Role of Sulfate on Wild Rice Health

Governor’s Task Force on Wild Rice Meeting 2
October 11, 2018
Presenters

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Wild rice (*Zizania palustris*) is an annual aquatic plant that grows in lakes and rivers of north-central North America.

Wild rice populations are sensitive to chemical/physical/biological processes including...

- Competing vegetation
- Water clarity
- Waterfowl predation
- Water level fluctuations
- Water chemistry

Our presentation today concerns only how water chemistry (sulfur in particular) affects wild rice.
A combination of factors impact which chemicals are present in sediment where wild rice grows.
Hydrology matters:

- How are **chemicals transported** to the rice rooting zone in different hydrologic scenarios? experimental vs. natural; rivers vs. lakes; ground water vs. surface water
Sediment reactions matter:

- *How do chemicals change form and combine with each other to create conditions toxic to wild rice?* bacteria, solid vs. porewater, balance of inputs among S, C, Fe, O.
Plant physiology/toxicity/life cycle matters:

- *Where and when are rice plants sensitive to sulfide?* Which portion of the plant (roots, stem, leaves)? Which portion of the annual life cycle is sensitive? How are successive generations impacted by sulfide?
Goals

- Present state of science
- Identify areas where interpretations differ or experiments/observations are inconclusive
- Identify knowledge gaps in state of science
- Discuss next steps, moving forward

No expectation of full resolution or complete consensus: building blocks for shared understanding
Introduction, points of agreement, and recent research on mechanisms

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Nate Johnson
Associate Professor of Civil Engineering
University of Minnesota Duluth
What is known?

• Some historic knowledge: Moyle 1947 – *no large stands of wild rice found in waters containing more than 10 mg/L sulfate*
• Literature for studies based on other plants (not much study on wild rice)
• Much knowledge gained from recent studies commissioned by legislature through MPCA and other follow-up studies
Tradeoff between experimental control and realism

From: Petersen & Kemp, The role of enclosed experimental ecosystems ("mesocosms") in ocean science
Recent wild rice studies encompass a range of experimental/observational scales.

Modified from: Petersen & Kemp, *The role of enclosed experimental ecosystems ("mesocosms") in ocean science*
What is (generally) agreed upon?

Form of sulfur (reactions)

• Sulfate (in surface water or ground water) is essentially not toxic at concentrations seen in MN Wild Rice waters
• Elevated porewater sulfide is potentially toxic
• In some cases, sulfate can be converted to sulfide in sediment; *(notable exceptions when groundwater “upwelling” is important)*
• Control of sulfate from dischargers would be very expensive
What is (generally) agreed upon?

How does sulfur get to rice (hydrology)

- Both groundwater and surface water can input chemicals to rice rooting zone *(discussion to follow)*

- Contributions of groundwater are not well-characterized; difficult in light of natural variability
What is (generally) agreed upon?

Where/when is rice sensitive to sulfide (toxicity/ecology)

• Some concentration of sulfide is toxic to wild rice (discussion to follow)
• Most sensitive life stage is not known; juvenile survival and seed production both impacted
• Chronic impacts (sulfate-induced or other factors, e.g. water level, water clarity, predation, etc.) can cause slow decline in populations
Recent UMD research on wild rice

• Working at scales between lab and field mesocosms

• Studying mechanisms of how sulfide interacts with rice roots in sediment, especially over course of wild rice annual life cycle

• Combination of Fe, C, S additions to mesocosms
Recent UMD findings

• Sulfide impacts different stages of rice growth: juvenile survival, plant reproduction

• Cumulative impacts matter (over time); plant populations and chemical accumulation

• Near-root geochemistry may be decoupled from porewater chemistry ‡ plant / geochemistry feedbacks

• Iron additions partly ameliorates impact of $\text{SO}_4$ in mesocosms; not completely, especially at early life stage
MPCA’s scientific research on the role of sulfate on wild rice

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Ed Swain
Minnesota Pollution Control Agency
MPCA’s Research on Wild Rice

• 2011 legislature provided funds to re-evaluate the existing sulfate standard of 10 mg/L.

• MPCA’s mandate is to identify a numeric standard that is protective of wild rice.
  – EPA suggests that protection of a species can be achieved at toxin concentrations that have a 10% negative impact (an EC_{10}).

• Use multiple lines of evidence to identify a protective concentration.
Significant peer-reviewed publications

1. Myrbo et al. 2017. Sulfide generated by sulfate reduction is a primary controller of the occurrence of wild rice (*Zizania palustris*) in shallow aquatic ecosystems.


5. Fort et al. 2014. Toxicity of sulfate and chloride to early life stages of wild rice (*Zizania palustris*).


8. LaFond-Hudson et al. 2018. Iron sulfide formation on root surfaces controlled by the life cycle of wild rice (*Zizania palustris*).

Key findings from research

– Sulfate is not toxic to wild rice.
– Sulfide in sediment porewater exerts significant control over wild rice presence and density across Minnesota.
– Porewater sulfide is usually derived from sulfate in the surface water.
– Porewater sulfide levels are controlled equally by surface water sulfate, sediment iron, and sediment organic carbon levels.
– A small proportion of surveyed sites (6%) develop less sulfide than expected, and therefore would be candidates for a site-specific sulfate standard (as provided for in the Clean Water Act).
Recent wild rice studies encompass a range of experimental/observational scales

Modified from: Petersen & Kemp, The role of enclosed experimental ecosystems ("mesocosms") in ocean science
Hydroponic Experiments Yield Protective (EC10) Concentrations (Pastor, Fort)

EC_{10} Values (μg/L)
- 299 (mean initial concentration)
- 160 (time-weighted arithmetic mean)
- 71 (time-weighted geometric mean)

Growth (relative change in dry weight)

Sulfide (micrograms per liter)
- 160

Plot showing growth and sulfide concentration across three tests.
Conclusions from Hydroponic Experiments

• Sulfate not toxic at observed surface water concentrations.
• Sulfide toxic at some observed pore water concentrations, depending on experimental set-up.
Recent wild rice studies encompass a range of experimental/observational scales.

Modified from: Petersen & Kemp, The role of enclosed experimental ecosystems ("mesocosms") in ocean science
Outdoor Mesocosms tell us how sulfate turns into sulfide, and how that affects wild rice (Pastor)

Pastor et al. grew wild rice for 3 years in 5 levels of sulfate:

control, 50, 100, 150, 300 mg/L

(6 tubs per treatment)
Conclusions from outdoor experiments in tubs

• More sulfate in surface water produces more porewater sulfide.

• Wild rice seedling emergence, seedling survival, biomass growth, viable seed production, and seed mass all declined with increasing sulfate additions and hence increasing porewater sulfide.
Recent wild rice studies encompass a range of experimental/observational scales.
Wild Rice Field Survey (Mybro)
Wild Rice Field Survey

**Surface water**
- Na, K, Mg, Ca, Fe
- SO₄, Cl
- Alkalinity, pH, conductivity, Total P, Total N, Ammonia, Nitrate + Nitrite, transparency

**Bulk Sediment Chemistry**
- Acid-Volatile Sulfide
- Total carbon, phosphorus, nitrogen, sulfur
- Phosphorus fractionation
- Simultaneously-Extracted Metals:
  - Fe, Cu, Zn, Co, Ni, Mn, Mo, Se, As, B

**Porewater**
- Sulfide
- Na, K, Mg, Ca,
- SO₄, Cl
- Total P, Total N, Silica
- Ammonia, Nitrate + Nitrite
- DOC (dissolved organic carbon)
- Fe, Cu, Zn, Co, Ni, Mn, Mo, Se, As, B

**Other Sediment Properties**
- Water
- organic matter
- carbonate content
- Organic grain size
- Wild rice phytolith presence/absence
Statistical analysis identified three variables that control wild rice (Myrbo et al. 2017)

- **Porewater sulfide**
  - Probability of wild rice presence declines
  - Probability of dense wild rice declines

- **Water transparency**
  - Wild rice needs light to get to the water surface, where it can get more light & much more oxygen.

- **Water temperature**
  - Colder is better
Multiple Lines of Evidence

Hydroponic Experiments (Fort et al.)
- EC10 of average initial sulfide concentrations
- EC10 of time-weighted arithmetic average of sulfide concentrations
- EC10 of time-weighted geometric average of sulfide concentrations

Hydroponic Experiments (Pastor et al.)
- EC10 of percent filled seeds
- EC10 of # of plants germinated

Outdoor mesocosm experiment (Pastor et al.)

Field survey endpoints (Myrbo et al.)
- Visual identification of graphed reduction in proportion of sites with wild rice (N=96 or 108)
- Change-point Analysis in stem density (waters with wild rice, N=67)
- EC10 of presence/absence (transparent sites, log-transformed sulfide, N=96)
- EC10 of presence/absence (all sites, log-transformed sulfide, N=108)*

Sulfide Concentration (micrograms/liter)
Even though sulfide comes from sulfate, there is a poor relationship between them.
Porewater sulfide is controlled equally by three variables

- Sulfate in surface water
- Iron in sediment
- Organic carbon in sediment
Outliers from the proposed MPCA equation

- Sites with sulfate less than calculated standard ←
- Sites with sulfate greater than calculated standard →
- Sites with sulfide greater than MPCA’s protective sulfide concentration ↑
- Sites that under-produce sulfide, perhaps because of upwelling groundwater (6% of all sites)

**Porewater sulfide (micrograms/liter)**

**Ambient Sulfate / Calculated Sulfate Standard**

- sample with no wild rice observed
- sample with less dense rice (< 10 stems / m²)
- sample with denser rice (≥ 10 stems / m²)
MN business point of view
MN Business Point of View

• Only industry is currently challenged by the existing 10 mg sulfate/L standard
  – Only mines and 1 power plant have sulfate standards to protect wild rice
  – Only mines have been required to monitor wild rice presence and health downstream

• Cost to comply with 10 mg sulfate/L standard for industries will be OTOO $10-100 MM/site (NPV)!
Groundwater

• Groundwater is a much bigger factor than has been previously been acknowledged
  – NE MN groundwater: high iron, low sulfate
  – SW MN groundwater: high sulfate, low iron (glaciers)

• Groundwater interaction with wild rice waters is not well understood, particularly for individual wild rice waters
  – e.g. lakes vs. headwater streams
Life Stage and Affected Parts

• Most organisms are most affected by toxic chemicals in the early life stages
  – US EPA guidance requires testing on neonates and juveniles

• MN Business believes that germination and mesocotyl growth are most affected
  – Other plant parts are not in contact with anoxic sediment or sulfide
  – Once wild rice produces “green” parts, photosynthesis produces oxygen which can offset toxicity of sulfide
Life Stage and Affected Parts

- Most experiments assumed that sulfate and sulfide are acute toxicants
- Some very recent experiments (LaFond-Hudson et al, September 2018), suggest that sulfate/sulfide may act as chronic toxicants
- More research needed to determine the mode of action of sulfate/sulfide toxicity.
Sulfide Toxicity

• While sulfide can be toxic to wild rice, the level at which sulfide is toxic is in dispute
• MN Business believes that sulfide may be toxic only above 3,200 µg/l, based on standard toxicity testing exposing only the germinating seed and mesocotyl
• In the presence of iron, sulfide may be toxic only above 7,800 µg/L (Fort et al 2017)
• Very few wild rice waters have concentrations of sulfide in porewater above that level
• Therefore, sulfide may not be the controlling factor in wild rice presence and health.
Wild Rice Protection

• MN Business believes that protection of wild rice should take into account all known factors which could affect wild rice.

• MN Business notes that single species protection plans in Minnesota typically involve management plans specific to regions of the state or even specific water bodies. E.g.:
  – Walleye in Mille Lacs Lake
  – Gray Wolves in NE MN
  – White tail deer
Wild Rice Protection

• Wildlife management plans encompass all known factors affecting the presence and health of the species
  – Takings and possession limits
  – Disease control
  – Habitat improvement

• Wild Rice protection should similarly encompass all known factors
Summary, final thoughts, questions

Panel & Task Force
Extra Johnson slides follow
Two relationships involved in quantifying sulfate impacts to rice

- **Surface water sulfate ⇔ rooting-zone sulfide**
  - Messy relationship in field data, clear trend in mesocosms
  - Reactions matter: allowed reactions to occur in mesocosms, (but for only one combination of Fe, C, O and everything else)

- **Rooting-zone sulfide ⇔ plant effects**
  - Isolated rooting-zone sulfide from other factors in lab studies (tight control)
  - Measured rooting zone sulfide in-situ (with other elements, Fe, S, C, O, etc.) for both mesocosm, field observations
• Rooting-zone sulfide – related to surface water sulfate

![Graph showing correlation between porewater sulfide and surface water sulfate]
• Rooting-zone sulfide – related to plant effects
Porewater sulfide † wild rice effects

• Relating porewater sulfide to plant health/reproduction is the state of practice based on established research: borne out in hydroponics, mesocosms, and field observations

• Recent studies show that there are additional (near-rooting zone) nuances that result in seasonal and life-stage variations in the sensitivity of rice to sulfide...
1. Vegetative growth: net oxygen flow towards sediment

2. Fe(III)-O phase: accumulates on root when plant is growing
1. Seed production: net e- flow towards root

2. Fe(II)-S phase: accumulates on root when plant is not growing
Extra MPCA slides follow
MBLR Equations

MBLR120 Sulfate = 0.0000121 x TOC^{-1.197} x TEFe^{1.923}

where sulfate is expressed as mg/L, Total Organic Carbon (TOC) as percent dry weight, and Total Extractable Iron (TEFe) as mg/kg

<table>
<thead>
<tr>
<th>Study Site</th>
<th>State ID</th>
<th>Sediment Total Organic Carbon (%)</th>
<th>Sediment Iron (µg/g)</th>
<th>MBLR-Calculated Sulfate (mg/L)</th>
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</thead>
<tbody>
<tr>
<td>Little Round Lake</td>
<td>03-0302</td>
<td>27.5</td>
<td>3,069</td>
<td>1.2</td>
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<td>Elk Lake</td>
<td>15-0010</td>
<td>10.2</td>
<td>8,480</td>
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<td>Rice Lake</td>
<td>18-0053</td>
<td>35.6</td>
<td>50,389</td>
<td>186</td>
</tr>
</tbody>
</table>
Equation-Based; “Fixed” Standard: Considerations

• Many commenters expressed concern with equation approach
• Equation-based standards are not new, but are less common
• Enhanced precision Û additional data collection
Accuracy of the MPCA’s proposed equation (Myrbo’s 108 field sites)

**False Negatives** (10 of 108 = 9% of all samples)
- Wild rice in 4 of 10 = 40% of false negatives
- Denser rice in 2 of 10 = 20% of false negatives
- Denser rice in 2 of 4 = 50% of false negatives with wild rice

**True Positives** (29 of 108 = 27% of all samples)
- Wild rice in 14 of 29 = 48% of all true positives
- Denser rice in 7 of 29 = 24% of all true positives
- Denser rice in 7 of 14 = 50% of true positives with wild rice

**True Negatives** (62 of 108 = 57% of all samples)
- Wild rice in 44 of 62 = 71% of all true negatives
- Denser rice in 38 of 62 = 61% of all true negatives
- Denser rice in 38 of 44 = 86% of true negatives with wild rice

**False Positives** (7 of 108 = 6% of all samples)
- Wild rice in 5 of 7 = 71% of all false positives
- Denser rice in 5 of 7 = 71% of all false positives
- Denser rice in 5 of 5 = 100% of false positives with wild rice

- ● sample with no wild rice observed
- ○ sample with less dense rice (< 10 stems / m²)
- ○ sample with denser rice (≥ 10 stems / m²)
Percent of waterbodies misclassified

Potential sulfate standard (mg/liter)
Field survey of lakes & streams
(Amy Myrbo)

• 108 different lakes and streams sampled for surface water, sediment porewater, & sediment.
• 67 of 108 waterbodies had wild rice.
• When field crew couldn’t find wild rice, sediment was sampled at waterlilies, since they often co-occur with wild rice.
• Sampling sites with and without the species of interest is a standard method in conservation biology to discover what variables control favorable habitat (via binary logistic regression).
• 65 variables measured at each site.
Probability that wild rice is present declines as porewater sulfide increases.
Probability that wild rice is dense declines as porewater sulfide increases.
So porewater sulfide can only be predicted by considering all 3 variables simultaneously.
Probability of wild rice presence as a function of sediment iron

Probability of wild rice presence (%)

Sediment Iron (micrograms per gram)
Probability of wild rice presence as a function of Sediment Total Organic Carbon

- Model fit
- 95% C.I.