Effects of enhanced sulfate concentrations on wild rice populations:
results from a mesocosm experiment

A Report
presented to
Minnesota Pollution Control Agency
by
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Introduction

This report is one part of a larger study—the Wild Rice Sulfate Standard Study—coordinated by the Minnesota Pollution Control Agency (MPCA) on the effect of elevated sulfate concentrations on wild rice. Minnesota currently has a water quality standard of “10 mg/L sulfate - applicable to water used for production of wild rice during periods when the rice may be susceptible to damage by high sulfate levels.” (Minn. R. 7050.0224, subpart 2). In 2010, the MPCA initiated a multi-year effort to clarify implementation of the state’s wild rice sulfate standard, which had recently come under increased questioning and contention. Based on a review of available studies and information, the MPCA determined that additional studies were needed to evaluate the effects of sulfate on wild rice before a revision to the numeric sulfate standard could be considered. In 2011 the Minnesota Legislature provided funding to gather this additional information in the Legacy Amendment Bill (Laws of Minnesota, 2011, First Special Session, ch. 6, Art. 2, Sec. 5(j)).

Wild rice is an important aquatic plant in parts of Minnesota, particularly northern Minnesota. It provides food for waterfowl, is also a very important cultural resource to many Minnesotans, and is economically important to those who harvest and market wild rice.

The goal of the overall Wild Rice Sulfate Standard Study is to enhance understanding of the effects of sulfate on wild rice and to inform a decision as to whether a revision of the wild rice sulfate standard is warranted. The Study consists of several research efforts that have been conducted by several groups of scientists at the University of Minnesota campuses in Duluth and the Twin Cities under contract with the MPCA. The data collection phase of the study was completed in December 2013, and is documented in individual reports, along with associated data, from the researchers working on each component of the study.

The primary hypothesis driving the Study has been that if elevated sulfate has a negative effect on the growth of wild rice it is mediated through the formation of hydrogen sulfide in the rooting zone of wild rice, and that elevated iron would mitigate the toxicity of the sulfide by forming insoluble iron sulfide compounds.

The Study components include:

- **Field study of wild rice habitats** to investigate physical and chemical conditions correlated with the presence or absence of wild rice stands, including concentrations of sulfate in surface water and sulfide in the rooting zone.
• **Controlled laboratory hydroponic experiments** to determine the effect of elevated sulfate and sulfide on early stages of wild rice growth and development.

• **Outdoor container experiments utilizing natural sediments** to determine the response of wild rice to a range of sulfate concentrations in the surface water, and associated sulfide in the rooting zone, across the growing season.

• **Collection and analysis of rooting zone depth profiles of dissolved chemicals at wild rice outdoor experiments and field sites** to characterize sulfate, sulfide, and iron in the rooting zone of wild rice.

• **Sediment incubation study** to explore the difference ambient temperature has on the rate that elevated sulfate concentrations in water enter underlying sediment, convert to sulfide, and later release sulfate back into the overlying water.

The MPCA will review the results from individual reports along with existing monitoring data, other relevant scientific studies, pertinent ecological, cultural and historical information, and the original basis for the wild rice sulfate standard to determine if a change to the current wild rice sulfate standard is warranted, and what that change might be. If change(s) are proposed, they would be adopted into Minnesota Rules via the administrative rulemaking process and subject to U.S. EPA approval before the changes could be implemented.

This report focuses on the outdoor container experiments utilizing natural sediments to determine the response of wild rice to a range of sulfate concentrations in the surface water, and associated sulfide in the rooting zone.

**Background**

Northern wild rice (*Zizania palustris*) is one of four species in the genus *Zizania*, which are the only native aquatic grains in North America. The range of northern wild rice (hereafter wild rice) is centered across the Great Lakes region but is most abundant in the rivers and lakes of the watersheds of Lakes Superior and Michigan in northern Minnesota, Wisconsin, and Ontario. Because of its widespread distribution and tendency to form monotypic stands, wild rice has great potential to influence the food supply for waterfowl, muskrats, and other members of the food web. In addition, the native Ojibway people of the watersheds of Lakes Superior and Michigan teach that they were led to this region to find “the food that grows upon the water”,

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which is wild rice. The Ojibway identify their origins with wild rice and consider themselves “people of the rice” (Vennum 1988). Therefore, the productivity, perpetuation, and restoration of wild rice are of great ecological and cultural significance.

Wild rice is an annual plant. It grows in both lakes and rivers in water between 0.3 and 0.67 m depth where there is some water flow. Native stands of wild rice grow in waters that are circum-neutral pH, of low conductivity and hardness, and generally low in nutrient concentrations. In lakes, the most common sediment is an organic-rich silt, but the sediment types ranges widely (Day and Lee 1990). Sediment in the riverine habitat also ranges widely and may be higher in mineral sediment in the main channels than in backwaters (Meeker 1996). Seeds germinate in the spring and first develop a mesocotyl, or primordial shoot, and a radical, or primordial root. The mesocotyl then grows above the sediment surface, where it develops into a green shoot with a primordial leaf in late spring and early summer. The plant is now at the seedling stage. When the shoot of the seedling reaches the water surface, the plant generates a long narrow leaf which floats atop the water surface; this stage is therefore called the floating leaf stage. Photosynthesis by the floating leaf is used to expand the root system and the beginnings of an aerial shoot which emerges from the leaf axil of the floating leaf and the stem below the water surface. Once the aerial stem and the first aerial leaf emerge, the floating leaf dies and the plant grows taller, putting out additional aerial leaves until late July or early August. Nutrient uptake is very rapid during this stage, and approximately 60-70% of the plant’s annual requirement for nitrogen, the most limiting nutrient to both vegetative growth and seed production in most environments, is taken up then (Grava and Raisanen 1978, Sims et al. 2012a, b). In late July or early August, vegetative growth slows and the plant begins to produce a flowering shoot containing male (pollen producing) flowers above female (seed producing) flowers below. Wild rice does not self-pollinate well; instead, as for most graminoids, pollination is largely by wind although bees and flies occasionally visit the male flowers to gather pollen (J. Pastor, *personal observations*). During the seed production and ripening stage, there is another burst of nutrient uptake from the sediment and the lower vegetative leaves begin to senesce as the nutrients they contain are translocated to the ripening seeds (Grava and Raisanen 1978, Sims et al. 2012a, b). Seeds ripen in late August and through September, although the first two weeks of September are commonly the period of peak ripening. The seeds contain a long awn, which helps stabilize them vertically when they are dispersed into the water and thereby allow them to
drill into the sediment (Ferren and Good 1977, J. Pastor personal observations). After seed dispersal, the plant dies and its stem, leaf, and root litter are returned to the sediment. Delays in the release of nitrogen from these litters in subsequent years may be responsible for the population oscillations of 3-5 year periods often seen in wild populations (Pastor and Walker 2006, Walker et al. 2010, Hildebrandt et al. 2012).

In a survey of the distribution of aquatic plants in relation to water chemistry, Moyle (1944, 1945) found that wild rice and its abundance falls off rapidly at sulfate concentrations higher than 10 mg · L⁻¹. However, understanding whether sulfate itself or some other co-varying property of these lakes is responsible for the distribution and growth of wild rice requires additional experimental studies designed to test specific mechanistic hypotheses.

Sediments of wild rice lakes are almost always anoxic and consequently can have a high potential for reducing sulfate to sulfide under certain conditions. Because sulfate reduction is more thermodynamically favorable than methanogenesis (Capone and Kiene 1988), increasing concentrations of sulfate are likely to increase the rate of sulfide accumulation in sediment. High sulfide levels can inhibit root growth of wetland plants (Koch and Mendelssohn 1989, Koch et al. 1990, Lamers et al. 2002, Guerts et al. 2009), including white rice (Oryza sativa; Gao et al. 2003; Armstrong and Armstrong 2005). If root biomass is reduced by sulfide, then the plant’s ability to take up limiting nutrients, especially nitrogen, will be impaired (Gao et al. 2003, Armstrong and Armstrong 2005). Sulfide may also directly inhibit the ability of roots to take up nitrogen (DeLaune et al. 1983), but this remains a hypothesis requiring further testing.

Here, we report on experiments conducted in outdoor stock tank mesocosms (containers) with wild rice growing in sediments from a natural wild rice lake. Various amounts of sulfate were added to the overlying water achieve several target sulfate concentrations. While hydroponics experiments test the effects of sulfate and sulfide on individual plant growth, these mesocosm experiments test the effects of added sulfate and associated reduction to sulfide on populations of wild rice where the plants are allowed to grow from the germinating seed to maturity through several generations.

Methods

These experiments were conducted at the University of Minnesota Field Studies Research Station on Jean Duluth Road in Duluth, MN. These experiments are based on a similar
experimental technique first used by Walker et al. (2010) to examine population cycles in wild rice.

The experiments were begun in 2011 with funding from the Environmental Protection Agency to the Natural Resource Management Division of the Grand Portage Reservation. The Reservation and the EPA requested that J. Pastor begin these experiments to investigate the effects of sulfate concentrations and loadings on wild rice growth and population dynamics. The experiments were intended to be long-term experiments continued for as many years as funding allows. Long-term (multi-year) experiments were considered essential for understanding the effects of sulfate loadings on wild rice population dynamics for several reasons. First, wild rice populations typically oscillate in a 3-5 year period; this oscillation appears to be caused by delays in nitrogen cycling due to the slow decay of wild rice litter (Pastor and Walker 2006, Walker et al. 2010, Hildebrandt et al. 2012). However, we have no understanding of how sulfate and sulfide (to which sulfate may be reduced in the anaerobic sediments of rice beds; Tanaka et al. 1968; Jacq et al. 1991) affects population growth at different parts of the 3-5 year cycle. Second, cumulative loadings over several years or decades may have long-term effects not seen in a short-term, one-year experiment. Third, it is possible that over time the population may become more dominated by plants adapted to high levels of sulfate or sulfide.

In July 2013, the EPA and Grand Portage Reservation discontinued funding of these experiments and agreed that the Minnesota Pollution Control Agency would take over funding as part of the Wild Rice Sulfate Standard Study for as long as funding would be available. Funding from MPCA began on July 1, 2012 and has continued to December 31, 2013. We will report data from all three years of the experiment to provide a multi-year context for the data collected under MPCA funding. Beginning in February 2014, Minnesota Sea Grant will assume funding of these and other experiments designed to further investigate the biogeochemical habitat of wild rice, as per a proposal by J. Pastor, Nathan Johnson, and Jim Cotner from the University of Minnesota Duluth and Twin Cities campuses.

Great care was taken that the maintenance of these experiments, the methods used and basic data collected did not change as a result of these changes in funding levels and funding agencies. This consistency in measurements and data collection was accomplished by having Mr. Bradley Dewey, a technician for the last 28 years in Pastor’s lab, oversee the experiments, sample analyses, and data entry and analysis as well as supervise undergraduate student field and
laboratory crews. Many students on the field and laboratory crews were involved in the experiments for at least two years, which helped maintain continuity of maintenance and data and sample collection. Finally, many of the techniques used in this experiment had been perfected by Dewey and Pastor during our on-going eight year study of population cycles in wild rice (Walker et al. 2010, Sims et al. 2012a,b, Hildebrandt et al. 2012). We will indicate where new measurements were added as funding priorities and our understanding of wild rice population responses grew, but no measurements have been discontinued nor have any methods begun in 2011 changed during these three years.

In late spring of 2011, polyethylene stock tanks (Rubbermaid #4242, 378 L capacity, 132 cm long x 79 cm wide x 63 cm deep) were fitted with overflow drain pipes and buried to ground level (Fig. 1). The drain pipes are connected to 20 L polyethylene overflow buckets adjacent to each tank (mesocosm). Water tables are set by the inflow to the drain pipe at 23 cm above the sediment surface. The tanks were leveled and then partly filled with 10 cm of clean washed sand covered with 12 cm of surface sediment collected from natural wild rice bed in Rice Portage Lake (Minnesota Lake ID 09003700, Latitude 46.70˚N, Longitude 92.70˚W) on the Fond du Lac Band of Lake Superior Ojibway Reservation in Carlton County, Minnesota. Rice Portage Lake is approximately 832 acres, of which approximately 120 acres are wild rice beds (Minnesota Department of Natural Resources 2008). Ten to twenty cm of sediment over sand mimics the rooting depths we have observed in natural wild rice lakes. The sediments were mixed in a large stock tank prior to distribution. Prior analysis of three volumetric samples of the mixed sediment indicate a homogenous material (% C = 12.18 ± s.d. 1.00, % N = 1.07 ± s.d. 0.02; Walker et al. 2010). Sediment bulk density was 0.27 g · cm⁻³ ± s.d. 0.01 (Walker et al. 2010). These values are comparable to those of other wild rice beds (Keenan and Lee 1988, Day and Lee 1989). No new sediment has been added to the stock tanks since.

The tanks were immediately filled with water after sediment additions to prevent the sediment from drying. Water was added cautiously from a garden hose to prevent redistribution and suspension of sediment. During the growing season, water levels were maintained at 23 cm above the sediment surface by weekly additions of water to the drain pipe heights or by allowing water to drain through the pipe into the overflow buckets. Water used to fill and supplement tank levels is obtained from a nearby well. Rainfall N concentrations as NO₃-N and NH₄-N ranged from 0.2 – 1.99 mg · L⁻¹ while the NO₃-N and NH₄-N concentrations in the well water are
always < 0.2 mg · L⁻¹ (Walker et al. 2010). Sulfate concentrations in the tanks with no additional added sulfate (see Results below) averaged 7 mg · L⁻¹ and were always less than 10 mg · L⁻¹. The sediments comprise an inoculation source for microbes and a background supply of nutrients for plant growth source. The sediments and plant litter remain submerged year round with water levels set at approximately 20 cm in late fall.

Wild rice was planted once in late spring 2011 from seeds obtained from the Grand Portage Reservation from Swamp Lake, a 91 acre lake in Cook County (Minnesota Lake ID 16025600, Latitude 47.85° N, Longitude 90.58° W). Seeds from each year’s crop were allowed to fall unimpeded into the tanks to provide the seed source for the next year’s population; no further seeding from external seed sources was done.

End-of-season plant density in Minnesota wild rice lakes monitored by the 1854 Treaty Authority average 40 plants per square meter (Vogt 2010). Accordingly, the seedlings were thinned to this density (30 plants per tank) in late spring or early summer in 2011 and 2012 before the floating leaf stage was achieved. In spring of 2013, plexiglass barriers were installed across one end of the tanks (Fig. 1); on the outer side of the plexiglass barrier all plants were removed in order to study how the presence or absence of wild rice affects sulfate transformations (to be presented in a separate report by N. Johnson). Accordingly, the plants on the inner side of the barrier were kept at the same density per m² as in previous years, which required that the population on this side of the barrier be thinned to 28 plants per tank. Peepers were installed on both sides of the plexiglass barrier in 2013 in order to measure geochemical profiles of sulfur and iron species vertically through the sediment profile; details can be found in a spate report by N. Johnson and will not be discussed here. Care was taken that the installation and extraction of the peepers did not disturb any plants. The numbers of seedlings removed from each tank during thinning in 2012 and 2013 were counted as estimates of seed germination and early seedling success.

Tanks were treated in late June 2011 with different amounts of sulfate to achieve several target sulfate concentrations beginning immediately after installation and seeding of the tanks. There are six replicate tanks per sulfate treatment, for a total of five sulfate treatment levels, totaling 30 tanks. The water volumes in the tanks from sediment surface to drain pipe height were determined when filling the tanks. Nominal water column sulfate concentrations of 0, 50, 100, 150, and 300 mg SO₄ · L⁻¹ were chosen as target treatment levels. The levels chosen bracket
both the existing 10 mg · L⁻¹ Minnesota statutory standard for wild rice waters and the EPA drinking water standard of 250 mg · L⁻¹. Sulfate was added to the water filled tanks as solutions of sodium sulfate (Fisher Chemical S421. Na₂SO₄). To do this, a determined mass of sodium sulfate sufficient to bring the volume of water in each filled tank to near the desired nominal sulfate concentration was weighed and dissolved in 1 to 2 liters of water from the tank. The mixture was then added back to the tank with mild mixing. Samples of the water column were taken weekly and analyzed for sulfate concentration using a Lachate Autoanalyzer. When necessary, the sulfate concentration was adjusted to near the desired nominal concentrations with appropriate amounts of 10 g · L⁻¹ sulfate stock solution and well water. In all cases stock solution was diluted with tank water before adding to the tanks to prevent abnormally high sulfate contact with the plants. In order to not overload the tanks with excessive sulfate concentrations, we kept the solution concentrations slightly below the desired nominal concentrations. Here we report plant responses relative to the nominal concentrations in experimental treatments as conservative estimates of the level of sulfate associated with anomalous growth responses relative to controls. Actual concentrations of sulfate producing anomalous growth may be lower than reported here.

In 2011 and 2012, five plants in each tank were randomly chosen in early summer for detailed measurements throughout the growing season and the five plants were sampled at the end of the growing season. In late August to September, ripe seeds from these plants were collected every two or three days by gently removing ripe seed, leaving unripe seeds behind for the next collection date. The seeds from each individual plant were placed in a paper envelope and marked with the tank identification number; the same envelop was used at each collection time. At the end of the growing season these five plants were harvested by gently excavating the root system from the sediment and used for measurement of length, weight, root:shoot ratios, and nutrient analyses. In addition, all aboveground plant material was collected from each tank and weighed along with a subsample taken to determine wet:dry ratios for moisture correction after drying at 60° C. All aboveground plant material except for the five sample plants were returned to each tank. All stems in each stank were counted at the time of harvesting the aboveground plant biomass to determine end of growing season plant density.

Seeds from each of the five sampled plant were separated into filled (viable) seeds and empty (nonviable) seeds, counted and weighed. A subsample of seeds collected in 2011 and 2012 were dried at 60° C for determination of moisture content to convert wet weight to dry
weight. The five sample plants were separated into root and shoot (stem + leaves), and then weighed. Root:shoot ratios and seed weights and numbers from the five sampled plants were applied to total aboveground population weights and total plant numbers to determine total root and seed biomass and total population biomass in each tank.

In 2013, significant seedling mortality in all tanks after thinning but before the floating leaf stage precluded this sampling of individual plants. Instead, the condition of all plants in each tank were noted weekly. The height of each plant was measured twice during the growing season. Harvesting and drying seeds from five sample plants in each tank as was done in 2011 and 2012 would greatly decrease the number of viable seeds returned to the sediment for the following growing season. Instead, all seeds were harvested from each and every plant in the tanks and sorted as described above each collection day and were returned without drying to the tanks within 24 hours of collection. Not drying the seeds maintained their viability for future populations. To determine wet-dry conversion ratios for these seeds, additional seeds were collected at the same collection times from an adjacent experiment on wild rice (Walker et al. 2010) for moisture determination after drying them at 60°C as was done in 2011 and 2012. After all seeds were collected, all plants were clipped at sediment surface and weighed with a small subsample taken for dry weight determination. All plant material was then returned to the tanks.

The effects of sulfate levels on these plant attributes were tested by regressing the attribute against nominal sulfate concentrations. Spreadsheets containing raw data are provided in the Appendices.

Results

Different sulfate concentrations were maintained amongst the five treatments (Fig. 2), but at levels slightly below the nominal concentrations. The sulfate concentrations sometimes decreased after large rainfall events. Note that the control tanks without any external sulfate additions had average sulfate concentrations of 7 mg SO₄ · L⁻¹. These background concentrations (presumably from decomposing sediment, rain, and well water added to maintain water levels) are similar to the State of Minnesota sulfate standard of 10 mg SO₄ L⁻¹ applicable to waters used for the production of wild rice. Nonetheless, in all graphs and tables we designate this treatment as 0 or Control, meaning no added sulfate.
Higher sulfate concentrations were associated with decreased total biomass in all three years (Fig. 3 and Table 1; see figure legends for \( r^2 \) and \( p \) levels), although the decline was not significant for 2013. Underlying this decrease in population biomass were statistically weak declines in shoot (stem plus leaves) and root weights of individual plants with increasing sulfate concentrations (\( p < 0.10 \)). The weaker relationships between root and shoot weights, respectively, associated with increasing sulfate levels were caused by the larger standard deviations of these plant properties within treatments than the standard deviations of population biomass. Apparently, the variability in root and shoot weights between individual plants averaged out at the tank (population) level. Root and shoot weights of individual plants were highly correlated (\( r = 0.998, p < 0.001 \)) and root: shoot ratios were nearly constant between 0.210 and 0.224. Therefore, while the amounts of root and shoot productions were significantly affected by sulfate levels, the proportional allocation of production between roots and shoots was not.

Plants grown in the control tanks had white or light tan roots, but plants in the tanks amended with sulfate had blackened roots (Fig. 4). Visual estimates of the proportion of blackened roots increased progressively from approximately 50% in the tanks with 50 mg \( \cdot \) L\(^{-1} \) SO\(_4\) to 100% in tanks with 300 mg/l sulfate. A sample of roots from a one plant from one control tanks and one plant from a 300 mg \( \cdot \) L\(^{-1} \) SO\(_4\) tank were sent to the Limnological Research Center, School of Earth Sciences, University of Minnesota for analyses of Fe and S concentrations by scanning X-ray fluorescence (Amy Myrbo, personal communication). The sample was subdivided into seven subsamples from the blackened roots and five subsamples from the tan control roots. The blackened roots from the 300 mg \( \cdot \) L\(^{-1} \) SO\(_4\) tank had 28.3% Fe by weight (s.d. 9.8) and 13.4% S (s.d. 4.6), for an S:Fe of 0.50 (s.d 0.15). These values were all much greater than roots from the control tanks, which averaged 5.0% Fe (s.d. 3.9%), 0.34% S (s.d. 0.29%), with an S:Fe of 0.11 (s.d. 0.08). Therefore, it is likely that the blackening was caused by precipitation of some form of iron sulfide, most likely amorphous or less crystalline forms such as pyrrhotite (Fe\(_7\)S\(_\alpha\)-FeS) whose composition more closely matches the S:Fe ratio of 0.5 than the more crystalline pyrite (FeS\(_2\)).

Seed weights in the control tanks remained relatively constant during the three years, but mean seed weights the 300 mg SO\(_4\) \( \cdot \) L\(^{-1} \) tanks declined significantly by 12% in 2011, by 21% in 2012, and by 50% in 2013 compared to seeds from plants in control tanks (Fig. 5 and Table 1).
The declines in seed weights were significant \( p < 0.055 \) for 2011 and 2012, but only weakly so for 2013 \( p = 0.122 \) for 2013.

Although the total number of seeds produced (both viable and empty) did not change significantly across all sulfate concentrations, the proportion of viable seeds from each plant (Fig. 5 and Table 1) remained relatively constant during all three years on control plants (55 – 60%) but declined to 48% in 2011, to 40% in 2012, and to 31% in 2013 on plants grown at 300 mg SO\(_4\) L\(^{-1}\). These declines were statistically significant for all three years.

In spring of both 2012 and 2013, the number of seedlings which emerged from the sediment declined significantly \( p < 0.01 \) with increased sulfate levels (Fig. 6 and Table 1). In addition, the subsequent survival of those seedlings remaining after thinning to the prescribed density of 30 plants per tank also declined with increased sulfate levels (Fig. 6). Mortality of seedlings was especially high in late June and early July just prior to the floating leaf stage (Fig. 7). The number of surviving seedlings was not correlated with the number of seedlings removed by thinning \( p > 0.10 \), so the magnitude of thinning itself had no effect on seedling survival. The number of surviving seedlings was also not correlated \( p > 0.10 \) with the production of straw litter from 2012, so the decline in seedling survival was not an artifact of inhibition by thatch accumulation.

**Discussion**

Measures of wild rice growth consistently decreased as the concentration of sulfate in surface water increased in these tanks, especially during the second and third years of the experiment. These measures include the production, viability, and germination rate of seeds and survival of juvenile seedlings. This association of higher sulfate concentrations with reduced seed production and seedling survival suggest that continuous inputs of elevated sulfate concentrations into wild rice waters could be associated with progressive declines of wild rice population density.

Blackened roots are often observed in white rice (Oryza sativa) populations subjected to enhanced sulfate levels; the blackening may be caused when sulfate is reduced to sulfide in the anaerobic sediments by microbial sulfate reducers and then precipitated as iron sulfides (Tanaka et al. 1968, Jacq et al. 1991, Gao et al. 2003). Coating of roots with iron sulfide may inhibit nutrient uptake in some wetland plants (DeLaune et al. 1983, Gao et al. 2003, Armstrong and
Armstrong 2005). Jorgenson et al. (2012) found that orange plaques on wild rice roots are sometimes iron hydroxides, but the blackened roots of our plants suggests that iron sulfides were being precipitated.

There appears to be a steady decline in properties of plants over the span of the three years, especially the proportion of viable seeds from plants grown in waters with concentrations of 300 mg SO₄ · L⁻¹. These proportions declined from 48% in 2011 to 40.1% in 2012 to 31% in 2013. At the same time, the proportion of viable seeds in the control tanks remained relatively constant at 55-60%. The decline in viable seeds associated with higher sulfate concentrations is well outside the 5% natural variability over these three years in the control tanks.

**Conclusion**

Enhanced sulfate concentrations in waters containing experimental wild rice populations were associated with depressed measures of plant performance, most especially in seed production. The rate of depression of seed production and seedling emergence with increasing sulfate steepened over time.

**References**


Table 1. Association of sulfate concentrations (in mg SO₄·L⁻¹) with wild rice properties. Values are means of samples from 6 tanks with standard errors in parentheses.

<table>
<thead>
<tr>
<th>Property</th>
<th>Year</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed wt (mg)</td>
<td>2011</td>
<td>24.6(1.24)</td>
<td>22.9(1.1)</td>
<td>22.8(0.8)</td>
<td>21.2(0.9)</td>
<td>21.1(1.26)</td>
</tr>
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<td></td>
<td>2012</td>
<td>27.8(0.9)</td>
<td>28.1(1.1)</td>
<td>26.2(1.0)</td>
<td>25.7(1.2)</td>
<td>21.8(1.2)</td>
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<td>2013</td>
<td>29.7(1.1)</td>
<td>29.5(1.9)</td>
<td>36.0(5.6)</td>
<td>28.8(0.9)</td>
<td>15.7(3.6)</td>
</tr>
<tr>
<td>Viable seeds (%)</td>
<td>2011</td>
<td>57.7(3.9)</td>
<td>55.9(4.5)</td>
<td>57.2(3.9)</td>
<td>52.6(3.9)</td>
<td>48.2(4.2)</td>
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<td></td>
<td>2012</td>
<td>55.1(3.5)</td>
<td>48.9(3.8)</td>
<td>50.6(3.7)</td>
<td>49.2(4.0)</td>
<td>40.1(3.0)</td>
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<tr>
<td></td>
<td>2013</td>
<td>60.6(3.8)</td>
<td>62.8(7.0)</td>
<td>61.2(5.7)</td>
<td>55.6(3.3)</td>
<td>31.2(6.8)</td>
</tr>
<tr>
<td>Viable seeds (count · m⁻²)</td>
<td>2011</td>
<td>710.1(69.2)</td>
<td>841.8(101.6)</td>
<td>674.6(58.3)</td>
<td>646.2(116.0)</td>
<td>586.2(84.1)</td>
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<td></td>
<td>2012</td>
<td>1442.5(296.1)</td>
<td>1021.2(210.6)</td>
<td>1001.0(120.3)</td>
<td>863.0(152.7)</td>
<td>744.1(84.7)</td>
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<td></td>
<td>2013</td>
<td>147.1(41.0)</td>
<td>196.6(73.9)</td>
<td>67.0(31.1)</td>
<td>255.5(63.3)</td>
<td>40.5(12.4)</td>
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<td>Plant Biomass (g · m⁻²)</td>
<td>2011</td>
<td>47.38(3.54)</td>
<td>55.32(10.55)</td>
<td>49.99(2.97)</td>
<td>50.65(4.69)</td>
<td>34.89(1.24)</td>
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<tr>
<td></td>
<td>2012</td>
<td>117.25(7.48)</td>
<td>138.24(15.84)</td>
<td>127.49(8.62)</td>
<td>102.42(3.54)</td>
<td>95.64(10.35)</td>
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<td></td>
<td>2013</td>
<td>15.21(4.18)</td>
<td>16.12(5.26)</td>
<td>7.10(2.90)</td>
<td>36.09(16.37)</td>
<td>5.61(1.52)</td>
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<tr>
<td>Number of Seedlings</td>
<td>2012</td>
<td>183.0(17.0)</td>
<td>163.0(16.9)</td>
<td>148.7(12.0)</td>
<td>144.3(12.6)</td>
<td>120.8(18.4)</td>
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<td></td>
<td>2013</td>
<td>162.8(10.8)</td>
<td>177.8(33.8)</td>
<td>140.5(18.3)</td>
<td>142.5(20.3)</td>
<td>60.3(8.3)</td>
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<td>Seedling Survival (#)</td>
<td>2012</td>
<td>28.2(4.7)</td>
<td>27.5(2.1)</td>
<td>26.0(3.4)</td>
<td>24.8(3.8)</td>
<td>18.2(1.2)</td>
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<tr>
<td></td>
<td>2013</td>
<td>16.5(1.6)</td>
<td>12.0(3.1)</td>
<td>5.2(2.1)</td>
<td>11.8(3.2)</td>
<td>3.0(0.9)</td>
</tr>
</tbody>
</table>
Fig. 1. Schematic of the stock tanks used as mesocosms (Ed Swain, artist).
Fig. 2. Sulfate concentrations in the overlying water in the mesocosms.
Fig. 3. Declines in plant biomass associated with increasing sulfate concentrations in the water column.
Fig. 4. Blackened roots from plants grown in tanks with 300 mg SO₄ \cdot L^{-1} (left) compared to tan roots from plants grown in control tanks (right).
Fig. 5. Declines in seed production associated with increased sulfate concentrations in the water columns.
Fig. 6. Declines in seedling survival with increased sulfate concentrations in the water column.
Fig. 7. Decline of seedling density in one of the 300 mg SO$_4$ · L$^{-1}$ tanks compared with a control tank after seedling density in both tanks had been thinned to 28 seedlings in mid-June, 2013.