# SUMMARY

## COMMENTS ON MINNESOTA POLLUTION CONTROL AGENCY

## WILD RICE SULFATE / SULFIDE RULE MAKING SUGGESTIONS

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# 1.0 SUMMARY

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 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. Multiple examples of each of these influences can be observed occurring independently or, as is sometimes the case, concurrently. One example which is highlighted in following sections is increasing cattail dominance in areas once dominated by WR. Based on historical, and current data and observations during laboratory and field experiments, as well as direct field-scale application via WR restoration activities, maintaining an appropriate water depth for WR and managing competing vegetation should be the first two objectives for maintaining waters for increased WR growth, health, and abundance.

Comments and examples contained in this document will focus specifically

- Overall, as also discussed in following sections of these comments, controlling competing vegetation in waters intended for WR production is critical for maintenance of desired WR growth, distribution, abundance, and productivity.
- WR will grow to harvestable densities in a variety of sediment, porewater, and overlying water conditions and chemical characteristics. Initial measurement of typical sediment and porewater nutrients (nitrogen, phosphorus, sulfur, organic carbon, etc.) can indicate acceptable conditions for WR growth. Also, once familiar with the 'general appearance' of sediment in which WR has been observed growing in harvestable densities, the 'general appearance' can indicate a sediment conducive to WR growth.
- 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 / absence of standing water, prairie potholes are unlikely to be acceptable habitat for WR production, regardless of chemical characteristics of overlying water and sediment porewater.
    - 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.
- In this case WR and water lilies do occur in the same general area; however, a distinction between higher density locations of each plant appears evident. Therefore, simply stating that the presence of water lilies is an indicator of acceptable WR habitat is overgeneralized.

- Based on available data and consideration of factors beyond control specific to microbial H2S synthesis, application and enforcement of a sediment porewater sulfide protective limit is arguably beneficial to WR distribution in MN.

# 2.0 INFLUENCES ON WILD RICE GROWTH, HEALTH, AND ABUNDANCE

As suggested at the public meeting in Virginia, MN, on October 24, 2017, 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 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. Multiple examples of each of these influences can be observed occurring independently or, as often is the case, concurrently. One example which is highlighted in following sections is the increasing area of cattail dominance in areas once dominated by WR. Based on historical, and current data and observations during laboratory and field experiments, as well as direct field-scale application via WR restoration activities, maintaining an appropriate water depth for WR and managing competing vegetation should be the first two objectives for maintaining waters for increased WR growth, health, and abundance.

## 2.1 PHYSICAL INFLUENCES – WATER DEPTH

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 (**Figures 1-3**). Current, on-going studies indicate that, under these experimental conditions, WR seeds germinated and grew at a depth from surface of 160 cm (~ 5.3 ft). However, WR plants were not able to achieve floating leaf, aerial, or the later phenological reproductively mature (viable seed-bearing) stages within the growing season (**Figures 4-7**). Since WR is an annual (described below), if this scenario continues to occur, the viable seed source in the sediment will more likely be depleted, resulting in elimination of WR from that area or water resource.

Phenological development refers to the life stages of higher plants (angiosperms) from germination of the seed until death / senescence of the plant either after a single season (annuals; such as WR), two seasons (biennials), or multiple growing seasons (perennials). Unlike terrestrial grains, the shoot in WR emerges from the seed before the root (Aiken et al. 1989). WR uses this germination strategy because light and carbon dioxide availability limit early development of the seedling, as opposed to water availability in terrestrial plants. Seedlings rely on limited food reserves in the seed as they grow toward the surface of the water where light will not be limiting. Photosynthesis will only be optimized when light availability is not limited by overlying water, and when carbon dioxide levels increase by ~ 40x as the plant emerges into the atmosphere.

Weir and Dale (1960) give a good account of the development of the plant. Three forms of leaves develop – submerged, floating, and aerial – which differ in anatomical characteristics likely related to their physiological environment. The submerged leaves are thin and lack a cuticle, the outer waxy covering present on leaves of terrestrial plants. No stomata (tiny openings on the cuticle for gas exchange) are present. These leaves also have abundant air passages at this time which are known as lacunae, and greatly reduced conducting tissue (the vascular bundles or veins) which transport both water and sugars. The lacunae presumably help oxygenate the interior of the leaf and prevent the build-up of potentially problematic products common under anoxic conditions; while the veins are not as critical since water is not limiting and photosynthesis is not yet optimal. Two to three submerged leaves are normally formed while the plant grows to the surface. In late May to early June, the floating leaves appear. These differ from the submerged leaves by having a cuticle with stomata on the upper surface of the leaf (Hawthorn and Stewart 1970) and a well-developed mid vein on the lower surface which does not have a cuticle. Gaseous exchange is now possible with the atmosphere and the cuticle essentially makes the leaf 'waterproof' enabling the leaf to tolerate wetting from wave action. Two to three floating leaves are produced before the first emergent leaf emerges. These leaves have a well-developed vascular system with associated lacunae having compartments separated by diaphragms. Gaseous exchange is no longer an issue and now the plant concentrates on obtaining needed nutrients from the sediment while aerating the underwater organs via the lacunae and thus enhancing energy production via aerobic respiration. Most biomass production occurs in the aerial stage of development. The ratio of root:biomass also increases (Thomas and Stewart 1969) at this stage of development as the plant becomes better anchored. Another occurrence at this stage is that the shoot apex (vertical growing tip) changes from vegetative to reproductive growth (Weir and Dale 1960), triggering the rice plant to begin grain formation. The timing for this event in Northwestern Ontario varies depending on the environmental characteristics (depth, nutrients) of the individual sites and the depth tolerance of the seed source involved (Counts and Lee 1988), but would likely occur in most sites, including northeast Minnesota, towards the end of June with flower formation evident in early July.

Phenological development is directly dependent on water depth; the greater the water depths, the longer it will take a WR plant to reach the surface (Thomas and Stewart 1969). Additionally, the longer it takes for the plant to reach the surface of the water, the longer it will remain under photosynthetic and respiratory stress, decreasing its likelihood of survival. Equally as important as overall plant survival is achievement of reproductive maturity – the point at which the plant releases its (viable) seeds prior to complete senescence. The depth effect will vary with the WR stand. In most WR stands there is a depth gradient from the shore outward. Plants at the outer edges, where water is deeper, develop more slowly than WR plants in areas with shallower water. Wild rice can still be in the floating leaf stages at the outer edges while the rice near shore is flowering. Depending on the configuration of the rice stand, large sections may be adversely influenced well into the summer from increases in water depths that may not adversely influence WR in shallower water. Decreased light penetration due to increased water depths would also decrease tillering and ultimately grain yield. Bloom et al. (2001) reported yields in the same transects in Rice Lake, MN, declined > 8x from 1383 lb / acre in year 2000 to 170 lb / acre in year 2001 as average water depths increased from 26 inches (66 cm, 2.17 ft) to 43 inches (109 cm, 3.58 ft). They also showed that in both years, grain yield and biomass were negatively correlated to water depth increases. Marcum and Porter (2006) reported that the number of mature seeds per panicle and total seed yield decreased in controlled experiments as depth increased from 15 – 30 cm (0.5 – 1.0 ft) to 46 – 61 cm (1.5 – 2.0 ft). No WR seedlings were able to reach the surface of the containers when planted with a water depth of 76 cm (2.5 ft). Specific examples of water depth influences on WR development from more recent experimental and open-water (field) conditions are detailed in **Figures 1-9.**

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 poorly 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. Some varieties survive by reducing growth and initiating metabolic processes that enable the plant to tolerate temporarily increased water depth. Other varieties elongate by internodal growth returning the leaves to the water surface as quickly as possible. It is likely this is the strategy used by WR. Using rafts with suspended buckets containing WR plants, Stevenson and Lee (1987) showed that although WR was able to tolerate increases of up to 50 cm (1.64 ft), increases in water depth of 15 – 30 cm (0.5 – 1.0 ft) caused decreases in total dry weights, number of tillers, and grain yield. The more severe response to depth increases under natural conditions were attributed to decreased / decreasing nutrient levels versus the fertilized treatments in their rafts. They suggested that 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). As water levels increase, the plants elongate to reach the surface. If the water levels then recede, the leaves and stems are more susceptible to breakage (Thomas and Stewart 1969).

Finally, consideration has to be given to the water depth during winter. If water depth decreases to an extent that the water in the rice areas freezes throughout the water column, 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 at the shallowest edges of the wetland, but is present in the deeper sections (Aiken et al. 1989).

### 2.2 Biological Influences – Competing Aquatic Vegetation

Another adverse influence in WR areas is development of problematic densities of competing aquatic plants (**Figures 1-3, 10-12, 15**). Specific herbicides may be used to control problematic aquatic plants. Mechanical harvesting of competing vegetation has also been used as a control practice by cutting off culms which supply the rhizomes with oxygen (Lee 1986a). A more substantial problem has been increased coverage of WR areas by narrow leaf cattails tolerant of similar water depths as WR. Cutting or harvesting as a cattail control method has been effective, and is currently being tested in a cattail dominated area (which was dominated by WR multiple years ago) in Ontario on the Seine River near the Minnesota border (**Figures 1-3**).

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). Properly managing water depths in lakes that once contained WR has been effective. In some cases, WR seed likely remained in the seed bank due to secondary dormancy (Atkins 1986). In other cases, volunteer WR appeared in commercial amounts once competing cattails were removed with a 'cookie-cutter' (blades mounted on barge) at Long Point on Lake Erie (Lee 2001). One major restoration project was the re-establishment of southern WR into a contaminated site on Lake Ontario (Lee 2004). The site had also been invaded by carp; WR production was only possible once carp had largely been removed.

Overall, as also discussed in following sections of these comments, controlling competing vegetation in waters intended for WR production is critical for maintenance of desired WR growth, distribution, abundance, and productivity.

### 2.3 Major Chemical Influences

Some initial research describing WR in relation to water chemistry are Moyle's (1944; 1945; 1956) descriptions of WR in relation to water chemistry in Minnesota. 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. It is noteworthy that these observations led to the development of Minnesota's regulation concerning the discharge of sulfates. Under the Class 4A use classification for Agriculture and Wildlife, Minnesota's water quality standard states: '10 mg / L sulfate - applicable to water used for the production of wild rice during periods when the rice may be susceptible to damage by high sulfate levels.' (Minn. R. 7050.0224, subpart 2). Moyle's observational work has led to a number of studies concerning the importance of sulfate. Paulishyn and Stewart (1970) reported WR growing in Manitoba in waters with sulfate ranging from 2 – 170 mg l-1. Rogalsky et al. (1971) advised that levels of 200 mg l-1 sulfate were acceptable for WR paddies. In a study in the Mississippi River in Minnesota, Lee and Stewart (1981) showed that WR grew well in waters with levels of 30 mg l-1 and showed that sulfate in the water varied seasonally at one sampling site from 5 – 120 mg l-1.

Controlled experiments that examine the effects of sulfate have also been conducted. 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.

Currently, a debate has been initiated that the problem is not aqueous sulfate, but rather the production of hydrogen sulfide in the sediment and sediment porewater related to aqueous sulfate. This was previously postulated by Grava (1973) and Grava and Rose (1975) who suggested that sulfides could form in WR paddies and adversely influence WR when sulfate was added as a fertilizer as ammonium sulfate, or an algicide as copper sulfate (Grava 1977).

Other water quality variables have also been described for waters in which WR grows. 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). Whether eutrophication is a causative factor or correlated factor is not currently defined. Jorgenson (2013) showed that WR could grow in both eutrophic waters with seasonal total phosphorus concentrations reaching 1500 µg l-1 and non-eutrophic waters with total phosphorus concentrations of only 170 µg l-1. 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.

Wild rice obtains most of its nutrients from the sediment. Nutrients in the rhizosphere of the WR plant are more influenced by plant growth (Jorgenson 2013). Seasonal nutrient concentrations in the WR roots seem to be correlated to those in the stems and leaves (Lee and Stewart 1983) suggesting that sediment characteristics around the roots are translocated to the rest of the plant. For commercial purposes, concentrations needed for paddy production related to fertilizer requirements are well documented (Oelke et al. 1982; Marcum 2006). These concentrations are determined using traditional soil science methods: drying and grinding, followed by analysis of filtered supernatants released with specific extracts. Day and Lee (1989) outlined methods used to classify sediments suitable for growing WR.

Their procedure was to extract nutrients from the soils while still wet and express concentrations on a volume rather than a weight basis. This procedure corrected for the variations in bulk densities observed within and between lake sediments. Other studies have used total concentrations of nutrients (requiring digestion) to describe soil characteristics (Aikens et al. 1992) or extracts on dried soils (Lee 1979). Microwave digestion is the more common procedure (LUEL 2012). Sediment porewater nutrient concentrations are another method of comparing nutrient availability, but do not estimate the replenishment ability of nutrients from soil particles. There is also the question as to whether pore water concentrations determined by centrifuging samples are comparable to those obtained from 'peepers,' i.e., simple sediment porewater sampling devices. Investigations by Mayer et al. (2002) showed that pore water values obtained from 'peepers' in a highly eutrophic wetland compared well to those obtained by Lee (2001). The following ranges for parameters (mg l-1) in pore water and for uncapped total values in sediment from WR areas (**Table 1**) was developed using data from Jorgenson (2013) for a mesotrophic and eutrophic WR wetland and values for a highly eutrophic area growing WR studied by Lee (2001) and Mayer et al. (2002).

**Table 1.** Concentrations of sediment pore-water characteristics measured from a highly eutrophic area.

| **Parameter** | **Pore Water Range (mg $l^{-1}$)** | **Total Range (µg $g^{-1}$) or (%)** |
|---|---|---|
| Ca | 29.1 – 150 | 0.8 – 4.6 % |
| Ba | 0.057 – 0.16 | 47.7 – 178.0 (µg $g^{-1}$) |
| Fe | 0.009 – 15.0 | 0.54 – 2.7 (%) |
| K | 0.11 – 5.2 | 0.06 – 0.90 (%) |
| Mg | 5.3 – 34.5 | 0.16 – 1.3 (%) |
| Mn | 0.001 – 1.03 | 107 – 555 (µg $g^{-1}$) |
| P | 0.003 – 7.5 | 0.05 – 0.15 (%) |
| S | 0.10 – 38.5 | 0.45 – 1.25 (%) |
| Sr | 0.074 – 1.2 | 21.5 – 136 (µg $g^{-1}$) |
| $NH_4$ | Up to 150 | Total N – Up to 1.28 (%) |

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.

Values for metals and sulfate levels in solution have been investigated using hydroponic methods by Lee and Hughes (1997) and Lee and Hughes (2000). Values listed below are for lowest observed effects for leaf and root areas in range finding experiments (**Table 2**). More recent laboratory studies exposing WR seeds under hydroponic conditions suggest that adverse responses are observed at aqueous sulfate concentrations exceeding 1500 mg / L (Fort et al. 2014).

**Table 2**. Lowest observed effect levels for specific elements, and sulfate, in solution from Lee and Hughes (1997; 2000)

| Parameter | Lowest Observed Effect (Leaf) (mg $l^{-1}$) | Lowest Observed Effect (Roots) (mg $l^{-1}$) |
|---|---|---|
| Al | 1.0 | 1.0 |
| Cu | 1.0 | 1.0 |
| Cd | 0.01 | 1.0 |
| Pb | 1.0 | 1.0 |
| Hg | 1.0 | 1.0 |
| $SO_4$ | 1500 | |

Values for the same metals in pore water in the above publications were generally below detection levels (mg l-1): Cu (0.002); Cd (0.001); Pb (0.005). Al was reported above detection levels (0.005), with average values ranging from 0.005 – 0.012 mg l-1. All metals were therefore below levels where any adverse influence on WR should occur. In terms of sulfate, the highest values in the pore water would be approximately 120 mg l-1, below levels considered detrimental in the above studies.

## 3.0 Wild Rice in MN Prairie Potholes

The Prairie Pothole region of MN extends throughout approximately the central-western portion of MN. These bodies of water are remnants of glacial activity, are 'land-locked' (not a part of a riverine system), are fairly small re: surface area (contrasted to 'lakes' and reservoirs as would be commonly defined), and are predominantly surrounded by agricultural activity; the portion of MN in which prairie potholes are located tends to have terrestrial soils suitable for cropland. Since prairie potholes are isolated from flowing systems and are relatively small re: surface area, other than water fowl (for hunting purposes) they tend to not be managed as a resource for aquatic plants; in this case, specifically WR.

Currently, prairie potholes have been included in a debate about their suitability as water resources for WR habitat. 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.

### 3.1 Physical Influences – Water Depth

Prairie potholes in MN as described above are unlikely water resources capable of sustaining perpetual, harvestable densities of WR due to their typical non-management of water depth, arguably the more important characteristic of WR waters requiring management for a sustainable WR population. Standing water presence and absence, and levels in the case of presence, in potholes can be hydrologically influenced by localized groundwater levels, as well as precipitation events. Due to weather-related and overall climatic influences, acceptable WR habitat may not be available in specific potholes, or entire regions of potholes; periodic years lacking standing water, with contrasting periodic years of increased standing water depth can result in depletion of any viable WR seeds that may have been present in the pothole sediments. This depletion of any viable WR seeds would presumably occur under the following scenario – during years of increased water depth, (viable) WR seeds may germinate, but not have the ability to achieve reproductive success due to excessive water depth. A visual example of how this scenario may influence WR abundance may be inferred via **Figures 5-7, 13-16**. **Figures 13-16** are of a pothole which historically has been observed without standing water.

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 / 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.

### 3.2 Biological Influences –Competing Vegetation

As mentioned above, potholes are not typically managed for WR productivity; specifically, in terms of maintaining stable and appropriate water depth, and management of aquatic vegetation which may complete with, and out-compete, WR for scarce resources – of particular interest is light availability. Adverse influences from aquatic vegetation competing with WR for scarce resources can be exacerbated in the presence of non-controlled water depth. Under conditions of water depth in excess of optimal WR conditions (0.5-3.0 feet), competition for scarce resources such as light can decrease the rate of development and overall potential for achieving reproductive success. Continued occurrence of WR seed germination followed by lack of reproductive success can result in depletion of the viable WR seed source. Examples of a lake and pothole, respectively, in which competing aquatic vegetation may be a hinderance to WR abundance are included as **Figures 10-12, 15, 16**. Additionally, in the event that a prairie pothole 'dries up,' the area which would have been considered aquatic may become dominated by terrestrial plants. This further decreases the likelihood that a prairie pothole exhibiting this hydrological scenario is acceptable habitat for WR production.

Overall, 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.

# 4.0 Water Lilies as an Indicator of Acceptable Wild Rice Habitat

## 4.1 Physical Influences

Several examples exist of aquatic plants, both rooted and non-rooted, which can occur in areas of aquatic systems with conditions favorable for WR plants. Currently, water lilies (lily pads) have been suggested as an indicator of acceptable WR habitat in aquatic systems; although, water lilies can also grow in areas lacking preferential conditions for WR – specifically, water lilies can grow in water sufficiently deep to be counter-productive to WR abundance. 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.

One particular WR water this association may be observed is Lake Monongalia in Kandiyohi County, MN (**Figure 10**). In this image, both WR and water lilies can be observed in the same general area; however, there appears to be a distinction between populations of each plant; and although some water lilies occur within the WR areas, a separation between higher densities of each plant appears to be evident. In addition to the image provided as **Figure 10**, additional images of near-shore WR areas with associated water lily areas are available.

Furthermore, some areas nearer shore tend to be dominated by water lilies; although water nearer shore tends to be shallower, which is preferred for WR growth and abundance. Water lilies can therefore be indicators of acceptable habitat for WR; however, in this system, in this area, a separation of higher densities of lilies and WR appears evident. For multiple reasons (competition for resources, potential allelopathic influences), the overall inference from this observation may contraindicate water lilies as indicators of acceptable WR habitat. This observation may also be an example of 'ring effect' as described in section 2.1. However, if this is the case nearer-shore habitat may not be suitable for WR growth due to the likelihood of seed desiccation during winter.

In this case WR and water lilies do occur in the same general area; however, a distinction between higher density locations of each plant appears evident. Therefore, simply stating that the presence of water lilies is an indicator of acceptable WR habitat is overgeneralized.

# 5.0 Might the 120 µg / L Sulfide Porewater Standard Help / Improve Wild Rice Distribution in MN?

A component of the current debate about developing site-specific standards for sulfate in discharge waters is application of a sediment porewater sulfide protective limit of 120 µg / L, which may 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 factors beyond control specific to microbial H2S synthesis, application and enforcement of a sediment porewater sulfide protective limit is arguably beneficial to WR distribution in MN.

## 5.1 Physical Influences – Water Depth

As described multiple times previously, water depth should be one of the first, if not the first, consideration(s) when determining acceptable aquatic habitat for WR growth and abundance. The synthesis of hydrogen sulfide (H2S) in sediment porewater is dependent on the absence of oxygen in the area of H2S generation. In general, shallower water is more easily mixed during disturbance events, and therefore the likelihood of oxygenating the entire water column increases as water depth decreases. 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.

Microbial metabolic rate can also be controlled by temperature of the surrounding medium; as a general rule, as the temperature decreases, microbial metabolic rate decreases, which can influence H2S synthesis. Factors associated with microbial synthesis of H2S are unlikely to be predictable due to factors beyond control – temperature fluctuations; precipitation event frequency, duration, and intensity; and the actual density of microbes capable of H2S synthesis.

## 5.2 Biological Influences –Aquatic Plants – Wild Rice

Wild rice has the capability to oxygenate its root zone (aka rhizosphere) through a process known as radial oxygen loss (ROL), thus resulting in more chemically oxidizing conditions in the area immediately surrounding, and in contact with, its roots. ROL can be a controlling influence on the presence of chemically reduced elements and compounds, one of which is H2S. As WR typically germinates during late-spring, water column oxygenation due to mixing influences (spring melt, temperature changes, storm events) tends to be high, which can be a limiting factor for H2S synthesis in nearer-surface sediments. As WR plants mature, their ability to, and extent of, maintain ROL increases and may decrease the likelihood of adverse influences from reduced chemical species (i.e., H2S) on WR health, abundance, and distribution.

In addition to influences on H2S synthesis via ROL by WR, since H2S synthesis is driven via microbial activity, maintenance of an appropriate type and density of sulfur / sulfate reducing microbes is necessary for H2S synthesis. ROL from WR plants could influence the overall microbial assemblage, further decreasing the potential for microbially mediated H2S synthesis.

## 6.0 Potential Influences from Iron Plaque Formation on Wild Rice Roots

As previously referenced, WR plants can release oxygen into the rhizosphere through a process known as radial oxygen loss (ROL) promoting more chemically oxidizing conditions in the rhizosphere. This can aid in protecting the roots from chemically reduced elements and compounds, such as ferrous iron (Fe2+) and hydrogen sulfide (H2S). Under certain conditions, iron can precipitate onto root tissue as oxidized iron (iron oxy-hydroxides), or reduced iron (iron sulfide – FeS). Oxidized iron precipitates on root tissue have been observed to occur during the plant's life stage(s) in which growth is the more dominant activity. 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 when 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. Regardless of the speciation of iron, these precipitates have been commonly referred to as 'iron plaques.'

A proposed mechanism of iron plaque formation is detailed in Jorgenson (2013), and potential influences on plant physiology and reproduction in terms of nitrogen uptake and translocation is detailed in LaFond-Hudson (2016). The general suggestion is that iron plaque formation on roots of WR plants exposed to increased aqueous sulfate is a potential source of decreased nitrogen content of WR seeds produced by exposed plants. 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.