ix] Since UMD (and occasionally FEL) researchers chose to report sulfide values in uM (micromoles) instead of more conventional mg/l or µg/L values, values have been converted for clarity and consistency.