State of Minnesota

Minnesota Pollution Control Agency

In the Matter of Proposed Amendment to Minnesota Rules Chapters 7050 and 7053, Relating to Minnesota Rules 7050.0130, 7050.0220, 7050.0224, 7050.0470, 7050.0471, 7053.0135, 7053.0205, and 7053.0406,	MPCA Rebuttal Response to Public Comments
OAH Docket # 80-9003-34519	
Revisor ID 4324	December 1, 2017

MPCA Rebuttal Response to Public Comments Submitted During the Post-Hearing Public Comment Period.

I. Introduction

This document supplements information provided in the Minnesota Pollution Control Agency's (MPCA or Agency) Response to Comments Submitted during the Pre-Hearing Public Comment Period and at the Public Hearings, dated November 22, 2017 (Response to Comments).

This document contains the MPCA's detailed responses to public comments submitted during the post-hearing comment period following the final public hearing on November 2, 2017. The MPCA reviewed those comments and is addressing them in this Rebuttal Response to Public Comments Submitted During the Post-Hearing Public Comment Period (Rebuttal Response). The subjects of many of the public comments received were addressed previously in the MPCA's November 22, 2017 Response to Comments so that in this Rebuttal Response the MPCA will only respond in detail to those comments not previously addressed or that require a more complete response than was previously provided.[1]

The MPCA's Rebuttal Response consists of this document and a spreadsheet (Attachment 1) that identifies the comments received and the MPCA's response.

The next section of this document explains and discusses areas where the MPCA is proposing to rule language changes. The following section offers a response to comments. To the extent possible, the MPCA responds to some comments with short responses directly in the Attachment 1 spreadsheet in the column titled "MPCA Response." Where a more detailed response is necessary, the discussion is provided in this document.

The comment topics addressed in detail in Part III of this document are:

A. Scope of the Propose Rulemaking

B. Beneficial Use Comments

C. Waterbody Identification Numbers (WIDs)

D. Future Identification of Additional Class 4D Wild Rice Waters

[1] To meet the deadline for submitting post-hearing comments, MPCA focused on responding to comments available for review through November 17, 2017.

E. Application of Wild Rice Sulfate Standard to Streams

F. Comments on Specific Waters Proposed or Not Proposed as Wild Rice Waters

G. Comments on Developing the Magnitude of the Standard

H. Comments on the Duration, Flow Rate, Frequency, and Seasonality

I. Comments on NPDES Permitting

J. Sampling and Analytical Methods

K. Procedural Concerns

II. Proposed and Planned Rule Changes

Some commenters identified specific changes to the proposed rule language. In some cases, the MPCA agrees that the rule should be revised and has provided proposed revised language below. In other cases, the MPCA agrees that the rule language should be revised but is not ready at this point to provide specific language.

Rule Part: 7050.0130, Subp. 2b.

The MPCA plans to remove proposed rule language 7050.0130, Subp. 2b. (lines 1.11 to 1.12), the definition of cultivated wild rice water. EPA provided a comment stating, “Minn. R. § 7050.0130, Subpart 2b. Cultivated Waters. EPA’s understanding is that the surface waters to which the proposed rules apply are those waters identified specifically in the proposed rules at Minn. R. § 7050.0471 and that none of the waters identified as wild rice waters at Minn. R. § 7050.0471 include sub areas that meet the definition of “cultivated waters.” Unless otherwise specified in rule, EPA considers the Class 4D wild rice use (wild rice use) and criteria to be applicable to all waters identified in Minn. R. § 7050.0471.” The MPCA agrees with EPA’s interpretation and comment and has not (and will not in future) identify cultivated wild rice areas as Class 4D wild rice waters, because such waters do not need a sulfate standard. Therefore, we plan to remove the proposed definition.

Rule Part 7050.0220, Subpart 1, B (1 – 4) ; 7050.0220, Subpart 3a.

In several locations (line 2.19, line 2.22, line 3.2, line 3.8, and line 5.14), the MPCA added rule language to clarify that certain kinds of waters may hold multiple use classes and that the sulfate standard would apply to those waters if they were also specifically listed as Class 4D waters. The proposed rule language here was “4D when applicable to a wild rice water listed in part 7050.0471”. EPA provided a comment that “4D is always applicable to water bodies listed in Minn. R. § 7050.0471 and so the phrase ‘when applicable to a wild rice water listed in part 7050.0471’ is superfluous. To avoid confusion as to whether there might be instances when 4D would not be applicable to a wild rice water listed in Minn. R. § 7050.0471, EPA recommended that the language be revised to simply say ‘4D for water bodies listed in part 7050.0471.’” The MPCA agrees with this comment and will make the recommended change.

Rule Part 7050.0220, Subpart 3a, A

In several locations (line 3.11, line 3.18, line 4.5, line 4.13, line 5.2, and line 5.16), the rule language contains tables that include columns listing use classes; the columns are then filled in with the related standards for each use class. Because the wild rice sulfate standard was originally listed within (as a subset of) Class 4A, it was delineated within these lists with the words "wild rice present". The MPCA proposed to retain the structure of the tables and replace the language "Sulfates, wild rice present, mg/L" with "sulfate in a wild rice water" and the 10 mg/L standard with a reference to the equation. EPA provided a comment that the rule language should have a column heading for the 4D use class. The MPCA agrees that this structure would be clearer and will work with the Revisor to determine the feasibility of making such a change.

<u>Rule Part 7050.0220, Subp. 6c</u>

As earlier mentioned, the MPCA proposes to delete the definition of "Cultivated wild rice water." With the deletion of that definition, there is no need to reference the term in the definition of "Wild rice waters", particularly since wild rice waters are defined to be those water bodies identified in part 7050.0471. Therefore, the MPCA plans to delete the sentence at lines 2.3-2.4 that reads, "Wild rice waters do not include cultivated wild rice waters."

<u>Rule Part 7050.0224, Subp. 5, B</u>.

A comment from EPA recommended that the first sentence (lines 7.22-7.24) be revised to clarify that the annual average concentration of sulfate is that in the surface water. The MPCA agrees and is proposing to change the rule language to read:

> 7.22 B. The annual average concentration of sulfate <u>in the surface water of</u> a wild rice water must not exceed
> 7.23 the concentration established as the calculated sulfate standard under subitem (1) or alternate
> 7.24 sulfate standard under subitem (2) more than one year out of every ten years.

<u>Rule Part 7050.0224, Subp. 5., B, (1)</u>

This rule part contains the equation that is the primary option used to derive the numeric sulfate standard. EPA commented that "it is not possible to say with certainty that the relationships between sediment pore water sulfide and total organic carbon and total extractable iron used to calculate protective water column sulfate concentrations remain valid outside the range of the data used to develop the criterion." Comments from Nathan Johnson also raise this issue, stating "I would like to encourage the MPCA to carefully consider the range over which their empirical equation that relates the quantity of sulfide realized as a function of sediment iron, sediment carbon, and surface water sulfate…It is possible that a limitation on the model predictions could be imposed on this basis which would not allow high sulfate concentrations to be calculated by the model if the statistical strength of the model's predictive abilities towards the edge of the domains is limited.

Using the proposed equation to extrapolate to very high surface water sulfate concentrations (higher than those observed commonly in the observational dataset) represents a potential instance of applying the model beyond an appropriate domain of applicability. The same could be said for sediment carbon and iron."

The MPCA understands the concerns raised – namely that the equation is of unknown validity outside of the range of data used to develop it. "EPA recommends that potential input parameter values be constrained to reflect the range of concentrations observed in the studies upon which the criterion is based." The MPCA believes it is appropriate to respond to this concern by setting constraints on the implementation of the equation that would ensure that the equation is protective. The MPCA is proposing that input values of carbon cannot be lower than the minimum value in the range of data used to develop the equation, because carbon enhances sulfide production. The MPCA is proposing that input values of iron cannot be higher than the maximum value in the range of data used to develop the equation because iron removes sulfide from porewater.

The MPCA is proposing that output values of sulfate cannot be higher than the maximum value in the range of data used to develop the equation, 838 mg/L.

The constraint on sulfate is appropriate because observed sulfate levels were an input to the development of the equation, and the equation is of unknown validity outside the range used to develop it.

Such an approach will help assuage commenter concerns about exceedingly high sulfate levels that may result from the equation. The MPCA understands that this limitation will may raise more concerns for other commenters. The MPCA notes that this limitation only applies to one of three possible mechanisms to develop the numeric sulfate standard – the equation. While the equation is the primary mechanism for setting a sulfate standard, the alternate standard and a site-specific standard approach will also be available for appropriate conditions and could result in numeric standards that are higher or lower than calculated by the equation.

The MPCA is considering where and how to make such rule language changes (likely either here or in Subp. 5. B. (1)(d)) needed to implement this change.

<u>Rule Part 7050.0224, Subp. 5., B, (1),(a – c)</u>

These rule parts describe how sediment samples are to be collected and analyzed, based on the Sampling and Analytical Methods that are incorporated by reference. EPA provided comments that by adopting the methods by reference, "Minnesota may hamper its ability to respond to unforeseen technical issues that may arise as new sites are visited and for which application of the methods as written may lead to results that do not adequately protect the wild rice use as it occurs in a given water." EPA suggested various rule changes in pages 10 – 11 of its comment letter; the suggestions place more language directly in the rule but then do not incorporate the methods by reference.

Additionally, commenter Norman Miranda noted that "The dilemma I see for utility managers regardless of whatever protective limit is adopted is to convince their respective City Council and rate payers that a very limited number of samples and sample locations yielded adequate and conclusive data to justify a significant capital investment…I believe MPCA is on the right track offering a consistent sampling regiment of a fixed number of samples at a prescribed location array…I believe at least two sampling events conducted in appropriate but separate locations need to be conducted by the MPCA. I realize the MPCA has limited financial resources to conduct extensive sampling and analysis in multiple locations for every discharger. However, to offer some flexibility, I think the Rule should include a provision that municipalities/permitted facilities be given the opportunity to conduct additional sampling/testing beyond two events that would be required under the Rule. The ground rules for this additional sampling could include:

1. Regulated party must submit a plan for MPCA approval showing proposed alternative sample locations.
2. Sampling must follow MPCA "Sampling and Analytical Methods" and be conducted by approved lab/consultant.
3. Sampling/testing to be done before or concurrent with MPCA sampling as not to delay MPCA's schedule.
4. Cost of additional sampling events to be the responsibility of the Regulated Party.

In return I believe there should be language where the MPCA will give the Regulated Party's data set the same weight if all conditions are followed."

The MPCA does agree that some flexibility may be needed as more sampling occurs, and appreciates that many permittees want to do more sampling (and perhaps sooner) than the MPCA plans to undertake. While the MPCA has planned to do most sampling with our own resources, we have always planned to allow the use of data submitted by other parties (whether regulated/permitted parties or others) if it meets our requirements.

A primary goal of incorporating the sampling methodology into the rule was to provide clarity so that others can conduct sampling and to ensure that the sampling, which is foundational to the developing of a numeric sulfate standard, is completed consistently and accurately. The MPCA believes this is an important goal and will continue to incorporate the methods by reference. Changes to the methods will need to be made through rulemaking.

However, MPCA is proposing a rule language change at lines 8.6, 8.11, and 8.13 to require that analysis and sampling happen consistent with the methods, rather than requiring exact adherence to the methods. This will allow some flexibility if, for example, an analytical method is slightly updated. The MPCA is also proposing to add language that the sediment samples are collected in areas where wild rice is growing or may grow within the wild rice water.

The proposed rule language would then read:

> Where:
>
> (a) organic carbon is the amount of organic matter in dry sediment. The concentration is expressed as percentage of carbon, as determined ~~using~~ consistent with the method for organic carbon analysis in Sampling and Analytical Methods for Wild Rice Waters, which is incorporated by reference in item E;
>
> (b) iron is the amount of extractable iron in dry sediment. The concentration is expressed as micrograms of iron per gram of dry sediment, as determined ~~using~~ consistent with the method for extractable iron in Sampling and Analytical Methods for Wild Rice Waters;
>
> (c) sediment samples are collected ~~using~~ consistent with the procedures established in Sampling and Analytical Methods for Wild Rice Waters; and

The MPCA is then proposing additional related changes, likely to be codified as rule part 7050.0224, Subp. 5., E. which would read as follows:

> For each wild rice water identified in 7050.0471, the methods for selecting sediment sampling sites and for collecting, processing and analyzing sediment samples must be documented, including all QA/QC. Where methods are used that are consistent with but different from those specified in Sampling and Analytical Methods for Wild Rice Waters, the intended methods and how they will be used to calculate the numeric sulfate standard must be submitted to and approved by the Commissioner prior to sample collection.

The incorporation by reference would then be moved to Subp. 5., F.

The MPCA believes this change will allow flexibility when other parties wish to undertake sampling of wild rice waters needed to calculate a protective sulfate value, while ensuring the necessary consistency. The MPCA believes sampling by others could occur at any time; if MPCA sampling has already occurred, the intended methods should describe how both the MPCA gathered data and any additional data will be used in concert. Regardless of the method employed, it is intended that all sampling be documented as required by this rule language. The MPCA will make the final determination about the numeric sulfate standard for any given water body.

Rule Part 7050.0224, Subp. 5., B (2)

The MPCA proposes to change the phrase "ambient sulfate concentration" found in this rule part at lines 8.19 and 8.23 to "surface water sulfate concentration" to be consistent with the rule language change suggested by EPA for line 7.22.

The MPCA received several comments about the alternate standard. This section responds to many of those comments by describing how MPCA envisions that the alternate standard procedure would work and setting forth some proposed rule changes.

This alternate standard procedure develops a replicable approach to developing an alternate standard for areas where the equation does not fit – where there is high sulfate but low porewater sulfide. Some commenters (e.g., Mining Minnesota) have stated that the alternate standard procedure is unclear and creates confusion. They have said that the "Sampling Methods do not include a clear description of the purpose of the porewater sampling", and that the language "create[s] substantial confusion as to what water quality standards [will] actually be applied by the MPCA in any given circumstance."

The MPCA envisions that the alternate standard would be used in places where sediment and surface water sampling has been completed, the equation indicates that the calculated numeric standard is being exceeded in the surface water of the wild rice water, but there are indications that porewater sulfide may not be above the 120 µg/L protective threshold. These indications may be, for example, information about groundwater upwelling or evidence of thriving wild rice (see p. 67 of the TSD). In these situations, if MPCA has done the sediment sampling the MPCA may choose to go back to do porewater sulfide sampling; MPCA also envisions that a permittee may do porewater sulfide sampling and request that the alternate standard approach be used to develop the numeric sulfate standard.

One of MPCA's goals for this rule language was to set out a procedure that is sufficiently defined in rule to be approved by EPA as an alternate methodology to the equation for specifying a numeric sulfate standard. This would obviate the need for each individual sulfate standard developed via the alternate method to be submitted to EPA as a site-specific standard for their approval. In their comment letter, EPA noted that "The only situation where states would not need to submit any new or revised water quality criteria to EPA for review and approval would be where states have adopted and EPA has approved a 'performance-based' standard that relies on regulatory adoption of a process (i.e., a criterion derivation methodology) rather than a specific outcome (i.e., a concentration limit for a pollutant) . . . when such a performance-based approach is binding; sufficiently detailed; and contains suitable safeguards to ensure predictable, repeatable outcomes, EPA's approval of such an approach can also serve as approval of the outcomes as well. If a state's approach is not sufficiently detailed or lacks appropriate safeguards to produce predictable outcomes, EPA review of a specific outcome remains necessary." EPA's comments indicate that they do not find the current rule language to have sufficient specificity to meet this threshold, and suggest that MPCA could add sufficient detail to satisfy the requirements.

Other commenters (USS, Mining Minnesota, etc.) also felt that the alternate standard was vaguely described. The MPCA intends to provide more clarity and meet EPA's requirements for a performance-based rule by revising the rule language. As stated in the TSD on page 70, "it is likely that the maximum increase in porewater sulfide concentrations as a result of increased sulfate would be proportional to the increase in sulfate…With this understanding, a conservative alternate standard would be an increase in the observed ambient sulfate that is proportional to the degree that 120 µg/L is greater than the observed maximum porewater sulfide concentration. For instance, if the observed porewater sulfide was 80 µg/L and the observed surface water sulfate was 110 mg/L, a conservative sulfide standard would be 165 mg/L sulfate (120/80 * 110 mg/L)."

The MPCA plans to revise the rule language. The rule language currently reads (line 8.18 to 8.25):

(2) The commissioner may establish an alternate sulfate standard for a wild rice water when the ambient sulfate concentration is above the calculated sulfate standard and data demonstrates that sulfide concentrations in pore water are 120 micrograms per liter or less. Data must be gathered using the procedures specified in Sampling and Analytical Methods for Wild Rice Waters, which is incorporated by reference in item E. The alternate sulfate standard established must be either the annual average sulfate concentration in the ambient water or a level of sulfate the commissioner has determined will maintain the sulfide concentrations in pore water at or below 120 micrograms per liter.

The MPCA's planned revision, subject to review by the Revisor, would be

(2) The commissioner may establish an alternate sulfate standard for a wild rice water when the ~~ambient~~ surface water sulfate concentration is above the calculated sulfate standard and data demonstrates that sulfide concentrations in pore water are 120 micrograms per liter or less. Data must be gathered ~~using~~ consistent with the procedures specified in Sampling and Analytical Methods for Wild Rice Waters, which is incorporated by reference in item E. The alternate sulfate standard ~~established must be either the annual average sulfate concentration in the ambient water or a level of sulfate the commissioner has determined will maintain the sulfide concentrations in pore water at or below 120 micrograms per liter.~~ is determined by calculating the ratio of measured sulfide, in micrograms per liter, to 120 micrograms per liter and applying that ratio to the surface water sulfate as follows $\frac{120}{porewater\ sulfide} * surface\ water\ sulfate.$

The EPA notes that MPCA must also have supporting documentation specifying how much sulfate and sulfide data is sufficient to describe the empirical relationship between the two in the specific wild rice water. This information is contained in the methods incorporated by reference.

The MPCA believes this revision ensures the process is sufficiently repeatable and detailed to qualify as a performance based standard that does not require individual EPA review. If EPA does not agree, the rule language provides helpful additional clarity but MPCA will submit alternate standards through the EPA's site-specific standards process; MPCA does not find that language about that process is needed in the rule either in this section or in the section about the site-specific standard.

Rule Part 7050.0224, Subp. 5., E.

This rule part contains the incorporation of Sampling and Analytical Methods for Wild Rice Waters by reference. It is important to note that documents incorporated by reference have the standing of rule and should not be viewed as guidance. They are fully enforceable. EPA provided many detailed comments on the Sampling and Analytical methods. Additional detailed comments were provided by other commenters such as Mining Minnesota.

MPCA intends to review those carefully and may make changes to the methods. Some of these changes are likely needed to reflect the prior rule language change that sediment and porewater sampling and analysis must be completed in a manner "consistent with" the methods document. MPCA will be reviewing to see where the methods document can contain broader language – such as by not specifying exactly how samples are to be dried and pulverized if that is not intrinsic to the resulting calculation – to respond to comments about the methods being overly specific.

However, the MPCA believes that in many cases the level of detail that is requested by EPA or other commenters is inappropriate to include as binding language in rule, and many commenters seem to believe that parts of the methods document are already overly restrictive. EPA seems to have intended these comments to apply to a "technical guidance" document not incorporated by reference and purported to have the same standing as rule language. However, technical guidance would not have the same standing unless expressly incorporated by reference. The MPCA will also consider the need for a Standard Operating Procedure and additional detail as a useful guide for sampling, but such a detailed document would not be incorporated in rule. It would be available to others who wish to do sampling in order to help them develop their alternative sampling method or protocol as needed for approval by the MPCA.

Rule Part 7050.0471, Subp. 2.

This rule part sets out that MPCA will solicit information to identify new Class 4D wild rice waters in the Triennial Standards Review, and provides an illustrative example of the types of evidence that should be provided. EPA suggests that MPCA should provide additional details about how this review would be accomplished and the type of information that would be needed. MPCA agrees that additional details would be helpful, but will best be included in the public notice process for each triennial review.

Other commenters raise concerns that the types of evidence that MPCA lists is overly restrictive. A response to these comments can be found in the response to the topic “Listing of Waters”. However, in re-reading the rule language, the MPCA notes that the statement that the evidence “must demonstrate the wild rice beneficial use exists” is somewhat restrictive. It is the MPCA’s responsibility to demonstrate, based on available information, that the wild rice beneficial use exists or has existed. Furthermore. The MPCA does not intend to limit the evidence that commenters provide as part of the triennial review process, but instead to clearly lay out the demonstration that the MPCA will need to make as part of any rulemaking process to add Class 4D wild rice waters. In order to clarify this, the MPCA is proposing the following rule change:

11.18 Subp. 2. Triennial review and future identification of wild rice waters. As part of each triennial review of water-quality standards
11.19 conducted under Code of Federal Regulations, title 40, section 131.20, the commissioner
11.20 must solicit evidence that supports identifying additional wild rice waters in rule. Identifying additional waters in rule must be based on ~~The~~
11.21 evidence ~~must demonstrate~~ that supports a demonstration that the wild rice beneficial use exists or has existed on or after
11.22 November 28, 1975, in the water body, such as by showing a history of human harvest or
11.23 use of the grain as food for wildlife or by showing that a cumulative total of at least two
11.24 acres of wild rice are present. Acceptable types of evidence include:
12.1 A. written or oral histories that meet the criteria of validity, reliability, and
12.2 consistency;
12.3 B. written records, such as harvest records;
12.4 C. photographs, aerial surveys, or field surveys; or
12.5 D. other quantitative or qualitative information that provides a reasonable basis
12.6 to conclude that the wild rice beneficial use exists.

Rule Part 7050.0471 – List of Waters

The MPCA is proposing three changes to the proposed list of Class 4D wild rice waters. The reasons for these changes are addressed in the section of this document about specific wild rice waters. The MPCA is proposing to remove the following waters from the list of wild rice waters in Subp. 3., C.

Line 16.21 (42) Mud Lake St. Louis 69-0652-00

Line 17.1 (49) Round Lake St. Louis 69-0649-00

In addition, MPCA is proposing to split the Embarrass River WID 04010201-577 into two separate WIDs – one from Embarrass Lake through Esquagama Lake and the other from Esquagama Lake to the St. Louis River. Both stretches will receive new WID numbers to identify them. The MPCA proposes to list the WID from Embarrass Lake through Esquagama Lake as a Class 4D wild rice water. The MPCA does not have sufficient information to list the segment from Esquagama Lake to the St. Louis River as a Class 4D wild rice water and will therefore track it as an insufficient information water.

III. Detailed Rebuttal Responses

A. Scope of the Proposed Rulemaking

Many commenters have expressed concerns about the scope of the proposed rulemaking, asserting that it is too narrowly focused and therefore somehow fundamentally flawed. MPCA has responded to specific and general comments about scope in the 11/22/17 Response to Comments and elsewhere in this Rebuttal Response. It may be useful to also note here, however, that the number and volume of comments received prior to, during and after the Administrative Hearings for this rulemaking speaks to the reasonableness of the MPCA's decision to focus the scope of this rulemaking as it has. Given the complexity of the science of sulfate, sulfide and wild rice; the extensive interest in both wild rice and in the activities that may result in sulfate discharges; and the immediate need to address the difficulties in interpreting and implementing the existing wild rice sulfate standard it was reasonable for the MPCA to focus the scope of this rulemaking as it has.

Aquatic Life Standard

Some commenters (MCEA) also stated that "the SONAR generally proceeds on the assumption that if a water body or discharge does not need limits on the discharge of sulfate to protect wild rice, it does not need any limit on sulfate discharges at all. In other words, it is presumed that if there is no wild rice to be protected, that any amount of sulfate may be allowed because any amount of sulfate is presumed to be harmless to fish and other aquatic life. However, at high concentrations, sulfate is harmful to a number of aquatic uses. While probably in most cases the 10 mg/L sulfate standard is more stringent than necessary to protect uses other than wild rice, Minnesota should not throw out its only numeric sulfate standard without establishing standards to protect other uses. Doing so would have the effect of weakening protections for aquatic life from sulfate pollution."

MPCA has never made an assertion that it is not necessary to consider sulfate impacts on other beneficial uses. Rather, MPCA has explained its reasonable decision to limit the scope of this rulemaking to the effects of sulfate on the wild rice beneficial use (see Cover memorandum to the MPCA's 11/22/17 response to Comments). In fact, a sulfate standard to protect aquatic life is on the MPCA's list of potential future water quality standards development and rulemaking efforts (see https://www.pca.state.mn.us/water/mpca%E2%80%99s-proposed-water-quality-standards-work-plan-2018-2020). The SONAR does not speak to other uses because the purpose of this rulemaking was to revise and clarify the sulfate standard related to wild rice.

B. Beneficial Use Comments

Several commenters had detailed comments concerning the beneficial use and how the MPCA has designated waters. Many of the comments are addressed here, but they also overlap with comment addressed in the section discussing water body identification numbers (WIDs).

Background

Discussions related to the Clean Water Act often use three terms that include the word "use." These are – beneficial use, designated use, and existing use. It is helpful to understand these terms in order to better understand our response to comments which follow.

Beneficial use and designated use are generally used interchangeably. The MPCA refers to the wild rice "beneficial use," while EPA and other commenters might refer to the same concept as the "designated use."

According to EPA, designated uses "specify goals and expectations for how each water body is used. Typical designated uses include:

1. Protection and propagation of fish, shellfish and wildlife
2. Recreation
3. Public drinking water supply
4. Agricultural, industrial, navigational and other purposes."[2]

The terms "designated use" and "beneficial use" both refer to the goals and expectations that Minnesota has set for the use of a water body. Minnesota has identified 7 beneficial use classes which are listed in Minnesota Rules 7050.0140.

A critical goal of the CWA, as stated in section 101(a)(2) of the Act, is to ensure that all waters are fishable and swimmable. "The national goal in CWA section 101(a)(2) is water quality that provides for the protection and propagation of fish, shellfish, and wildlife and recreation in and on the water where attainable" (80 Fed. Reg. 51024, Aug.21, 2015). Thus, these fishable, swimmable goals are known as the "101(a)(2)" beneficial uses. In Minnesota, CWA section 101(a)(2) beneficial uses are protected in Class 2 of the beneficial use classes in Minnesota Rules Chapter 7050.

States may also identify beneficial uses other than 101(a)(2) beneficial uses. These other beneficial uses often include protecting water quality for drinking water, industrial use, or agriculture. Minnesota has identified 7 beneficial use classes in Minnesota Rules 7050.0140. The wild rice beneficial use of "use of the grain as food for humans and wildlife" is one of these other beneficial uses and is found in Class 4 (Minn. R. 7050.0224), which protects waters supporting agriculture and wildlife uses.

Some beneficial uses (such as Class 2) are designated to apply to all water bodies in Minnesota, while other beneficial uses are designated to apply to specific water bodies (such as the Class 1 use for drinking water, which only applies to a subset of Minnesota waters).

A single waterbody may be designated as having more than one beneficial use. For example, a single waterbody may be designated as having both Class 2 and Class 4 beneficial uses.

In 1973, the Class 4 wild rice beneficial use was initially designated to apply to "water used for production of wild rice." In a 1998 rulemaking, the MPCA specifically identified a list of 24 selected wild rice waters to which the use and the narrative standard of specifically applied. See Minn. R. 7050.0470. However, other specific waters to which this category applied were never identified. Therefore, up to this point designating the specific waters to which this beneficial use applies has been a case-by-case determination. With this rulemaking the MPCA is specifically identifying, by WID, those waters that have been designated as having the wild rice beneficial use.

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[2] https://www.epa.gov/standards-water-body-health/what-are-water-quality-standards#designated

Under the CWA, the term "existing use" means that a designated beneficial use actually existed (was attained) in a water body at any time on or after November 28, 1975 (the date on which the CWA became effective). The concept of existing use is somewhat confusing because to establish an "existing use" you must consider a time period that starts in 1975 and continues to the present. If at any point in time from November 28, 1975, to the present a designated beneficial use existed in a water body, that beneficial use is an "existing use."

Beneficial Use, Designated Use, Existing Use

Some commenters suggested that MPCA confused the idea of a designated use and an existing use. The Fond du Lac band stated "The CWA protects both "designated" and "existing" uses of water bodies…

"Designated uses" are "those uses specified in water quality standards for each water body or segment whether or not they are being attained." 40 C.F.R. § 131.3(f). Designated uses are not dependent on whether or not conditions currently support the use."

The MPCA has not confused the concepts of "designated use" and "existing use." The MPCA used the existing use concept to identify waters where the Class 4D wild rice waters beneficial use would be designated (the beneficial use was previously "waters used for production of wild rice" and is now "Class 4D wild rice waters"). As stated above, the term "existing use" means that the designated beneficial use actually existed in the water on or after November 28, 1975. The MPCA is designating 1300 waters by WID as Class 4D wild rice waters where the beneficial use of wild rice has existed in the water on or after November 28, 1975. The two concepts are tied together, but are not used inappropriately by the MPCA.

When the wild rice sulfate standard was originally adopted, it was clearly intended to apply to a subset of Minnesota waters – those *used* for wild rice production -- not all Minnesota waters (SONAR, p. 20). A plain language reading of the original beneficial use description, which references use for wild rice production, supports the MPCA's reasonable reliance on this concept in specifying the Class 4D wild rice waters. It is also reasonable for the MPCA to identify waters where the Class 4D wild rice waters beneficial use applies as those waters where the wild rice beneficial use has existed in the water on or after November 28, 1975.

Class 2 Use vs Class 4 Use

Many commenters indicated that the wild rice beneficial use was inappropriately placed in the Class 4 Agriculture and Wildlife Use Class in 1973; and should be reclassified as a Class 2 aquatic life beneficial use because they assert it is a 101(a)(2) use under the CWA. For example:

- The Fond du Lac Band of Lake Superior Chippewa commented that the MPCA should have considered tribal recommendations to "elevate the unique qualities and characteristics beyond simply 'food.'"
- The Minnesota Center for Environmental Advocacy asserted that the wild rice beneficial use is a 101(a)(2) use because wild rice is properly seen as a form of wildlife, wild rice is closely related to propagation of wildlife, and because the collection of wild rice can be considered a form of "recreation on the water."
- Water Legacy commented that when a "designated use" pertains to fish, shellfish, recreation or wildlife, the use has special protection under Section 101(a)(2) of the Clean Water Act.

The MPCA has repeatedly asserted and provided an affirmative demonstration in the SONAR and the Response to Comments dated November 22, 2017, that the wild rice beneficial use is appropriately retained as a Class 4 use, related to agriculture and wildlife uses; it is not a Class 2 use. The MPCA established this beneficial use through rulemaking in 1973 and rule amendments in 1997. When the Class 4A wild rice beneficial use was adopted in 1973 it clearly did not apply to all waters, which is evidence of the fact that this beneficial use is not and should not be interpreted as a CWA section 101(a)(2) use. As noted on pp. 33-35 of the SONAR, in this rulemaking the MPCA is clarifying the existing Class 4 beneficial use; the MPCA is not removing the existing Class 4 beneficial use, nor designating a new wild rice beneficial use. This effort is focused on protecting the specific wild rice beneficial use of use of the grain as a food source for humans and wildlife, not aquatic life more generally as do CWA 101(a)(2) uses. Furthermore, while wild rice is a food source for wildlife, it is not the only food source and it is therefore not reasonable to conclude that the Class 4D wild rice beneficial use is "necessary for protection and propagation of fish, shellfish and wildlife." (Fond du Lac Band, p. 24)

Identifying Waters

The MPCA also received comments that the agency was removing a designated use or existing use as part of this rulemaking when it failed to identify certain waters as wild rice waters. The comments referred to all waters listed in Appendix B of MDNR's 2008 *Natural Wild Rice in Minnesota* report and the I854 Treaty Authority's 2016 and 2017 lists of wild rice waters. The Friends of the Boundary Waters comment letter asserts that "MPCA is removing an "existing use" because MPCA has proposed a list of wild rice waters that omits many waters despite evidence that wild rice grows or has grown in them." The Fond du Lac Band's comment letter argues that the MPCA is removing a designated use when it did not include all of the waters included in Appendix B of the MDNR's 2008 report *Natural Wild Rice in Minnesota*. The Band commented that "the more than 900 excluded water bodies have the 'designated use' of wild rice waters because that use was 'specified in water quality standards' for those waters, when the state designated all surface waters in the state as Class 4A waters used for the production of wild rice." Commenters also disputed the MPCA's statement in the SONAR that the MDNR inventory in Appendix B was not developed for regulatory use. The Fond du Lac Band commented that the MPCA actually used the list for regulation of water quality when it used the list to review water discharge permits to evaluate if they discharged to wild rice waters.

The MPCA does not agree that all surface waters in the state are class 4A waters used for production of wild rice. The existing Class 4A rule has a sulfate standard that is only "applicable to water used for production of wild rice." This language is a modifier that serves to limit the scope of the waters to which the standard applies- not all Class 4A waters, but just those waters that are "used for production of wild rice." This modifier establishes a new sub-class of Class 4A, clearly demonstrating that not all Class 4A waters are wild rice waters.

The MPCA also does not agree that the presence (or evidence of past presence) of any amount of wild rice is indicative that the Class 4D wild rice beneficial use is an existing use in that water body. This topic is covered in depth in Section 6D of the SONAR and in the MPCA's Response to Comments dated November 22, 2017.

Finally, the MPCA does not agree that presence of a waterbody in the inventory found in Appendix B of MDNR's 2008 *Natural Wild Rice in Minnesota—A Wild Rice Report Study Report to the Legislature* is sufficient to demonstrate the beneficial use of the grain as a food source for wildlife and humans. The MDNR report was not developed for regulatory purposes and the MDNR is not a regulatory agency under the Clean Water Act. Although the MDNR report is the most comprehensive statewide inventory available, it has some limitations with respect to the MPCA's need to identify Class 4D wild rice waters subject to the wild rice sulfate standard. For example, the report does not consider density or acreage estimates for all the wild rice stands and it contains only limited information on streams. (see discussion in SONAR pp.42-51 and Response Exhibit N.28 e-mail from Ray Norrgard about DNR inventories). MPCA's evaluation of 1854 Treaty Authority Waters was discussed in MPCA's November 22, 2017 response to comments in Attachment 1.

The discussion cited by a commenter detailing how the MPCA conducted the permit review process to determine if waters were "water used for production of wild rice" shows that the MPCA did NOT treat the 2008 MDNR list as definitive or presumptively valid. As noted there, the MPCA reviewed multiple wild rice records and databases maintained by the MDNR (as was done to establish the list of Class 4D wild rice waters for this rulemaking), and in many cases, required permit applicants to conduct a survey of wild rice in the receiving waters. If the MDNR 2008 list was definitive, then additional surveys would not have been needed.

Other commenters (Mining Minnesota, etc.) have stated that "MPCA has elected to over-designate waters", and that "MPCA does not have statutory authority to designate waters that contain no wild rice as 'wild rice waters' subject to the requirements of Minn. R. ch. 7050." As described here, the MPCA has identified those waters where wild rice is an existing use as wild rice waters. Some of those waters may not have wild rice today, but under the CWA must be protected if the use has existed since November 28, 1975.

C. Waterbody Identification Numbers (WIDs)

Mining Minnesota commented that the application of the standard to the entire WID is inappropriate because it does not require wild rice presence within the WID, and because application of the standard to an entire WID is overly broad.

The comment letter suggests that the identification of a wild rice water should require the actual presence of rice in the waters over a defined period (4-6 years) and that an opportunity for public comment should be required before identifying wild rice waters.

The MPCA does not agree that actual presence of rice is required for identification of a Class 4D wild rice water. Class 4D wild rice waters identified in the proposed rule are the lakes, reservoirs, streams and wetlands where the MPCA has concluded that the beneficial use has existed since November 28, 1975. (November 28, 1975, is a key date in the Clean Water Act. Any beneficial use that a water body actually attained on or since that date is an existing use, and water quality should be such as to ensure that existing use is maintained.) The MPCA agrees that the public should have an opportunity for public comment when wild rice waters are identified. The current public comment period provides this opportunity for the MPCA's proposed Class 4D wild rice waters, and rulemaking is required for any future listing of wild rice waters. Rulemaking always includes an opportunity for public comment.

The commenter also raised concerns that the use of WIDs gives the agency "virtually unfettered discretion to identify and regulate 'wild rice waters' after the rulemaking process has been completed. There are no objective criteria included in the Proposed Rules for determining WIDs, and there is no public process by which interested parties can provide MPCA with information as to how to determine WIDS or their boundaries other than in the cumbersome rulemaking process."

WIDs are unique numeric identifiers assigned to surface waters that are used throughout the MPCA's permitting, water assessment and monitoring programs. This use of unique identifiers for lakes and stream reaches is well established in Minnesota. The MPCA has been using this system of unique lake and stream identifiers since 2001 for its water quality assessments and the MDNR has assigned the DOW numbers (the same as lake WIDs) for lakes since at least 1968. See SONAR Part 1D pp.39-41 for background on the reasonable scientific and hydrological bases for decisions on how WIDs are assigned. Unique numeric identifiers for lakes and stream reaches are essential in Minnesota where there may be many waters in the state with identical names.

The MPCA disagrees that the use of WIDs provides the agency with undue discretion to change where the wild rice standard applies. WIDs are an important component of the MPCA's water programs. For example, they are used to identify impaired waters for public review and reporting to EPA. (Note that for the impaired waters list they are known as assessment unit IDs or AUIDs. The MPCA is moving towards the WID nomenclature in all contexts. The AUID and WID numbers are the same.) The MPCA is committed to documenting WIDs with numeric sulfate standards on the Agency's website, and plans to provide map layers or other tools to make the geographic boundaries of WIDs more accessible. As noted in the EPA comments "EPA emphasizes that modifications to a WID number are only permissible as long as the designation of the WID as a wild rice water is not removed for the entirety of a WID or any subpart of a WID previously approved as a wild rice water". Otherwise, the modifications require rulemaking. The MPCA agrees with EPA's statement that rulemaking is required for any WID modification that may result in removal of a wild rice water from any part or subpart of a WID. Although it may be perceived by the commenter as cumbersome, this rulemaking process will provide the public process by which interested parties can provide comments.

The MPCA will address the comments about application of the standard to the entire WID in three parts: first, applicability of the standard to WIDs in lakes, wetlands and reservoirs; secondly, applicability of the standard to stream and river WIDS; and thirdly, the MPCA's plan for exceptions to the proposed approach.

1. The MPCA's decision to apply the standard to the entire WID for lakes, wetlands and reservoirs was straightforward and is reasonable because in most cases water moves and mixes throughout the entire waterbody. Therefore, discharge to any part of the WID will affect sulfide production in every other part. There are some limited cases where one part of a lake, such as a bay, may be hydrologically isolated, and will not mix with the rest of the waters of the lake. In these cases, the state has a mechanism to assign more than one WID to a lake or reservoir; and each hydrologically separate part of the water body is assigned a unique WID. In these limited cases, the MPCA will make a separate determination of whether each part of the waterbody is a wild rice water. See SONAR Part F. p.93 for further discussion and an example.

2. MPCA's decision to apply the standard to an entire WID for streams and rivers was more complex, and the MPCA considered many alternatives before deciding to apply the standard based on documented presence of the wild rice beneficial use at some point in the WID and to have the standard applicable to the entire WID. Briefly, the alternatives MPCA considered were:

    a. Applying the standard within a distance range from where the beneficial use is present or had been previously documented (MPCA at one time considered 800 meters upstream and downstream of where the rice was located). However, this proved unworkable because further investigation of sources used to identify wild rice waters showed a lack of evidence detailing the exact location of the rice. In some cases, this was due to how information was collected, but it is also because wild rice is known to move around within a water from year to year.

    b. Basing the identification of where the standard applies on the presence of suitable conditions in the wild rice water that would support wild rice. This idea was rejected as it would be very difficult to implement because of the variability of conditions for wild rice growth or the presence of other factors that could limit the growth of wild rice (e.g., wild rice will not grow where water levels vary too widely.)

    c. Establishing wild rice waters at a level smaller than a WID. This would require either subdividing existing WIDs into smaller units or establishing a wholly separate system of WIDs for wild rice waters. While it would be possible to request WID splits to better identify where wild rice might be present within an existing stream WID, it would not be reasonable to do so in every case. The WID is used by the MPCA as the main administrative designation used to assess whether a stream reach may be impaired for a variety of parameters such as dissolved oxygen, sulfate, nutrients and various toxic substances. While a series of smaller WIDs might better represent the location of wild rice, smaller WIDs would like make it more difficult for the MPCA and others to collect representative samples to characterize conditions for other parameters and would also create additional administrative and monitoring burdens. See SONAR Part 6F pp. 93-96 for further discussion.

After considering alternatives, the MPCA decided to establish wild rice waters at the WID level. This choice was reasonable because, as discussed in the SONAR at 39-41, the existing WID nomenclature provides a consistent, accessible, and reliable system to identify specific portions of streams and rivers as wild rice waters.

3. The MPCA recognizes that there may cases where the presence of wild rice within a large or very diverse WID does not justify the application of the standard to the entire WID. The MPCA had originally suggested a proposed amendment for Minn. R. Chapter 7053, which allowed the commissioner to determine that an effluent limit is not necessary under certain circumstances. These circumstances generally relate to the location of a discharge within the wild rice water; e.g., discharge from a facility only affects part of a wild rice water where there is no wild rice. Or there may be specific hydraulic or substrate conditions in the part of the WID that dischargers affects that would prevent growth of wild rice regardless of sulfate levels. Some commenters objected to this provision in the rule, and the EPA also suggested the removal of this provision. In Part IV of the Cover Memorandum to the November 22, 2017 response, MPCA proposed to remove this proposed provision from part 7053.0406, subpart 1. Even with the removal of the proposed amendment to Minn. R. Chapter 7053, there is an approach for situations where rice is not and cannot grow within part of a WID. In these situations, the MPCA can split the WID and conduct a use and value determination (see response to topic area 1.6 in Attachment 1 in MPCA's November 22, 2017 Response to Comments) to remove the wild rice beneficial use from the WID that does not support the beneficial use.

D. Future Identification of Additional Class 4D Wild Rice Waters and Triennial Standards Review

The proposed rule specifies that the sulfate standard applies only to Class 4D wild rice waters, and that those waters must be specified in rule. MPCA has proposed rule language requiring the Commissioner to solicit information about wild rice waters as part of the triennial standards review process that is mandated by the Clean Water Act. The MPCA would then, as a separate process, undertake rulemaking to add any waters to the list of Class 4D wild rice waters to which the standard applies.

Several commenters have raised concerns about the proposed rule language regarding soliciting future evidence for listing waters as Class 4D wild rice waters.

First, one commenter (Fond du Lac Band) stated that "the State admits that its methodology for identifying existing uses may fail, because it provides a process for parties to add water bodies to its list in the future by proving that a water has been used for wild rice in the past". The MPCA's acknowledgement that the list of Class 4D wild rice waters is likely to be incomplete and need to be updated does not mean that the methodology is a failure. The Clean Water Act has a rebuttable presumption that 101(a)(2) uses apply "unless states and authorized tribes show those uses are unattainable" (Water Quality Standards Regulatory Revisions, 80 Fed.

Reg. 51020 (Aug. 21, 2015).) There is no such presumption for non-101(a)(2) uses, such as wild rice, so it is not unreasonable or unexpected for states to designate such beneficial uses as they are found.

One commenter, WaterLegacy, stated that the Minn. R. 7050.041 Subp. 2 “proposed rule section requiring that the commissioner must solicit evidence that supports identifying additional wild rice waters as part of triennial review is, at best, superfluous.” The MPCA agrees that we could solicit information about water bodies to add to the list of Class 4D wild rice waters without the proposed language. However, particularly given concern by Tribes and stakeholders about the lack of additions to the list of [WR] waters since their initial promulgation in 1998, the MPCA felt it was important to make our intent clear. Namely, that we are interested in gathering more information about waters that are not presently identified as proposed Class 4D wild rice waters due to a lack of information and will be specifically asking for the public to provide information for consideration in future rulemaking.

Other comments (Cleveland-Cliffs) found that the “MPCA’s guidelines in proposed part 7050.0471 for how to demonstrate that the beneficial use exists in a water provide no further clarity. The proposed rule simply lists non-mandatory types of evidence that can be used to establish the beneficial use.”

In this proposed rule language, the MPCA felt that it was important to give those people who wish to provide information to support future listing of waters some kind of goal or target for the information that they should be providing; therefore, the proposed rule language describes what the evidence must show in order for it to be used in development of a SONAR for a future rulemaking. The criteria and types of evidence listed mirror the process the MPCA went through to develop the proposed list of waters in this rulemaking. In developing the list of proposed wild rice waters the MPCA used not only a history of human harvest, but other evidence that rice was present in sufficient amounts (acreage, density) to support the beneficial use. The MPCA did not rely solely on human harvest history as one commenter (MCEA) implies.

Other commenters (e.g. Cleveland-Cliffs) are concerned that the criteria are insufficient because they do not require showing a history of harvest and density and acreage, which the commenter believes the legislative language requires. The MPCA responded to that concern in our November 22, 2017 Response, noting that Laws of Minn. 2011, 1st Special Session, ch. 2, article 4, section 32 states: The criteria shall include, but not be limited to, history of wild rice harvests, minimum acreage, and wild rice density. The MPCA has correctly interpreted the legislative directive to mean that all of these criteria can be considered in evaluating whether a water is a wild rice water to which the standard applies, but that the determination of a waterbody being a wild rice water does not require that the waterbody show a history of harvest and a certain acreage of rice and a certain density of rice. The usual statutory construction of the term “include” is that an illustrative example follows.

Commenters (WaterLegacy) stated that “the proposed rule adds no requirements that would increase the likelihood that additional wild rice waters would be listed in rulemaking. It would provide no benefit to citizen stakeholders or tribal rights holders who seek to protect wild rice.” The MPCA believes the proposed rule does provide such benefit by providing an illustrative example of the types of evidence that parties interested in adding a water to the Class 4D wild rice waters list should be gathering.The MPCA believes the proposed rule language strikes a reasonable balance by articulating the criteria that the MPCA used to develop the list of wild rice waters proposed in this rule and setting that forth so that all interested parties know what kind of information they should be gathering to support a listing, without being overly restrictive about acceptable evidence.

Some commenters are concerned that additional Class 4D wild rice waters can only be added through rulemaking. WaterLegacy states that the MPCA’s proposal of this provision “underscore[s] that – irrespective of evidence – it will not add any wild rice water prior to additional rulemaking" and implies that this is a flaw in the rulemaking.

Another commenter, Cleveland-Cliffs, states that "under the plain language of the proposed rule, a water could be regulated as a wild rice water simply on the basis of a person telling the MPCA that he or she observed a single animal (i.e., wildlife) eating some wild rice (i.e., using the grain as food). The result of MPCA's "criteria" would be either (a) that effectively *all* waters containing any amount of wild rice would be listed (because it seems likely that any water with even the smallest amount of wild rice has experienced at least one instance of an animal or human eating wild rice at some point since 1975), or (b) MPCA staff will exercise "best professional judgment" to draw the line between those waters that are "in" and those waters that are "out" of the Rule's reach, creating an inherent risk of arbitrary application of the rule."

The opposing nature of these comments demonstrates why it is reasonable, as the MPCA has proposed in the rule, to add Class 4D waters only through rulemaking. A rulemaking process, including a SONAR and public comment, will allow a full discussion of the evidence for identifying a water as a Class 4D wild rice water. The MPCA staff will exercise best professional judgement about what waters to propose as Class 4D waters, but that judgement will be subject to public review and comment, thereby preventing arbitrary application of the rule or a sulfate standard.

Some commenters are concerned about the types of evidence that the MPCA described as being needed to support the future listing of waters. Commenters were especially concerned about the two acre threshold mentioned. The MPCA believes that two acres of wild rice is sufficient, without additional corroborating evidence, to show that the beneficial use exists in the waterbody. If the MPCA is provided evidence that two acres of rice exist, or has existed since November 28, 1975, we would propose to add the waterbody to the Class 4D wild rice waters. However, if there is no evidence of two acres of wild rice, we would want to look at multiple lines of evidence to see if the beneficial use exists or has existed. A demonstration of two acres of wild rice is not required to identify a water as a Class 4D wild rice water.

Other commenters (1854 Treaty Authority) raised concerns that the list of criteria show that "[a]ny additions will undergo a more burdensome and scrutinous process than waters currently being proposed. To add waters, evidence could include meeting the minimum level of two acres of wild rice in a water, past or current human harvest, or other evidence of wild rice presence (oral histories, written records, photographs, field surveys, etc.)" The MPCA does not believe this is the case; the intent was for future criteria or evidence to be the same as the evidence that the MPCA used to propose Class 4D wild rice waters in this rulemaking. (See SONAR, pg 58 – 64).

Specific to the criteria about oral histories, WaterLegacy mentioned that "Oral histories of wild rice harvest are particularly salient to protection of tribal Treaty resources and are often referenced in tribal comments. Although the SONAR and MPCA's hearing presentations may suggest that MPCA 'recognizes the validity of written or oral histories about wild rice,' the proposed rule text belies this assertion. Written or oral histories about wild rice are only 'acceptable' as evidence if they 'meet the criteria of validity, reliability, and consistency.' No other form of evidence must meet these criteria to be considered 'acceptable.'" As stated in the SONAR (pg 62 and Exhibit 33), the MPCA drew these criteria from the way in which oral evidence was presented in the court case Zuni Tribe of New Mexico vs. United States. This strikes a reasonable balance that allows MPCA to accept important information from oral history and tradition while mitigating the potential for an erroneous listing based on hearsay.

E. Application of Wild Rice Sulfate Standard to Streams

MPCA received several comments asserting that the proposed equation should not apply to streams. The rationale stated was that sulfate does not convert to sulfide as readily in a stream as it does in a lake, because streams typically have more oxygen present in comparison to lakes (MESERB and Hall memorandum). The commenters are assuming that the greater oxygen that may occur in the surface water of streams penetrates the sediment and produces low porewater sulfide concentrations that do not conform to predictions. MPCA did investigate this question while developing the structural equation model (SEM) that was published as Pollman et al. (2017, Response Exhibit N.4). To test to see if there is a difference between the variables that control sulfide in streams and lakes, the residuals of the predictions were examined. Residuals are the difference between the observed sulfide and the predicted sulfide concentration. Residuals for both groups (lakes and streams) were normally distributed. Using a t-test to evaluate whether the mean differences between the two groups is significantly different from zero yielded a non-significant probability of $p = 0.63$. Therefore, the ability of SEM to predict sulfide was similar for lakes and streams, and there is no evidence that another variable not included in the model, such as oxygen, was influencing sulfide in stream sediment porewater differently between the two waterbody types.

If elevated oxygen were efficiently oxidizing sulfide in stream sediment, one would expect sulfide to have been consumed and not measureable. However, sulfide was measureable at all stream sites sampled during the MPCA-sponsored field work. Out of 232 sulfide measurements at lakes and streams, only three were below the lab's reporting limit of 11 micrograms per liter (i.e., near zero), and those occurred in two lakes, Carlos Avery (one measurement) and Height of Land (2 measurements, separated by a year). These low sulfide concentrations were probably the result of low availability of sulfate in the overlying water, as all sulfate concentrations from these lakes were below the lab's reporting limit of 0.5 mg/L sulfate.

Overall, these results are consistent with the premise that wild rice tends to grow at sites in waterbodies that accumulate organic matter in the sediment. Bacteria that colonize the accumulated organic matter consume all available oxygen, which allows the accumulation of sulfide.

Another commenter asserted that the "agency's field data is vastly skewed toward still water (27 streams compared to 81 lakes), and that the data has been molded into a mathematical expression that does not account for the differences between lakes and streams." (Mesabi Nugget). The assertion is immaterial, considering that the MPCA analysis (first paragraph above) found that the mathematical expression is not affected differently by lakes and streams.

Mesabi Nugget also suggested the MPCA should have collected data on water movement, and cited the repeated presence of healthy rice in Second Creek as evidence that the MPCA's equation is flawed. MPCA staff disagree with his conclusion that the equation is flawed. A detailed discussion of an alternative numeric standard approach that can apply to unique situations, such as Second Creek, can be found in the MPCA Technical Support Document (TSD) beginning on page 67.

F. Comments on Specific Waters Proposed or Not Proposed as Class 4D Wild Rice Waters

Representatives from three mining operations in northeastern Minnesota (ArcelorMittal Minorca Mine Inc. (ArcelorMittal), Cleveland-Cliffs, Inc. (Cliffs), and U. S. Steel – Minnesota Ore Operations (U.S. Steel)) submitted specific comments on individual waters proposed for inclusion in Minnesota Rules 7050.0471 as a Class 4D wild rice water. Comments on individual waters were also submitted on behalf of Northeastern Minnesotans for Wilderness and WaterLegacy. The specific proposals and MPCA's responses are summarized below.

<u>ArcelorMittal</u>

In its November 22, 2017 comment letter, ArcelorMittal provided information and recommendations on White Lake (69-0571-00) and the lower portion of the Embarrass River. ArcelorMittal requests that the MPCA:

- Remove White Lake, WID 69-0571-00 from the Proposed Rule, (Minn. R. 7050.0471, Subp. 3.C. (74)); and
- Remove the Lower Embarrass River from WID 04010201-577 (Minn. R. 7050.0471, Subp. 3.C. (17), thereby redefining the WID to only include Embarrass Lake to Esquagama Lake.

White Lake. White Lake (69-0571-00) was proposed as a wild rice water based on the initial listing of this lake in the March 24, 2016 version of the 1854 Treaty Authority's list of wild rice waters (SONAR Exhibit 24). In retrospect, the MPCA should also have included "Permittee" as a reference source applicable to this water based on the results from the December 29, 2011 wild rice survey conducted by Barr Engineering for ArcelorMittal and Figures 2 through 6 attached to the November 22, 2017 comment letter.

The background imagery of Figures 2 through 6 is of particular interest. Figure 2 background imagery is attributed to a 2010 aerial photograph from the U.S. Department of Agriculture's Farm Service Agency (FSA). In this photograph, along the northwest shore of White Lake just above the site label "60 sqft", there is a land extension that juts out into the lake. (This land extension is even more evident in another aerial photograph in the online historical aerial photographs collection from the University of Minnesota's John R. Borchert Map Library at: http://maps.dnr.state.mn.us/landview/historical_airphotos/projects/stl/y1981/stl_014_199.jpg). These two aerial photos show a more or less continuous extension of land jutting out into the lake. Comparing it to the background imagery in Figures 3 – 5 (2013) and Figure 6 (2016), one can see that the land jut becomes an island in the lake. The cause for this apparent difference is likely due to an increase in the water level of the lake. MPCA contends that the sparse number of wild rice plants observed during these surveys may have been associated with the elevated water levels that are reflected in these aerial photographs. Another possible contributing factor affecting the in-lake wild rice may have been elevated lake sulfate concentrations (two lake samples collected on August 18, 2011, each with 123 mg/L sulfate). MPCA staff do not agree with ArcelorMittal's request to remove White Lake from the proposed Class 4D wild rice waters list.

Embarrass River. The Embarrass River WID 04010201-577 is approximately 14.5 miles long with about 70 percent of its length being between Esquagama Lake and the St. Louis River. Based on the September 2017 Barr Engineering Embarrass River survey characterizing the sediment within this reach, MPCA agrees that redefining (splitting) WID 04010201-577 would be appropriate. MPCA will split this WID into two separate WIDs – one from Embarrass Lake through Esquagama Lake and the other from Esquagama Lake to the St. Louis River. The former will continue to be proposed as a Class 4D water and the latter will be added to the MPCA wild rice database as an Insufficient Information water.

Cleveland-Cliffs

Cliffs' November 22, 2017 comment letter (pages 19 – 22) questions the reasonableness of listing six individual waters as proposed Class 4D wild rice waters. A seventh waterbody noted, Dunka River, is considered an Insufficient Information water and is not being proposed as a Class 4D water. The specific concern with this water relates to the Dunka River entry in SONAR Attachment 5 that lists it as a proposed wild rice water. MPCA staff acknowledge this discrepancy and would like the hearing record reflect this acknowledgment.

MPCA's responses to the proposed Class 4D issues raised by Cliffs are summarized as follows.

Day Brook. The wild rice beneficial use of Day Brook (WID 07010103-542 in St. Louis and Itasca Counties) is discussed in MPCA's November 22, 2017 Post Hearing Response, Attachment 1 at page 17. The Permittee reference source of wild rice information came from the Barr Engineering technical memorandum with the subject heading *2011 Wild Rice Survey for Hibbing Taconite Company* dated December 22, 2011.

Mud Lake (69-0652-00) and Round Lake (69-0649-00). The primary reference source cited by the MPCA for both of these lakes is a November 9, 2011 Barr Engineering technical memorandum with the subject heading *Wild Rice Field Survey for United Taconite LLC*. Both lakes were surveyed once on August 19, 2011. The other reference source cited by MPCA for these lakes is the 1854 Treaty Authority wild rice waters list (SONAR Exhibit 24) although the 2011 Barr survey results were the primary reason these two lakes were added to the 1854 list.

At the time of the survey, a limited number of wild rice plants was observed. Field estimates of the cumulative total of wild rice plants were 65 and 95 for Mud Lake and Round Lake, respectively. The sulfate concentration in Mud Lake based on three samples averaged about 19.6 mg/L. Round Lake had sulfate concentrations less than 1 mg/L in all four of the samples that were collected.

Generally speaking, the 2011 wild rice production levels in northeast Minnesota lakes was characterized by the 1854 Treaty Authority as being "fair". The amount of wild rice on Mud and Round Lakes observed in 2011 fall way short of what would be considered as being "fair."

The nearest lake with 2011 water level records is Stone Lake (69-0686-00) which is about one-half mile south of Round Lake. For calendar year 2011, the water level in Stone Lake ranged from 0.4 to 1.1 feet below the lake's ordinary high water mark, with the lowest water levels occurring in June of that year. If lake water levels were below the ordinary high water marks for Mud and Round Lakes, water levels do not seem to be a factor influencing the limited amount of wild rice observed in these two lakes. That is not to say that 2011 was not a poor growing year in the natural wild rice cycle for these two lakes; it is difficult to say based on the one-time survey results.

Based on the above considerations, MPCA staff plan to remove Mud Lake (69-0652-00) and Round Lake (69-0649-00) from the list of proposed Class 4D waters and will maintain them in the MPCA wild rice database as insufficient Information waters pending the collection of additional wild rice information.

Perch Lake (69-0688-00). MDNR files in the St. Paul office contain a fisheries survey for Perch Lake (69-0688-00) from August 28 – 29, 1968. Wild rice density was assigned a density rating of "2". The emergent vegetation density rating scale in use at this time assigned the following numeric ratings: 4 for lush; 3 for moderate; 2 for scattered, and 1 for sparse. In addition, the survey noted "wild rice was concentrated in two areas on the northwest shoreline". The MDNR 2008 report (SONAR Exhibit 21) estimated wild rice coverage to be 32 acres. While the September 2017 Northeast Technical Services survey referenced provides useful information building upon the "wild rice story" specific to this waterbody, it does not alter MPCA's position on Perch Lake. MPCA maintains that Perch Lake (69-0688-00) should be proposed as a Class 4D water.

St. Louis River Segments. Cliffs identifies two separate entries for the St. Louis River – rule as proposed line number 58, St. Louis River WID 04010201-644 and line number 59, St. Louis River WID 04010201-631 and one entry for the St. Louis River/Estuary WID 04010201-532.

St. Louis River WID 04010201-631 is already in Minn. R. 7050.0471, subp. 1 as a wild rice water. This is the headwaters reach of the St. Louis River downstream of Seven Beaver Lake to the west side of Section 36, Twp.58, R.13. The next downstream WID, 04010201- 644, extends from the east line of Section 35, Twp. 58, R.13 to the Partridge River. There are four separate locations within this reach with wild rice identified by the 1854 Treaty Authority.

St. Louis River/Estuary WID 04010201-532 is the river reach from Mission Creek to the Oliver Bridge. In 2013 the UofM/MPCA wild rice field study had a sampling station within this reach that reported mid-summer wild rice stem counts averaging between 11.8 and 31.2 stems per square meter. Permittee wild rice surveys in this WID reach also reported wild rice. Barr Engineering conducted these permittee-sponsored surveys at the request of PolyMet Mining. These surveys were conducted in 2009, 2010, 2014, and 2016. Wild rice densities encountered within WID 04010201-532 during these surveys ranged from 1 to 5 at numerous sample site locations (see wild rice density classification description table below).

Wild Rice Density Classification	Description
1	<10% Wild Rice Coverage
2	10 – 25 % Wild Rive Coverage
3	25 – 50 % Wild Rice Coverage
4	50 – 75% Wild Rice Coverage
5	>75% Wild Rice Coverage

MPCA maintains the proposed Class 4D classification for these reaches of the St. Louis River and Estuary, as well a proposed Class 4D WID reach of the St. Louis Estuary (2) WID 04010201-533 (Oliver Bridge to Pokegama River).

Embarrass River. Cliffs questioned the reasonableness of proposing two Embarrass River WIDs as Class 4D waters: WID 04010201-577 (Embarrass Lake to the St. Louis River) and WID 04010201-579 (Headwaters to Embarrass Lake). [See also the discussion above regarding WID 04010201-577.]

There were a series of permittee sponsored wild rice and water quality monitoring surveys conducted by Barr Engineering for PolyMet Mining Inc. over the period 2009 – 2016 (SONAR Exhibit 30). These reports were evaluated by MPCA and the survey findings provided additional evidence supporting the Class 4D classification proposal for these two WID reaches of the Embarrass River.

Cliffs questioned whether one wild rice harvester trip on the Embarrass River was sufficient information to list the river as a wild rice water (see SONAR Exhibit 22). MPCA views one harvester trip to be more than adequate in support of the proposed Class 4D listing.

<u>U.S. Steel.</u>

Little Sandy Lake (69-0729-00) and Sandy Lake (69-0730-00). MPCA has provided Post Hearing response and additional exhibits concerning these two lakes (see MPCA November 22, 2017 Post Hearing Response – Attachment 1 at page 16 and MPCA Post Hearing response exhibits N. 28, N.35 – 37, and N. 39).

Little Sandy Lake and Sandy Lake are prime examples of waters where significant wild rice was present post November 28, 1975 but currently are experiencing a greatly diminished wild rice population. Since available information documents this existing use after the 1975 date, it is reasonable to propose that these two lakes be classified as Class 4D wild rice waters.

<u>Northeastern Minnesotans for Wilderness.</u>

The November 22, 2017 comment letter submitted by Northeastern Minnesotans for Wilderness offered support for inclusion of White Iron Lake (69-0004-00) as a wild rice water. MPCA staff acknowledge the statement of support and appreciate the comments.

WaterLegacy

Dark Lake (69-0790-00). Dark Lake is currently being maintained as an Insufficient Information water in the MPCA wild rice database. Dark Lake was not listed in MDNR's 2008 inventory of wild rice waters (SONAR Exhibit 21) but was included by the MDNR in response to the 2013 Call for Data (SONAR Exhibit 29). Dark Lake was among the waterbodies surveyed and sampled by University of Minnesota as part of the MPCA sponsored wild rice field survey.

In their comment letter, WaterLegacy provided comments on Dark Lake (page 37). MPCA's November 22, 2017 Response to Comments – Attachment 1 at page 15 discusses the reasons why Dark Lake (and Dark River) are not being proposed by the MPCA for Class 4D use classification during this rulemaking.

Upper Partridge River. WaterLegacy (at page 38) states that the portion of the Partridge River east of Colby Lake is not being proposed as a wild rice water. This is not the case. MPCA is proposing Partridge River WID 04010201-552 (Headwaters to St. Louis River) as a Class 4D wild rice water. The portion of the Partridge River east of Colby Lake is included in this WID.

G. Comments on Developing the Magnitude of the Standard

The MPCA continued to receive detailed comments relating to the development of the protective sulfide level and the resulting equation for calculating the numeric sulfate standard. Some of these comments included information on new studies, or more information on ongoing studies, while others related to data analysis and statistical approaches. Many of these comments were submitted by multiple commenters or referenced across comment letters.

1. Hydroponics Studies

   MPCA received multiple comments concerning the use of hydroponic studies in developing the proposed standard. In particularly, many commenters submitted results of a new hydroponics study conducted by Fort Environmental Laboratories in November 2017. The main discussion of this study is provided in the executive summary of the expert comments on behalf of the Iron Mining Association attached to comments submitted by a coalition of mining companies and Minnesota Power. The comments state:

   "Fort Environmental Laboratories conducted another hydroponics study in November 2017 (unpublished) in response to the MPCA speculations that the water depth was not deep enough in the previous Fort hydroponics study. The study design is substantially the same as that used in the published Fort et al 2017 study, but the water depth was increased from 1 cm to 6 cm. The study was conducted from November 3, 2017 to November 13, 2017. The study was conducted using Good Laboratory Practices, addressed all of the recommendations of the Peer Review Committee, and met all acceptability criteria. Results from the most recent study, as well as previous Fort et al studies confirmed:

   - That sulfide was not toxic to wild rice at concentrations observed in Minnesota wild rice waters;
   - That adequate oxygen was not present at sufficient levels in the test media to support detoxification based on the hypoxic environment, as speculated by the MPCA in their rejection of the 2017 Fort et al study.

- Rather complexation with Fe is the primary mitigating factor in terms of sulfide toxicity. Thus, the results suggest that detoxification of sulfide in the Fort et al. were also the result of Fe complexation rather than detoxification by the plant itself.
- The November 2017 study provides even more evidence that MPCA unreasonably rejected the published 2017 Fort et al study and should have given much more weight to its results."

The study design for the new, unpublished study, was not substantially the same as that used in the published Fort et al. 2017 study, as claimed. The study was 10 days instead of 21, was conducted in the dark, and did not include some biological endpoints such as mesocotyl (stem) length. The study does not have any bearing on whether the wild rice seedlings in Fort et al. (2017) were able to detoxify sulfide because the young seedlings in Fort et al. (2017) were afforded access to the elevated oxygen of the atmosphere. Rather, the new study repeated a similar exposure reported in Pastor et al. (2017). Both of these studies germinated seeds for 10 or 11 days in anoxic, dark conditions against a range of sulfide concentrations. Both studies showed that germination is not a growth stage that is very sensitive to elevated sulfide. The fact that the new Fort study showed that adding iron reduces the toxicity of sulfide has no bearing, despite assertions, on the reduced toxicity of sulfide when seedlings have access to the atmosphere. The new study did not even report the same biological endpoints, such as mesocotyl length, but rather just reported germination rate.

Furthermore, MPCA did not "reject" the 2017 Fort et al study. In fact, MPCA reviewed that study and included the results in the TSD (pp. 33-34, 37-38). The fact that the MPCA put less weight on that study in establishing the proposed protective sulfide concentration is not evidence that the Fort et al study was rejected or ignored. Notably, MPCA also gave less weight to some of the MPCA-sponsored studies TSD pp. 33-34, 38-39).

2. Mesocosm Studies (Dr. Pastor)

Multiple commenters (Water Legacy, Fond du Lac) stated that the MPCA's proposed equation would not result in defensible levels of sulfate because the equation treats iron as protective. These comments refer to research done using outdoor mesocosms by Dr. John Pastor at the University of Minnesota, Duluth; Dr. Pastor himself submitted comments and his research is discussed here based on his comments.

First, Dr. Pastor stated that "Our recent research at the University of Minnesota Duluth demonstrates that sulfide, not sulfate, is toxic to seedlings of wild rice. The MPCA proposes that iron can protect wild rice by precipitating with the sulfide. However, the addition of iron to mesocosms with high sulfate concentrations did not entirely mitigate the toxic effects of sulfide to seedlings. Our research also demonstrates that precipitation of iron sulfide on wild rice roots can inhibit nutrient uptake needed to ripen seeds, so iron sulfide can have negative effects on wild rice sustainability. Setting sulfate limits based on the level of sediment iron is premature and is not reasonable. (p. 2)…The net effect of sulfate additions to wild rice populations is to drive the populations to extinction within 4 or 5 years at high concentrations of sulfate (300 mg/l), even when iron was added to the sediments."

First, the MPCA wants to emphasize the first part of Dr. Pastor's comment. While other comments (Hansel attachment to Expert Comments) state that 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, the hypothesis that porewater sulfide impacts the presence and density of wild rice is supported by the mesocosm results published in Pastor et al. (2017), as Dr. Pastor mentions above, and by the field data published by Myrbo et al. (2017), and the presentation of Myrbo's data on pages 51-52 of the TSD.

Turning to iron plaque formation, the only information the MPCA has on this issue is a four-page non-peer reviewed progress report (Pastor, 2017, N.34) that indicates that exposing sediment from Rice Portage Lake, which would have an equation-calculated numeric sulfate standard of about 34 mg/L (TSD, page 92). The only evidence presented by Pastor (2017) that iron plaque can inhibit nutrient uptake was performed at a treatment concentration of 300 mg/L, which is over eight times greater than the average sulfate concentration calculated for that mesocosm exposure using the equation under the proposed standard (34 mg/L). Thus, it may be true that deleterious forms of iron sulfide can form when sulfate concentrations occur that are much higher than would be allowed using the MPCA's proposed equation. Regarding the ineffectiveness of added iron: First, it is not clear how to calculate, or whether it is possible to calculate, what the equation-based standard would be after the addition of iron (the iron additions are of unstated quantity and form, and there are many chemical forms of iron). Therefore, it is unknown whether the failure of iron addition to protect wild rice is consistent or inconsistent with the equation.

Secondly, Dr. Pastor noted that "In addition, the MPCA's model assumes that concentrations of sulfide, sulfate, reactive iron and organic matter are in a steady state. This is not a reasonable assumption, especially once sulfate loading increases from various sources of pollution." (p. 2) He also commented that "the amount of reactive iron in a localized area will decline with increased sulfate loading, just as a checkbook balance declines when withdrawals increase without a matching increase in deposits. MPCA's model does not demonstrate that natural inputs of iron would replenish the reactive iron in the sediment commensurate with sulfate discharge. The model assumes, without evidence, that iron input will remain at a rate sufficient to ameliorate sulfide toxicity from the additional sulfate without creating additional adverse consequences for wild rice survival." (p.6)

This comment expresses a misunderstanding of the assumption of steady state and how an increase in sulfate in a given wild rice water will affect the prediction of the new sulfide concentration, once the water body reaches a new steady state. First, it is common and reasonable for scientists to assume that porewater sulfide is in a steady state with the controlling variables of sulfate, TOC, and iron (TSD p. 43 and Pollman et al. (Response Exhibit N.4). Because the MPCA's equation was fit to real observations in natural systems at steady state, the equation describing those relationships predicts the effect of an increase in sulfate at a particular combination of TOC and iron as modeled by waters in a steady state with similar TOC and iron, but higher sulfate. The assumption that the waters in the calibration data set are in steady state includes the assumption that there is a continuous supply of iron to the waterbody from its watershed, so that it can be assumed, contrary to Dr. Pastor's comment, that iron input will remain at a rate sufficient to ameliorate sulfide toxicity.

Finally, Dr. Pastor commented that "Both historic field data and the recent field surveys performed by the University of Minnesota as part of the Wild Rice Sulfate Standards Study demonstrate that concentrations of sulfate in surface water above 10 mg/L proposed in the MPCA's flexible standard may not adequately protect wild rice." (p. 2)

The evidence cited by Dr. Pastor for this assertion are two: (1) that most lakes with wild rice currently have low sulfate, and (2) that Sandy Lake has lost most of its wild rice even though its current sulfate concentration (cited as 95 mg/L, but actually 125 mg/L when MPCA study site Sandy-1, influenced by an incoming low-sulfate stream is not included) is only slightly higher than the average calculated standard (from 10 MPCA samplings), which is 79 mg/L.

Regarding (1), the correlation of wild rice with low sulfate does not indicate cause and effect between sulfate and wild rice, which is what the 10 mg/L standard was based on. The MPCA-sponsored research clearly demonstrated, in peer-reviewed publications, that the true cause and effect is more complicated, and that the production of porewater sulfide is primarily responsible for the presence and absence of wild rice in Minnesota (Myrbo et al. 2017, Response Exhibit N.2). As documented in Myrbo et al. there is no statistically significant relationship between sulfate concentration and wild rice occurrence, whereas there is a highly significant relationship with porewater sulfide.

Regarding (2), first, MPCA is proposing to use the lowest calculated standard, not the average. Second, sulfate concentrations declined significantly in recent years due to sulfate mitigation efforts by nearby Minntac. The wild rice was mainly lost by 2004 (according to a draft EIS titled Minntac Water Inventory Reduction) when sulfate concentrations were much higher than observed by the MPCA in 2013. The draft EIS cites a pre-Minntac sulfate concentration of 7.6 mg/L. Thus, the loss of wild rice in Sandy Lake is consistent with exceedances of equation-calculated standards, and the observations do not support Dr. Pastor's comment.

3. Field Surveys and Data

Many commenters raised concerns about the MPCA's use of field survey data. For example, one commenter (Hansel attachment to Expert Comments), stated that "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 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."

The MPCA did not ascribe all ill effects on wild rice to sulfide and sulfide alone. On page 23 of the TSD, MPCA summarizes its investigation into the multiple factors that control wild rice:
"Performing *multiple* BLR with more than one variable demonstrated that porewater sulfide is one of three primary independent variables correlated with wild rice occurrence (Myrbo et al., in press-1): porewater sulfide, water transparency, and water temperature. The statistical analysis strongly supports the conclusion that sulfide independently affects wild rice presence and absence ($p=0.001$; Table 1-3), which implies that limiting sulfate availability has the potential to protect wild rice from elevated sulfide." As MPCA noted in the 11/22/17 Response to Comments (p. 3) "the fact that other factors than sulfate…also affect wild rice does not by itself negate the need for or reasonableness of a revised sulfate standard to protect wild rice from *sulfate* impacts."

This commenter continues to note that "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." MPCA finds that the data are remarkably consistent, except for the results of Fort et al. (2017), as presented on pages 33-34 of the TSD.

Some commenters were specifically concerned about the field data and the related analysis to develop the protective sulfide concentration. Comments from the Great Lakes Indian Fish and Wildlife

Commission (GLIFWC) discussed the MPCA's visual examination of the proportion of waterbodies with wild rice present, noting that "The graphical method used to identify 120 ug/L of pore water sulfide as the 'protective concentration' is conceptually flawed and cannot be used to identify a change in response of rice to sulfide concentration. The 'dip' at 120 ug/L of sulfide, identified in Figure 1-5 of the FTSD and Figure A7-3 of Appendix 7 of the FTSD, is an artifact of the number of samples with a concentration near 120 ug/L. The dip does not represent a response of rice to sulfide." (p. 3)

This commenter also stated that "The field-data based methods used to identify 120 ug/L of pore-water sulfide as the 'protective' level are either faulty (the visual examination of graphical representation) or generate highly variable results and are data-set dependent (EC10 on logistic regression and change-point analysis). The field survey data sets were not collected in a statistically rigorous manner and are not adequate to identify any particular 'protective' level of sulfide using these methods." (p. 7)

It may be true that the graphical method is conceptually flawed. Regardless, it is still a useful analysis. The MPCA relied on multiple lines of evidence from quantitative analyses of the MPCA-sponsored hydroponic, mesocosm, and field data, the central tendencies of which tend to cluster near 120 µg/l, albeit with relatively large 95% uncertainty ranges (TSD Table 1-8, page 33).

The commenter asserts that the field survey data sets were not collected in a statistically rigorous manner, without actually stating an actual problem with the data set. The easiest and most common conformance to a "statistically rigorous" dataset would be to sample sites randomly, so as to be probability-based. This issue was addressed in the MPCA June 2014 report that was peer reviewed. The MPCA wrote (p. 21):

Statisticians recommend that surveys be probability-based when the point of the survey is to characterize the population being sampled. Probability-based surveys allow survey results to be extrapolated back to a larger population. The 2012-2013 Field Survey was purposefully not probability based, in that the point was not to characterize the population of wild rice production waters, but rather to explore the effect of elevated sulfate on the chemistry of the porewater of actual and potential wild rice habitat. If wild rice habitats had been sampled probabilistically, most of the sites would have had very low sulfate concentrations and little would have been learned about the effect of elevated sulfate. To ensure that the Study included samples from waters with elevated sulfate concentrations, the survey sites were intentionally not chosen in a random manner.

After presentation and interpretation of several databases, MPCA concluded (p. 23):

In summary, the 2012-2013 Field Survey of lakes has a sulfate frequency distribution that is intermediate between the probability-based USEPA survey and the 513 sulfate values that were available for the 1,290 wild rice lakes identified by the DNR (2008). The intermediate position means that the Field Survey sampled more high-sulfate lakes than would be expected if only known wild rice lakes were sampled, but fewer than would be expected if all lakes in the state were sampled probabilistically. Given that wild rice does not occur naturally in all lakes of the state, and that a major goal of the Field Survey was to assess the effect of elevated sulfate on wild rice, the site selection approach used for the Field Survey could be just right. The intent of the sampling was to find variation in sulfate while maintaining all other parameters suitable for wild rice growth (water transparency, water depth, pH, alkalinity, hardness, etc.). If this was accomplished, then the Field Survey could be interpreted as functioning as a sampling of a natural experiment that can be used to evaluate the effect of sulfate on wild rice.

Therefore, the MPCA sampled a range of lakes that was appropriate to answering the question of the effect of sulfate (and consequently sulfide) on wild rice. The MPCA data were analyzed by Myrbo et al. 2017 (Response Exhibit N.2), in which logistic regressions were presented and used to support the conclusion that porewater sulfide is a primary controller of wild rice presence and absence. During the journal's peer review process, the representativeness of the dataset was not raised as a concern by the reviewers. It is therefore not reasonable for GLIFWC to claim that an EC10 derived from the same logistic regression is not valid. Similarly, there is no reason that change-point in wild rice density should not be analyzed on the same dataset.

Another commenter (Bock attachment to Expert Comments) raised concerns about the MPCA's use and analysis of the field data. First, this commenter stated that "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." Dr. Bock asserts that there are many waterbodies that exceed the protective sulfide level of 120 µg /L that possess healthy stands of wild rice. The only information on the health of the stands is the density, which the MPCA has shown continuously declines above 120 µg /L (TSD, pp 50-52).

This commenter goes on to state that "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." However, Change-point analysis, when restricted to identifying the single largest reduction in wild rice density, finds a significant reduction in wild rice density at 112 micrograms per liter, from an average of 68 stems per square meter below 112, to 34 stems per square meter above 112. This analysis was independently confirmed (presented in the GLIFWC comments).

Third, this commenter says that "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." MPCA notes that toxicologists fit dose-response data to a variety of curves, so it is incorrect to say that the data do not fit the assumptions of the statistical model.

This commenter also analyzed the field data and finds "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."The only metric available to assess the "health" of wild rice is the density of the rice in the waterbody. The MPCA demonstrated in TSD Appendix 9 that the density of rice decreases significantly above 120, so 300 µg/L would not be protective of the health of wild rice. Therefore, MPCA reasonably excluded the alternative threshold of 300 µg/L as demonstrated in TSD Appendix 9.

Finally, commenters continued to question the MPCA's use of field data from waters that are not being proposed as Class 4D wild rice waters in order to determine protective levels of sulfide and sulfide. The MPCA used procedures commonly used by conservation biologists to identify habitat requirements for species, which require the sampling of habitat that does not support the species of interest (page 8 of the TSD):

> "Binary logistic regression (BLR) is the classic method for scientists to identify environmental variables that control the suitability of habitat for a particular species of interest (Hosmer and Lemeshow, 1989; Peeters and Gardeniers, 1998; van der Heide et al., 2009). BLR is "binary" in the sense that it classifies field sites as having, or not having, the species of interest—in this approach, the density of the species is irrelevant to the classification. Conservation biologists use binary information (presence/absence) in the analysis of habitat suitability; density is rarely used because representative density data are difficult to obtain and density can be a function of factors unrelated to the long-term suitability of the habitat."

See also Attachment 1 of the MPCA's 11/22/17 Response to Comments.

4. <u>Effect Concentration</u>

Some commenters raised specific questions about the effect concentration chosen by the MPCA.

NCASI states that "It is unclear from the TSD why MPCA first selected the EC20 for the wild rice response effect level of interest, and later decided to use the EC10." (p. 1). This issue was discussed in detail in the TSD (pages 31-32). The choice of EC10 was a risk management decision by the MPCA.

Others (Richard, attachment to expert comments) stated that "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 MPCA is not aware that Pastor calculated an EC10.

Several commenters (USFS, Fond du Lac Band, Tuominen) suggested that the MPCA should have considered using an EC5 or NOEC concentration rather than an EC10 concentration in establishing the protective sulfide level. Other commenters (NCASI) suggested that the use of an EC10 approach was overly conservative and an EC20 should have been used. The reasonableness of the EC10 approach compared to a higher EC (EC20-EC50) is discussed in detail in the TSD (pp. 31-32). This section focuses on the reasonableness of MPCA's use of EC10 concentrations rather than an EC5 or NOEC calculation.

The effect concentration concept in general is explained on pp. 31-32 of the TSD, as well as the history of MPCA's analyses of effect concentration (EC). The proposed protective sulfide concentration of 120 µ/L is based on a visual observation of the field data with corroborating evidence provided by change-point analysis of the field data and EC10 calculations from the hydroponic, mesocosm and field data.

Commenters stated that MPCA did not adequately discuss its choice to rely on an EC10 in the development of the protective sulfide level rather than EC5 calculations. The Fond du Lac band suggested an EC5 and compared it to the "extirpation coefficient" of five used by EPA in developing a benchmark conductivity standard. Fond du Lac suggests that "the EC5 or even the 'no effect' concentration (NOEC) is the reasonable protective concentration, when holistically considering the ecology of wild rice, its vastly diminished geographic range, its natural annual variability in production, and the adverse effects of other well-known stressors such as hydrologic alterations, invasive species, and climate change."

MPCA's use of EC10 calculations in the development of the protective sulfide concentration is reasonable because in a toxicological study, the tail ends of the dose-response curve are not as reliably estimated as the center of the curve (such as an EC50). The closer to the tail end of the curve (such as towards an EC0) you get, the less certain you are in the estimation. A no effect concentration is often represented as an EC5 or EC10, and these protective values were considered. The EC10 was chosen because it could be estimated more reliably than an EC5, but still represent a concentration that would elicit minimal effect.

5. <u>Sulfate/Sulfide Model</u>

Comments were received on the MPCA's model of the interactions surrounding sulfate and sulfide formation.

One commenter (Hansel) stated that "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 conceptualization of the hydrogeologic conditions is an accepted scientific approach. Diffusion of sulfate in surface water into the sediment porewater has been demonstrated in the peer-reviewed literature by the few sulfate addition experiments (both purposeful and natural) that have been made, where it has been noted that sulfide increases in the underlying sediment (Little Rock Lake, Wisconsin (Response Exhibit N.42)), a lake in the Experimental Lakes Area, north of International Falls, Minnesota (Response Exhibit N.41), and two lakes and a wetland receiving sulfate drainage from the iron range in Minnesota (Response Exhibits N.43 and N.44).

It would have not been reasonable to collect local groundwater samples from field survey sites, since installing wells is time-consuming and expensive. It was a major expense for the MPCA to install wells adjacent to Second Creek for the intensive study conducted by Ng et al. (2017). Rather than collecting empirical data, MPCA relied on the peer-reviewed scientific literature to inform its conceptual model, as noted above and in the TSD (Section 1D).

Another commenter, NCASI, found MPCA's model generally reasonable but noted that other models could be used, stating: "Finally, with respect to MPCA's reliance on the empirical sulfate model, we note that representation of the basic concepts of H2S formation (i.e. dependent upon available carbon and sulfide) in the model appears reasonable. Nonetheless, some widely used water quality computer simulation models (e.g., Water Quality Analysis Simulation Program, or WASP) predict H2S in porewaters using an approach that incorporates the underlying mechanisms that control sulfur chemistry, rather than relying on purely statistical relationships. Such a mechanistic approach could improve upon MPCA's empirical model, especially for predictions at locations not represented in the derivation of the empirical model."

The MPCA considered using a mechanistic model and determined that an empirical model would meet the needs of the state better (TSD, p. 41-43). In addition, the peer review panel recommended use of empirical modeling, in particular structural equation modeling.

6. Equation Development

Some commenters (Roberts) raised specific concerns about how the MPCA developed the probabilistic equation with the MBLR. This includes comments that "The reasons for changing from a deterministic equation to a probabilistic one are not fully explained in the TSD. The main reason given in the TSD is that it is supposed to avoid a phenomenon called re-transformation bias, sometimes also called back-transformation bias. This phenomenon occurs when a linear equation is fitted to logarithmically transformed data…The TSD provides no explanation of how the MBLR approach overcomes this bias. In fact, the claim that the MBLR approach overcomes the re-transformation bias actually is subject to serious doubt, because the derivation of the MBLR equation starts from a regression formula applied to log-transformed data. (That regression formula is presented in subsection (c) below.)" (pg 6)

The reasons for the change in equation approach are noted in the TSD as the commenter asserts, and explained in more detail in supporting information to the TSD, particularly the Pollman et. al. journal article (2017, Response Exhibit N.4). Transformation bias becomes an issue when the dependent variable is initially transformed to better fit the underlying assumption of linearity inherent in linear regression modeling. The bias is imposed on the back-transformation of predicted dependent variable values to their original (un-transformed) form because the back-transformation typically does not explicitly account for the effect of model residuals (model error) on the predicted and subsequently back-transformed value.

With MBLR, the transformation of the dependent variable is categorical and binary, with the two categories delineated by a threshold value of the original dependent variable. The MBLR model predicts the likelihood or probability that a given set of values for the independent variables will exceed the threshold value; it does not predict the threshold value. The threshold value is determined separately and external to the MBLR, and there is thus no back-transformation and associated bias relevant to the MBLR modeling.

In addition, the MBLR-calculated sulfate concentrations are more accurate than SEM-calculated numbers (16% misclassification rate for MBLR vs. 26% for SEM; TSD page 49), consistent with the elimination of back-transformation bias. Also, note that the peer-reviewed article by Pollman et al. (2017, Response Exhibit N.4) recommends the use of MBLR over SEM for predictions to avoid the back-transformation bias.

The commenter also stated that "Whether or not the decision to set p = 0.5 is protective of wild rice is much more debatable, however. Accepting it would mean that we were settling for a 50% chance of wild rice being protected at the EC10 level that was recommended by the peer review panel. This seems inadequate for protecting wild rice. Therefore a lower probability would be needed to be protective of wild rice. The TSD provides no discussion or citation to support the assumption that a 50% chance of protecting wild rice would be sufficiently protective. Absent a compelling rationale to the contrary, simple logic suggests that a lower probability would be needed to be protective of wild rice." (pg 7) The discussion that addresses the degree of protection set by p=0.5 is discussed in the TSD (p. 46) and more extensively in Appendix 8 of the TSD (pp. 123-126).

7. Error Rate

Some commenters raised questions about the error rate – particularly in how it was described and discussed. One commenter (NCASI) stated that "MPCA's error rate analysis focuses on the relationship between pore water sulfide concentration and water column sulfate concentration, rather than the relationship between sulfate (the target of criteria and management) and the wild rice response. Therefore, the error rates presented are likely underestimates of the overall false positive and false negative error rates" (p. 2)

Although it might be a worthy goal to calculate error rates that extend from sulfide to the presence or density of wild rice, it is not practical, and therefore not a reasonable goal. It is not practical because wild rice does not appear in a waterbody just because sulfide is low. Because of environmental variables that have not been studied rigorously, and therefore are poorly understood, there are many waterbodies with low porewater sulfide but no wild rice population. Beyond presence and absence, wild rice is infamous for having wild swings in density from year to year even in a well-established wild rice water. Because there are other variables aside from sulfide that control wild rice presence and density, it is not possible to calculate error rates that relate the variables that control sulfide (sulfate, TOC, and iron) to wild rice.

This commenter also notes that "As an additional consequence, the comparison made to the error rates estimated by the state of Vermont in their nutrient criteria development document, which include the relationships between nutrient concentration and biological responses (see TSD at pp. 62-63), does not seem to be an "apples-to-apples" comparison." (p. 3) Similarly, another commenter (Richards) states that "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. 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 never claimed that the Vermont approach was the same as ours, only that it was an approach used (which we then used to help evaluate our approach). The Vermont approach for lakes relates phosphorus to phytoplankton density. Vermont is able to do this because all waterbodies have phytoplankton, which will grow to greater density when more phosphorus is available. The fact that MPCA's approach to developing a standard was different than Vermont's does not mean that the use of error rate analysis as a tool to help evaluate MPCA's proposed standard is inappropriate. It is appropriate for Vermont to take an integrated approach, which allows for compliance with, say, phosphorus standards combined with the biological response, which would be the invertebrate community in a stream. If monitoring the invertebrate community shows that phosphorus is too high, the community will presumably recover fairly rapidly after phosphorus is decreased. However, it would be inappropriate to try to detect a decline in health of a wild rice population by monitoring, given the naturally chaotic fluctuations in wild rice density. By the time that wild rice is definitely harmed by elevated porewater sulfide, the sediment reactive iron would have been overwhelmed by sulfide, and recovery would take many years. Facilitating the recovery of wetlands with sulfidic sediment is problematic, and has rarely been studied (TSD, p. 100).

8. Effect of Sulfide on Wild Rice

Many comments received express general skepticism that sulfate (because of its relationship to sulfide) is an important controller of the presence of wild rice.

One commenter (Tedrow attachment to Expert comments) focuses extensively on the idea that competing vegetation in waters (particularly water lilies) and water depth control are an important factor for wild rice growth, implying that these are more important factors than sulfide. These comments seemed to be based on a misconception regarding the goal of the current rulemaking. The goal is not to manipulate the environment to encourage wild rice growth. Rather, the goal is to develop a sulfate standard so that the wild rice beneficial use is not impaired by porewater sulfide, regardless of any other factor that might be affecting wild rice. The MPCA acknowledged in the TSD that many other factors affect the success of wild rice in shallow aquatic systems (TSD, pp 23-31), and has also addressed comments related to this topic in its 11/22/17 Response to Comments. Also, regarding the commenters reference to water lilies and competition with wild rice, MPCA agrees that abundant water lilies can exclude wild rice from habitat that would otherwise be suitable for wild rice. Nevertheless, the presence of waterlilies can also be used as a sign that a site has a high probability of being suitable habitat for wild rice in the absence of water lilies.

Another commenter (Hansel) states that "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. 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 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."

The reference to Berndt et al. is misleading, in that it concerns the St. Louis River, which has no wild rice habitat in the section studied. The experiments that added sulfate and showed increases in sulfide imply to the observer that decreases in the sulfate load would cause a decrease in sulfide: sulfate increases caused sulfide increases in the underlying sediment (Little Rock Lake, Wisconsin (Response Exhibit N.42)), a lake in the Experimental Lakes Area, north of International Falls, Minnesota (Response Exhibit N.41), and two lakes and a wetland receiving sulfate drainage from the iron range in Minnesota (Response Exhibits N.43 and N.44). It is highly likely that decreasing sulfate loading will decrease sulfide production. Be that as it may, the primary benefit of water quality standards is to protect waters from excessive increases.

9. Use of Conservative Assumptions

Several commenters asserted that the MPCA's standard is based on a number of overly conservative assumptions (Alexandria Lakes Sanitary District, MESERB) without providing specific detail about the assumptions. The implications are that these conservative assumptions compound through the rulemaking, resulting in a sulfate standard that will be overly stringent.

Cleveland Cliffs provided the following specific comment on MPCA assumptions associated with the standard; the substance of this comment is also largely echoed by USS.

"Furthermore, the protocol unreasonably proposes to apply the lowest sulfate standard to be the water body's sulfate standard. This introduces an additional level of conservatism for two reasons:

1. MPCA has not specified that only areas of the water body capable of supporting wild rice based on criteria such as water depth and sediment type be sampled. Therefore, the water body specific sulfate standard could be designed to control pore water sulfide in areas incapable of supporting wild rice and therefore wild rice would not benefit from implementation of the standard.

2. Statistically, the lowest sulfate standard approximates the 20th percentile of the distribution of possible sulfate standards. In brief, 4/5 samples, or 80% will have higher standards. We can combine the probabilities associated with the EC10 and the 20th percentile by multiplication as such: 10% x 20% is 2 %. That means that 2 % of the potential population of wild rice could be affected while 98% are predicted to be unaffected. This is much more conservative that limiting the effects to a 10% level specified by the EC10. This pattern is repeated because additional conservative inputs have been added, such as the currently recommended sulfide threshold of 120 µg/L, which is a factor of 10 lower than the NOEC from both the Fort et al. (2017) as well as the newly conducted Fort November of 2017 results. The final probability is the product of the individual probabilities. For example, if we take that 95% confidence level of the Ec10 and apply that to the 20th percentile sulfate standard for a sulfide standard that is over a factor of 100 too low. The true level of conservatism is 5% x 10% x 20% x 10% or in other words 0.01%.

Conclusions: Conservatism on the order of one one-hundredth of a percent or more is not reasonable, and therefore the use of the lowest calculated protective sulfate value for a water body is not reasonable. We recommend using some type of averaging of the results. (p. 12-13)"

This comment claims that the MPCA makes three conservative (i.e., overprotective) choices and that these three choices in combination compounds the conservatism into a standard that is exceedingly overprotective. These three choices are:

1) the choice of the EC10 (as opposed to a less protective level such as the EC20);

2) the requirement that the protective sulfate standard for a waterbody will be based on the lowest calculated protective sulfate value from five samples from the waterbody; and

3) the fact that a 120 µg/L protective threshold for sulfide concentration is too low when compared to the NOEC from the Fort et al study.

The MPCA maintains that none of these three choices is overly conservative and thus there is no compounding of conservatism when these choices are made in combination. Defenses of each of these three choices as reasonable and appropriate (and not overly-conservative) – the EC10, the five sample requirement, and the 120 µg/L protective sulfide threshold – are each addressed elsewhere in these comments and in other rulemaking support documents. MPCA's choice of EC10 over EC 20 is discussed in detail in the Technical Support Document pp. 31-32. The use of the lowest calculated protective sulfate value is discussed in detail in the SONAR (p. 88) and the reasonableness of 120 µg/L is discussed in the SONAR (pp. 66-72). One other point that the commenter seems to make is that the conservatism is further compounded by using the lower bound of the 95% confidence interval around the EC10 point estimate. This, however, is not what the MPCA did. The MPCA used the EC10 point estimate itself, not the lower bound of the confidence interval around that estimate.

The MPCA has adequately demonstrated through the SONAR, TSD, and multiple responses to comment that sulfide is a factor that impacts wild rice and that the proposed rule is reasonable to protect the wild rice beneficial use from that adverse impact.

H. Duration and Related Flow Rate; Seasonality; Frequency of the Proposed Standard

Several commenters raised concerns about the duration of the standard – proposed as an annual average – and the flow rate that the MPCA proposed as the critical flow condition to evaluate in effluent limit reviews. Comments also were received about a seasonal component to the rules and the proposed one-in-ten year frequency of the standard. Most of these comments involved questions and concerns about how these elements of the rule proposal would affect the effluent limit review process and how they would allow (or not allow) higher levels of sulfate discharge from permitted facilities.

<u>Duration of the standard and related flow rate</u>

Duration: Several commenters raised concerns that the MPCA's proposed standard, by including an annual average duration and a related 365Q10 flow rate for effluent limit review, asserting that this approach would allow for high sulfate discharges that could harm wild rice. (MCEA, USFS, 1854 Treaty Authority)

Some of these comments seem to confuse a water quality standard and an effluent limit. As noted on page 96 of the SONAR and MPCA Hearing Exhibit L7, a standard applies in a water body to protect a specified beneficial use; an effluent limit applies to the discharge of a permitted facility and are an important tool in ensuring that a water quality standard is met in the receiving water(s) to which the facility discharges.

As noted on pages 15 and 79 of the SONAR, the duration and frequency of a standard are important components of understanding how a standard will be applied.

Commenters have also raised concerns that the annual average would allow some time periods of quite high sulfate discharge. For example:

- Water Legacy stated: "In practice, the MPCA would allow every sulfate discharger to use year-round dilution based on averaging of snow melt and other highest water flow conditions even if the discharge were taking place during the driest week of the year, when far less flow would be available to dilute sulfate pollution. MPCA's proposed rule would relax pollution limits based on annual average flow even in shallow streams, common natural habitats for wild rice, which may have little or no flow available to dilute pollution."

- The 1854 Treaty Authority, also raised concerns that "dischargers could potentially 'flush' their systems and release high concentrations of sulfate during certain times of the year, and attempt to reduce or stop discharges during other times" and stated that this kind of discharge regime would be a problem.
- The Fond du Lac Band expressed concern that the annual average allows high levels of sulfate to be discharged to wild rice waters while the mesocosm experiments "suggests that there actually may be a discrete time in the growing season when wild rice plants are exceptionally vulnerable to the effect of sulfate loading and reduction to sulfide." The Nature Conservancy expressed a similar concern.

Fond du Lac Band and the Nature Conservancy assertion that wild rice has periods in its life cycle during which it is exceptionally vulnerable does not change the reasonableness of the proposed annual average duration. Myrbo et al. (Exhibit 18 of rulemaking) showed that there is no significant seasonal trend in porewater sulfide over the wild rice growing season. Since porewater sulfide does not vary significantly over the growing season, then protecting for sulfide during all periods of the year is also protective of sulfide over any single period. Consequently, protecting for sulfide over an annual average period is protective of all periods of the wild rice life cycle including any period during which the wild rice may be most sensitive. This further demonstrates the reasonableness of the proposed annual duration.

Water Legacy also stated that "MPCA attempts to justify use of an annual average since sulfate is not a direct toxicant upon wild rice. However, other pollutants controlled by water quality standards are not direct toxicants. Discharge limits for mercury, for example, are set to prevent the methylation of mercury and the bioaccumulation of mercury in the aquatic food chain. Mercury monitoring and effluent limits are generally based on a daily maximum and a calculated monthly average."

MPCA does routinely interpret the duration of mercury surface water quality standard as a 30 day average during NPDES permitting in order to protect for bio-geochemical processes with multi-year effects such as mercury bioaccumulation in the aquatic food chain. An annual average standard does not imply that effluent limits will always be set on an annual basis. Water Legacy appears to be confusing effluent limits and water quality standards in this instance.

Pages 79-81 of the SONAR and 91-94 of the TSD provide extensive discussions of MPCA's conclusion that proposing an annual average duration for the wild rice sulfate standard is reasonable. Expressing the standard as an annual average does mean that at times the concentration of sulfate in the waterbody might be higher than the calculated sulfate standard, so long as the average over the year is at or below the standard. As described in the SONAR and TSD, a longer averaging time is appropriate for the wild rice sulfate standard because sulfate is not a direct toxicant, and the negative impact of elevated sulfate occurs over time, not in a matter of days or weeks. As noted in the TSD, page 94, "temporary high sulfate concentrations are not the direct cause of negative effects on wild rice". Specific to the concerns raised about elevated sulfate discharges during dry periods of the year, the TSD specifically explains how the scientific evidence supports MPCA's conclusion that short-duration increases in sulfate concentration will not impact the wild rice beneficial use, so long as the annual average is maintained.

The reasonableness of MPCA's proposed annual average frequency is bolstered by the 11/22/2017 comment letter from EPA, which states on page 5 "Based on the information provided by Minnesota as part of the public notice for these rules, the proposed criterion appears to be scientifically defensible and protective of the wild rice use."

Flow rate: The proposed rule language regarding the applicable flow rate for evaluating the need for effluent limits to ensure discharges are protective of the standard is found in two places: 7050.0224, Subp. 5D and 7053.0205, Subp. 7E, with a definition of 365Q10 at 7050.0130, Subp. 2a. The language states that "discharges of sulfate in sewage, industrial waste, or other wastes affecting class 4D waters must be controlled so that the numeric sulfate standard for wild rice is maintained at stream flows that are equal to or greater than 365Q10." This proposed rule language mirrors language elsewhere in Minnesota Rules that specify other flow rates applicable to the evaluation of potential impacts to other standards (see 7Q10 language at Minn. R. 7050.0210, Subp. 7 and 30Q10 language at 7053.0135, subp. 4 and 7053.0205, subp. 7B. Similar concepts related to 122Q10 are at 7050.0150 supp. 4A and 4BB and 7053.0255 Subp. 1A and 1G).

MPCA is proposing to use the 365Q10 flow as that protective stream flow rate to use in the effluent limit review and development. 365Q10 means the lowest average 365-day flow with a once in ten-year interval. This flow rate is calculated specific to the receiving water of concern. Built into the choice of using the 365Q10 protective flow is the assumption that high flow rates after snow melt will average out low flow rates during late summer and thus protect wild rice from sulfate over a 365 day period.

The 365Q10 flow rate is a proposed variable in the mass balance formula used to calculate effluent limits. The mass balance formula allows the MPCA to reasonably calculate the assimilative capacity of the receiving water to receive the pollutant load from the discharger and thus determine the need for effluent limits for the discharger. Pages 98 through 102 of the SONAR address the mass-balance approach and the reasonableness of the proposed 365Q10 flow rate.

EPA provided comments stating that "it is unclear whether Minnesota intends for water quality-based effluent limits (WQBELs) to apply when receiving waters flows are less than 365Q10" and recommends that proposed 7050.0224, subp. 5D be clarified. MPCA absolutely intends that once established a water quality-based effluent limit will apply to the permitted discharge as specified by the effluent limit, regardless of the receiving water flow rate at a given time. Given that this proposed rule language identically mirrors other rule parts in Chapters 7050 and 7053 regarding, MPCA will address these recommendations for enhanced clarity in a future rulemaking when the other similar rule parts can also be addressed.

Commenters also expressed concern that the use of a 365Q10 flow in setting effluent limits would not be protective of the beneficial use, and MPCA should instead use the 7Q10 analysis used to evaluate toxic pollutants. This comment is analogous to concerns expressed about the annual average duration proposed for the standard. In both cases, as explained above the MPCA's proposed approach is scientifically defensible for the wild rice sulfate standard because sulfate is not a direct toxicant. For direct toxicants, concentration at low receiving water flows is a concern because point source have the greatest impact on stream composition at those flows and short-duration exposures to direct toxicants can impact the beneficial use. This same concern is not present for sulfate and wild rice, where the impact occurs over a longer timeframe.

<u>Seasonality</u>

The concept of "seasonality" is a further consideration of the duration of the water quality standard. As noted on page 20 of the SONAR, implementation of the existing wild rice sulfate standard has at times included an interpretation of the "period when rice may be susceptible to high sulfate levels" as being the growing season.

MPCA recognized the need to examine this interpretation in light of new scientific information, and included in the SONAR a specific discussion of the seasonality concept (pp. 81-82). The 2011 legislature also referenced seasonality in their specific rulemaking charge to MPCA.

Given that sulfide can form from elevated sulfate alt any time of the year, MPCA is proposing an annual average duration for the standard rather than a duration limited to a specific season. In other words, MPCA is proposing that the standard apply in all seasons. In general, most commenters supported the MPCA's proposal to have a standard that applies year-round.

Mesabi Nugget provided the following comments about the annual duration of the standard and introducing the concept of seasonality and/or temperature dependence to the equation.

"The equation fails to account for seasonal trends in porewater sulfide...Minnesota has never had a year-round sulfate limit for the protection of wild rice. This reflects the reality that wild rice is not a perennial and only grows for less than half of the calendar year. Accordingly, it does not make sense to remove the existing seasonality language and apply the sulfate standard year-round…MPCA is claiming that wild rice is equally susceptible to sulfate at all times of the year…MPCA arbitrarily discounted the only research on this topic and proceeded as though its data supported nothing but a year-round standard, with the calculated effects of a summer discharge being treated just like a sulfate discharge in the dead of winter. In 2013 DeRocher and Johnson provided research to MPCA showing significant temperature-dependent differences in the rate of sulfide creation in sediment. Their sediment incubation study indicates that in cold water, additions of sulfate take several weeks to show any increased porewater sulfide, and then it takes only a few weeks to go back to previous sulfide levels once the sulfate additions have ended."

As noted above, the SONAR provides a discussion on the reasonableness of the annual duration and the concept of seasonality beginning on page 81; pp. 91 – 94 also address these topics. The MPCA did not discount the 2013 DeRocher and Johnson study and in fact cites this work when discussing the reasonableness of the annual duration of the standard. MPCA has acknowledged that sulfate conversion to sulfide is slower in cold temperatures. However, MPCA explained that is does not have sufficient scientific information to quantify this difference in a way that could be incorporated into a proposed water quality standard. Specifically, the SONAR (excerpted below) provides the following justification of the reasonableness of not incorporating temperature dependence into the proposed equation.

"…the MPCA lacks sufficient scientific information to quantify the lower winter diffusion rates and thereby develop a ratio or other numeric approach to allow higher sulfate levels in the winter. The MPCA also does not know if an approach that allowed higher sulfate levels in the winter would be protective over the long term. Because of this, is it reasonable to have a standard that applies all year, not just seasonally."

John Hall also provided comments interpreting the Pastor et al. 2017 study that asserted:

"These data clearly indicate that a single season exposure does not cause adverse effects, even at the highest concentrations. These results show that the duration must be greater than one year to show an effect…The criteria duration necessary to protect wild rice is at least two years."

Other commenters are clearly concerned that even an annual duration is too long, because there may be effects at shorter duration.

The SONAR provides a reasonable justification for the annual duration using data from the Pastor et al. 2017 beginning on page 79:

"In this case, it was not until the third year of the experiment that wild rice growth and reproduction was significantly affected by the 100 mg/L treatment (Pastor et al., 2017). This mesocosm experiment conducted by Pastor et al. (2017) demonstrated that porewater sulfide is directly proportional to the long-term (annual) average sulfate concentration (Myrbo et al. Exhibit 36)."

Although statistically significant effects were not observed until year 3, it is not reasonable to assume that a 3-year average would be protective, for the following reason: The mesocosm experiment was not designed to evaluate the frequency or duration of exceedances. Rather, the mesocosms evaluated the cumulative impact of sudden increases to new elevated concentrations, from a base exposure that was very low—the sediment was taken from a wild rice lake with an average sulfate concentration of less than 3 mg/L. An experiment designed to address this issue might have first exposed the sediment to a sulfate concentration closer to the calculated standard of 34, and then observed the effect of an increase above the standard. Accordingly, the MPCA made a judgements of protective frequency and duration values, partly informed by the mesocosms experiment.

Given the available data, an annual average for a standard that applies at all times, (not just seasonally), is a reasonable choice.

Frequency of the standard

The proposed sulfate water quality criterion to protect wild rice waters has an exceedance frequency of once in ten years. This means that a water body would not be considered impaired until the numeric sulfate standard is exceeded in a second year out of ten.

Some commenters (Hall) found this frequency to be excessively conservative, stating that the mesocosm experiment: "results show that wild rice has the ability to recover even when plant growth was virtually eradicated after multiple years of exposure to extremely high levels of sulfate. If wild rice can begin recovery from this extreme condition, it should be apparent that recovery would be complete within two years after an exposure that only causes slight effects…These observations support a return frequency of once in three years."

Other commenters (Fond du Lac Band) expressed concern that the proposed frequency is not protective, stating that: "Dr. Pastor's experiments were not designed to determine what that frequency might be...MPCA cannot assume that this natural resilience of wild rice will be realized if an anthropogenic disturbance such as excessive pollutant loading occurs. The only existing data that is relevant to that issue are the latest mesocosm results (Pastor progress report, June 2017), where only about half of the high sulfate treatment mesocosms rebounded when the sulfate loadings ceased."

Still others (WaterLegacy) stated that: "even if sulfate was elevated over an entire year, the proposed rules would only consider this an "exceedance" of the standard if the discharger violated the wild rice sulfate standard for more than one year out of ten."

The MPCA believes that a shorter standard frequency (one to three years) is not protective. The MPCA agrees that the objective of the mesocosm study was not to determine a protective frequency in which to express the proposed standard; however, this does not mean the MPCA's evaluation of the data from the mesocosms and other lines of evidence is not scientifically defensible. Additionally, with regards to Mr. Hall's claim that wild rice did "recover", there was a notable decrease in wild rice density after five years of elevated sulfate concentrations followed by two years of reduced exposure. This finding suggests that multiple consecutive years of increased exposure reduced the potential of the wild rice to produce viable seed heads for future plant establishment. There is not sufficient information indicating a one in three-year frequency is protective for the use and propagation of wild rice. The reasonableness of applying a one in ten year frequency is available on page 82 of the SONAR. Again, the MPCA's choice is a reasonable balance and the one in ten year frequency is reasonable and protective.

I. Comments Related to NPDES Permitting

Many commenters felt that the MPCA has not provided enough detail in the implementation sections of the proposed rule and supporting documents. For example, EPA suggests the inclusion or development of specific procedures. And, operators of permitted facilities provided comment that the lack of detail prevents them from fully understanding their future effluent limits and thus the cost implications of the rule. This is perhaps best illustrated by the comment from the Minnesota Chamber of Commerce, which states "The technical support document (TSD) and the Statement of Need and Reasonableness (SONAR) both have economic and socioeconomic impacts, but do not include all the factors that would be assembled in a complete cost analysis of the proposed rule. The MPCA estimates that, at a minimum, 130 permitted facilities will be evaluated for the possibility of requiring additional permit limits to protect wild rice under the new rule. Without an understanding of the feasibility and cost of meeting these new limits, it is difficult for these 130 facilities to plan for future development and commit capital investment into their facilities."

More general comments about the Agency's obligations around cost analyses are provided in the section of this Rebuttal Response related to procedural issues. This response section addresses the MPCA's effluent limit permitting process and why it is reasonable for the MPCA to not have specific details about effluent limits available at this time.

As noted in the November 22, 2017 Response to Comments, the MPCA understands that dischargers want clarity about how the standard will affect them, and we are sensitive to comments that the MPCA should strive to fully understand and articulate the implementation details of a rule prior to adopting the rule. In the case of water quality standards, the impact on permitted facilities comes through development of an effluent limit specific to a facility that ensures the permitted facility will not cause or contribute to a violation of the water quality standard. Effluent limit setting requires evaluating multiple factors as described beginning on page 96 of the SONAR.

There are approximately 1000 facilities in Minnesota that hold water discharge permits. Site-specific data is required to evaluate the need for an effluent limit at each facility, and these issues are addressed in an individualized permitting process. This data is not immediately available for all facilities and it takes time to gather this data.

This time and data need is inherent to the difference between water quality standards and effluent limits, and is not unique to the proposed revisions to the wild rice sulfate standard. As explained in Part 6G, pp. 96-99 of the SONAR, evaluating the need for and (as needed) determining a water quality based effluent limit requires data specific to the discharge being evaluated and the receiving water(s) being discharged to. Data needs unique to the proposed rule revisions are the sediment iron and carbon (or porewater sulfide) data.

Collecting all the data necessary to calculate all effluent limits statewide would take at least ten to fifteen years, even if the sediment data were not needed. Necessary steps such as gathering five years of effluent data to evaluate and set effluent limits combined with the 10-year surface water monitoring schedule to gather surface water data cumulatively add up to the necessary data not being available for some permitted discharges until at least ten to fifteen years after rule promulgation. The MPCA does plan to prioritize data collection based on factors such as those mentioned in the EPA comments, Appendix 2 – the likelihood of sulfate impacts (because of type and location of dischargers) and permitting schedules.

It is unreasonable to delay this rulemaking for ten to fifteen years to provide total certainty regarding future effluent limits for specific facility discharges and the exact future costs. In addition, every facility is unique and detailed engineering is needed to estimate the costs of installing any treatment system.

This is why the MPCA provided general effluent limit considerations and the range of costs detailed in the SONAR. A delay such as would be necessary to gather data and estimate the cost for all potentially affected facilities is particularly unreasonable given that while the rulemaking would be delayed the existing sulfate standard would remain in place and need to be addressed as required by the Clean Water Act and federal regulations.

NDPES Effluent Limit Expression

Commenters also raised questions about how the MPCA plans to express effluent limits, and several asked that this information to be placed in rules. For example, Mesabi Nugget made the following comment: "It appears MPCA may have committed a drafting error when preparing the rule language for public notice. The agency says that water quality-based effluent limitations (WQBELs) for sulfate will typically be expressed as a 12-month moving total mass. MPCA SONAR (July 2017), p. 105. However, the corresponding rule language does not appear to reflect this policy decision made by MPCA. The rule language should be updated to properly reflect the mass limit approach."

MPCA has not committed a drafting error. There are two general ways to express an effluent limit: as a mass limit or a concentration limit. The choice to express a limit as a mass limit or concentration is a decision that is made at the time that an effluent limit is developed for a specific discharge. The MPCA intends to fulfill its statutory responsibility to protect water quality standards and designated uses through requiring the most appropriate and protective effluent limits. At this time, based on our knowledge, the MPCA would prefer that effluent limits be expressed as 12 month moving mass totals. However, the MPCA may use other approaches as necessary to ensure protection of the water quality standard. MPCA expressly noted this intent in the sentence that follows the sentence quoted in the comment above, which reads "Concentration-based limits will also be included in the permit if need is demonstrated" (SONAR, p. 105).

More generally, it is not needed or reasonable to specify in rule the exact manner in which effluent limits must be expressed for every permitted discharge that may need a limit to protect the beneficial use. Data that the MPCA does not currently have for every facility, such as sulfate concentrations in the discharge and the receiving water(s), are key to informing the MPCA's decision on which approach is needed to protect the beneficial use in the receiving water(s).

There is extensive EPA guidance and MPCA past practices for effluent limit setting that will be evaluated and used as appropriate. This flexibility is important for setting individual facility limits, and is part of why the MPCA is not providing more detail in the rule such as the suggestion by EPA to specify a flow rate for the relatively rare situation of isolated waterbodies. MPCA may take the approach suggested by EPA, but putting detail for such a specific situation in rule is unnecessary.

It is reasonable for the agency to define key variables such as the 365Q10 in the rule and indicate the general limit-setting approach in the SONAR. It is unreasonable for the agency to know with total certainty the exact limit-setting approach for all wastewater plants, which would be needed to put exact limit-setting approaches into rule.

Sulfate Fate and Transport

Joe Mayasich provided comments on the limit-setting approach outlined in the SONAR specifically related to sulfate fate and transport in the environment. The comments criticized a lack of a discussion of specific sulfate fate decay rates in the SONAR, and provided the specific comment below on sulfate transport.

"The proposed Rules erroneously assume that 100% of the sulfate load/concentration discharged from permitted facilities' outfalls reach wild rice habitat via surface water transport, and then erroneously assert, with a simplistic equation, that the 'protective' level of biogeochemically produced Sulfide (i.e. 120 µg/L) can be achieved by reducing just the load/concentration of just the point-source-discharged, surface-water-transported Sulfate."

The MPCA has not assumed that 100% of the sulfate discharged from a facility will reach the wild rice habitat in downstream wild rice water(s). As noted in Part 6G of the SONAR, the first step in conducting an effluent limit review is determining if a discharge will cause, have the reasonable potential to cause, or contribute to an exceedance of a water quality standard (SONAR p. 97, also 40 CFR 122.44). This step is often referred to as the "reasonable potential" analysis. The MPCA effluent limit reviews of sulfate discharges from permitted facilities will consider factors such as flow dilution, water body type, water flow path, and site-specific sulfate decay rates in this "reasonable potential" analysis. Sulfate fate and transport is a complex environmental phenomenon and it is not possible to simplify sulfate fate in the environment to a singular half-life decay rate applicable statewide. The MPCA expects to treat sulfate transport in the environment conservatively during limit setting to be suitably protective and simplify the limit setting process. If quality evidence suggests sulfate is not transported conservatively then the MPCA is willing to consider that evidence in the limit setting process.

Regarding the second part of this comment, MPCA has not asserted that the protective sulfide level can only be achieved by controlling point source discharges of sulfate to surface waters. This comment again confuses key differences between water quality standards and permit effluent limits. Standards apply in the water body to protect the beneficial use. The need for and details of an effluent limit is established by first determining if a discharge has "reasonable potential" to cause or contribute to an exceedance of a standard applicable in the receiving water(s). If the discharge does have reasonable potential, the effluent limit must then be set at a level that controls the pollutant so that the facility does not cause or contribute to an exceedance. This requirement of the Clean Water Act does not assume that controlling the discharge will by itself ensure the water quality standard will be achieved, and MPCA has not made such an assertion.

Singular conservative assumptions in the implementation strategy will cumulatively result in excessive over-protection and unnecessarily low effluent limits

John Hall and other commenters provided comments on the limit-setting approach outlined in the SONAR, specifically the concept of individual conservative effluent limit setting assumptions compounding into excessively conservative assumptions when considered cumulatively. These commenters did not rigorously distinguish between the concept of compounding conservative assumptions in the science underlying the standard development and the concept of compounding conservative assumptions in the implementation of the standard. The MPCA addresses the concept of compounding conservative assumption in the science behind the standard development elsewhere in this document (Section K) and will address the concept of compounding conservative assumptions in the implementation of the standard below.

We maintain that none of the individual assumptions in the implementation section of the SONAR is overly conservative and thus there is no compounding of conservatism when these choices are made in combination. For example, the choice of the 365Q10 as the receiving water flow rate during the limit setting process is reasonable and not overly conservative. We did not choose an unnecessarily conservative receiving water flow rate such as the 7Q10 (defined in Minn. Rule 7053.0135) because choosing that extremely low receiving water flow rate would have been overprotective of the duration and frequency of the proposed standard. Since every individual implementation assumption is not overly conservative, there can be no compounding of individual conservative assumption and thus there is no cumulative compounding of conservation assumption in the implementation of the standard. We maintain that the proposed implementation strategy is reasonable, is appropriately protective of the water quality standard and will not result in unnecessarily low effluent limits.

Implementation Timeline

Commenters (Friends of the Boundary Waters) also raised concerns that high levels of sulfate would be allowed until the MPCA gathers data and sets a numeric sulfate standard – essentially leaving waters without a standard. As noted in our Response to Comments, data gathering will be needed regardless of whether MPCA moves forward with the proposed equation based rule or chose to implement a single standard. In either case, data is needed on sulfate in effluent and sulfate in surface waters in order to implement discharge limits. The addition of the need to collect sediment data to implement the equation based standard does not substantially change the timeline.

Other commenters had concerns that an increase in sulfate loading could occur prior to the setting of a numeric sulfate standard. As raised by MCEA: "MPCA has rejected the alternatives of keeping the 10 mg/L standard in place while data are collected and also the alternative of specifying that there shall be no net increase in sulfate discharges until a numeric standard is developed that can be used to set protective effluent limits…sulfate loadings cannot be relied on to stay constant until new permit limits are calculated. Dischargers are not generally discharging the full amount of pollutants that their NPDES permits allow them to discharge and, thus, there is frequently room for increasing the flow or discharges of particular pollutants without obtaining a new permit."

The commenter is correct that most dischargers do not discharge at the full levels authorized by their NPDES permit; in MPCA's experience, most dischargers prefer to operate with a degree of buffer between their actual and permitted discharges. The MPCA felt that the concept of "no net increase" was not implementable primarily because of very limited existing data on sulfate effluent concentrations and on how much a permittee is operating below their maximum permitted levels and how sustainable that operation is.

Implementation of a "no net increase" provision would require defensible numerical methods for defining a baseline that correctly characterizes the concentration or load the facility is currently discharging. Several methods could be used, but nearly all would result in the same outcome: a disincentive to reduce loading below maximum authorized levels. For example, the current actual discharge baseline could be defined as the average effluent concentration recorded during the previous five years. A permit condition, or limit, would then be derived from this baseline. During the next permit cycle, the permittee would strive to operate below this baseline in order to remain in compliance with permit conditions. At the subsequent permit review, the new five years of data would be used to readjust the no net increase baseline lower to comply with the previously determined no net increase baseline. In this hypothetical scenario, it would be nearly impossible to not reduce discharge during every reissuance, and as a result, permittees would be tacitly encouraged to always discharge at maximum authorized levels. Another potential result of this scenario is that effluent limits for affected facilities could ultimately be reduced to a level where violations would be frequent and unavoidable.

J. Sampling and Analytical Methods

Multiple commenters provided input on the sampling and analytical methods incorporated by reference into the rule. The goal is to set forth methods that are sufficiently clear as to result in a consistent development of a numeric sulfate standard via the equation or alternate standard, while not constraining flexibility that may be needed to adapt to the differing conditions of a given wild rice water and different lab abilities and does not affect the ultimate result.

The MPCA chose to incorporate methods by reference because the sediment or porewater sampling and analysis are fundamental to the development of a numeric sulfate standard (through either the equation or the alternate standard) and we anticipate that permittees or other parties may want to conduct sediment or porewater sampling themselves. Some commenters (MCEA) raised concerns that parties other than the MPCA should not be allowed "to do sampling that determines the applicable water quality standard under state law." Others (Water Legacy) suggested that allowing such sampling is an "invitation to mischief".

Incorporating the sampling and analytical methods by reference makes them enforceable and ensures that the MPCA is able to accept only information with results that are consistent with the results that would be received if the MPCA itself conducted all sampling and analysis. To ensure quality data, the MPCA is also requiring outside parties to submit a sampling plan if they want to collect and analyze data in a way that is consistent with but does not exactly follow the incorporated methods. The MPCA will assess data quality before any use of the data occurs. MPCA will be responsible for documenting the final numeric sulfate standard for each water and will not document or enforce a result that arises from data that does not conform to the rule's methods.

Comments on the sampling methodology generally were in the areas of clarity and flexibility. For the methods on where and how to collect sediment and porewater within wild rice waters, commenters seemed to want more clarity; for the analytical methods, commenters tended to want more flexibility.

The MPCA is considering some rule changes based on these comments; more information is provided in the section on planned and proposed rule changes. The MPCA also plans to develop detailed guidance of best practices or standard procedures that can be used for sampling and analysis in order to provide a "recipe" for those who want such details.

<u>Sampling Methods are for Wild Rice Waters</u>

Some comments seemed to conflate the sampling methods – particularly discussion of where to sample within the wild rice water – with the identification of the wild rice water. For instance, one commenter (Cliffs) states that "the use of water lilies as indicators of suitable wild rice habitat is scientifically flawed." Another commenter (MESERB) stated that the "The list of areas within wild rice waters that must be sampled is overly broad. Wild rice propagates through seed. The Agency should look for more than the presence of waterlilies, other plants or areas with a certain water depth to demand testing. An upstream source of seed should also be required. Similarly, if conditions that preclude establishment of wild rice are present, such as waters that are not clear or that support a population of carp, sampling should not be required." It is important to note that the sampling methods are to be deployed in waters that the MPCA has already identified as Class 4D wild rice waters. Therefore, the waters are known to demonstrate or have demonstrated the wild rice beneficial use since November 28, 1975.

The sampling methods are about getting the best characterization of sediment iron and carbon or porewater sulfide in waters that have already been determined to be wild rice waters. EPA's comments in Appendix 2, particularly comment #3, raise concerns that requiring or allowing sampling to be constrained to areas of obvious wild rice habitat within the wild rice water may bias the sampling. The MPCA will consider making changes to the method document to address this concern.

Sediment and Porewater Sampling Methods

Commenters (1854 Treaty Authority) rightly noted that "The design of this sampling would be crucial: where does sampling occur, how many samples are taken…it is also likely that sampling results in each water would give a range of sulfate values…under the proposed approach… However, guidelines could lead to inconsistent implementation." The goal of the methods document is to set out requirements for sampling, not guidelines, in order to have the most consistent implementation possible. As noted in the rule, the equation-based sulfate standard must be set at the lowest sulfate number obtained based on the sediment iron and carbon values found via sampling.

Similarly, commenters (MCEA) noted that "having a standard based on sampling of each site requires, at a minimum, a standard sampling protocol that rigorously controls for the spatial variability of iron and carbon in the sampled environment." Another commenter specifically mentioned the high spatial variability in iron and TOC in Twin Lakes. The TSD (Chapter 3) and Hearing Response to Comments discusses the variability of sediment TOC and iron, and the reasonableness of the methods proposed for sampling wild rice waters to collect data for use in calculating a standard for the waterbody.

The MPCA has adequately shown that the required 25 sediment samples is sufficient to characterize the spatial variability of iron and carbon, and the use of the lowest resulting sulfate value is sufficiently protective. Comments from EPA have suggested that MPCA consider providing more specificity about transects, specifically information like lengths and distances, and the MPCA will consider this and may make changes to the sampling methods. In particular, EPA Appendix 1 comments number 3, 4, 6, 11, and 13 suggest additional clarity that the MPCA will consider.

There was also some confusion among the commenters about the relationship between sampling for sediment iron and carbon, and porewater sulfide. Some stated that the sampling methods do not include a clear description of the purpose of the porewater sampling and others seemed to believe that all of these parameters would be collected at all times. To be clear (this is also discussed in the portion of this document on proposed and planned rule changes), the MPCA envisions that the vast majority of the sampling will be only for sediment iron and carbon. Porewater sulfide will only be collected if there is a reason to believe that using the alternate standard approach to developing a numeric sulfate standard would be appropriate. Other commenters (GLIFWC) noted that "The procedures do not make it clear how the porewater sampling effort can occur in conjunction with the sediment core sampling. The document states that the sediment sampling must be done before the porewater sampling. It then states that the porewater sampling must be done no later than 4 hours after the sediment cores are taken. Given that the sediment sampling is done first, how will the MPCA determine what is an undisturbed sediment for the purpose of porewater sampling?" The MPCA will review the methods document and add clarity as needed.

Commenters (Mining Minnesota) also stated that "[i]t is also unclear how to interpret porewater sulfide data. The MPCA Sampling Methods include direction that two porewater samples be collected at each of five transects used for the previous sediment sampling for a total of ten porewater samples per 'wild rice water.' It is unclear, however, which porewater sulfide value will be considered relevant for compliance. Is it the lowest of the ten values in the dataset, an average, or some other value? If sulfide values in the same location differ by hundreds of micrograms per liter or more, how will that data be evaluated and for what purpose? How will results be interpreted if they differ from the calculated sulfate standard based on sediment iron and total organic carbon data?" Porewater sulfide data would only be used to establish a numeric sulfate standard via the alternate standard procedures. Once that numeric sulfate standard is set, that sulfate standard would be used to determine attainment of the standard and in effluent limit review. However, MPCA does agree that the rule and method do not adequately explain how to use the multiple porewater sample values to develop a sulfide level for use in the alternate standard. The MPCA will clarify.

Use of Sediment Data to Develop Sulfate Level

The rule language directs that "the calculated sulfate standard is the lowest sulfate value resulting from the application of the equation to each pair of organic carbon and iron values collected and analyzed" consistent with the methods document. Several commenters state that it is not appropriate to use the lowest calculated sulfate level rather than an average. The Technical Support Document discusses the detailed reasonableness of using the lowest calculated value of sulfate derived from the analysis of five composite sediment samples. (See page 87 of the TSD). Briefly, though each of the five values that are calculated from the five paired data sets of sediment TOC and iron is protective of wild rice, the lowest value represents the most sensitive condition for the wild rice in that waterbody. It is reasonable to protect for the beneficial use based on applying that lowest calculated sulfate value.

Analytical Methods

The document incorporated by reference also includes methods for analyzing the collected sediment to determine the iron and carbon levels and analyzing the collected porewater to determine the sulfide level. Commenters provided some very detailed and technical comments on the analytical methods in particular. These issues are more detailed than MPCA can fully investigate and respond to in the time allotted for the post-hearing comment and rebuttal periods. In addition, EPA posed some detailed questions concerning the analytical methods. MPCA is therefore responding broadly here. We will continue to consider the comments on the methods and the need for changes to the methods document prior to adoption of a final rule and will work to provide additional information to EPA and others as needed.

In general, comments about the analytical methods seemed to focus around the need for more flexibility – allowing for analytical methods that would provide comparable results while not requiring certain steps that are not consistently available at every analytical lab. The MPCA believes that the proposed rule language change to require analysis be conduct "consistent with" rather than "using" the specified methods will provide an appropriate level of flexibility and will be reviewing the analytical methods for similar types of revisions.

Many comments were received about the availability and need to follow specific procedures for drying sediment samples, sieving samples, method blanks and various other specifics of sediment sample preparation and analysis. The MPCA will review these comments and consider revisions to the methods as needed.

Comments (Mining Minnesota) were also received about the availability of the methods. "Because MPCA is specifying an analytical method that must be used under the Proposed Rule for porewater sampling, the MPCA should also consider whether commercial laboratories are willing to perform the specified method, and if laboratories become available, whether they are able to conduct the testing within the required detection limits and QA/QC standards."

Particular concerns were raised about the method for porewater sulfide analysis. Mining Minnesota noted that two methods have been used in the past; the two methods have a different distillation step; they state that "MPCA has incorporated Method E as the sole approved porewater sampling methodology without regard to its historical purpose or commercial availability… approximately 30 separate laboratories in the United States and

Canada were contacted, and none were able to conduct a Method E analysis." They note that most labs could analyze sulfide using a third method, which has higher reporting limits. Another commenter states that "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 MPCA will consider the need to specify a method detection limit in the incorporate document; the MPCA envisions that if a MDL is specified, multiple methods able to meet that limit could be used.

Ramboll also notes that they have "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 ug/L sulfide. 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. It is concerning that dischargers have limited knowledge on the accuracy and precision of the state laboratory execution of Method 4500-S2- E Sulfide and has no information on what to expect for interlaboratory variability." In analyzing samples for the MPCA, the Minnesota Department Health (MDH) and the Science Museum of Minnesota labs both avoided the problem mentioned here--the potential for false positives due to naturally colored water--by separating the sulfide from the water sample prior to quantification. Standard Method 4500--S2-E, used by MDH, first separates the sulfide from the sample via gas dialysis, and only then quantifies the sulfide via colorimetric methods. The Standard Methods book states, "The automated methylene blue method (E) is similar to Method D. A gas dialysis technique separates the sulfide from the sample matrix. Gas dialysis eliminates most interferences, including turbidity and color." Standard Methods notes that this method can accurately quantify sulfide as low as 2 μg/L sulfide, lower than the MDH reporting limit of 11 μg/L sulfide.

K. Procedural Concerns

Several comment letters include assertions regarding purported failures of the MPCA to meet legal/procedural requirements of the Administrative procedures Act, SONAR content requirements, and Minnesota Statutes Section 116.07, subd. 6. The comments allege that MPCA failed to:

- Adequately cite its statutory authority to adopt rules
- Include economic information in the SONAR
- Give due consideration to economic factors
- Consider feasibility and practicability
- Properly assess alternatives

The following paragraphs address each comment in turn.

Statutory Authority: U.S. Steel has commented that the MPCA could have cited additional statutory provisions to demonstrate its authority for the present rulemaking. The agency appreciates that U.S. Steel acknowledges and identifies that the rulemaking is also authorized under other authorities in addition to those specifically cited in the SONAR. Minn. Stat. 14.131 establishes the requirement for a statement of need and reasonableness and delineates general content requirements. Additionally, Minn. R. 1400.2070 (not 1400.0270) presents additional content requirements, providing specifically that the statement must include:

> D. a citation to the agency's grant of statutory authority to adopt the rule and, if the grant of authority was made after January 1, 1996, the effective date of the agency's statutory authority to adopt the rule;

Minn. R. 1400.2070, subp. 1.D. This is to assure that agencies have the necessary statutory authority to promulgate a rule and that the rule is lawful. Subpart 2.D. of the rule refers to information required by other law to be included in a SONAR. The agency complied with the requirements of both the statute and the rule. Neither requires an exhaustive listing of all agency rulemaking authorities nor is specific mention of the rule, Minn. R. 1400.2070, required SONAR content. The MPCA demonstrated that it has the necessary authority for the present rule amendment and cited sufficient statutory authority for the rulemaking.

<u>Economic information included in the SONAR and used to inform development of the standard:</u> In its November 22, 2017 Response to Comments the MPCA responds to the multiple comments about how and to what extent MPCA included economic information in the rule development and SONAR. This included whether the separate study MPCA has underway, funded by the Legislative Citizen Commission on Minnesota Resources, provided information to inform development of the standard.

<u>Due consideration given to economic factors:</u> A number of comments (USS, Cleveland-Cliffs, ArcelorMittal) suggest that the MPCA failed to consider cost and economic factors as required by 14.131 or that the analysis was insufficient. While it is true that MPCA did not title a section of the SONAR as "Consideration of Economic Factors," it is also true that the MPCA gave due consideration to economic factors as required by statute. In fact, the specific SONAR citations provided on page 9 of the USS comments demonstrate that cost considerations <u>were</u> part of MPCA's thinking in developing the proposed rule and SONAR.

USS on pp. 8-10 of its comments also cites recently completed examples of MPCA rulemaking as evidence that MPCA has recognized its obligation to consider economic impacts, and implies that these are in contrast to the rulemaking at hand. The SONAR's content readilyrefutes this assertion. The cost and enhanced economic analysis components of the SONAR for this rulemaking span pages 145-195 and 209-216; and the full regulatory Analysis section spans pages 143 - 218. Due to differences in economic impacts, formats and changes in statutory requirements direct comparisons of SONARs cannot provide a meaningful measure of whether costs were appropriately considered in any individual rulemaking. However, an examination of the SONARs mentioned by for the earlier rulemakings shows that:

- The Regulatory Analysis for the Tiered Aquatic Life Use rulemaking was 17 pages, and the "consideration of economic factors" spanned eight pages.
- The Regulatory Analysis for the Variance rulemaking was six pages, and the "consideration of economic factors" is three paragraphs.
- The "consideration of economic factors" section for the 1997-98 Great Lakes Initiative rulemaking was two pages.

The number of pages in SONAR for the present rule containing discussion of costs and economics exceeds the combined total of the above-identified SONARs. MPCA has fully met the requirements of Minn. Stat. § § 14.131 and 116.07, subd. 6. The fact that MPCA integrated its consideration of economic impacts throughout the Regulatory Analysis for this SONAR rather than limiting them to a section titled "consideration of economic factors" is not evidence that the requirement of due consideration was not met.

ArcelorMittal and USS also claim that MPCA has not met the statutory requirements under Minn. Stat. 14.131 and 115.43 to illustrate the benefits of implementing the proposed rules and that MPCA must directly compare economic costs to benefits. Minn. Stat. 115.43 does require the agency to give due consideration to economic factors and take into account any taxes on municipalities. As demonstrated above, the MPCA has done this for this rulemaking. The APA does not require an explicit balancing of costs and benefits; in fact, Minn. Stat. 14.131 never explicitly mentions the idea of the benefits of a proposed rule (merely the costs of not implementing a rule). In addition, the Tribes in particular would note (as they have in consultation with the MPCA) that it is nearly impossible to quantify the benefits of wild rice and that this results in an uneven balance between easily monetized financial costs and difficult to monetize but very real benefits

Cliffs also claims that the CWA does not prohibit MPCA from evaluating the cost of compliance and references the agency's statements regarding the role of economics in determining water quality standards. The MPCA is on record as stating that the cost of compliance is not a determining factor in *setting* a water quality standard. The content of pages 143-218 of the SONAR demonstrate that the MPCA has considered costs as required by law. The MPCA cannot and should not act as many commenters suggested and simply determine the standard is unreasonable because it is expensive to implement.

A number of commenters have suggested that the MPCA can and should simply delete the existing wild rice standard, that the proposed rule amendment is solely a policy decision, and that the MPCA would be authorized to delete the existing standard without adopting a replacement. . Both the MPCA's response to Comments and the EPA's November 22, 2017 comment letter address this. EPA's comments directly contradicts assertions that MPCA can simply delete the existing wild rice sulfate standard without a replacement and meet its obligations under the CWA Section 303(c) and 40 CFR 131.11(a), and that the proposed revisions to the wild rice sulfate standard are in some way a "policy decision" and not a legal obligation.

<u>Consideration of Feasibility and Practicality:</u> USS asserts that MPCA has not given due consideration to the feasibility and practicability of the proposed rules, and references Section 404 of the Clean Water Act, Webster's Dictionary and the variance discussion in the SONAR as evidence of this lack of consideration. MPCA disagrees with these comments. Consideration of feasibility and practicality is about the proposed rule revisions, not the original adoption of a wild rice sulfate standard. As noted in the Response to Comments and above, MPCA cannot demonstrate that removing the existing wild rice sulfate standard, without a replacement approach, would be protective of the wild rice beneficial use. Therefore the consideration becomes the feasibility and practicality of the proposed revisions as compared to the existing rule.

In citing definitions of "practicable," the commenter references Section 404 of the Clean Water Act. This reference is misguided, since this proposed rulemaking involves the requirements and authorities of Section 303(c) of the Clean Water Act (see EPA comment letter); Section 404 is not relevant to this particular rulemaking.

Finally, the comments note that a condition for granting a variance is a finding "that attaining the designated use and criterion is not feasible" and suggests that MPCA's acknowledgment of the likely need for an applicability of variances therefore proves the rule is not feasible. This argument conflates two separate concepts: the feasibility and practicality of the rule revisions themselves and the feasibility of imposing specific permit conditions as needed to be protective of the adopted standard. These are not the same thing, as MPCA has repeatedly demonstrated throughout the SONAR and rulemaking record. In fact, the availability of variances as a tool to address economic impacts to permitted facilities is evidence that the proposed rule revisions are feasible and practical even though sulfate treatment technologies are currently limited and costly.

<u>Minnesota Statutes 14.127:</u> Mesabi Nugget's submittal includes a request for a statement from the MPCA acknowledging that Minn. Stat. 14.127 protections apply to them and that the Proposed Rule will not apply to

Mesabi Nugget until the rules are approved by law enacted after the agency determination or disapproval by the Administrative Law Judge. Such a statement is not required. The MPCA made the determination required by Minn. Stat. 14.127 in the SONAR as is noted in Mesabi Nugget's comment. The statute does not require the agency to make the requested acknowledgement and the statute speaks for itself as to its applicability and effect. Further, the statute requires that a business or city submit a statement claiming a temporary exemption from the rules before protections under 14.127 are triggered.

AGU PUBLICATIONS

JGR

Journal of Geophysical Research: Biogeosciences

RESEARCH ARTICLE
10.1002/2017JG003787

This article is a companion to Myrbo et al. (2017), https://doi.org/10.1002/2017JG003788 and Pollman et al. (2017), https://doi.org/10.1002/2017JG003785.

Key Points:
- Sulfate loading to freshwater ecosystems may alter aquatic plant communities when sulfate is reduced to sulfide in the anoxic rooting zone
- The occurrence of self-sustaining wild rice populations is mainly controlled by pore water sulfide concentrations
- Even if pore water sulfide is low, wild rice is less likely to be found if the surface water is turbid or warm

Supporting Information:
- Table S1
- Table S2
- Table S3
- Data Set S1
- Figure S1

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Sulfide Generated by Sulfate Reduction is a Primary Controller of the Occurrence of Wild Rice (*Zizania palustris*) in Shallow Aquatic Ecosystems

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Abstract Field observations suggest that surface water sulfate concentrations control the distribution of wild rice, an aquatic grass (*Zizania palustris*). However, hydroponic studies show that sulfate is not toxic to wild rice at even unrealistically high concentrations. To determine how sulfate might directly or indirectly affect wild rice, potential wild rice habitat was characterized for 64 chemical and physical variables in over 100 sites spanning a relatively steep climatic and geological gradient in Minnesota. Habitat suitability was assessed by comparing the occurrence of wild rice with the field variables, through binary logistic regression. This analysis demonstrated that sulfide in sediment pore water, generated by the microbial reduction of sulfate that diffuses or advects into the sediment, is the primary control of wild rice occurrence. Water temperature and water transparency independently control the suitability of habitat for wild rice. In addition to generating phytotoxic sulfide, sulfate reduction also supports anaerobic decomposition of organic matter, releasing nutrients that can compound the harm of direct sulfide toxicity. These results are important because they show that increases in sulfate loading to surface water can have multiple negative consequences for ecosystems, even though sulfate itself is relatively benign.

Plain Language Summary Research in the 1940s and 1950s found that wild rice grew best in low-sulfate Minnesota lakes, but it was not known why. The correlation was a puzzle, since sulfate is not very toxic to plants or animals. This study found that the problem is sulfide, not sulfate. Sulfate can be converted into toxic levels of sulfide in the soil in which wild rice germinates and roots. Wild rice is an annual plant that must sprout each spring from seed that was dropped the previous fall into wet soil. Anaerobic microbes in the soil make sulfide from sulfate in the overlying water. Lakes, streams, and wetlands that have high concentrations of dissolved sulfide in the sediment therefore have a low probability of hosting wild rice. The study also found that wild rice prefers high-transparency water and cold winters.

1. Introduction

Minnesota is unique among U.S. states and Canadian provinces in having a water quality standard that regulates sulfate (SO_4) to protect wild rice, *Zizania palustris* and *Zizania aquatica*. The more common wild rice species in Minnesota, *Z. palustris* (northern wild rice), is an annual emergent aquatic grass that forms monocultures in shallow freshwaters (wetlands, lakes, and rivers) in the area of the Laurentian Great Lakes (Minnesota, Wisconsin, Michigan, Ontario, and Manitoba). Wild rice is culturally important to multiple groups in Minnesota, especially Ojibwe, Dakota, and other Native Americans, and also provides habitat and food for waterfowl and other wildlife (Vennum, 1988). In 1977, the Minnesota legislature voted to make wild rice the Minnesota state grain. Minnesota Rule 7050.0224, promulgated in 1973, seeks to limit the exposure of wild rice to SO_4 concentrations exceeding 10 mg L^{-1} (0.1 mmol L^{-1}). This value was based on empirical research that correlated water chemistry to aquatic plant assemblages and included the observations that no large populations of *Z. palustris* occur in waters exceeding 10 mg L^{-1} SO_4 and that stands are uncommon where SO_4 concentrations exceed 50 mg L^{-1} (Moyle, 1944, 1945). In addition, Moyle (1956) noted that plantings of wild rice seed in high-SO_4 regions generally failed. A larger unpublished Minnesota Department of Natural Resources data set also shows that sites with reported wild rice presence (DNR, 2008) are generally correlated with surface water SO_4 below 10 mg L^{-1} (Figure S1 in the supporting information).

We report here on a multiyear field survey that was part of a larger study (Myrbo et al., 2017; Pastor et al., 2017; Pollman et al., 2017) designed to reevaluate the 10 mg L^{-1} SO_4 standard by testing potential mechanisms by which SO_4 might be harmful to wild rice. SO_4 is a relatively nontoxic and unreactive compound under aerobic conditions. Pastor et al. (2017) and Fort et al. (2014) have shown that SO_4 is not directly toxic to wild rice at concentrations up to 1,600 mg L^{-1}, which exceeds concentrations in virtually all natural surface waters of the upper Midwest (Gorham, Dean, & Sanger, 1983). The U.S. Environmental Protection Agency's nonmandatory drinking water standard of 250 mg L^{-1} SO_4 (2.6 mmol L^{-1}) is based on taste rather than toxicity (EPA, 2010).

1.1. Potential Effects of Elevated Sulfate and Sulfide in Freshwater Systems

SO_4 concentrations in most freshwaters are less than a few percent of the mean concentration in seawater (2,800 mg L^{-1} (29.1 mmol L^{-1})). We surveyed 108 different lakes, streams, and wetlands across Minnesota, where the median SO_4 concentration in lakes is 10 mg L^{-1} (10th and 90th percentiles of 0.2 and 285 mg L^{-1} (MPCA, 2016)). In the much higher SO_4 concentrations of marine waters, it is well established that SO_4 can diffuse into sediment and be converted by microbial sulfate reduction (MSR) to potentially toxic sulfide that influences the presence and absence of rooted macrophytes, such as seagrasses (Borum et al., 2005; Ingold & Havill, 1984; Koch & Erskine, 2001; Lamers et al., 2013).

Despite a long-standing assumption that SO_4 is benign (Pester et al., 2012; Schindler, 1986; Urban et al., 1994) and plays a negligible role in freshwater biogeochemistry (e.g., Capone & Kiene, 1988), there is evidence that SO_4 availability in freshwaters can control the concentration and therefore the toxicity of hydrogen sulfide (H_2S) in sediment pore water to plants and animals (Bagarinao, 1992; Kinsman-Costello, O'Brien, & Hamilton, 2015; Lamers et al., 2013; Wang & Chapman, 1999). The chemical species of H_2S varies with pH; below pH 7, H_2S dominates, and above pH 7, the bisulfide ion (HS^{-}) dominates. For simplicity in this discussion we refer to the sum of the two species as sulfide.

1.1.1. Sulfide Toxicity to Freshwater Plants

Remarkably little attention has been given to the potential toxicity of sulfide in sediment pore water, even after Bagarinao (1992) concluded in a major review that sulfide had been "largely overlooked as an environmental factor for aquatic organisms." In a discussion of sediment toxicity testing, Wang and Chapman (1999) also observed that the biological implications of sulfide in sediments are poorly understood and "all too often ignored." They suggested that sulfide may be more important than ammonia in determining sediment toxicity to organisms and made a suite of recommendations to fill the knowledge gap, including the measurement of sulfide in undisturbed sediments. Kinsman-Costello et al. (2015) measured sulfide in undisturbed sediments and concluded that the potential toxicity of pore water sulfide is likely shaping the plant and animal communities of freshwater ecosystems. Lamers et al. (2013), in a review of sulfide toxicity to aquatic plants, pointed out that traditional toxicity testing generally neglects the chemistry of the rooting zone. Simkin, Bedford, and Weathers (2013) showed that pore water sulfide in a wetland controlled the distribution of plants more than did nutrients.

The toxicity of elevated sulfide to freshwater plants was first recognized in paddy-grown white rice (*Oryza sativa*) in the 1950s (Lamers et al., 2013; Pearsall, 1950). Rice paddies and other water-saturated soils present a profound challenge for rooted plants because of the chemical changes caused by the absence of oxygen and resulting potential toxicity of the pore water (Ponnamperuma, 1972). Anaerobic decomposition of organic matter results in elevated pore water concentrations of ammonia, organic acids, and variable concentrations of sulfide and ferrous Fe, depending on the availability of SO_4 and Fe. The interaction of Fe and the S cycle is complicated (Hansel et al., 2015), but because under anoxic conditions sulfide forms an insoluble precipitate with Fe, elevated pore water sulfide concentrations occur when Fe availability is relatively low (Ponnamperuma, 1972; van der Welle et al., 2006). It is thought that the iron sulfide precipitates are relatively inert and that only sulfide dissolved in pore water is potentially toxic. The concentration of sulfide in pore water is the balance between production and competing fates of sulfide, including precipitation with metals such as Fe, oxidation by oxygen introduced by bioturbation or by release from the roots of macrophytes (Armstrong & Armstrong, 2005), and by downward advection of surface water due to groundwater movement or transpiration by dense macrophyte stands (Bachand et al., 2014).

1.1.2. Geochemical Consequences of Enhanced Microbial Sulfate Reduction

Increased SO_4 availability can allow increased anaerobic decomposition of organic matter, releasing the inorganic nutrients that generally limit growth of higher plants (N, P, and K) (Lamers, Tomassen, & Roelofs, 1998; Myrbo et al., 2017; Weston et al., 2006, 2011). Enhanced decomposition breaks down particulate and dissolved organic carbon (DOC) in the sediment, which can increase DOC and dissolved inorganic carbon (DIC) in the overlying water (Myrbo et al., 2017).

In addition to supporting organic matter mineralization, MSR production of sulfide causes a cascade of reactions that can alter ecosystem functioning independent of any toxicity to plants and animals. First, sulfide can participate in redox reactions, chemically reducing Fe, which converts Fe from solid phase Fe(III) oxyhydroxides to water-soluble Fe(II) (Hansel et al., 2015). The dissolution of the Fe oxyhydroxides releases sorbed ions into solution, including phosphate and trace metals (Caraco, Cole, & Likens, 1993; Davranche & Bollinger, 2000; Søndergaard, Jensen, & Jeppesen, 2003). Second, sulfide can precipitate dissolved metals, including essential plant nutrients Fe, Cu, and Zn, decreasing their bioavailability and leading to nutrient deficiency (Kirk, 2004; Lamers et al., 1998; Neue & Bloom, 1989; Neue & Lantin, 1994). In systems unpolluted with heavy metals, the sulfide precipitate is overwhelmingly dominated by iron sulfide compounds, consisting of a range of stoichiometries and minerals (Schoonen, 2004), which we here term "FeS compounds." Third, the conversion of SO_4 to sulfide entails the production of DIC, or alkalinity, an effect not fully appreciated in freshwater systems until the mechanistic consequences of acid rain were investigated in the 1980s (Baker, Brezonik, & Pollman, 1986; Cook et al., 1986; Schindler, 1986). Alkalinity is thought to be a major factor influencing the distribution of aquatic species, including macrophyte species (Moyle, 1945; Vestergaard & Sand-Jensen, 2000). In addition, elevated alkalinity may further enhance decomposition, producing a positive feedback to the effects of SO_4-driven mineralization (Geurts et al., 2009; Roelofs, 1991).

1.2. Multiple Plausible Negative Effects of Elevated Sulfate and Sulfide Production on Wild Rice

The purpose of this study was to examine the multiple ways that increases in SO_4 concentration and sulfide production can change the biogeochemical functioning of freshwater ecosystems and potentially negatively affect the growth and reproduction of wild rice populations. Before any field data were collected for this study, alternative hypotheses regarding the most likely negative effects were identified (MPCA, 2011), so that appropriate data would be available to test them. The primary hypotheses of how increases in surface water concentrations of SO_4 could harm wild rice populations include direct toxicity by elevated pore water sulfide; reduced bioavailability of Fe, Cu, or Zn; and increased P bioavailability promoting the growth of phytoplankton and macrophytes that compete for light and space. Additional variables were quantified so that exploratory data analysis could be pursued in addition to evaluation of the primary hypotheses. Surface water, pore water, and sediment physical and chemical properties were collected from 108 different sites, both with and without wild rice present, to inform evaluation of these multiple interrelated hypotheses.

2. Methods

2.1. Study Area

2.1.1. Physical Environment

SO_4 concentrations in surface water vary with geology and climate (Gorham et al., 1983) in a northeast-southwest gradient across Minnesota. Bedrock and bedrock-derived glacial deposits of northeastern Minnesota (the "arrowhead") comprise slowly weathered crystalline materials, generally low in S, and surface waters are dilute (specific conductance of $<$140 and often $<$30 μmho cm^{-1}; Gorham et al., 1983). Within this area of naturally low SO_4, iron-mining activities in the "Iron Range" district of northeastern Minnesota have created an "island" of lakes and streams elevated in SO_4 (Figure 1), a result of the weathering of sulfide minerals in waste rock piles and tailing basins. The surficial geology of southwestern Minnesota, in contrast, is derived from marine shales and carbonates that are relatively S-rich and readily weathered: surface waters often exceed 500 μmho cm^{-1} and may exceed 7,000 μmho cm^{-1} (Gorham et al., 1983). SO_4 concentration is positively correlated with conductivity above about 200 μmho cm^{-1} and 10 mg L^{-1} or 0.1 mmol L^{-1} SO_4 (Figure 7B in Gorham et al., 1983). Overprinting this geological pattern is a strong climatic gradient with warmer, drier conditions toward the southwest (toward the northern Great Plains) and colder, more humid conditions to the northeast (toward the Laurentian Great Lakes), which enhances the conductivity gradient: more evaporative conditions in the southwest serve to concentrate surface waters and further increase ionic

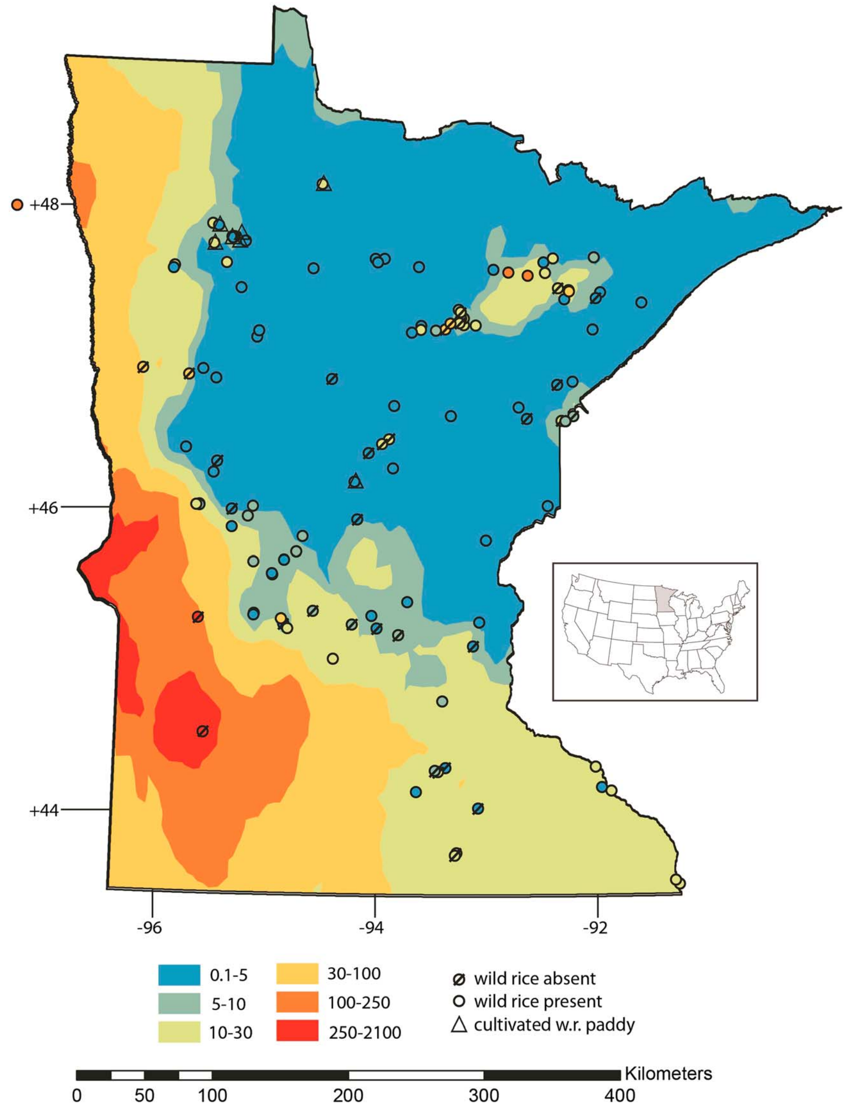

Figure 1. Map of Minnesota showing field sites overlain on kriged contours of average surface water SO_4 concentrations from 4,998 waterbodies (data from MPCA and DNR databases). The symbols are filled with the color corresponding to the site's surface water sulfate concentration. Site to the northwest of the Minnesota map is within the state of North Dakota, 40 km west of the border with Minnesota (see text). Sites where wild rice was not found have a diagonal line through the symbol.

strength, while moister conditions in the northeast maintain low ionic strength in surface waters (Gorham et al., 1983).

2.1.2. Habitat Preferences of Wild Rice

Although the scientific literature contains many assertions concerning the environmental preferences of wild rice regarding water and sediment quality (e.g., DNR, 2008, 2016; Lee, 2002; Moyle, 1944; Moyle & Krueger, 1964; Pillsbury & McGuire, 2009), sometimes little evidence was presented to support the putative preferences. The shallow freshwaters in which wild rice is found are usually relatively transparent because wild rice and other rooted macrophytes do best if they can photosynthesize as they grow each spring from the sediment to the water surface (Scheffer, 1998). Otherwise, any particular habitat preference of wild rice, such as specific chemical ranges of the sediment or surface water, is challenging to identify. Since wild rice is an opportunistic annual plant, a primary habitat requirement is periodic environmental disturbance (Grime, 1977) that keeps perennial plants such as water lilies from controlling the space and light of the shallow waters in which these species cooccur (Pillsbury & McGuire, 2009). Wild rice does not seem to have specific sediment requirements, as it has been observed growing in a variety of substrates (Aiken et al., 1988; Lee,

1986) within its range. Annual plants are adapted to exploit environments intermittently favorable for rapid plant growth and to maximize seed production (Grime, 1977). Wild rice produces between 25 and 150 relatively large seeds per stem (Eule-Nashoba, Biesboer, & Newman, 2012). Seeds buried in the sediment can survive for up to several decades until conditions are again favorable for germination and growth (DNR, 2008). *Zizania palustris* seeds germinate at low rates unless they have been exposed to near-freezing temperatures for at least 3 months (Kovach & Bradford, 1992), although the environmental cues for subsequent germination are poorly understood, aside from elevated temperature.

Because *Z. palustris* does not normally self-pollinate and is wind-pollinated (which implies that the pollen source must be relatively nearby; Friedman & Barrett, 2009), does not reproduce asexually, and has an annual life cycle (Aiken et al., 1988), in this analysis the presence of wild rice plants is taken to mean that the water-body hosts a successfully reproducing and self-sustaining population. A few of the sites in this study may have experienced recent watershed changes such that steady state has not yet been reached among environmental variables and wild rice reproduction, but the balance of the 108 sites should have been in steady state at the time of sampling. The population of wild rice in a given lake can exhibit large fluctuations from year to year, which has been attributed to disturbances such as abrupt increases in water level (DNR, 2008) and to cyclical changes in N availability (Walker et al., 2010). Wild rice fills a unique ecological niche, in that there are virtually no other annual aquatic macrophytes across the Great Lakes region (Eggers & Reed, 2011).

2.1.3. Site Selection

The goal of the field survey was to identify and sample potential wild rice habitat across a gradient of SO_4 concentrations. Potential wild rice habitat was defined simply as shallow water (30–120 cm deep, though some sampled sites had water as shallow as 5 cm) that was sufficiently transparent to support rooted aquatic macrophytes. Turbidity from dense phytoplankton blooms or suspended solids may effectively exclude rooted macrophytes due to light limitation (Scheffer, 1998).

Most Minnesota lakes that host wild rice populations have low SO_4 concentrations; the median concentration of wild rice lakes is 1.8 mg L^{-1}, and the 75th percentile is 3.6 mg L^{-1} (MPCA, 2014). If only those waters hosting abundant wild rice were sampled, most of those would be low in SO_4, and little would be learned about how elevated SO_4 concentrations (or other important variables) might affect wild rice. Conservation biologists commonly identify important habitat variables through binary (i.e., is the species present or absent at a given site?) logistic regression, which requires the sampling of sites that do not support the species of interest (e.g., Carroll, Zielinski, & Noss, 1999; Peeters & Gardeniers, 1998; van der Heide et al., 2009). Consequently, potential wild rice habitats with a range in SO_4 concentrations were sampled without regard to whether the waterbody hosted or was known to host wild rice. An effort was made to sample waterbodies that covered the range of SO_4 concentrations across Minnesota and especially to sample sites in high-SO_4 regions that had reports of recent or historical presence of wild rice. This strategy also resulted in the sampling of a high-SO_4 wild rice site in the North Branch of the Turtle River (198 mg SO_4 L^{-1}), 40 km into North Dakota from northwestern Minnesota (Figure 1). A gradient of SO_4 concentrations was sampled by identifying potential wild rice habitat in two areas of elevated SO_4, the "island" of elevated SO_4 in the Iron Range region of northeastern Minnesota and waters naturally elevated in SO_4 to the west and south of the known range of wild rice (Figures 1 and S1). Nutrient (N and P) availability (reflecting natural soil fertility as well as agricultural runoff) also increases to the west and south, potentially supporting phytoplankton growth sufficient to exclude macrophytes, including wild rice. Consequently, in western and southern Minnesota, DNR lake databases were screened for the presence of water lilies (*Nuphar variegata* or *Nymphaea odorata*), the occurrence of which indicates transparency sufficient to support rooted macrophytes that also often cooccur with wild rice. In an analysis of DNR aquatic plant surveys from 1,753 shallow lakes, we found that the odds of finding wild rice where there are water lilies are 26 times the odds of finding wild rice where there are no water lilies, with a 95% confidence interval of 20–36 times (neither wild rice nor water lilies = 968; wild rice but not water lilies = 60; no wild rice but water lilies = 272; both wild rice and water lilies = 453).

With these considerations, water bodies were selected for sampling based on preliminary data including average water depth, presence of water lilies, conductivity or SO_4 concentration, and geographic distribution (i.e., to sample widely across the state). Both rivers and lakes were sampled, as were seven different cultivated wild rice paddies. Within a given water body, the field team chose a location for sampling based on a decision tree (Table S1 in the supporting information).

2.2. Field Methods

Sampling occurred in August–September 2011 and June–September 2012 and 2013. Sampling efforts were focused in late summer to capture physical and chemical conditions when wild rice plants were maturing and when identification of wild rice is most certain; voucher specimens of wild rice were always taken. Samples were collected from an anchored canoe or small boat, except in the cultivated wild rice paddies when the water was too shallow to float a canoe, and samples were collected on foot. A Hach model HQ40d or Hydrolab Quanta sonde, calibrated daily, was used to measure temperature, specific conductance, dissolved oxygen, and pH in the surface water. Water transparency was determined with a 1 m long Secchi-tube (Water Monitoring Equipment & Supply, USA), and apparent color was measured using a Hach model CO-1 color test kit. Surface water samples (later split into separate subsamples; see below) were collected by a technician wearing long nitrile gloves in two 2 L amber Nalgene bottles that had been previously triple rinsed with deionized water and rinsed 3 times with water from the site before filling. Water samples were stored on ice.

Short (~50 cm) sediment cores with ~10–20 cm of overlying water were collected at eight undisturbed locations at least 1 m apart around the boat using an HTH corer (Pylonex, Sweden) with a 7 cm diameter polycarbonate barrel. A piston was inserted in the bottom end of each core as it was retrieved. Cores were kept upright and shaded prior to sample processing.

Aquatic macrophytes were identified and percent cover estimated within a plastic hoop 1 m in diameter placed at 4 locations around the boat. In 2012 and 2013, the number of stems of wild rice inside each hoop was also quantified.

Pore water samples were obtained from cores processed on shore using 10 cm Rhizon™ filters (pore size 0.12–0.18 μm) (Rhizosphere.com, Netherlands; Shotbolt, 2010) inserted vertically into the core tops, following extrusion of overlying water, and connected to evacuated serum bottles with PVC/polyethylene tubing and a stainless steel needle. Three separate cores were sampled, one for nutrients (nitrate + nitrite, TP, and TN) and DOC (70 mL), a second for metals (50 mL), and a third for dissolved silica, Cl, and SO_4 (30 mL). A fourth core was sampled for pore water sulfide, but in this case, the serum bottle was preloaded with 0.2 mL of 2.0 N zinc acetate, 0.5 mL of 15 M sodium hydroxide, and a stir bar, flushed with a nitrogen atmosphere, evacuated, and preweighed. Air was flushed from the Rhizon-tubing assembly with sample pore water using a second evacuated bottle before the needle was inserted into the sulfide sample bottle. In 2011 only pore water samples for sulfide and metals were collected. Pore water pH was measured on a fifth core by inserting the probe of a Hach model HQ40d pH meter into the sediment to a depth of 5 cm and allowing the reading to stabilize.

A composite sediment sample was collected from the uppermost 10 cm of the sixth, seventh, and eighth cores, placed into a stainless steel bowl, and stirred under nitrogen atmosphere to homogenize. A 50-mL subsample was placed in a polypropylene sample bottle along with 1.0 mL of 1.0 N zinc acetate for analysis of acid-volatile sulfide (AVS). The headspace of the AVS sample bottle was flushed with N_2 and the bottle capped; that bottle was placed in a larger glass jar and that jar flushed with N_2 and tightly sealed. The sample was immediately placed in a cooler with dry ice to freeze. The remaining composited sediment was placed into a polycarbonate container and stored on ice for later analysis.

Water subsamples were taken from the large amber Nalgene bottles by a technician wearing nitrile gloves. Sulfuric acid (5 mL of 10%) was immediately added to subsamples for the analysis of P, TKN (total Kjeldahl nitrogen), ammonia, and nitrate + nitrite in 250 mL polyethylene bottles. Nitric acid (2.5 mL of 20%, to acidify to $pH < 2$) was immediately added to subsamples for the analysis of total metals in 250 mL polyethylene bottles. Samples for dissolved metals were subsampled in 250 polyethylene bottles and subsequently filtered using a 0.45 μm filter and preserved with nitric acid in the laboratory. Samples were stored on ice.

2.3. Laboratory Methods

Surface water, pore water, and most sediment analyses were conducted by the Minnesota Department of Health Environmental Laboratory (MDHEL) in 2012 and 2013, following standard methods. In 2011 other laboratories conducted the analyses (University of Minnesota Soils Laboratory (UMNSL), St. Croix Watershed Research Station (SCWRS), and Gustavus Adolphus College (GAC)); methodological differences are noted, and the laboratory is identified where relevant.

Surface and pore water samples were analyzed for anions (Cl and SO_4) by ion chromatography on a Dionex ICS-3000 (MDHEL), Fe, Ca, Na, Mg, and K by inductively coupled optical emission spectrometry on a Varian 715-ES (MDHEL), Mn, Cu, Zn, Co, Ni, Al, As, and Se by inductively coupled mass spectrometry on a Perkin Elmer Elan DRCE (MDHEL), and DOC by UV-persulfate oxidation on a Tekmar-Dohrmann Phoenix 8000 (SCWRS and MDHEL). N and P were measured by colorimetric methods (on a Lachat Quikchem Flow-Injection Autoanalyzer) at SCWRS and MDHEL following cadmium-reduction (nitrate and ammonia) or dual alkaline-persulfate digestions (TP and TN). Silica was measured colorimetrically at SCWRS on the Lachat Autoanalyzer and at MDHEL on a Beckman Coulter DU 800 UV/VIS spectrophotometer. Pore water sulfide was analyzed colorimetrically on each lab's Lachat Autoanalyzer following in-line acid distillation and NaOH trapping (SM 4500-S2). Alkalinity was measured by potentiometric acid titration (MDHEL) or as DIC by acid digestion and IR detection (SCWRS). DIC was converted to alkalinity using pH, temperature, and specific conductance of the surface water.

Sediment samples from 2011 were analyzed by combustion for total carbon (TC), total nitrogen (TN), and total sulfur (TS) using, respectively, a Tekmar Phoenix 8000 CO_2 analyzer, an Elementar Vario Max N analyzer, and a LECO sulfur analyzer (UMNSL). Samples from 2012 and 2013 were analyzed for CHN on a Costech 4010 Elemental Analyzer (UMN Stable Isotope Laboratory). Total inorganic carbon (carbonate) was analyzed by coulometric titration on a UIC CM5015 CO_2 coulometer, while water and organic matter content were determined by loss-on-ignition methods (Heiri, Lotter, & Lemcke, 2001) in the UMN LacCore facilities. Sediment AVS was analyzed colorimetrically, as above for pore water sulfide, following acid-distillation and in-line alkaline trapping (SM 4500-S2; Hsieh & Shieh, 1997).

Sediment phosphorus was extracted from freeze-dried sediments following methods of Engstrom and Wright (1984) for total-P and Hieltjes and Lijklema (1980) for P fractions (NH_4Cl-extractable, NaOH-extractable, HCl-extractable, and residual (organic)-P). The P extracts were measured colorimetrically by flow-injection autoanalyzer (SCWRS). Extractable iron and trace metals were quantified from a 0.25 g homogenized freeze-dried sediment subsample incubated in 0.5 M HCl for 30 min at 80°C. The samples were centrifuged, decanted, and analyzed by ICP-MS at GAC. This extraction releases metal oxyhydroxides, sulfides, and loosely bound phases from the sediment without appreciably attacking the silicate matrix (Balogh et al., 2009).

2.4. Data Subsets and Statistical Analysis

From 2011 to 2013, 260 site visits were conducted in 108 different natural waterbodies, including lakes, small streams, backwaters of the Mississippi River, and wetlands, plus 7 different cultivated wild rice paddies. For a variety of logistical reasons, the full suite of samples could not be collected on some site visits. Three subsets of the field data were identified for the analyses reported here. A subset that consists of all of the samples from natural waterbodies (excluding the cultivated paddies) with virtually complete analyses (surface water, pore water, and bulk sediment; $n = 194$) was termed for internal purposes Class D. Pollman et al. (2017) used Class D to develop a structural equation model to elucidate key variables that govern the concentration of sulfide in sediment pore water. A second subset, Class S, of 51 samples from 7 stream and 8 lake sites was each sampled 3 to 5 times from 27 May to 19 September 2013, to provide a data set to assess seasonality in variables. A third subset, Class B, was used for conducting probabilistic analyses to identify the most likely parameters controlling the presence and absence of wild rice and to examine Spearman nonparametric correlations among field variables. Class B consists of one sample from each site ($n = 108$) and excludes samples collected in 2011, which were analyzed with slightly different lab methods. Although Class B was not created as a random sample of Minnesota waterbodies, the frequency distribution of SO_4 concentrations is intermediate between a probability-based survey conducted by USEPA (MPCA, 2016) and a list of known wild rice water bodies, which are overwhelmingly low-SO_4 lakes (MPCA, 2014). Therefore, the Class B data set is reasonably representative of potential wild rice habitat, and binary logistic regressions can be used to approximate the probability of wild rice occurrence as a function of field variables such as pore water sulfide. The Class B data set is used in all analyses presented here, with two exceptions: (1) Figures 3a–3c present the full Class D data set, plus for comparison, data from the cultivated wild rice paddies; (2) Statistical analysis was performed with the software package R version 3.2.3.

To identify variables associated with the presence and absence of wild rice, we relied on binary logistic regressions (BLR), using the glm function in R. BLR does not require normally distributed data, but

Table 1
Field Variable Correlation With the Presence-Absence of Wild Rice, Assessed Through Binary Logistic Regression (BLR), Plus Spearman Correlations Between Variables[a]

	Binary logistic regression (BLR) correlation with field variable			Spearman correlation with field variable (rho)				
Field variable	Log transformed?	*p* value	Correlation direction	Wild rice density (only wild rice sites) *n* = 67	Pore water sulfide	Sediment AVS	Water transparency	Water temperature
pw K	Y	0.0008***	Negative	−0.27*	0.46***	0.11	−0.10	0.33***
pw sulfide	Y	0.0012***	Negative	−0.31**	1.00	0.29**	−0.07	0.17
Water depth (m)	N	0.0028***	Negative	0.07	0.11	0.08	0.22*	0.19*
Transparency (cm)	N	0.0031***	Positive	0.11	−0.07	−0.13	1.00	−0.08
sw TN	Y	0.0054**	Negative	−0.12	0.22*	0.08	−0.61***	0.23*
sed Se % dry	N	0.0059**	Negative	0.12	0.08	0.27**	−0.21*	0.13
sw Temp	N	0.0077**	Negative	0.08	0.17	0.11	−0.08	1.00
pw Fe	Y	0.0109*	Positive	0.20	−0.58***	0.00	0.04	0.09
sw pH	N	0.0200*	Negative	−0.14	0.28**	0.08	−0.05	0.35***
sw TP	Y	0.0353*	Negative	0.15	0.05	0.29**	−0.58***	0.27**
Latitude	N	0.0376*	Positive	−0.04	−0.06	−0.09	0.13	−0.51***
sed TS % dry	Y	0.0483*	Negative	0.20	0.40***	0.42***	0.03	−0.08
sw K	Y	0.0922		−0.03	0.29**	0.21*	−0.18	−0.08
sed AVS % dry	Y	0.1317		0.02	0.29**	1.00	−0.13	0.11
sw sulfate	Y	0.1475		−0.10	0.44***	0.45***	−0.07	0.04
sed TP % dry	N	0.2697		−010	0.07	0.30**	−0.14	0.14
sw alkalinity	Y	0.2786		0.25*	0.22*	0.26**	0.11	0.17
pw TN	Y	0.2963		−0.30	0.31***	0.14	−0.20*	0.34***
pw NH4	Y	0.4505		−0.33**	0.33***	0.22*	−0.17	0.26**
sed Fe % dry	Y	0.4795		0.16	−0.35***	0.38***	−0.10	−0.06
pw DOC	Y	0.4865		0.08	−0.05	−0.1	−0.21*	0.09
pw Si	N	0.5548		0.03	0.33***	0.18	0.07	0.29**
pw TP	Y	0.6341		0.02	0.12	0.30**	−0.26**	0.26**
sed TN % dry	N	0.6807		−0.06	0.14	0.04	0.06	−0.06
sed water content	N	0.7274		−0.07	0.15	0.10	0.10	0.02
sed TOC % dry	N	0.7854		−0.10	0.10	0.02	0.10	−0.06

[a]Note. The variables are ordered by the significance of the BLR. The first 12 variables have BLR significance of $p < 0.05$. The additional 14 variables are listed because of their correlation with pore water sulfide, sediment AVS, or surface water transparency or temperature—or their notable lack of correlation with wild rice presence-absence (pw = pore water; sw = surface water; sed = sediment).
*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

nevertheless, we transformed some variables (Table 1) to achieve approximately normal distributions. BLR also yields the probability of occurrence at a given value of the variable. Spearman nonparametric correlations (rho) between field variables in Class B were examined as part of the effort to identify the major biogeochemical interactions in these shallow-water systems. Seasonality in a variable was assessed using linear mixed effects models, with time (fraction of a year) as a fixed variable and site as a random factor (to account for multiple samples per site). In the model developed for each field variable, we accounted for the seasonal cycle using the following equation (Crawley, 2007), where *A*, *B*, and *C* are fitted model coefficients:

$$y = A + B\ \sin(2\pi\ \text{time}) + C\ \cos(2\pi\ \text{time}) \quad (1)$$

3. Results and Discussion

3.1. Field Variables Associated With Wild Rice Presence and Absence

Of the 64 field quantified variables, BLR identified 12 that are associated with the presence/absence of wild rice at the 0.05 probability level or better (Table 1): pore water sulfide, K, and Fe (Figures 2a, 2b, and 2f); surface water temperature, TP, TN, and pH (Figures 2d, 2m, 2n, and 2o); sediment Se and TS (Figures 2c and 2p); water depth and transparency (not shown; Figure 2e); and latitude of the site (Figure 2g). These variables may be important in controlling the presence or absence of wild rice or may merely be correlated with one or more actual causative factors. Because we are primarily interested in factors that control presence or absence of wild rice, in contrast to the density of wild rice, we place primacy on the BLR results and use the Spearman

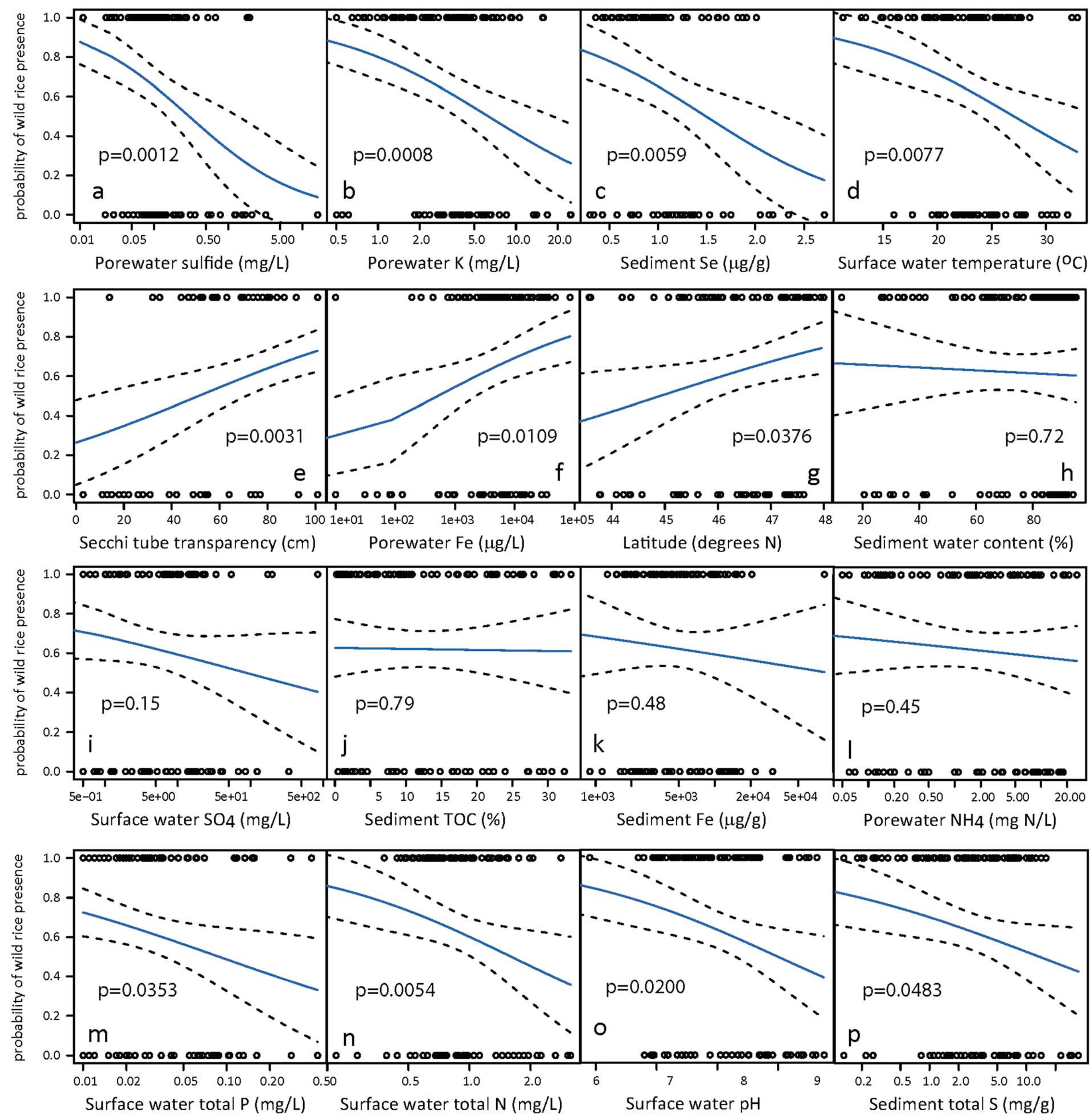

Figure 2. Binary logistic regressions for 16 variables found to be statistically significant in this study or indicated in the literature as important for wild rice habitat. The open circles indicate the value for a given parameter of each site used in the analysis; the circles at the top of a plot indicate sites with wild rice present; the circles at the bottom of a plot indicate sites with wild rice absent. The span of the dashed lines around the solid line indicates the 95% confidence interval.

correlations to help understand the relationships between environmental factors of interest. Wild rice density is negatively correlated with pore water sulfide, potassium (K), and NH_4 and positively correlated with surface water alkalinity (Table 1). There are likely factors controlling the density of wild rice at any particular location in addition to the variables measured, such as herbivory and hydrological disturbances.

One should be cautious in the interpretation of statistically significant associations between wild rice and field variables, in that true cause and effect are not necessarily obvious. For instance, is the toxic quality of pore water sulfide sufficient explanation for its negative associations with both the presence and density of wild rice? Do sites with greater density of wild rice have lower pore water sulfide because low sulfide allows wild rice to grow, or because plants release oxygen from their roots, oxidizing sulfide? The true explanation is likely a combination of the two mechanisms: elevated pore water sulfide can eventually extirpate a wild rice

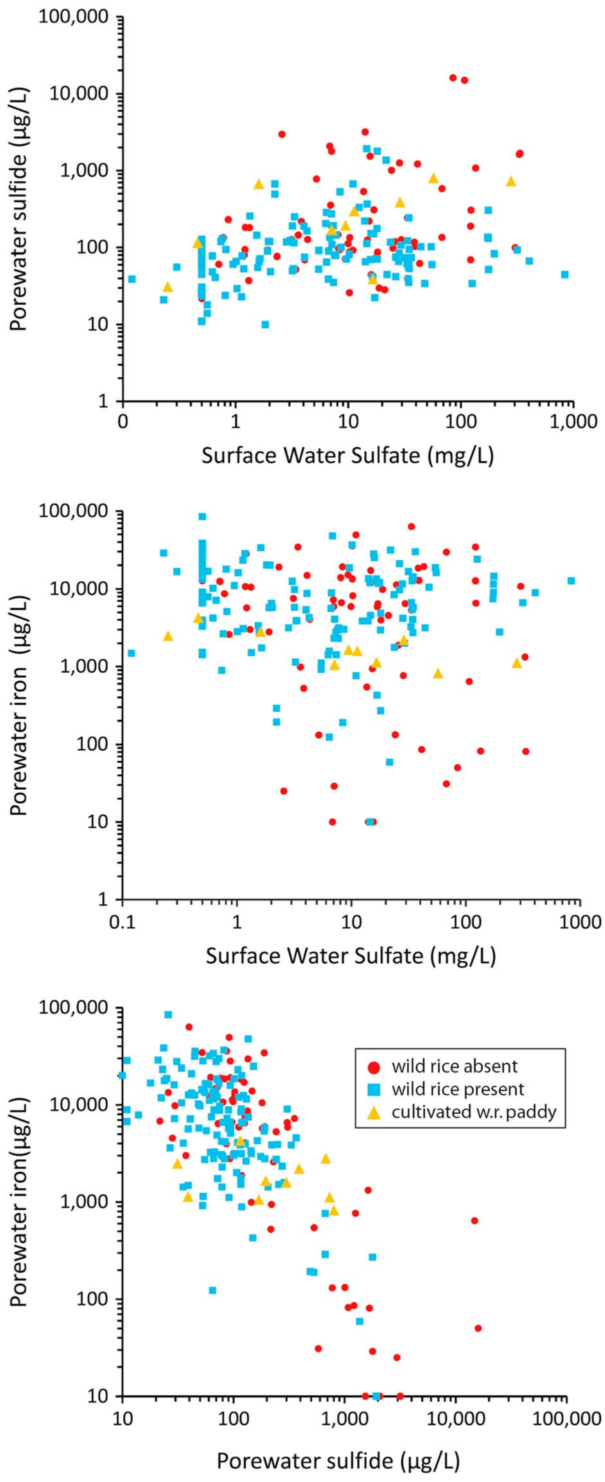

Figure 3. Class D data plus cultivated wild rice paddies (yellow triangles) showing sites with wild rice present (blue squares) and absent (red circles). Note that (a) pore water sulfide is not linearly related to surface water SO_4, indicating that additional factors besides SO_4 concentration contribute to the sulfide concentration in pore water; (b) wild rice can occur in sites over a wide range of surface water SO_4 values as long as pore water Fe is relatively high; and (c) pore water Fe and sulfide have a strong negative relationship, due to the energetically favorable formation of FeS compounds, and that wild rice mainly occurs where pore water sulfide is $<1,000$ μg L^{-1}.

population by reducing seedling survival and seed production, weight, and viability (Pastor et al., 2017), but oxygen release from roots when sulfide concentrations are low enough to allow wild rice to successfully reproduce drives sulfide to even lower concentrations than would have occurred without the plants (Myrbo et al., 2017). Although the negative correlation between pore water sulfide and wild rice density (rho = -0.31, $p < 0.01$, Table 1) could be interpreted to mean that lower sulfide allows a larger population to grow, it is also possible the correlation is partly caused by oxygen release from the wild rice roots, decreasing sulfide concentrations. This hypothesis is supported by the observation from a controlled experiment that acid-volatile sulfide (AVS) sediment concentrations were 30% lower when wild rice was present (Myrbo et al., 2017).

Of the 12 variables significantly associated with the occurrence of wild rice, we propose that nine are noncausal and merely related to one or more of three truly causative factors: (1) pore water sulfide, (2) water transparency, and (3) water temperature. These three variables are not significantly correlated with one another (Table 1), and there are plausible mechanisms for each to independently exclude wild rice from otherwise suitable habitat: (1) elevated sulfide reduces the growth of wild rice (Pastor et al., 2017); (2) low water transparency plausibly constrains the ability of a germinated wild rice seed to reach sufficient light before it runs out of endosperm energy (Aiken et al., 1988; DNR, 2008); and (3) warmer winter water temperature at lower latitudes may limit seed germination, as the seeds of *Z. palustris* must experience at least 3 months in cold water to break seed dormancy (Kovach & Bradford, 1992) (although summer temperatures were measured in this study, winter and summer temperatures would be correlated).

3.2. Pore Water Sulfide as a Causal Variable and Associated Correlated Variables

Our primary a priori hypothesis was that elevated surface water SO_4 can produce elevated pore water sulfide concentrations which, in turn, negatively affect potential wild rice habitat. A plot of pore water sulfide against SO_4, with wild rice presence noted (Figure 3a), suggests that wild rice is generally not found at sites with elevated sulfide. Of the sites with pore water sulfide greater than 1,000 μg L^{-1}, wild rice was present in 23% (3 of 13, with only a few plants found at 2 of those 3 sites). In contrast, wild rice was present at 74% of the sites where sulfide was less than 1,000 μg L^{-1}. Despite historical reports of *Zizania aquatica* in Minnesota, inspection of voucher specimens, following the taxonomy of Terrell et al. (1997), found only *Z. palustris* at all sites sampled. *Zizania aquatica* (Southern wild rice) is rare in Minnesota, but its range extends east through Wisconsin and the southern part of the Great Lakes region to New England and occurs in freshwater tidal marshes south to Florida and west to Louisiana (Terrell et al., 1997). The findings of this study, especially those related to temperature, may not apply to *Z. aquatica*.

Despite a strong relationship between sulfide and wild rice, the relationship between SO_4 and pore water sulfide among the 108 field sites was not a simple linear function. High surface water SO_4 did not always result in high pore water sulfide (above 1,000 μg L^{-1}), and some samples with surface water SO_4 less than 10 mg L^{-1} had pore water sulfide greater than 1,000 μg L^{-1} (Figure 3a). The 33 samples with surface water SO_4 below 1.0 mg L^{-1} all had pore water sulfide less than 200 μg L^{-1}, suggesting that surface water SO_4 might limit maximum sulfide production.

Wild rice was present at high and low SO_4 concentrations as long as pore water Fe was high and sulfide was low (Figures 3b and 3c): pore water Fe and sulfide each exert antipathetic control over the concentration of the other (Figure 3c; Pollman et al., 2017), and wild rice mainly persists in sites where pore waters are low in sulfide and high in Fe. Pollman et al. (2017) developed a structural equation model showing that variations in three variables (SO_4, sediment TOC, and sediment extractable Fe) contribute nearly equally to the observed variations in pore water sulfide among the sites in this study. Sulfate-reducing bacteria are simultaneously limited in their production of pore water sulfide by surface water SO_4 and sediment TOC (which are roughly proportional to available SO_4 and labile organic matter, respectively). Concentrations of sulfide in pore water are then constrained by the availability of pore water Fe, which is in turn controlled by the overall supply of Fe in the sediment (Pollman et al., 2017).

If pore water sulfide concentration is a causative factor controlling the presence of wild rice, as hypothesized, then other variables may be statistically significant because they either vary with the process of SO_4 reduction or with the sulfide itself. Pastor et al. (2017) showed that wild rice growth declines in proportion to sulfide concentrations in both hydroponic and outdoor mesocosm experiments. In a study of the Pastor et al. mesocosms, Myrbo et al. (2017) showed that the enhanced mineralization of plant litter associated with SO_4 reduction produced increases in surface water P and N, and increases in pore water sulfide caused decreases in pore water Fe. These effects from the controlled mesocosm experiment are consistent with correlations observed among sites in the field data between pore water sulfide and surface water N and pore water Fe (Table 1). Surface water P is not significantly correlated with pore water sulfide in the field data, despite dual mechanisms of P mobilization from sediment, (1) the interaction between sulfide and Fe (Caraco et al., 1993; Maynard, Dahlgren, & O'Geen, 2011; Smolders & Roelofs, 1993) and (2) mineralization of organic matter. The lack of correlation of surface water P with pore water sulfide in this field study is not surprising because given the wide variety of landscapes sampled, there is no reason that pore water sulfide would be proportional to the mass of Fe that has reacted with sulfide and released sorbed P. In SO_4 addition experiments where the P and Fe content of the sediment is held constant, there is often a significant correlation between pore water sulfide and mobilized P (Myrbo et al., 2017). In contrast to the lack of correlation of pore water sulfide with surface water P in this field study, AVS is significantly correlated with surface water P (rho = 0.30, $p < 0.01$, Table 1), presumably because AVS is proportional to the iron that has reacted with sulfide.

Elevated pore water sulfide is a product of SO_4 reduction-driven mineralization of sediment organic matter, which also releases the constituents of the decaying plant material, N, P, K, silica, and C (either as DIC, which increases alkalinity, or DOC) into sediment pore water and the overlying surface water (Myrbo et al., 2017). Consequently, the occurrence of wild rice is negatively associated not only with pore water sulfide ($p = 0.001$) but also with elevated pore water K ($p = 0.0008$) and surface water TN ($p = 0.005$, Table 1). Median concentrations of these variables are lower in wild rice waters compared to waters with no observed wild rice (88 versus 126 μg L^{-1} sulfide, 2.4 versus 4.3 mg L^{-1} pore water K, and 0.74 versus 0.95 mg L^{-1} TN, Table S3). Pore water sulfide is itself positively correlated with pore water K, TN, NH_4, and silica, surface water K, alkalinity, and pH, and sediment TS and AVS (Tables 1 and S4).

The strong evidence for the association of elevated pore water K with the absence of wild rice (Table 1) is interesting, as K is an essential plant nutrient, and therefore, it is unlikely that the association is based on toxicity to wild rice. Rather, it is likely that the association is a result of the simultaneous mobilization of K with the production of sulfide as plant matter is mineralized. Potassium does not bond covalently with organic compounds and is readily leached out of dead organic matter (Troeh & Thompson, 2005). Silica phytoliths dissolve as plant matter is mineralized, allowing additional K that had been trapped within the phytoliths to be released into sediment pore water (Nguyen et al., 2015). Wild rice and other wetland macrophytes develop abundant phytoliths that release dissolved silica upon decomposition (Struyf & Conley, 2009). Additional dissolved silica is likely released to pore water as epiphytic diatoms are mineralized. Pore water silica, K, and sulfide are all significantly correlated with each other (Tables 1 and S5). The negative correlation of pore water K with wild rice may be magnified by its additional positive correlation with elevated water temperature (Table 1), which plausibly accelerates dissolution of silica in organic matter (Gudasz et al., 2010; Kamatani, 1982).

AVS largely consists of solid-phase sulfide, which is not available to plants and therefore is not significantly associated with wild rice presence/absence ($p > 0.10$, Table 1). AVS is a measure of cumulative sulfide production, which is proportional to past mineralization of organic matter, consistent with significant positive correlations between AVS and pore water TP and NH_4, and surface water TP and alkalinity ($p < 0.01$, 0.05, 0.01, and 0.01, respectively, Table 1).

The negative association of sediment total-S (TS) with wild rice presence (BLR $p = 0.048$, Table 1) is likely the result of both TS and wild rice being controlled by sulfide production; the median TS concentration at sites with wild rice is 2.6 mg g^{-1}, compared to 4.1 mg g^{-1} at sites without wild rice (Table S3). Sediment TS is correlated with both pore water sulfide and AVS. The negative association of sediment Se with wild rice (BLR $p = 0.006$) is surprising, given that median Se concentrations are very low (0.9 and 1.2 μg g^{-1} in sites with and without wild rice, respectively). The slightly higher Se at sites without wild rice may be caused by the coprecipitation of Se and S by SRB, as shown by Hockin and Gadd (2003). Selenium is correlated with sediment total S (rho = 0.35; $p < 0.001$).

3.3. Water Transparency as a Causal Variable and Associated Correlated Variables

Lower water transparency is associated with a lower probability of wild rice occurrence (BLR $p = 0.003$). Transparency is not related to wild rice density (rho = 0.11, $p > 0.20$, Table 1); however, low transparency can apparently exclude wild rice (11 of the 12 sites with transparency <30 cm had no wild rice), but above that threshold, other variables control wild rice density. Low water transparency decreases photosynthesis of wild rice seedlings while growing to the water surface, which (a) decreases oxygen production that could otherwise be used to detoxify sulfide internally (Krüssel et al., 2014), or externally if released into pore water from the roots (Colmer, 2003); and (b) decreases the energy available for root development, enhancing vulnerability to a sudden increase in water depth, which can uproot an entire year's cohort (DNR, 2008). Wild rice is unusual among grasses in that the stem develops before the root, probably because the seedling may have to grow over 50 cm before reaching the water surface, at which time floating leaves are developed that can supply energy for root development (Aiken, 1986). If transparency is too low, or the water too deep, the energy stored in the seed can be insufficient for the seedling to reach the water surface.

Water transparency can be controlled by the density of phytoplankton, which was not measured in this study. However, transparency is highly correlated with the nutrient concentrations that control phytoplankton growth, total phosphorus (rho = −0.58, $p < 0.001$), and total nitrogen (rho = −0.61, $p < 0.001$). Transparency can also be controlled by colored dissolved organic matter, consistent with the observed significant correlation between transparency and water color (rho = −0.68, $p < 0.001$, Table S4).

3.4. Temperature as a Causal Variable and Associated Correlated Variables

Wild rice germinates at higher rates following longer exposure at temperatures closer to freezing (Kovach & Bradford, 1992), consistent with the significant association of wild rice absence with lower latitudes (BLR $p = 0.04$) and warmer summer water temperature (BLR $p = 0.008$; medians of 21.9° and 23.7°C with and without wild rice, respectively). Sites with warmer summer water temperatures generally also have warmer winter temperatures. Warmer and shorter winters would cause lower germination rates, ultimately reducing the probability that a population could persist over the long term. For this reason, the Minnesota Department of Natural Resources hypothesizes that climate change may push the range of wild rice farther north (DNR, 2008, 2016). However, the temperature control of wild rice germination has not been adequately investigated to rigorously assess this hypothesis. The observed association of wild rice with lower pH surface waters (BLR $p = 0.04$; median pH values of 7.8 and 8.5 at sites with and without wild rice, respectively) may be due simply to the correlation of wild rice with cooler surface waters: the solubility of CO_2 is higher in colder water, leading to lower pH (Spearman correlation between temperature and pH = 0.35, $p < 0.0001$).

Lower latitude sites are negatively associated with wild rice presence and density (Table 1). Lower latitude sites are correlated with higher water temperature (rho = −0.51, $p < 0.001$). Geological and land use gradients also correlate with latitude, producing correlations with surface water N and P (rho = −0.25 and −0.31, $p < 0.01$). Despite these correlations with nutrients, latitude is not significantly correlated with water transparency (rho = 0.13), so the general unsuitability of lower latitudes for wild rice may be a combination of warmer winters and some reduced transparency as a result of nutrient enrichment. Latitude is not significantly correlated with pore water sulfide (rho = −0.06).

3.5. Synergy Among the Three Causal Variables of Sulfide, Temperature, and Transparency

While temperature may control wild rice presence/absence through winter temperatures too warm to achieve high rates of seed germination in the spring, higher summer temperatures may act synergistically on variables correlated with pore water sulfide. Elevated summer temperatures likely enhance microbial activity, no matter which electron acceptor is respired by the dominant microbes (Gudasz et al., 2010). Water temperature is indeed correlated with pore water concentrations of the plant nutrients N, P, and K released by decomposition, which are also significantly correlated with either pore water sulfide or AVS (Table 1). Although sulfide production may be enhanced by elevated temperature, the correlation between sulfide and temperature is weak (rho = 0.17; $p > 0.05$).

The production of sulfide plausibly contributes to the significant correlation of six of the nine variables with wild rice occurrence: P and N in surface water, Fe and K in pore water, and S and Se in sediment. Water temperature is significantly correlated with three of these variables (P, N, and K), and therefore, synergistically reinforces their associations with the absence of wild rice. Elevated surface water P and N are further synergistically associated with the absence of wild rice because their release via MSR-driven mineralization also enhances phytoplankton growth, reducing light available to wild rice seedlings; P and N are highly correlated with reduced transparency ($p < 0.001$, Table 1).

In this data set greater water depth is negatively associated with the occurrence of wild rice, not because wild rice grows better in shallower water, which it may, but because of where field crews took samples when wild rice was not present. Following a decision tree (Table S1), when wild rice was not present, field crews usually sampled among water lilies, which, on average, were observed in slightly deeper water than wild rice (67 cm compared to 52 cm).

Finally, *multiple* BLR was employed to investigate the question of whether any of the correlated variables provided additional explanatory power for the occurrence of wild rice beyond the models with sulfide, transparency, or temperature as base predictors. Surface water temperature is predictive of wild rice presence independent of sulfide ($p = 0.03$). Pore water K improves models based on sulfide ($p = 0.02$) or temperature ($p = 0.004$) alone but provides no significant additional explanatory power beyond a model built on both sulfide and temperature ($p = 0.07$). Overall, multiple BLR analysis confirms that other variables provide no additional explanatory power and that the three base predictors are independent of each other.

Multiple BLR was used to model the probability of wild rice occurrence (WR presence) as a function of pore water sulfide (pw sulfide, in mg L^{-1}), water transparency as measured by the Secchi tube (trans, in cm), and water temperature (Temp, in °C):

$$\text{Logit}\,(\text{WR presence}) = 0.532 + 0.0183\,(\text{trans}) - 1.169\,(\log10\ \text{pw sulfide}) - 0.107\,(\text{Temp}) \tag{2}$$

where odds = exp(logit) and probability = (odds/(1 + odds)).

Pore water sulfide and water transparency are significant variables in the multiple BLR ($p = 0.012$ and $p = 0.016$, respectively), whereas water temperature is only marginally significant ($p = 0.056$). To visualize the interaction of the variables, a 3-D plot was constructed that predicts the probability of wild rice occurrence as a function of sulfide and transparency, while holding constant the marginally significant variable, temperature, at the median value of the Class B data set (23.2 C; Figure 4). Within the range of variables in Class B, modeled probabilities of wild rice occurrence range from a high of 89.8% (trans = 100 cm, which was the maximum of the measurement device, and pw sulfide = 11 $\mu g\ L^{-1}$, which was the reporting limit) to a low of 9.6% (trans = 3 cm, which was the minimum observed, and pw sulfide = 2,000 $\mu g\ L^{-1}$). Modeling was cut off at a sulfide concentration of 2,000 $\mu g\ L^{-1}$ because only three Class B sites had pore water sulfide greater than 2,000 $\mu g\ L^{-1}$ and none of the three hosted wild rice.

3.6. Field Variables not Evidently Associated With Wild Rice Presence and Absence

Some variables included among the initial hypotheses, or in the past cited as important attributes of wild rice habitat, were not found to be significantly associated with wild rice presence and absence. These include sediment TOC ($p = 0.79$, Figure 2j; DNR, 2008; Lee, 1986; Moyle & Krueger, 1964), flocculent sediment (quantified here as the sediment water content; $p = 0.72$, Figure 2h; DNR, 2008; Lee, 1986; Moyle & Krueger, 1964), sediment N ($p = 0.68$, Table 1; Carson, 2002; Walker et al., 2010), sediment P ($p = 0.27$, Table 1; Carson, 2002; DNR, 2008), and surface water alkalinity ($p = 0.28$, Table 1; Moyle, 1944).

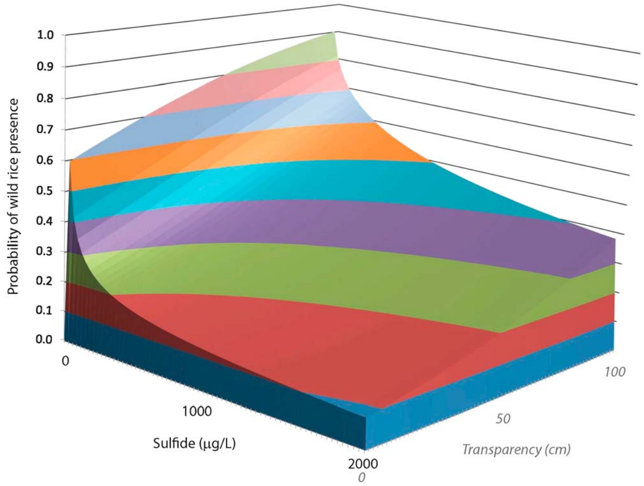

Figure 4. Visualization of the relationship, determined by multiple binary logistic regression, between water transparency and pore water sulfide in controlling the probability of wild rice occurrence at 23.2°C, the median temperature for all sites in the class B data set.

Reduced sediments are generally regarded as producing pore water constituents that are potentially toxic to rooted plants; potentially toxic agents, in addition to sulfide, include ammonia and Fe (Pezeshki & DeLaune, 2012). Analysis of the binary logistic regressions showed no evidence for ammonia toxicity (BLR $p = 0.45$, Figure 2l) or Fe toxicity (wild rice presence is positively correlated with pore water Fe, BLR $p = 0.01$, Figure 2f). As a product of decomposition, ammonia is correlated with pore water sulfide, K, and silica (Spearman correlations $p < 0.001$, Table S4).

The field data provide little evidence that elevated sulfide production excludes wild rice by producing Fe, Cu, or Zn deficiency (Table S3). The possibility that *low* pore water Fe limits wild rice growth is difficult to distinguish from sulfide toxicity because of the strong negative correlation between pore water Fe and pore water sulfide (rho = −0.58, $p < 0.0001$, Table 1). Hydroponic studies using chelated Fe in combination with elevated sulfide (Pastor et al., 2017) show reduced growth, implying that the mechanism is sulfide toxicity, rather than Fe limitation, assuming that the chelated Fe remains bioavailable in the presence of sulfide (Li et al., 2009). The median pore water Fe levels at sites with and without wild rice are similar, 5.7 and 5.9 mg L^{-1}, respectively; average levels are 11.0 and 7.0 mg L^{-1}, respectively, which produced the significant BLR positive correlation between pore water Fe and wild rice presence. On balance, there is little evidence to support the hypothesis that elevated sulfide reduces wild rice growth through Fe deficiency rather than by direct sulfide toxicity. Pore water Cu concentrations were often below the detection limit in the field sites sampled, precluding analysis of their correlation with wild rice presence and absence. The BLR for pore water Zn concentrations with wild rice presence and absence was not statistically significant (data not shown). Neither Cu nor Zn had a significant Spearman correlation with pore water sulfide (data not shown), unlike Fe.

3.7. Seasonality in Field Variables

Fifteen natural wild rice sites were sampled 3 to 5 times in 2013 to determine which field variables exhibit statistically significant seasonal trends that might be important to consider in the overall analysis, given that different sites were sampled in different months. Some variables conformed to expected seasonal trends, such as water temperature ($p = 0.001$), which increased in early summer, peaked about 1 August, and then declined. Some of the surface water variables exhibited monotonic increased concentrations over the summer (alkalinity, Na, Mg, and SO_4; $p = 0.0009$, 0.008, 0.021, and 0.05, respectively). One hypothesis that explains this observation is that waterbodies in Minnesota, after being diluted by spring snowmelt, in general become more concentrated as the summer progresses due to evaporation and, for waterbodies with shorter residence times, greater influence of groundwater flux. Cl and Ca had nonsignificant positive slopes ($p = 0.29$ and 0.37, respectively), whereas K exhibited no trend ($p = 0.48$). As expected, none of the solid-phase variables of the homogenized 10 cm long sediment cores showed any significant seasonal trends (e.g., sediment extractable Fe $p = 0.93$, sediment TOC $p = 0.96$). Among the pore water variables, only K and pH exhibited significant trends. Pore water K values decreased over the summer ($p = 0.006$), likely as a result of diffusion into surface water or uptake by growing rooted macrophytes. Pore water pH generally increased over the summer ($p = 0.003$), which may reflect loss of CO_2 as the sediment warmed over the summer, reducing the solubility of gases. The lack of seasonality in pore water sulfide ($p = 0.62$) indicates that sulfide concentrations were in steady state with the variables that exert primary control over its concentration, surface water SO_4, extractable Fe, and sediment TOC (Pollman et al., 2017). Of the variables controlling pore water sulfide, only SO_4 exhibited even marginally significant seasonal trends ($p = 0.05$, 0.93, and 0.97, for SO_4, extractable Fe, and sediment TOC, respectively). Despite the finding of seasonal variation in SO_4, Pollman et al. (2017) found that SO_4 is one of the primary variables that control pore water sulfide concentrations. Seasonal variation in SO_4 concentrations undoubtedly contributes to noise in the statistical relationship documented by Pollman et al. (2017). Myrbo et al. (2017) found that the microbial production of pore water sulfide in a wild rice sediment is proportional to the long-term average SO_4 surface water concentration.

3.8. Cultivated Wild Rice Paddies

Commercial wild rice paddies have been reported with healthy stands of wild rice growing under surface water SO_4 levels as high as 170 mg L^{-1} (Aiken et al., 1988). Our limited measurements in seven different paddies ranged from 0.3 mg L^{-1} to 279 mg L^{-1} SO_4, with a median of 8.3 mg L^{-1} (n = 7, Table S3). Natural wild rice waters in our study had a median SO_4 concentration of 4.1 mg L^{-1}. Surprisingly, median pore water sulfide in paddies was 182 μg L^{-1}, greater than the medians of natural wild rice waters (88 μg L^{-1}; n = 67) and waters without wild rice (126 μg L^{-1}; n = 41). The median sediment extractable Fe concentration of paddies (4.5 mg g^{-1}) is similar to that of natural wild rice waters (4.8 mg g^{-1}). In contrast, median sediment TOC is much higher in paddies (25.2%) than natural wild rice waters (9.1%). It thus appears that the greater TOC driving enhanced SO_4 reduction (Pollman et al., 2017) and driving median pore water Fe lower than in natural wild rice sediments (1.6 and 5.7 mg L^{-1}, respectively), despite the similar reservoirs of Fe in the sediment. Elevated production of sulfide coupled with the consumption of the available Fe may put some paddies on the brink of sulfide toxicity to wild rice.

However, the physical setting of cultivated wild rice paddies differs from that of natural lakes and streams in a number of important ways. Often paddies and their surface sediments are dewatered during the growing season through use of buried drainage tiles, allowing tillage after harvesting and enhanced aerobic decomposition of rice straw and roots, and possibly reoxidation of sulfide and reduced Fe. Prior to drainage tile installation in the 1980s, failure of wild rice crops was sometimes attributed to elevated sulfide (Grava & Rose, 1975; Gunvalson, personal communication, 2016). Water depth in paddies is also typically shallower (median of 30 cm) than wild rice habitat in natural lakes and rivers (median of 52 cm), allowing seedlings to emerge above water with less energy expended, photosynthesize sooner, and release oxygen from roots to oxidize sulfide. The use of nitrogen fertilizers may further enable seedlings to elongate more quickly through the water and into the air. Fertilized plants have been shown to be more resistant to sulfide toxicity than are control plants (Geurts et al., 2009). The production of sulfide would be inhibited if N fertilizers were applied as nitrate—but N fertilizers are applied as ammonia or urea (Oelke et al., 1997).

3.9. Conclusions and Implications

Analysis of an extensive suite of physical and chemical parameters from 108 different sites with potential wild rice habitat shows that pore water sulfide toxicity is a primary biogeochemical factor controlling the occurrence of wild rice populations in otherwise favorable habitat. High concentrations of pore water sulfide greatly decrease the probability that a wild rice population will be found in a waterbody. When pore water sulfide is low enough to support reproducing wild rice populations, however, it is likely that the relationship between sulfide and wild rice becomes more complicated and analysis of cause and effect more ambiguous. The variation in sulfide concentration is correlated to the density of wild rice, at least partially as a result of oxygen release from roots, and thus, not only does sulfide affect wild rice but wild rice affects sulfide.

Aside from low pore water sulfide, favorable wild rice habitat has long, cold winters and transparent surface water in the ice-free season. *Zizania palustris* seeds germinate at low rates if the winter is too warm or too short. The probability that wild rice seedlings successfully grow to maturity is reduced if photosynthesis is inhibited by low water clarity. Thus, pore water sulfide (itself a function of surface water SO_4, sediment Fe, and sediment organic matter (Pollman et al., 2017)), water temperature, and water transparency together largely determine wild rice presence and absence. These three factors are independent of one another but may act synergistically on other related processes.

In addition to generating sulfide, SO_4 reduction supports organic matter mineralization that releases nutrients and alkalinity to the surface water, which has the potential to change plant community structure even if Fe is high enough to keep pore water sulfide from reaching phytotoxic levels (Myrbo et al., 2017). Natural and anthropogenic SO_4 loading to freshwaters may thus strongly affect ecosystem composition and function, despite the low direct toxicity of SO_4 under oxic conditions.

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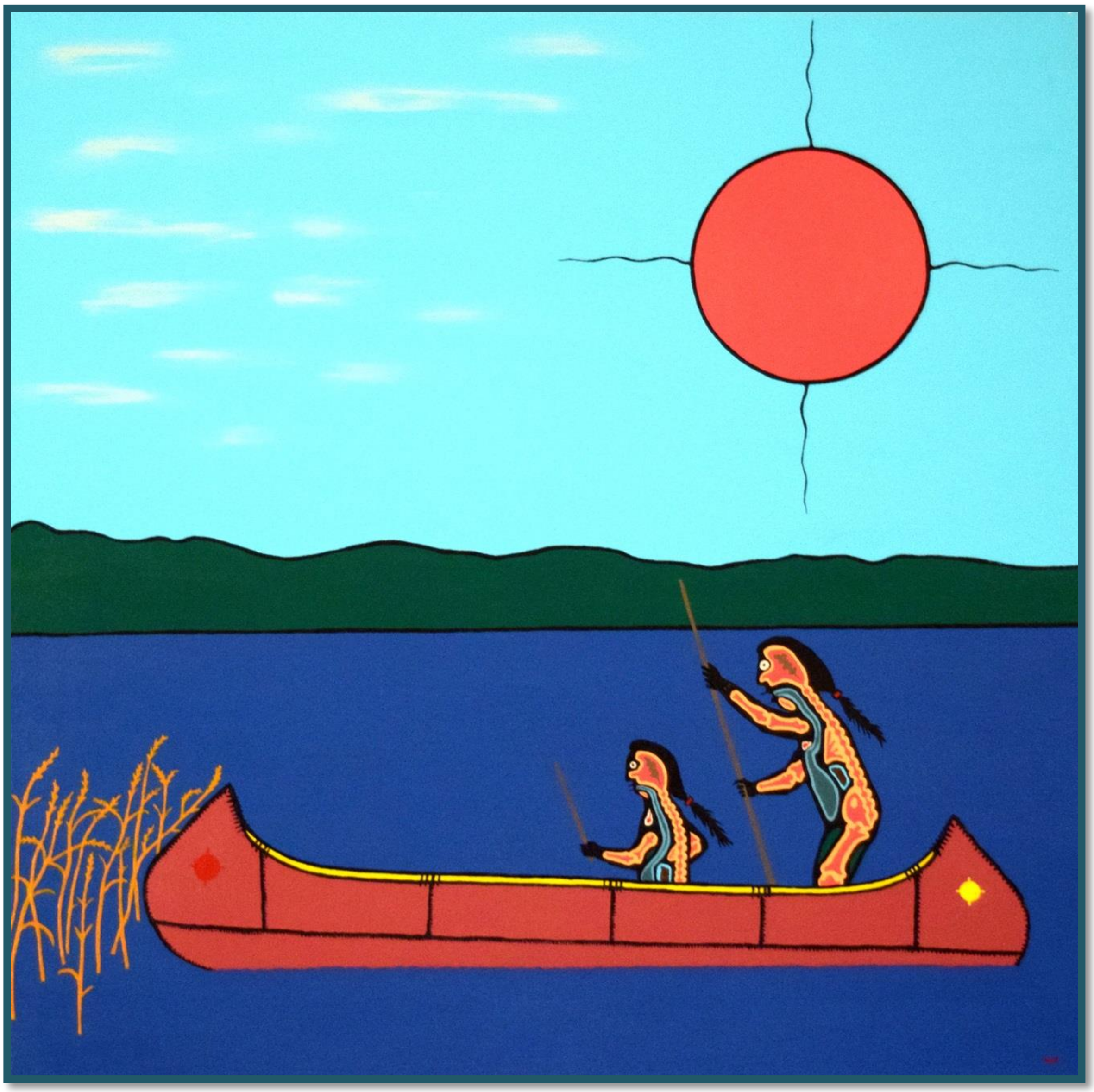
Wild Rice Monitoring Handbook

By Tonya Kjerland



Wild Rice Monitoring Handbook

Companion to *Wild Rice Monitoring Field Guide*

Electronic copies of this document and the *Wild Rice Monitoring Field Guide* are available at: www.seagrant.umn.edu/coastal_communities/wildrice

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“Because we can’t speak the same language, our work as scientists is to piece the story together as best we can. We can’t ask the salmon directly what they need, so we ask them with experiments and listen carefully to the answers. We stay up half the night at the microscope looking at the annual rings in fish ear bones in order to know how the fish react to water temperatures. So we can fix it. We run experiments on the effects of salinity on the growth of invasive grasses. So we can fix it. We measure and record and analyze in ways that might seem lifeless but to us are the conduits to understanding the inscrutable lives of species not our own. Doing science with awe and humility is a powerful act of reciprocity with the more-than-human world.”

~ From “Braiding Sweetgrass” by Robin Kimmerer

Preface

The methods described in the Wild Rice Monitoring Handbook have been designed to respect Native American, First Nation, and like-minded peoples' views on the sacred nature of wild rice.

The Handbook establishes a standardized method for measuring wild rice biomass and productivity. It is a comprehensive reference for designing wild rice surveys. The Handbook is a companion to the Wild Rice Monitoring Field Guide, which supports crews working to monitor wild rice populations. The Field Guide describes how to collect "core wild rice variables" and offers aid in identifying common aquatic plants that often occur with wild rice. The Handbook includes the field sampling protocols from the Field Guide, as well as generic wild rice biomass equations, information about the spiritual and cultural significance of wild rice, and a review of the biology of wild rice. It also presents a case study illustrating how data collected using these methods may be applied. It includes guidelines for setting up a monitoring plan, instructions for determining the number and location of sample points, instructions for creating site- or area-specific biomass equations, and blank field and lab data sheets. The Handbook also provides decision trees and tables to guide managers with decisions necessary to quantifying wild rice abundance and distribution.

The measurements recommended in the Field Guide and the Handbook will be most useful when taken over a series of years and used to assess trends on a given water body. These methods are not intended to establish relative condition or productivity between (or across) waters where wild rice grows. These are also not methods for identifying productive or unproductive waters with reference to wild rice.

These methods are designed to be flexible enough to allow for applicability in a range of situations and across a broad geographic range. For example, they may be used in different types and sizes of water bodies and also, with different species or varieties of wild rice.[1] Two species of annual wild rice are known to grow in Minnesota, Wisconsin, and Michigan, with hybrids occuring where their ranges overlap—*Zizania palustris* and *Zizania aquatica*. There is debate about whether these are two distinct species or varieties of the same species. One treatment of wild rice taxonomy further subdivided each species into two varieties (Aiken et al., 1988). *Zizania palustris* var. *palustris* is the variety most commonly found in the northern parts of the states and Canada where wild rice is harvested for food and commercial purposes.

The generic biomass equations are based on *Zizania palustris* var. *palustris* (northern wild rice). The core wild rice variables may be used with any species or variety of wild rice. If concerned about the accuracy of using generic wild rice biomass equations, consider developing site- or area-specific equations according to the instructions provided.

Aquatic vegetation survey manuals were consulted in order to create this Handbook, and these are listed in the References. The scope of this Handbook is principally wild rice, a unique emergent annual plant. The scope of other manuals usually is much broader, for example: surveys to identify a list of aquatic

[1] References for wild rice taxonomy: Aiken, et.al. (1988, pp. 21-38); Dore (1969, pp. 16-23); Meeker (1993, Ch. 3 in Ph.D. Dissertation).

plant species in addition to other organisms present in a lake, measurements of biodiversity, or locations of specific species (i.e. rare/endangered plants or plants considered a nuisance for recreational purposes). Another common purpose of aquatic vegetation survey manuals is to assess overall lake or wetland conditions and status, all of which are beyond the scope of this Handbook. The Field Guide offers a basic plant identification key for common plants that occur with wild rice.

The methods described in this Handbook would be relatively easy to adapt for use with aquatic plant manuals that have a broader scope by adding two parameters: a count of wild rice stalks in quadrats plus plant height of one sample plant per plot. By using Biomass Equation 1 and plugging in plant height, a measure of biomass would be obtained. The number of recommended sample points per water body (40) is in keeping with recommendations from most manuals reviewed or in some cases, considerably lower (some recommended up to 100 points).

Variables such as estimating wild rice stand area take more time to collect but enable computation of important variables for wild rice persistence including biomass per area, number of stalks per area and number of stalks per water body. Area of wild rice is straightforward to compute in shallow lakes where the entire lake is potentially wild rice habitat. In a study comparing emergent plant mapping of bulrush stands on five “deep” lakes (91 to 587 ha in surface area), Radomski et al. (2011) found that the time to carefully map bulrush on study lakes was about two to three 8-hour days per lake. Mapping wild rice would be expected to take less time because it usually grows in large contiguous stands.

Monitoring of lakes and rivers is an ongoing process and various agencies are measuring a range of parameters. Many agencies may already be collecting data related to water quality and sediment through existing agreements. Therefore, this Handbook does not attempt to define methods for collecting this type of data, which are well documented elsewhere. Instead, it provides recommendations for which parameters might be most important to measure if concerned about wild rice. How these parameters relate to the ecology of wild rice is unknown, but by establishing a standardized method for estimating wild rice growth, there is hope that new discoveries will emerge.

Overview

This Handbook establishes a standardized method for measuring wild rice biomass and productivity. These methods may be adapted to measure productivity for an entire lake, stream reach, or flowage.

Applications include:

- ✓ Monitoring wild rice productivity trends
- ✓ Relating trends to harvest, water quality, or weather
- ✓ Evaluating outcomes of management actions
- ✓ Informing adaptations to stressors such as climate change
- ✓ Evaluating success of restoration projects

This is a comprehensive reference for use in designing wild rice monitoring surveys and inventories, for analyzing data, and for communicating with others via a shared set of protocols. The Field Guide is a more portable version that focuses on field data collection.

GLOSSARY

Standardized method. A standardized method is one that defines procedures for collecting data in a statistically valid manner that can be easily reproduced and will provide consistent, accurate measurements each time, allowing trend analysis across years and locations.

This Handbook provides guidance about decisions that need to be made in order to quantify wild rice abundance and distribution. Use the "decision tree" charts and tables to choose which portions to incorporate. For example, the number of plots to sample is based on the amount of statistical precision you require and estimated biomass each year. Field and lab methods are explained in the Standard Operating Procedures. The Case Study illustrates some potential uses for the data collected. Helpful solutions for common concerns are included in the section, "Problems Faced When Doing Wild Rice Inventories and How to Solve Them." Appendix A includes data sheets for use in field and lab data collection.

These methods have been designed to respect Native American, First Nation, and like-minded peoples' views on the sacred nature of wild rice. Supporting the sustainability of natural wild rice populations is a primary goal of this project.

Summary of the Field Methods

- Stalk density with the quadrat frame.
- Water depth within the quadrat frame, or as close as possible.
- Sample plant height, measured one of two ways: either ABOVE WATER or TOTAL.
- Seed heads from the sample plant so the pedicels can be counted back in the lab.
- The names of other plant species within the quadrat frame.
- If creating a site- or area-specific biomass equation, collect whole wild rice plant (optional).
- Field notes.
- Related environmental variables (optional): sediment and water quality.

Cultural and Spiritual Significance

CONTEXT FOR BUILDING A COMMON GROUND

A valuable resource for all. Wild rice is significant to communities in northern Minnesota, Wisconsin, and Michigan. Historically, wild rice has been an important food source for thousands of years in these areas, and continues to be today. Wild rice is the only North American wild grain that produces substantial amounts of food for humans. Early European immigrants to the north country valued wild rice as a vital part of their food supply, and many non-Indians harvest wild rice. It is hoped that with more research, education, and outreach, the public will become more aware of this valuable resource and realize the importance of preserving natural stands of wild rice for future generations.

For Native American communities. Wild rice is as vital a cultural resource today as it was in the past. Wild rice is essential to many Native American communities - culturally, spiritually, socially, and economically. Many tribes of the region have long traditions of harvesting wild rice – Ojibwe, Lakota, Potawatomi, Menomonie, and Ho Chunk, among others.

Harvesting wild rice is a very important family and community activity. It provides a significant amount of food, and it is also a tradition that has been passed down through hundreds of generations. Passing along this traditional way of living is a way to connect people across time to their grandparents; a way to educate and strengthen young people in their awareness of who they are and where they come from.

For Ojibwe. Wild rice is featured prominently in the origin stories and traditions of one of the largest tribes in North America, the Ojibwe, also known as Chippewa, or Anishinabeg. Ojibwe nations are prominent in the states of Michigan, Wisconsin, and Minnesota. There are also many Anishinabe nations and related tribes in the Canadian provinces of Manitoba, Ontario, and Quebec.

The Ojibwe migration story tells of a time when Ojibwe ancestors lived in the east next to the ocean in the areas that are now called Maine and Nova Scotia. People of the Abenaki tribe in present day Maine still remember the ancient connections with Ojibwe people and have prophecies and stories that correlate with the Ojibwe stories. Both speak Algonquian-based languages.

Some say that prior to the Ojibwe migration, the Anishinabeg ("the people" in Ojibwe language) received a prophecy to move westward, where they would find "the food that grows on the water." Over many generations, the Ojibwe migrated west, where they found wild rice, or *manoomin*.

Contributing to community resilience. A cornerstone of social unity, the gathering and processing of wild rice during the early fall plays an important role in maintaining family and community ties. Wild rice harvesting is part of the traditional life ways of Anishinabeg that still follow the seasonal patterns of food availability. Another example is maple syrup, which is harvested by many in the early spring. Ricing is such an important activity that one of the months is named “manoominike-giizis”, or wild rice moon, which occurs in either August or September. Many people living in urban areas return to the reservation to help with wild rice harvesting and processing. Extended families and friends work together, and children learn from their elders. This provides a means of strengthening ties and passing along wisdom of all kinds, including how to rice and how to preserve the rice.

Economic benefits. Wild rice is also significant economically for tribes because sustainably harvested food constitutes a major portion of the diet. Numerous people still rely heavily on natural foods that they can harvest themselves such as wild rice, maple syrup, fish, deer, and moose. In addition, tribal governments gain financially through programs to harvest and sell wild rice. Each year families who are able to harvest wild rice are also able to supplement their income from its sale or trade, if they so choose.

In these ways, wild rice feeds the people, heals the people, and reunites the people. The preservation of wild rice for generations to come will have lasting benefits for everyone.

How to Respect Native Traditions When Conducting Wild Rice Studies

In order to be respectful of the cultural and spiritual significance of wild rice, there are important protocols to follow.

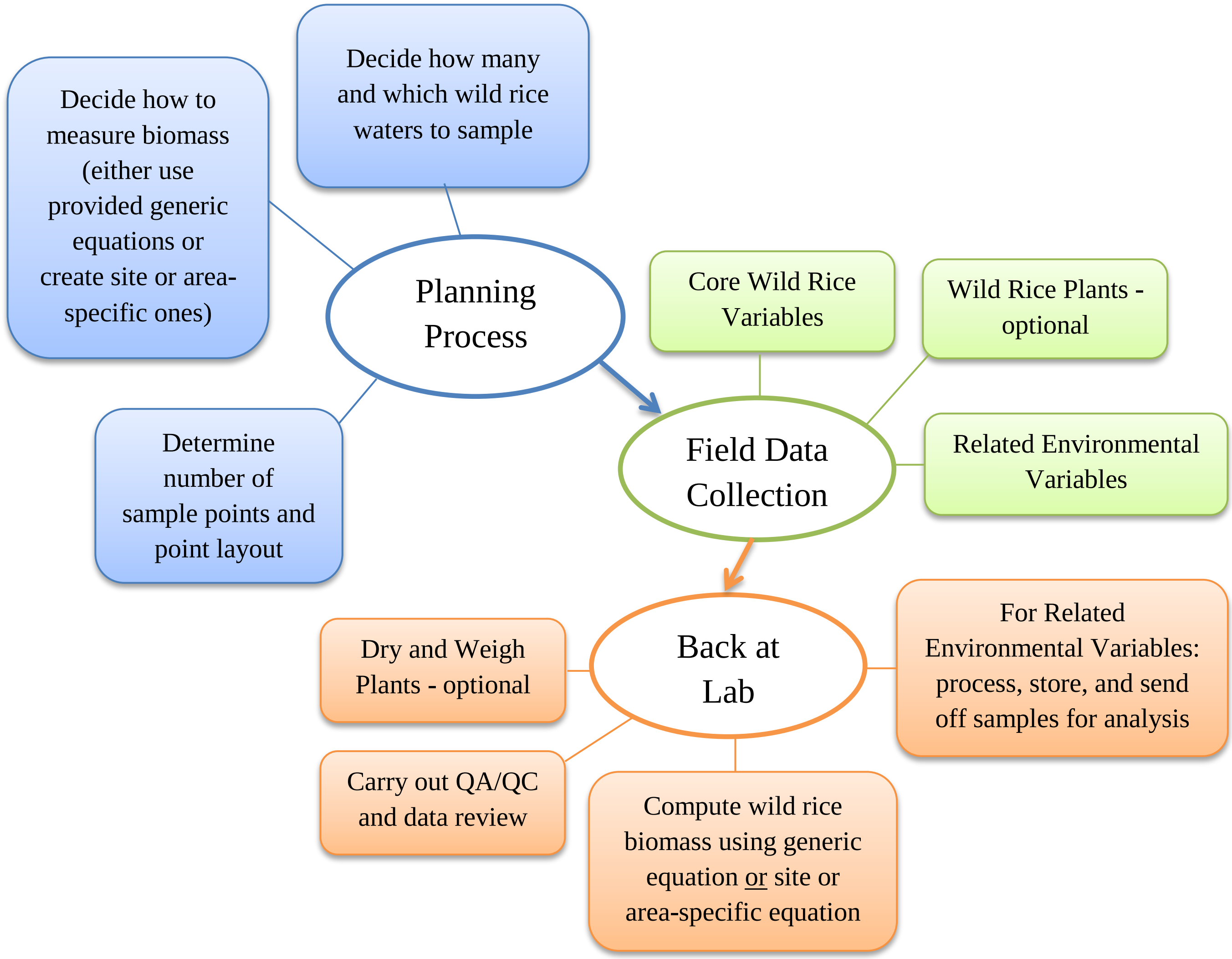
- Obtain appropriate permissions and know the cultural boundaries for research with wild rice
- Put down tobacco in the water before taking samples or collecting data
- Offer a prayer of gratitude and statement of your good intentions

The prayer can take many forms. Speak in your own words and according to your own religious traditions.

Sampling Design

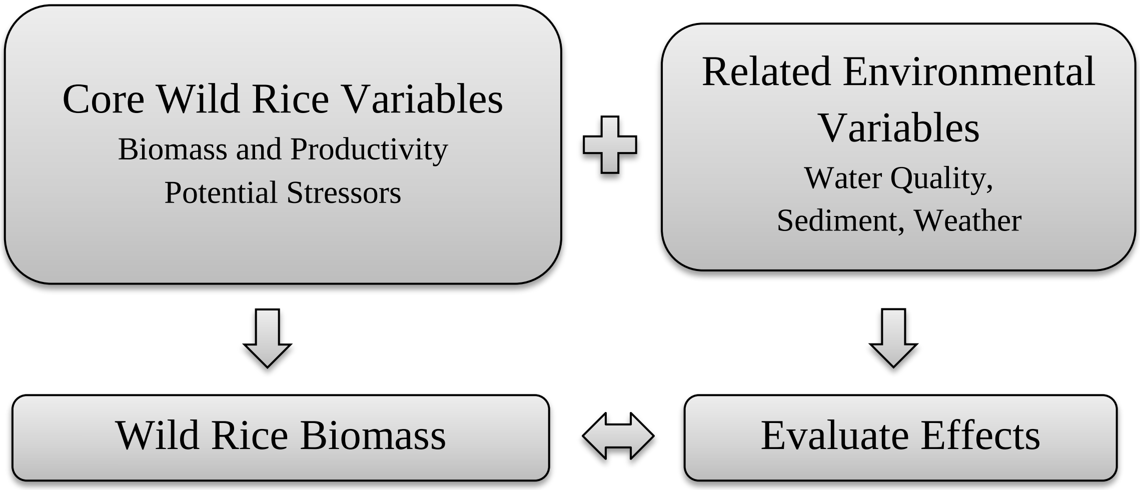
This overview recommends data to collect and provides guidance for designing the sampling plan. The main decisions to be made are: 1) how many and which wild rice waters to sample; 2) how to measure biomass; and 3) the number of sample points and point layout (see Figure 16).

Figure 1. Mental map of events in sampling process



Regulations pertaining to wild rice. Rules and protections for wild rice exist in many areas. If you are considering physically collecting wild rice plants, think carefully about whether this is necessary and then check into tribal, state, and other laws to determine if you need a permit to collect plants. Permits may also be required for collecting seeds and seed heads.

The sampling design includes two categories of variables, "Core" and "Related Environmental." The Core Variables are designed to accurately and objectively measure wild rice productivity (See **Table 1**). Measuring the related environmental variables will aid in evaluating trends and diagnosing problems.



CORE WILD RICE VARIABLES

The core wild rice variables are a set of carefully selected parameters that, taken as a whole, provide useful information to assess the health of wild rice populations. In addition, either plant height or seed number can be used to compute plant biomass by using a generic model.

GLOSSARY
Biomass is another name for the "weight" of an individual or group of organisms. This Handbook uses grams per square meter (g/m^2) as the measuring unit for wild rice biomass. If desired, whole lake production can be estimated by measuring or estimating the wild rice area.

Biomass is a commonly used measure of plant productivity that relates directly to important variables for wild rice, including plant health and number of seeds produced. Biomass estimates may be used to compare productivity for a single lake, flowage, or river reach from year-to-year; and, to compare general trends between different locations (increasing, decreasing, no change).

Collect the information listed in the first two columns of variables listed in Table 1 to adequately monitor wild rice populations. The last optional column requires collecting wild rice plants. Do this if you want to create a site-specific biomass equation. A chart comparing these two options is shown in Figure 2. A decision tree for deciding how to measure biomass is provided in Figure 3.

Table 1. Core wild rice variables

Core Wild Rice Variables		Optional
Biomass & Productivity (Annual Yield)	**Potential Stressors (Field Notes)**	**Plant weight measured directly**
Density (number of stalks per area)	Observed shoreline use	Plant dry weight
Average Stem Height	Observed water use	Number of viable (filled hulls) and non-viable seeds collected
Water depth	Brown spot fungal presence and severity index	Calculate new site-specific biomass equation
Number of potential seeds (# pedicels per stalk)	Animals, birds, pests, pathogens presence	Presence of worm holes in seeds (observed in the lab)
Presence of other plants co-occurring with wild rice (List)	Weather (current and past 2-3 days)	
Estimate of wild rice stand area	Other possible concerns for wild rice growth (i.e. pollutants)	

Estimating wild rice stand area. It is useful to create an approximation of the outline of areas where wild rice is found growing each year. Knowing this area is essential to computing overall biomass and for mapping challenges, such as interpolating values between sample points. Because using GPS to outline wild rice beds is subjective; the accuracy of area measurements may vary between surveyors. Areas may move considerably year to year due to the variability of wild rice growth. In order to standardize these approximations, it is recommended that whoever does the work be given clear instructions, make notes on what criteria they used to determine where to map and that the same crew assess each area each year. Because of GPS inaccuracy and field technician subjectivity associated with collecting this type of data, it should only be used as an estimate for comparing year-to-year variability *within* a specific waterbody. It is not intended to provide a mechanism for assessing relative condition or productivity *between* (or *across*) wild rice waterbodies.

Multiple methods for estimating the area of wild rice stands are described in Appendix B. The two methods recommended in these field sampling protocols and in the Field Guide were chosen due to their ease of implementation.

BIOMASS EQUATIONS

Generic equations. One way to measure biomass involves collecting wild rice plants, drying, and weighing them. However, this Handbook presents a short-cut way to approximate biomass that requires simple, non-destructive field methods and generic equations developed by collecting plants from a variety of different sources.[2] Generic biomass equations were created from pooled data of *Zizania palustris* var. *palustris* plants derived from six wild rice waters in Minnesota and Wisconsin (data

[2] See SOP #4 for details about how these biomass equations were created.

collected in 2011 and 2014.[3]) There are two generic equations. One relates biomass to plant height and the other relates biomass to seed number. Measure plant height and stem density in the field and collect seed heads and count number of potential seeds per unit area. By measuring these plant characteristics, and then plugging in the results to either of the two possible equations, a reasonably good estimate of biomass is obtained in a non-destructive way. Some managers may wish to develop their own biomass equations, and the Handbook also describes how to do this. Points to consider in making this determination are elaborated upon below.

Site- or area-specific equations. If resources are available, the most accurate biomass equation would be based on an individual water body. If not, it is suggested to use the generic equations presented in this Handbook. In order to develop a site- or area-specific biomass equation, it is necessary to collect wild rice plants, dry, and weigh them. This process involves collecting roots, stems, and seeds of one sample plant per quadrat; then drying and weighing the materials in order to determine their actual biomass, or dry weight. The sample plant is chosen by selecting the plant in each quadrat closest or close to a pre-marked corner. Number of stalks on this plant must be counted in the field. Ideally, plants would be collected from a minimum of 40 quadrats spanning a range of plant sizes from short to tall. This would allow the final equation to be useful over a range of different years. The data are then used to compute site-specific biomass equations according to instructions described in SOP #5.

When to Use Generic Biomass Equations and When to Create a New One

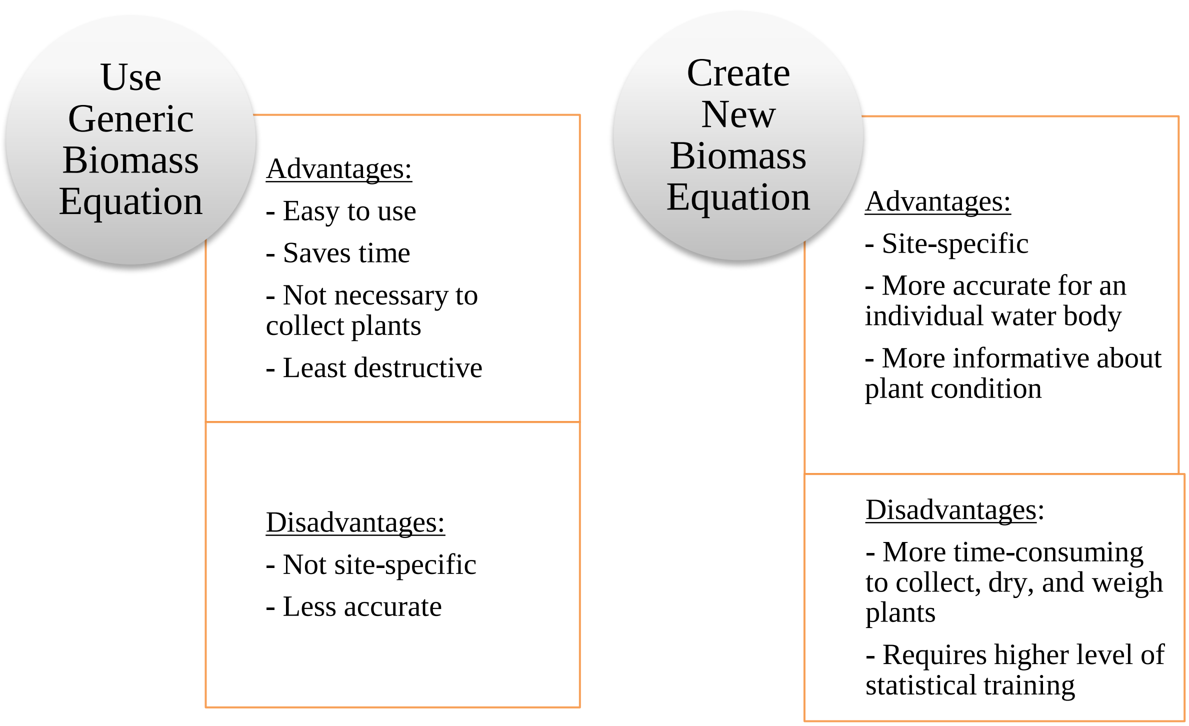


Figure 2. Advantages of creating site-specific biomass equations or using generic ones

[3] T. Kjerland analysis of data collected by Darren Vogt (2011) and Melissa Lewis (2014.)

Points to consider:

- Purpose of the study
- What levels of accuracy and precision are required
- Differences between wild rice stands and water bodies
- Alternative way to measure biomass
- Regulations and permitting

Purpose of the study. Clearly stating the study's purpose may help clarify which method to use:

  - If the purpose is to look at changes over time on a coarse-level, such as "increasing," "decreasing," or "no change," then using generic equations will suffice.
  - If the purpose is to explore what factors regulate year-to-year differences, such as water quality, use the generic equations.
  - If data collection might inform regulatory decisions, think about collecting whole wild rice plants and creating a site-specific or area-specific equation.
  - If the purpose is to accurately measure and compare biomass of wild rice plants in different water bodies, then it may make sense to create separate equations for each water body.

What levels of precision and accuracy are required? For most management studies, using the generic equations should be fine. But for more advanced studies, more accuracy may be required. The more accurate you are hoping to be with your estimate of biomass, the more you should be thinking about creating your own site-specific equation. In most cases, an equation should only need to be created once, and can be re-used in following years. The changes in morphological features of wild rice that are reflected in the generic biomass equations are expected to change slowly, but the rate is unknown and more research is needed in this area.

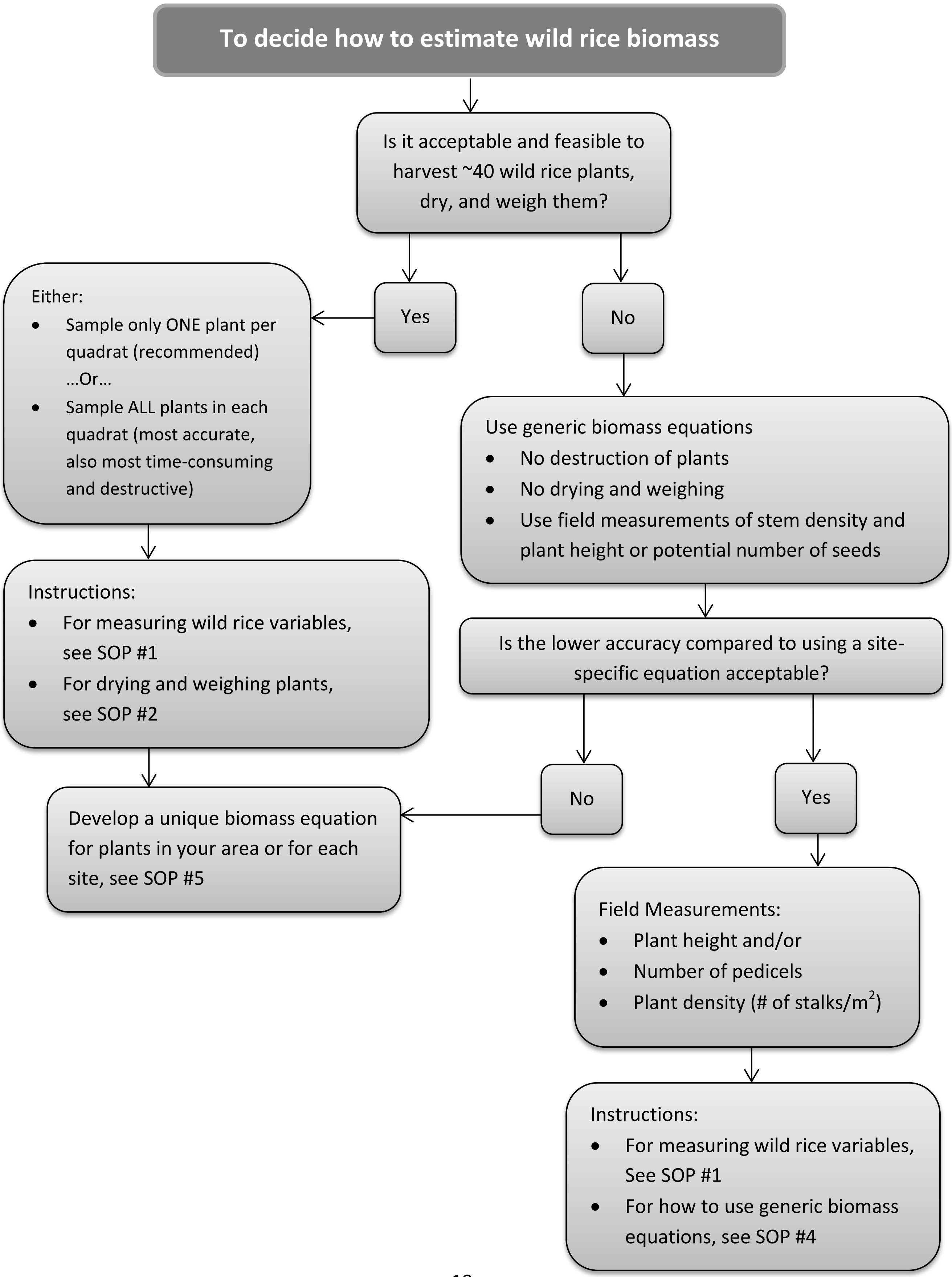
Differences between wild rice stands and water bodies. If it is not clear whether or not you need to develop your own biomass equations, one approach would be to conduct a pilot study of the wild rice in the water body you are measuring and compare results to the characteristics of the wild rice and associated water bodies used to create the generic biomass equations.

Data from the lakes used to create the biomass equations are provided in Appendix C.

Alternative way to measure biomass. The most accurate way to measure biomass would be to collect all of the wild rice plants within an area (e.g. quadrat) for drying and weighing. This method works well with some plant species and for scientific studies that demand high accuracy. In most situations for wild rice, this would be considered too destructive and time-consuming. For this reason, the methods in this Handbook recommend subsampling by selecting one plant per quadrat to measure.

Regulations and permitting. Rules and protections for wild rice exist in many areas. If you are considering physically collecting wild rice plants, think carefully about whether this is necessary and then check into tribal, state, and other laws to determine if you need a permit. Permits may also be required for collecting seeds and seed heads.

Figure 3. Decision tree for deciding how to estimate biomass



RELATED ENVIRONMENTAL VARIABLES

The "Related Environmental Variables" include additional laboratory measurements, water chemistry and sediment characteristics. Their purpose is to better understand the factors regulating wild rice growth. In order to link these two sets of parameters, it is important to sample them at the same locations and at the same time of year. The frequency of concurrent sampling of related environmental variables and core wild rice variables should occur at least every few years at a minimum of five (5) sampling points in each wild rice bed.

Standardized methods for measuring the related environmental variables are well established, and are not detailed in this manual (e.g. Elias et. al., 2008; see also Resources and Appendix D). Below is a list of the most important water quality and sediment parameters to consider for routine sampling in wild rice waters. This list is by no means exhaustive, but it is intended as a guide in cases where resources and/or time preclude comprehensive sampling.

Helpful Tip: A more complete list of variables to measure, rationale, estimated costs, and standardized protocols is provided in Appendix D.

Water Quality and Sediment Parameters

1. Parameters measured using electronic sensors (Temperature, dissolved oxygen, pH, specific electrical conductivity [EC25])
2. Alkalinity – Measure of acid-neutralizing capacity (ANC)
3. Transparency (secchi disk or transparency/secchi tube) – Trophic state indicator; proxy for color
4. Sulfate – Surface water concentrations can be biologically converted to toxic H_2S (hydrogen sulfide) gas in anoxic bottom water and in the sediment root zone.
5. Dominate Substrate Type - See Appendix D (Minnesota DNR, 2012, 1993).
6. Total Nitrogen (TKN) and Dissolved Inorganic Nitrogen (NH_4-N, $NO_{3/2}$-N) – Most likely limiting nutrient for wild rice
7. Total Phosphorus (TP) and Soluble Reactive Phosphorus (SRP) – Second most likely limiting nutrient for wild rice
8. Chlorophyll-a in open water

How to locate sample sites for related environmental variables. Reference (or "least impacted") sites located near wild rice sampling sites can help determine whether potential land use stressors or specific sources of pollution may be cause for concern. For this reason, the study plan might also include measurements of the related environmental variables at "reference sites" in addition to the places where concerns about negative impacts exist. In some cases, the reference sites may need to be located on a different water body, for example, when the whole lake is considered to be influenced by the potential cause for concern (i.e. entire lake surrounded by agriculture, residential development, etc)..

Suggested sites for measuring related environmental variables:

- Areas where **a major change in wild rice density** is noticed (to assess causes)
- **Stream inlets** (potential sources of nutrients and pollutants). In this case, also consider measuring in the middle of the lake and at an outlet site for comparison purposes.
- Land or water uses that could negatively impact wild rice stands, such as:
    - **Industrial discharges** (i.e. mining, power generation, etc.) – pollutant sources
    - **Waste water treatment facilities and sewage pond** discharges (leaching of phosphorus into water)
    - **Agricultural land** adjacent to water (nutrient, sediment, pesticide runoff)
    - **Roads and parking lots** (stormwater runoff, increased flashiness of storm water)
    - **Boating and jet skiing** (wakes uprooting wild rice plants, causing shoreline erosion, long tail boat blades chopping up wild rice and other plants, wave action re-suspending bottom sediments)
    - **Concentrations of homes** (lawn runoff, wakes from boats, herbicide use, clearing of plants for opening up water ways, leaching of nutrients from individual onsite sewage treatment systems, removal of shoreline vegetation that acts as a buffer strip)
- **Reference, or "least impacted" sites** for comparison

TIME AND LEVEL OF EFFORT INVOLVED

Allow more time the first year, and expect the time to lessen as field crews gain experience. The decision tree in Figure 4 illustrates a process for thinking about how many and which wild rice waters to sample based on the level of effort and time involved.

Many factors affect the time and effort involved, but as an example, the 1854 Treaty Authority reports that crews take 2-3 hours to measure the core wild rice variables for approximately 20 sample plots on 60 to 100-acre lakes. The estimated time to sample per point for these variables is about 3-5 minutes using a 0.5 m^2 quadrat. Assuming an additional 2 minutes to collect seed heads or whole plants and 2-3 minutes to travel between points, measuring 40 sample points should be completed in about 5-7 hours. Travel time to the water body and time to collect water or sediment samples (if part of the monitoring plan) should be added to estimate the total time required.

Helpful Tips
1) Try doing a dry run or pilot study of 5 points to determine the feasibility, number of sample points needed, and number of lakes to sample.
2) Take it slowly. Start in year 1 by only collecting the core wild rice variables on one lake. Add more variables and water bodies over time.
3) Practice using the GPS unit ahead of time on land and water.

Factors that may affect the time to complete sampling

- **Number of variables collected**. Sampling only the core wild rice variables takes 3-5 minutes per point.

- **Distance between points.** Distance may be adjusted in the initial setup of the sampling scheme; and should ideally be at least 30 m apart (MN DNR, 2012, Uzarski et al. 2014). See box, "Two-points-per-stop method" below for an exception to this rule, based on a sampling scheme of taking one set of measurements in a quadrat at the front of the boat, and one at the back.
- **Arrangement of quadrats.** Grid method is quickest because it involves following a straight line to navigate to points. Randomly-located points take longer to find.
- **Time to navigate to sample points.** The ability of the navigator to use the handheld GPS unit can be improved by practicing ahead of time.
- **Size of lake or river.** Affects the time to paddle from access point to sample points and the time to travel between points if they are spread further apart
- **Density of wild rice.** More dense rice takes more time to paddle through and more time to count rice stalks. In sparse areas, counting none to a few plants goes quickly (these type of sample points probably only take about 30 seconds).

TWO-POINTS-PER-STOP METHOD

Example from Fond du Lac Band of Lake Superior Chippewa:

If taking measurements at many GPS points is not feasible, consider halving the number of points and taking two measurements at each point or canoe stop. For example, rather than stopping at 40 different points, stop at 20 points and measure 2 quadrats. One measurement is taken by the person sitting at the front of the boat, and one in back.

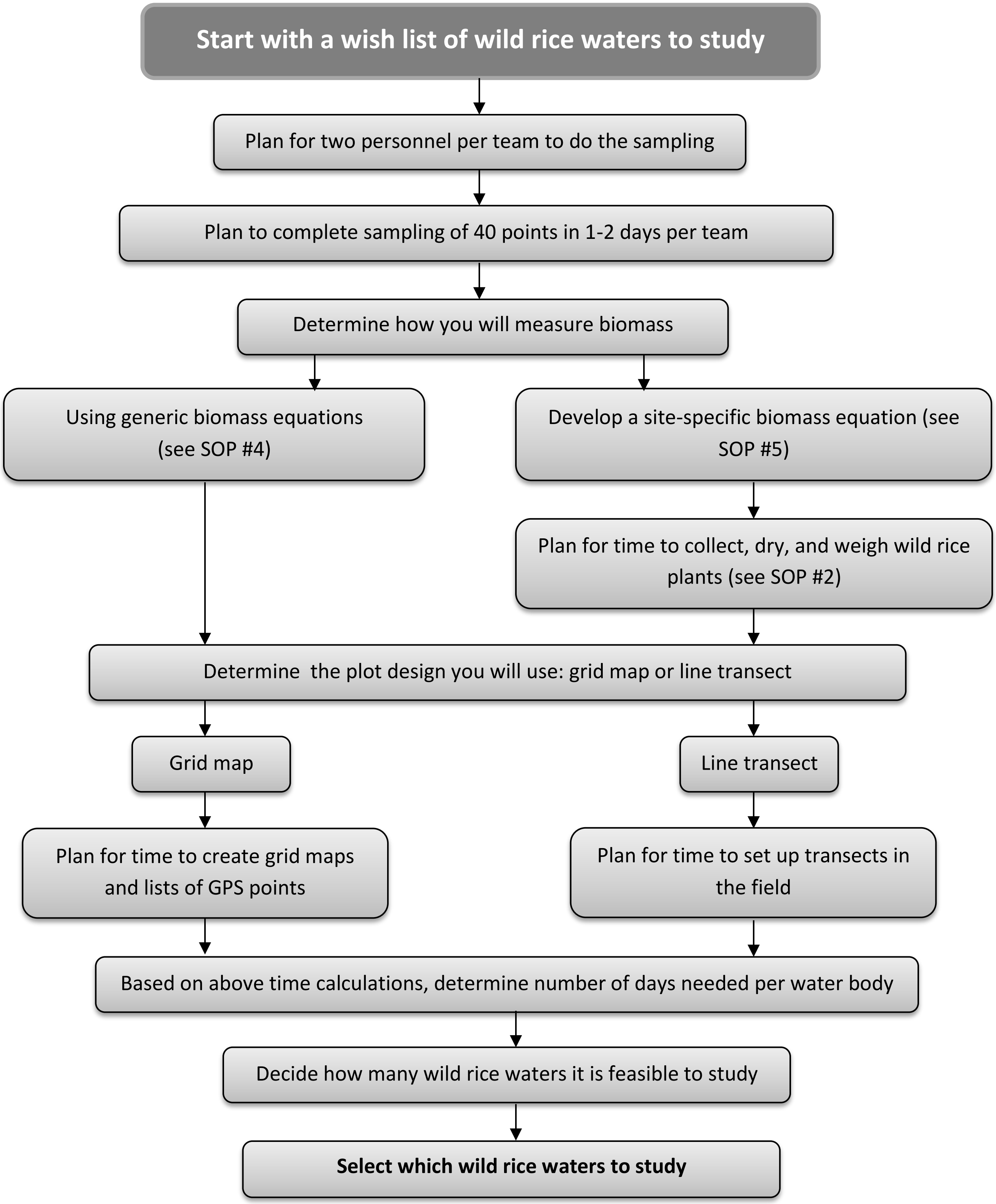
Be sure to be consistent about which side of the boat you take the measurements on (left or right each time.) This will avoid bias based in deciding which side looks "better."

By taking two measurements per stop, more data can be gathered in a relatively shorter period of time. This method may be a good choice if you are time limited, such as when also collecting data on related environmental variables.

The advantage of saving time should be weighed against the disadvantage that these two points are likely to be strongly correlated with one another due to proximity. The results will be more precise than if only 20 measurements were taken, but not as robust statistically as taking 40 samples that are the required distance apart. Analysis of the data collected by Fond du Lac Band of Lake Superior Chippewa in 2014 on Mud Lake showed positive correlations between two points collected per stop of 0.69 for plant height and 0.77 for stalk density.

Due to this strong correlation of paired quadrat points, the proper way to analyze paired points is to take their average and use this result as if it were one point for further analysis, such as for developing a new biomass equation. These averaged sample points will result in a lower variance compared to only sampling 20 points.

Figure 4. Decision tree for determining how many and which wild rice waters to sample



HOW TO DETERMINE THE `NUMBER OF SAMPLE POINTS

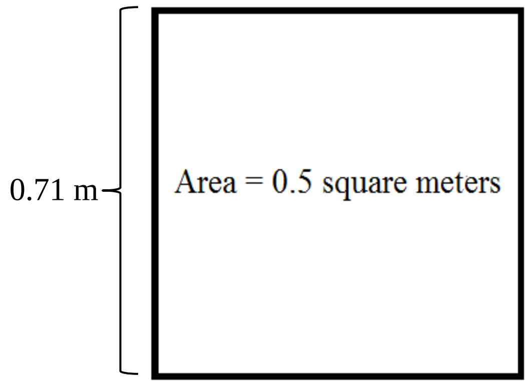
Three primary factors affect the number of sample points:

- ✓ Density of standing biomass
- ✓ Quadrat size
- ✓ Statistical precision desired

Size of area is a secondary consideration that may apply in some situations.

Density of standing biomass. The amount of sample points needed depends in part on the current year's biomass (units per area). More sample sites are needed in years when the rice is sparse to achieve the same level of accuracy as when rice is abundant.

Quadrat size. For reasons of efficiency, accuracy, and safety, the recommended quadrat is square, with a size of 0.5 m^2, which is 71 cm per side. A square-shaped quadrat is recommended because this is the shape used in many aquatic biomass studies and is easiest to construct and transport. See Appendix E for instructions on how to build a quadrat frame.



A 0.5 m^2 quadrat provides an efficient tradeoff between field
convenience and number of stems sampled in the typical range of stand densities in natural waters. This smaller size is also safest to prevent tipping when stalks are being counted from a canoe that is not equipped with anchors or outriggers.

Statistical Precision Desired. The level of statistical precision recommended is a standard error less than or equal to 20% of the mean. The number of sample points required under different sampling conditions and with different levels of precision are shown in Table 2. The shaded grey column is the recommended configuration. For more information, see Appendix F.


1854 Treaty Authority

Size of area. The same number of sample points should be used regardless of resource size. This recommendation is based on research showing size of a water body is not a factor in determining the number of sample points required for determining the amount of biomass or frequency of species occurrence (Downing and Anderson, 1985; Newman et al, 1998; MN DNR, 2012). However, for other reasons it may make sense to consider the area of wild rice, such as for mapping, for computing densities in different bays or separate wild rice beds, or for estimating overall amount of wild rice available. It is important to note that some scientists would argue that the level of sampling effort should be based upon size of the area sampled, and that this is a meaningful scientific debate.

Conduct A Pilot Study

For any size wild rice bed, consider using a pilot study to determine the number of points required. For example, for small areas (<10 acres) it may be that due to the homogeneity of the site, there is less variability among sample points, and therefore fewer points will be required to achieve the same level of statistical precision.

Zone technique for determining number of sample points. For large acreage wild rice waters with scattered beds of rice or deep lakes, consider using a zone technique for determining the total number of points to sample. Estimate the area of each wild rice stand (See Appendix B). Divide the water body into zones based on wild rice distribution. Sub-sample from the grid five points in each zone.

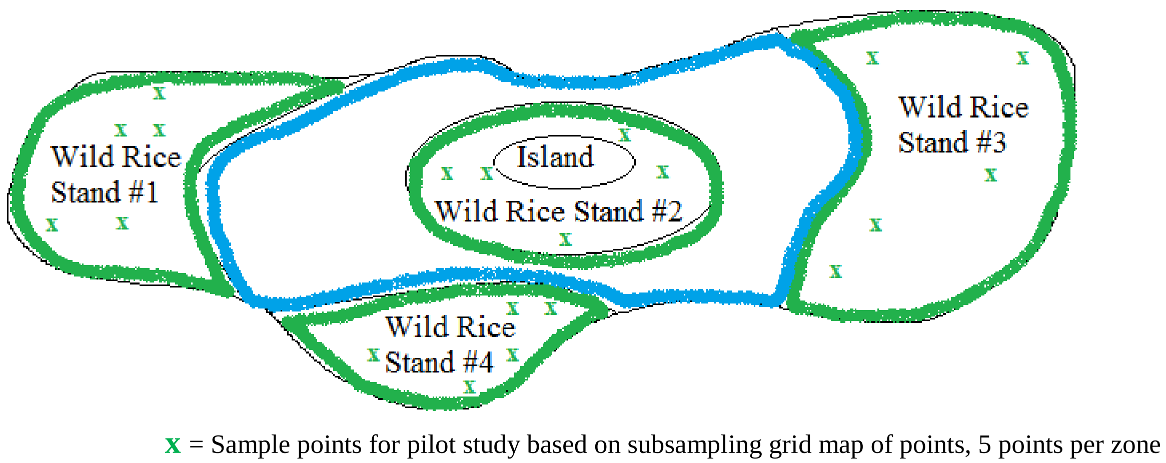


Figure 5. Zone technique for determining number of sample points using a pilot study

Use the average of the five points to determine the total number of points required that year for each stand based on Table 2. Below is an example showing the number of sample points required for each stand given hypothetical estimated averages for wild rice found during the pilot study.

Wild rice stand (zone)	Estimated average wild rice biomass (g/m^2) based on the pilot study samples	Number of sample points for each stand (zone)
1	116	20
2	80	24
3	125	20
4	25	40
		Total sample points = 104

Determine the number of sample points

The effect of quadrat size and statistical precision on the number of samples needed is shown in Table 2. The primary input for deciding number of sample points required is density of standing wild rice biomass. The number of sample points required to achieve the same level of precision will vary from year-to-year. While it may seem counterintuitive to estimate what you are trying to measure exactly, it's necessary because in low productivity years, there are more open water and low density areas. When there are more open water areas, it's necessary to sample more points to get an accurate measurement.

GLOSSARY

Density of standing biomass is the average wild rice biomass per area at the time of sampling.

Precision desired means the level of agreement of a set of measurements made on the same variable of interest. A high precision measurement will be very reproducible. Accuracy relates to the real life, "true" value of a variable. For example, your data for standing biomass may all be similar (very precise), but if you used an inaccurate lab balance to weigh your plants, or used wet weight instead of dry weight, your accuracy will be poor.

Standard error is a measure of the variability of the data; it is an estimate of the standard deviation.

STEP 1. Estimate wild rice biomass in the current year.

a) **Option 1: Use a pilot study.** A good way to estimate the current year's biomass is to do a pilot study of five points prior to sampling the entire water body to come up with a rough estimate. Use the methods described in this Handbook to measure the core wild rice variables and use one of the generic biomass equations from SOP #4 to compute biomass (See page 60 or page 61). . Use the generic equations to compute weight per stalk and multiply by the stalk density to get g/m^2. Use this rough estimate (average of 5 plots) and Table 2 to determine the number of sample points required for a particular year. Time to do the pilot study is well spent. Sample points from a pilot study can be part of the final data analysis and count toward the number of points needed in that year.

b) **Option 2: Use past experience.** Estimate the level of the current year's biomass based on past experience or existing data.

STEP 2. Find your estimated amount of wild rice biomass in Column 1 of Table 2.

STEP 3. Move across the corresponding row to find the number of sample points required to achieve the desired level of statistical precision.[4]

[4] A standard error no greater than 20% of the mean is recommended. This level of precision is built into this handbook's recommendations for the number of sampling points because it is an acceptable level of variability for aquatic plant biomass studies (Minnesota DNR, 2012; Downing and Anderson, 1985).

Decision Table: Connecting Biomass and Number of Sample Points

Table 2 demonstrates how the required sample size varies with quadrat size, wild rice biomass, and level of statistical precision. The recommendations from this Handbook's methods are shaded grey. The statistical basis for this table is explained in Appendix F.

Table 2. Number of Sample Points

	Quadrat area = 0.5 m^2			Quadrat area = 1.0 m^2		
Wild Rice Biomass (g/m^2)	Required sample size (25% error)	Required sample size (20% error)	Required sample size (15% error)	Required sample size (25% error)	Required sample size (20% error)	Required sample size (15% error)
10	38	59	105	34	53	94
20	28	44	78	23	39	70
25	25	40*	71	25	36	63
30	24	37	65	21	33	59
40	21	32	58	19	29	52
50	19	29	52	17	26	47
60	17	27	48	16	24	43
70	16	25	45	15	23	41
80	15	24	43	14	22	38
90	15	23	41	13	20	36
100	14	22	39	13	20	35
200	10	16	29	9	14	26

*Recommended number of sample points

Recommendations. Use a 0.5 m^2 quadrat to sample 40 points per water body for most situations. Analysis of wild rice historical data showed that sampling 40 points would achieve the recommended statistical precision in 80% of the years[5]. On larger lakes with multiple wild rice stands of differing densities it may be more efficient and accurate to use a zone technique to determine the number of sample points (See Figure 5). Using a larger quadrat size will result in more time to count stalks. Aiming for greater level of statistical precision will require sampling a greater number of points.

In a nutshell: When using a 0.5 m^2 quadrat, 40 points will give you good precision ~80% of the time. In sparse years, you will need to add more points (T. Kjerland analysis of data collected by Vogt, 2014).

Plan for extra sample points. For example, if sampling 40 points, identify up to 60 possible points. Why? Because: 1) there will be times when plots will be eliminated from sampling in the field upon discovery that those plots are not within suitable wild rice habitat in that year (i.e. water is too deep, plot is on shore, there is some obstruction, etc.); and, 2) 60 is the maximum number of plots recommended, even during sparse years (~15 g/m^2). For consistency across years, there is value in sampling the same points year-to-year, even when they contain no wild rice, as long as they remain in suitable wild rice habitat.

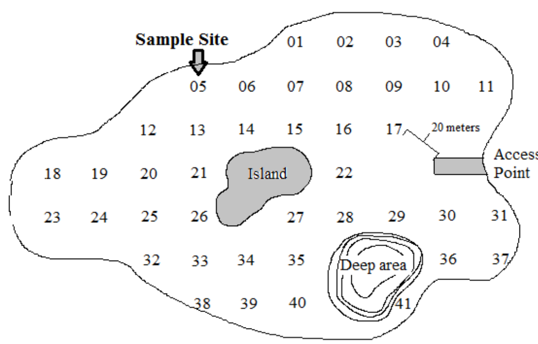
[5] T. Kjerland analysis of data collected by Vogt, 2014

How to Select Sample Point Locations

Two options are presented for designing the location of sample points: grid map and line transect. A third possibility is a variation on the grid map, which is to randomly subsample points from the grid. Related environmental variables should be located at the same points as wild rice, and/or in areas where potential stressors or pollutants are a concern. Several “reference” or “least-impacted” points should also be included for comparison purposes. See “related environmental variables” for details.

Recommended method: Grid map

Use mapping software (i.e. ArcGIS) to create a grid map of coordinates.



Advantages:

- Easy to set up
- Covers entire spatial area of interest
- Pre-selecting points avoids bias in the field
- Points can be re-sampled annually to monitor trends
- Simple to explain
- Easy to sample points in the field systematically
- Faster navigation to points along straight line

Disadvantages:

- If the variable of interest varies in a systematic way along lines of the grid, this method may potentially be biased. This is unlikely in most natural wild rice lakes.
- Points are systematically set up, in other words, not randomly. If this is a concern, one way to make the points more random is to set up more points than are required and subsample a list of random points.

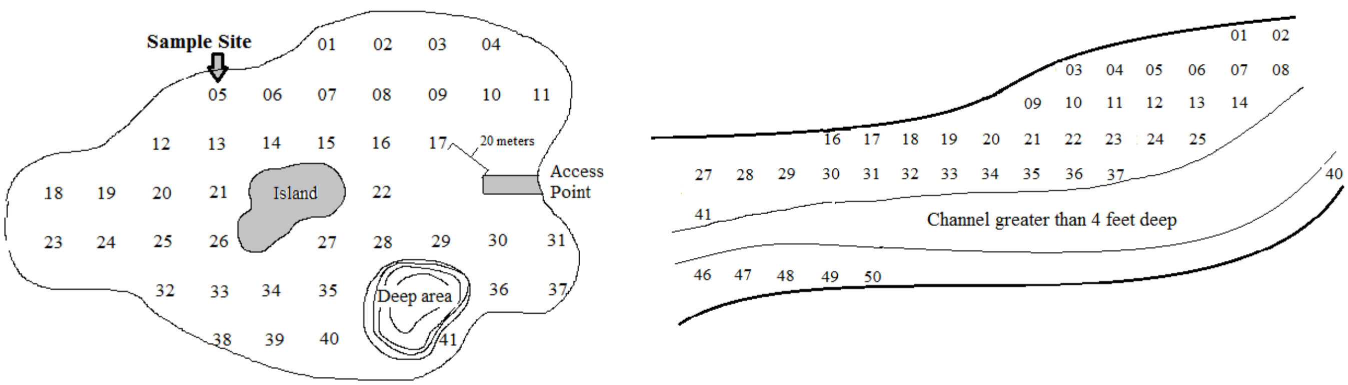
Helpful tips: If not all sample points in the grid are selected, try to cover the entire area of interest rather than clustering sample points. This will help avoid confounding factors that might exist in smaller areas (such as point-source pollutant discharges or nutrient inflows from streams) that might bias the overall study results.

Why completely random point placement is not recommended. For larger areas, there are several disadvantages: 1) random point placement can locate a large number of points in difficult to reach areas (such as the shoreline, which is often too shallow for canoe travel); 2) points may be clumped, leaving large areas under-sampled; 3) navigating to randomly located points greatly increases the field time needed to complete the sampling (Madsen and Wersal, 2012).

How to set up a grid map of sample points. Use ArcGIS or other mapping software to create a map and list of GPS coordinates for the sample sites. If sampling in deep lakes or areas where suitable wild rice habitat only covers a portion of the area, it will be helpful to create an outline of the sampling area first. A depth of four feet is recommended as the best known estimate for maximum rooting depth of wild rice. Due to the annual spatial variability in wild rice stands, use multiple years of historic data to gain a more accurate outline.

Make a waterproof copy of the map and list of coordinates for field use. Either laminate or print on water-resistant paper. If the GPS unit fails or satellite coverage is spotty, the map and list of coordinates can salvage the day. Including obvious shoreline landmarks on the paper map can be helpful if you need to navigate without a GPS unit. Without a working GPS unit, you won't know when you arrive at each point, but you can approximate the sampling grid using a paper map by trying to keep points a set distance apart and using landmarks. The paper map is also helpful for keeping track of which points have already been sampled, and to make notes on unusual things the crew sees while in the field.

Grid maps



LAKE **RIVER**

Figure 6. Grid map sample point design on a lake and a river

Other design considerations for setting up a grid map

1. Use a UTM coordinate system instead of a degree-based system. It will be easier to locate points based on the north and east coordinates (Madsen, 1999).
2. Use a round number for distance between grid points (i.e. 50 m) to make it easier to estimate the distance and find the next point in the field (Madsen, 1999).
3. Set the distance between grid points/quadrats to a minimum of 30 m, which will allow for the sampling points to be far enough apart to function as statistically independent samples. Distance between points/quadrats should also be based on the size of the area to be sampled and should ideally cover the entire area of <u>suitable wild rice habitat</u>. Practically speaking, sampling 40 grid points that are 50 to 250 m apart for core wild rice variables should be manageable in a day of field work.

4. Suitable wild rice habitat will include points up to four feet (~1.2 m) deep in August – October when wild rice sampling is done. These points may be deeper earlier in the season and may be different in rivers. More research is needed to test this depth as the maximum cut-off point. This depth cut-off point was established from an analysis of points sampled on 4 lakes in Minnesota and 2 lakes in Wisconsin (n=162)[6]. Points may be located either in the littoral zone or mid-lake (i.e. around islands).
5. Due to the accuracy level of most handheld GPS units, it's not expected that field crews will sample exactly the same spot each year, but rather that the sample site will consistently be within ~3-5 meters of the GPS coordinates (Minnesota DNR, 2012).

Table 3. Example of GPS coordinates list

Site ID	Longitude	Latitude	Sampled in 20XX
01	491355	5325676	x
02	490855	5325676	
03	490605	5325676	x
04	494105	5325926	

Alternative method: Line transects

The **line transect** method, another good option for selecting sample sites, involves selecting a random starting point for each transect and then laying out a transect line. Sampling is equally spaced along the line.

Advantages:

- Does not require mapping software to set up the sampling map
- Might reduce the time required to locate sample points in the field
- In a river, it may provide a better understanding of cross-section characteristics

Disadvantages:

- Works best when sampling in shallow shoreline areas
- If only sampling along shoreline of a lake, this method may inaccurately represent the wild rice growing in the middle of the lake
- Requires more training of field crew
- Likely to result in less spatial coverage of the water body being measured

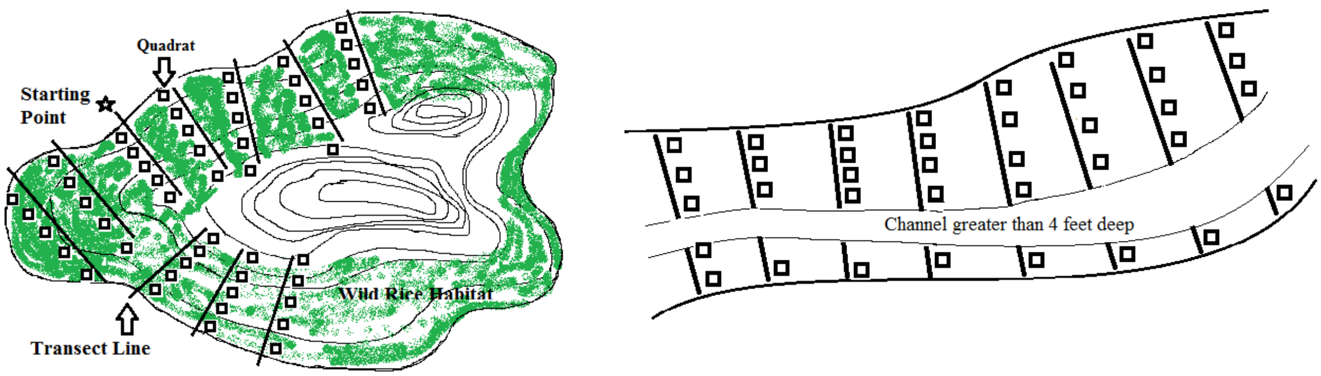
How to set up line transects. In a lake, transects should be set up perpendicular to the depth contours at regular intervals; preferably 30 m apart—but not less than 20 m apart. The transect end points will be where the water becomes too deep ($\geq$ 4 ft). Using a rope with floats can help to define transects. Sample quadrats at equally spaced 30 m intervals along the transect until you reach the end point (Uzarski, et al., 2014; Yost, et. al. 2013, Lee and Stewart, 1981). If the area is smaller, the quadrat

[6] These were the same lakes used for creating the wild rice biomass equations.

spacing may be reduced to 20 m. Record the GPS coordinates of the transect start and end points. In a river, transects should be located so as to be as representative as possible of the wild rice distribution, set at perpendicular angles to the flow.

Determine how starting points for transects will be identified randomly, either beforehand or once out in the field. Here are a few ideas for random placement:

- Prior to going out in the field, make a random mark on a map of the area. This point will identify the starting point for the first transect (not at the dock or access area). Separate each transect starting point 20 to 50 meters apart, depending on how large the area is to cover. Unless there is an obstruction, keep consistent spacing between transects; decide on a distance and repeat when setting up each transect. Record the distance used.
- In the field: Travel to the area where wild rice is growing and select a random starting point. Record the GPS coordinates of the start and end points of the transect.



LAKE **RIVER**

Figure 7. Line transect sample point design on a lake and a river

Dealing with areas that lack wild rice

Don't ignore open water areas if they are within suitable wild rice habitat. Because wild rice density varies spatially from year-to-year, open water areas that are suitable habitat for wild rice should be included in the sampling. The exception to this general rule will be when there has been no wild rice in those sites for a long time and it's not expected that wild rice will be able to grow there in the future (again, suitability of habitat is the deciding factor about whether or not to include the site). Documenting why sites are eliminated from sampling is a good practice for future reference. If sites are eliminated, new sites must be added in order to still have 40 sites. Sample points should ideally be selected prior to going out into the field to avoid the bias of picking the "best" points to sample.

Eliminating sites prior to field work. Sample sites may be eliminated prior to field work by comparing the grid map to a bathymetric map (one that shows the water depths). Since wild rice will not grow past a certain depth, sample points that are always deeper than the wild rice will grow can be eliminated prior to going out in the field. Estimates of maximum rooting depth range from 3 to 4 feet (0.9 to 1.2 meters). Use a depth of 4 feet as a cut-off level for sampling for most locations. If your wild rice plants are much taller on average, adjust the cut-off level accordingly.

In a Nutshell: Sample sites may be eliminated when they are not within suitable wild rice habitat.

Eliminating sites during field work. Sample sites may be eliminated when they are found to be too deep (greater than 4 feet), located on shore, sediment is unsuitable (cobbles, for instance), or there is some other type of obstruction to wild rice growth (e.g. floating mat of vegetation or a dock). If wild rice has been damaged or cut down for some reason, it is a good practice to make note of the damage, but don't include the site in the data analysis (unless you are particularly interested in this data).

IMPORTANT: If a sample point is **within suitable wild rice habitat**, and yet there is **no wild rice** present, **record a zero ("0")** for that plot. Do not skip the point because even a "zero" is a significant data point.

How to Determine Frequency of Sampling

Core wild rice variables should be collected annually, if feasible. Since wild rice populations vary considerably across time, annual monitoring will create a dataset that is most representative of actual wild rice yield. However, in some situations, for example when monitoring larger lakes it may be necessary to balance frequency of sampling and number of sample points to achieve your goals. In this case, one option is to select a few lakes to serve as index sites that will be monitored every year. Sample other lakes on a rotating basis (~ 2 to 3 years) to establish a baseline for those lakes.

Below are some options to consider for different situations:

- **Small lakes.** Ideally, all points on a smaller lake (or river reach) will be sampled every year if they are within suitable wild rice habitat (i.e. suitable depth, no obstructions, etc). Value has been found in sampling the same points each year (Vogt, 2014). However, a pilot study may be used to determine the number of points needed each year, and a random sample selected from available points used to determine that number.
- **Medium to large lakes.** An option to reduce field effort is to sample every other point every other year, while still sampling a minimum of 40 points. For example, sample odd-numbered sites in one year and even-numbered sites in the next year. A second option is to randomly subsample the grid points, but in this case you will want to make sure to cover the entire area of interest, and still sample 40 points. Another option is to divide the lake into zones and monitor several areas as baseline zones, but rotate monitoring of other zones per year as time and resources allow. Determine the number of sample points in each zone based on Table 2.

Record-Keeping

Be sure to keep track of the ArcGIS shape files, maps, and GPS coordinates associated with each sample point. Record metadata on how the maps were created (coordinate projections, etc.). If using line transects, keep a record of the GPS coordinates of the starting and ending point for each transect. These records will be useful for future spatial analysis. Other records that might prove to be useful include: 1) phenology of life stages of wild rice: When did seedlings begin to emerge? When did floating leaves appear? When did plants emerge out of the water? When did the seeds mature? and 2) phenology of water body condition/weather (i.e. ice-on date, ice-off date, lake levels, precipitation, etc).

SEQUENCE OF EVENTS DURING FIELD SEASON

Pre-field season preparations (June-July)

- ☐ Design monitoring plan and/or review prior year's plan
- ☐ Gather equipment and make sure it is working properly
- ☐ Train field crew
- ☐ Plan for cleaning boats when moving between water bodies to avoid spreading invasive species (very important!)

- ☐ Sample core wild rice variables when plants are mature
- ☐ Sample related environmental variables concurrently, if possible
- ☐ Decide on a labeling system ahead of time for sample points and collected plants
- ☐ In the field, store plants **on ice** in large zippered plastic bags

- ☐ Field season: mid-August to early-October
- ☐ If collecting plants for a biomass equation, collection should be completed by the end of September.

In the Lab:

- ☐ ASAP, move plants to paper bags and store in a dry location
- ☐ Identify other plants that were collected in 1-2 days to avoid decomposition and press right away if voucher specimens for later identification

As soon as possible:

- ☐ Oven-dry wild rice plants as soon as possible to avoid decomposition
- ☐ Oven-dried plants that have been left out overnight need to be re-dried. An oven-dried sample may increase in weight overnight by 5-10% through added moisture (Madsen, 1993)
- ☐ Process wild rice samples by either: 1) counting potential seeds (female pedicels) or 2) drying and weighing plants

After all data has been collected and recorded:

- ☐ Enter data into a spreadsheet (i.e. M.S. Excel)
- ☐ Clean the data (check for errors and/or outliers)
- ☐ Analyze the data (i.e. compute biomass and/or create site-specific equations)
- ☐ Upload data to a database if part of monitoring plan (i.e. AWQMS - WQX)
- ☐ Plan for next field season

Problems Faced When Doing Wild Rice Inventories and How to Solve Them

Cultural considerations and community concerns. Be aware that being out on the water conducting a wild rice study during harvest time may unsettle some people. Many Native Americans consider wild rice to be a sacred plant—and may not be comfortable with people paddling through the rice stands during harvest, especially if uprooting plants. Due to spiritual beliefs and negative experiences, some people will be disturbed by almost any type of scientific study being done on wild rice.

The methods in this Handbook have been designed to minimize effects on the wild rice plants. The recommended boat to use is a canoe. Using an airboat is too destructive; and, may offend people for cultural and spiritual reasons. Using a canoe with paddles will result in some bending of the wild rice plants when moving through dense patches, but soon after passing through the bed, the plants will usually stand up again and be as they were before.

<u>Here are possible solutions to consider</u>:

- Enlist help from tribal elders and leaders before any field surveys are conducted. Explain to them what you will be doing and why. Seek advice and listen to what they say.
- Take it slowly. This is a good way to build capacity—for know-how with the methods and for building trust and support within the community.
- Prior to the field season, consider notifying the local harvesting community through news media that you will be out in the lakes and rivers conducting a wild rice study and explain the benefits to the community and the safeguards you propose to protect the wild rice from damage.
- For collecting plants and seed counts, it is best to conduct the sampling when plants are mature (i.e. during harvest). If that is not possible, consider starting before the harvest. If you must wait until after harvest, don't wait too long, as senescing plants are hard to measure.
- Refer to the first pages of the Handbook for the quote from "Braiding Sweetgrass" by Robin Kimmerer. This passage illustrates a respectful, spiritual reasoning for conducting scientific studies.

In a nutshell: Proceed with awareness and respect when dealing with community and cultural concerns about doing wild rice studies.

Spatial variability. How to handle spatial variability is an important decision when designing a wild rice study. Wild rice often varies considerably in location annually—this is in a large part due to being an annual plant growing in a dynamic environment. Wild seeds tend to fall into the water near the parent plant, but there is still movement in wild rice beds each year due to many factors—wind and wave action, sediment transport and nutrient availability. As a result, wild rice plant distribution is not uniform across a given area, and dense patches are interspersed with open water. In addition, there are sometimes gaps along the edges of lakes.

While there are no strict rules for how to deal with wild rice spatial variability, it is important to think through how to handle this variability and to use consistent sampling methods from year-to-year and from one wild rice stand to the next.

One question that frequently arises is, "How do I handle open water areas?" The answer lies in thinking about the goals of the study, the historical distribution of wild rice, and the likelihood that open water areas may at some point contain wild rice. This last likelihood depends on the suitability of habitat, the seed source, and future plans for restoration. Suitable wild rice habitat is explained in depth in the section, "Biology of Wild Rice."

The section, "How to Determine Sample Point Location" explains more about making the decision about how to locate sample points, which is especially difficult for large areas of lakes that typically do not produce wild rice. This same section also provides guidance for the situation where there are separated areas of wild rice, such as isolated bays. When there are large areas of open water every year, it is often useful to do a baseline study in the first monitoring season to document the lack of wild rice presence and to assess the suitability of habitat. In subsequent years, sample plots might only be placed within areas that are known to produce wild rice consistently.

In a nutshell: Sample sites with zero ("0") rice should still be recorded and included in the data analysis when they are <u>within suitable wild rice habitat.</u>

Temporal variability. Wild rice varies annually in abundance, as measured by height, number of stalks, and biomass. Normal patterns of variability range from 3 to 7 years. The number of sample points required to achieve the same level of statistical precision will vary from year-to-year. In years when wild rice is sparse, there will be more areas with zero rice. Therefore, in order to accurately measure the wild rice present in sparse years, more sample points will be needed.

In a nutshell: In years of low biomass, more sample points will be needed to achieve the same level of statistical precision as in years of high biomass.

Sampling problems. Some sampling problems are predictable and can be mitigated or avoided, while others arise from unpredictable circumstances.

<u>The following are suggestions for avoiding problems:</u>

- **Site Access.** Access issues may occur in unfamiliar or less frequented areas, which could result in not enough time to complete the work as planned for the day.

  Avoid this problem: Do a dry run. Visit each of the sites ahead of time to assess the time it will take to drive there, load the equipment, and paddle to the wild rice areas.

- **Navigating using the GPS unit.** A common problem is difficulty navigating to the sample points on the water using a handheld GPS unit. This problem can be frustrating and greatly increases the amount of time needed to complete sampling.

  Avoid this problem:
  - Get to know your GPS unit ahead of time.

- Practice finding points on shore and on the water prior to starting the wild rice study.
- Keep in mind that arriving within 5 meters of the sample point is considered accurate enough.
- Features to look for when purchasing a new GPS unit include: waterproof, floats if dropped in the water, receiver capacity (to ensure it works well in remote areas), WAAS capability (to improve accuracy), waypoint capacity (number of points that can be stored), built-in electronic compass (to aid in navigation), a live tracking feature (for getting close to GPS points and for outlining wild rice stands). A helpful interface for uploading GPS points from ArcMap is called DNRGPS. This software is available free from the Minnesota DNR at: http://www.dnr.state.mn.us/mis/gis/DNRGPS/DNRGPS.html

- **Concerns about the time it will take.** At first there may be concerns about the amount of time and effort it will take to implement these methods. Experiencing the reality of doing the field work usually allays these concerns.

  Mitigate this problem:
  - Do a dry run or pilot study of 5 points to determine the feasibility, number of sample points needed, and number of lakes to sample.
  - Take it slowly. Start in year 1 by only collecting the core variables on one lake. Add more variables and water bodies over time.
  - Practice using the GPS unit prior to the start of field work on land and water.

- **Wind.** Windy days can be especially difficult for the sampling crew due to paddling against waves and maintaining a steady canoe while sampling.

  Avoid this problem:
  - Stabilize the canoe using an anchor in front and back or outriggers.
  - Don’t work on windy days.

- **Plant senescence.** Wild rice plants sometimes mature and reach senescence earlier than expected, or earlier on some lakes relative to others. This is more likely to be a problem when sampling must be done after the harvest season. Measuring plant height and counting stalks is more difficult when plants are beginning to rot and fall back into the water.

  Avoid this problem:
  - Sample prior to or during harvest, if possible.
  - If sampling must be done after harvest is over, be sure to get out there as soon as possible.
  - A judgment call may be needed for when the plants are too decayed for accurate sampling, especially when collecting plants for a creating a new biomass equation. Ideally, plants should be collected when they are ready to harvest and at their prime.

In a nutshell: Doing a practice run ahead of time to test out the equipment and methods will go a long way towards mitigating many sampling problems.

Standard Operating Procedures

SOP #1: Measuring Core Wild Rice Variables

(Source: Kjerland, T. 2015. *Wild Rice Monitoring Field Guide*. The University of Minnesota Sea Grant Program, Publication #SH15. ISBN 978-0-9965959-0-2).

For every waterbody, field crews will need to outline the area occupied by wild rice according to the method selected by the resource manager.[7]

Field crews will collect the following core wild rice variables in approximately 40 sample points per waterbody.

Variables for Generic Biomass Model:

- Stalk density within the quadrat frame
- Water depth within the quadrat frame or as close as possible
- Sample plant height (ABOVE WATER or TOTAL)
- Seed heads from the sample plant so the pedicels can be counted back in the lab
- The names of other plant species within the quadrat frame.

Variables for Site-Specific Biomass Model:

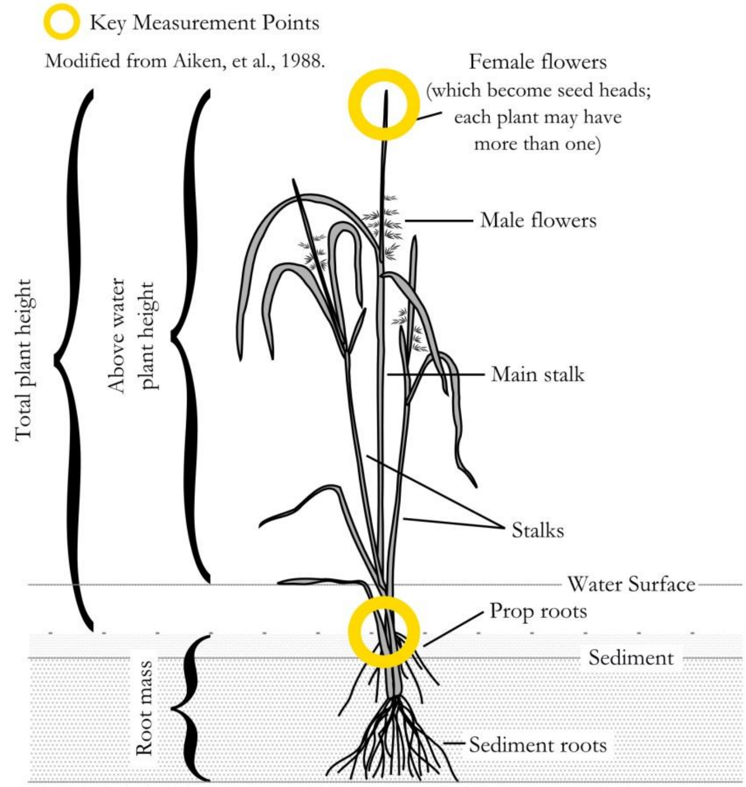
- Stalk density within the quadrat frame
- Water depth within the quadrat frame or as close as possible
- Collect entire sample plant collected so its dry weight can be determined back in the lab
- TOTAL sample plant height
- The number of stalks on the sample plant
- The names of other plant species within the quadrat frame

When conducting fieldwork, also note brown spot fungi information, shoreland and water use, weather information that might affect the data, and concerns for wild rice plant growth.

[7] See Appendix B, "Estimating Wild Rice Stand Area" or Step 12 in the field sampling protocols on Page 37.

Wild Rice Plants

Figure 8. Labeled illustration of wild rice plant



Prop roots are found on some plants, but not all. They are shorter and often colored differently compared to sediment roots—either more darkly or more lightly (even white). Prop roots are found above the sediment roots, and appear to be a second set of roots higher on the stalk. Prop roots do not have fine hairs.

Sediment roots are the lower roots on a wild rice plant that grow in the sediment, which every plant will have. Some plants will also have prop roots. Sediment roots have fine hairs for absorbing nutrients.

Equipment Needed

- ☐ Canoe
- ☐ Canoe cushions
- ☐ Paddles (3)
- ☐ Life jackets
- ☐ Drinking water and food
- ☐ First aid kit, hand sanitizer
- ☐ Hat and sunglasses, sunscreen, rain gear
- ☐ Cell phone, fully charged, in waterproof bag (for emergencies)
- ☐ Insect repellant
- ☐ Quadrat frame, 0.5 m^2, or 0.71 m x 0.71 m (one corner marked with colored tape, notch, or colored PVC elbow)
- ☐ Handheld GPS unit (fully charged, with spare batteries, ideally with tracking function)
- ☐ List of GPS points to sample printed on water-resistant paper
- ☐ Map of water body showing labeled GPS points, i.e. "grid map" OR if using transects, simply a map of the area (laminate or print on water-resistant paper)
- ☐ Metal box clipboard
- ☐ Device to measure water depth (e.g. secchi disk with chain or rope taped to meter stick or measuring rod—the measuring rod should rest on top of the secchi disk. This is needed to measure water depth in soft, flocculent sediments.)
- ☐ Permanent marker
- ☐ Water-resistant paper (for labels to put inside bags)
- ☐ Mechanical pencils
- ☐ Field data sheets printed on water resistant paper– see Appendix A
- ☐ Tape measure or meter stick (needed to measure wild rice plant height)
- ☐ Equipment for collecting water and/or sediment samples, if part of the sampling plan
- ☐ Wild Rice Monitoring Field Guide (includes Plant ID Key)
- ☐ Additional plant ID guides (for more comprehensive references)
- ☐ Permits, if needed
- ☐ Large (~2-gallon) zippered plastic bags (about 60) – for collecting seed heads and/or plants
- ☐ Large scissors (for collecting seed heads)
- ☐ Cooler with ice

Helpful Tip: Use a copy of the Field Data Sheet on page 102 to record data.

Field Sampling Protocol

1 **Locate Sample Points Using GPS Unit**
Referencing the map, navigate to the sample points using a GPS unit. If you are unfamiliar with this process or the GPS unit, practice ahead of time.

2 **Collect Water Quality and Sediment Samples…**
if required by your sampling plan. Do this BEFORE taking other measurements to avoid stirring up the sediment and contaminating samples.

3 **Place Quadrat Frame Over the Plants to Measure Stalk Density**
Lower the quadrat frame straight down over the wild rice plants to the side of the canoe next to the seat of the person in front (same side each time). When placing the frame, if there are any stalks leaning in or out (due to thick rice, wind, canoe movement, etc.) they should be moved in or out accordingly.

Avoid Sampling Bias

- Do not simply place the quadrat frame on an area that "looks good" or is easiest to measure. Instead, use a methodical, non-biased way of deciding where to place the frame.
- Navigate to within 5 meters (~16 feet) of sample point coordinates. Stop and quickly stabilize the canoe. Don't back up or paddle an extra stroke to reach a "better" area.
- Place the quadrat frame in the water next to the seat of the person in front. Use the same side of the boat each time.
- If taking two quadrat readings per sample point, decide ahead of time and be consistent about placing the frame. See "Two-points-per-stop" method described on page 21.

Skipping Sample Points

Sample points may be eliminated if they are not within suitable wild rice habitat. If sample points are skipped, add more sample points as needed to measure the required number of points. Reasons for skipping include:

- the water is too deep (greater than 4 feet for most locations)
- the point is located on shore
- there is an obstruction (e.g. a dock, floating mat of vegetation)
- the sediment is unsuitable

Record the reason for skipping on the Field Data Sheet

Having zero wild rice is not a valid reason to skip a sample point. If there is no wild rice in an otherwise suitable site, record is as “0” on the field data sheet along with the water depth and other plants. Don’t leave blanks because this would mean “data missing.” If wild rice has been damaged or cut down, make note and take photos, but don’t include this point in the analysis unless you are particularly interested in this data.

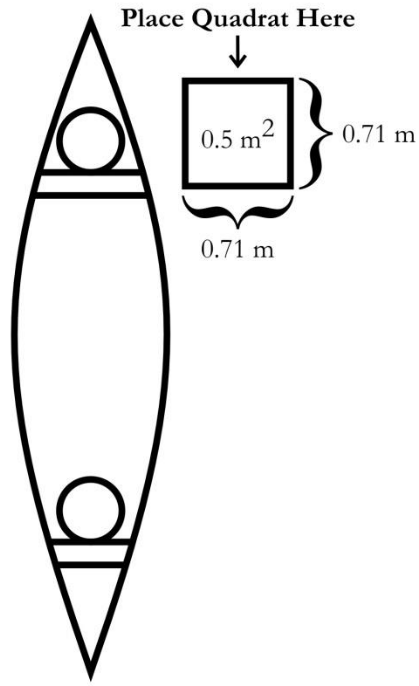


Figure 9. Quadrat placement

4 **Measure Stalk Density**

Count the stalks that are inside the frame. **Count stalks, not plants**. Individual plants may have stalks within and outside the frame.

5 Identify Other Plants in the Quadrat

- Use the Plant Identification Key in the Field Guide or other reference guides. Record the common name(s), using abbreviations if needed.
- If a plant cannot be identified, collect the plant for later identification
- Label a large, zippered plastic bag: Unknown #1, etc.
  1. Sample ID# & waterbody name
  2. Date & time of day
  3. Water depth
  4. Note observations about leaves, flowers, or fruits:
     - Emergent (above water, like wild rice)
     - Floating (floating on the surface)
     - Submersed (below the surface entirely)
  5. Color of flower
  6. Technician initials
  - Collect entire plant—flowers, fruits, roots, stems, leaves…everything.
  - Wash the roots carefully but thoroughly in the water
  - Remove sticks, bugs, etc., that are clinging to the plant.
  - Include a duplicate label on water –resistant paper inside the bag.
  - Store plants on ice in the cooler.

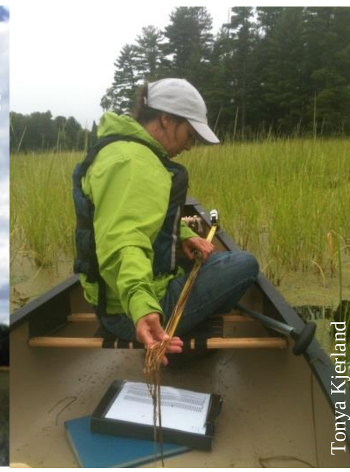
6 Select the Sample Plant

- Find the corner of the quadrat marked with colored tape, notch, or PVC elbow
- Select the wild rice stalk that is nearest to this designated corner. Whichever plant this stalk is growing from is the sample plant.
- This will be the plant you measure and either:
  - A) collect seed heads from, OR
  - B) collect in its entirety.

7 Measure Sample Plant Height

- Circle on the Field Data Sheet whether measuring in inches or centimeters
- Check box for which method used, and record plant height. Use one of the following methods:
  - A) Above water. Measure the sample plant's height from the water line to the top of the tallest stem.
  - B) Total. Uproot the plant and measure the distance from the top of the roots to the top of the tallest stem. If two sets of roots, measure from the top of the prop roots.

A) Above Water



B) Total

8 Measure Water Depth as close as Possible to the Sample Plant

Circle on the Field Data Sheet whether measuring in inches or centimeters. Use one of the following methods:

- A) Use a device for measuring depth and record the device type used. Measuring water depth can be difficult due to thick plant growth and soft lake bottoms that are hard to define. The recommended device is a secchi disk attached to a marked rope or chain, which can be allowed to settle to the bottom. Temporarily tape the secchi and its chain or rope to a meter stick or measuring rod, then allow the secchi to settle to the bottom so that the stick rests directly on top of the secchi disk.
- B) Measure water depth by uprooting the sample plant and measuring from the top of the roots to the water line on the plant. If there are two sets of roots, measure from the top of the prop roots (See page 38, Wild Rice Plants).

9 Collect Seed Heads OR Sample Plants to Take Back to the Lab for Analysis

A) Seed Heads from Sample Plant

To assess the potential number of seeds requires removing the seed head portion of the plants and then counting the tiny stalks that hold female flowers (called pedicels).

- Label a plastic zippered bag with the sample point ID #, water body name, and date.
- Include a duplicate water-resistant label inside the bag.
- Using a scissors, cut the stem below the seed head on every stem of the sample plant and place it in a plastic zippered bag, store on ice. Gather all of the seed heads on the sample plant.

*Back in the lab, to avoid decay, **remove seed heads from the plastic bags as soon as possible** and store in labeled **paper** bags to dry until ready to count pedicels. Counting pedicels is necessary to calculate the number of potential seeds and whole plant biomass.*

B) Entire Sample Plant and Count Number of Stalks

- Label a large (~2 gallon-size) zippered plastic bag with sample point ID #, water body name, plant height (indicate units), and date.
- Include a duplicate water-resistant label inside the bag.
- Holding the bag to catch falling seeds, carefully run your hand over the seed head to collect loose seeds.
- Pull the plant slowly up out of the sediment, trying to retain as many seeds and roots as possible.
- Gently wash the roots in the water, and pick off sticks, bugs, or other materials sticking to the wild rice plant.
- Fold the plant accordion style, trying to save as many seeds as possible, and place the whole plant in the bag. Store on ice.

*Back in the lab, within 24 hours, **remove the wet plants from their bags.** Repackage in labeled **paper** bags and store in a dry area. Note**

About Collecting Seed Heads

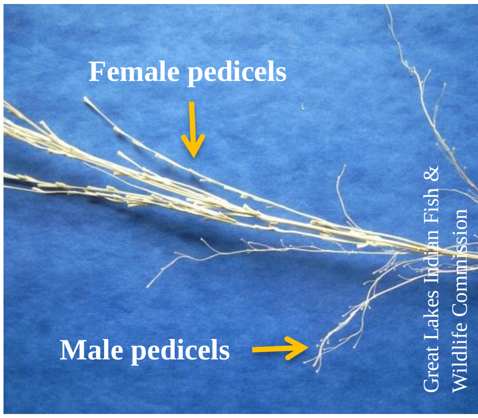
For information on processing the samples (i.e. counting potential seeds/female pedicels) see page 44, SOP #2 Drying and Weighing Wild Rice Plants. It is important to collect the entire seed head from every stalk on the sample plant and process them as soon as possible after returning to the lab.

About Collecting Wild Rice Plants

To create a site- or area-specific biomass equation, it's necessary to collect wild rice plants, dry and weigh them. These results are compared to stem height and seed number to develop the equation. Specific biomass equations are optional, as generic equations exist; see page 57, SOP #4 Using Generic Wild Rice Biomass Equations.

*Alternatively, allow plants to drip-dry on canvas in the lab. Tag them for later identification with folded-over "lab tape" or aluminum write-on tags.

Helpful tip: the female pedicels are larger and sturdier and located above the male structures on the stem (see photo, below left). Because seeds fall off regularly, counting pedicels is the best way to estimate total seed production. When counting pedicels, it is important to count only the female ones.



A) Female Pedicels on Seed Head

B) Collection of Wild Rice Plants

10 Record Field Notes

These observations will help reveal the environmental conditions that affect wild rice growth

- Complete weather and comments on the Field Data Sheet
- Note presence of animals, birds, pests, and signs of plant disease.
  Examples: Rice Worms (*Apamea apamiformis*), Muskrats, Ducks, Other Birds, Rusty Crawfish, Ergots, etc.
- Write legibly using pencil or waterproof ink!
- Important: Do not leave blanks on the datasheet. If the data cannot be collected, record the reason. A blank dataset means "data missing", whereas "zero" means "we looked and didn't detect this variable."

Wild Rice Worm

Wild Rice Worm and Seed

Ergots on Wild Rice

Muskrat Lodge

11

Record Brown Spot Fungal Disease Severity Within the Quadrat Frame

- Record the severity of brown spot fungal disease at five random sample points across the water body.
  SEVERITY INDEX: "0" = wild rice leaf is free of the disease; "low" = less than 1/3 of the leaf if covered; "high" = more than 1/3 leaf is covered. See images below.
- Make your best estimate, being as consistent as possible across the sites.

"Low" severity infection
LESS than 1/3 of leaf covered

"High" severity infection
MORE than 1/3 of leaf covered

12

Estimate Wild Rice Stand Area

Method A: Canoe or walk around the wild rice stand using a GPS with a tracking function to record points and get an outline (bare minimum points needed = 4; 5-sec. or shorter setting for tracking function recommended). The edge of the stand may be identified by moving to the open water where there is no wild rice and then defining the edge according to the most outlying stem. Even one stem is considered part of the wild rice stand. This is a relatively time-consuming method. If there are areas without wild rice, or areas in which wild rice is of differing densities, these areas may need to be treated separately. (Reference: Valerie Brady, Natural Resources Research Institute)

Method B: While completing sampling, the field crew uses a map of the water body printed on waterproof paper with a grid of GPS points. Throughout the day, the crew draws areas of 1) wild rice, 2) sparse rice, 3) open water, or 4) other vegetation. Later, using a transparent grid overlaid on the lake map, estimate area of wild rice in relation to total lake area. These polygons may also be digitized for use with mapping software. For purposes of making within lake comparisons, "Sparse wild rice" may be defined as areas with greater than one canoe length between rice stalks. (Reference: Darren Vogt, 1854 Treaty Authority)

About Estimating the Area of a Wild Rice Stand

It is useful to create an approximation of the outline of areas where wild rice is found growing each year. Knowing this area is essential to computing overall biomass and for mapping challenges, such as interpolating values between sample points. Because using GPS to outline wild rice beds is subjective; the accuracy of area measurements may vary between surveyors. Areas may move considerably year to year due to the variability of wild rice growth. In order to standardize these approximations, it is recommended that whoever does the work be given clear instructions, make notes on what criteria they used to determine where to map and that the same crew assess each area each year. Because of GPS inaccuracy and field technician subjectivity associated with collecting this type of data, it should only be used as an estimate for comparing year-to-year variability *within* a specific waterbody. It is not intended to provide a mechanism for assessing relative condition or productivity *between* (or *across*) wild rice waterbodies.

Multiple methods for estimating the area of wild rice stands are described in Appendix B. The two methods recommended in these field sampling protocols and in the Field Guide were chosen due to their ease of implementation.

Back in the Lab: Dry and Weigh Wild Rice Plants

Instructions are provided in Standard Operating Procedure #2, "Drying and Weighing Wild Rice Plants."

SOP #2: Drying and Weighing Wild Rice Plants

Helpful Tip: Use the lab data sheet provided in Appendix A for recording data.

Obtain permission first. Wild rice is considered to be a sacred plant by many Native Americans and like-minded people (Vennum, 1988). Pay attention to local cultural protocols and consult with tribal authorities to determine what is appropriate. At the end of the study, treat the plant materials with respect. Again, ask ahead of time about local cultural protocols and follow the advice of tribal leaders and elders for disposing of plant materials.

Rules and protections for wild rice exist in many areas. If you are considering physically collecting wild rice plants, think carefully about whether or not this is necessary and then check into tribal, state, and other laws to determine if you need a permit in order to collect plants. Permits may also be required for collecting seeds heads.

Equipment Needed:

Field (also included in equipment list for SOP #1)

- ☐ Large (~2-Gallon) plastic zippered bags (At least 60, enough for 40 plots plus 20 for collecting other plants if need for identification.)
- ☐ Permanent markers
- ☐ Mechanical pencils
- ☐ Extra water resistant paper for placing labels inside bags
- ☐ Cooler with ice
- ☐ Measuring tape or stick
- ☐ Lab tape or aluminum write-on wire tags for identifying plants (optional)

Lab

- ☐ Small paper bags (i.e. lunch bags), one per plant
- ☐ Permanent markers
- ☐ Pencils
- ☐ Data recording sheets for plant weight (see *Plant Weight Lab Data Sheet*)
- ☐ Large sink for washing plants, preferably with a sprayer that can be set to a gentle spray setting
- ☐ Drying oven or incubator
- ☐ Refrigerator
- ☐ Scientific balance, ideally accurate to 0.001 grams, but with minimum accuracy of 0.01 grams (properly calibrated)
- ☐ 2 large trays (~9"x13")
- ☐ Small plastic weigh boats
- ☐ Tweezers
- ☐ Magnifying glass

Collecting Wild Rice Plants

In order to compute a site- or area-specific biomass equation, collect entire wild rice plants as described in SOP #1 and the Field Guide. Wild rice roots account for approximately 10% to 15% of the total plant weight (T. Kjerland analysis of data collected by Vogt, 2014). Stalks usually account for between 65 and 75% of the total plant weight, and seeds may account for 10% to 25% of total plant weight.

Washing, Drying and Weighing Plants

Plants can be dried and weighed using a variety of methods. The methods presented in this section will produce accurate results and are the same as used to compute the generic biomass equation.

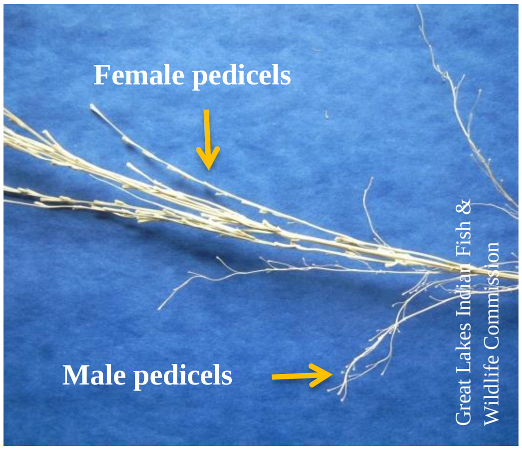
Timing. Ideally, plants will be dried as soon as possible after collecting, but they can be stored for up to several weeks if kept in a very dry location. Rather than keeping plants in a refrigerator where they are likely to decay quickly, wash the plants at once and put them into paper bags to air dry. When plants begin to decay they lose weight, and counting pedicels will become difficult.

Washing and Drying Plants

1. Have on hand a stack of small brown paper bags and a permanent marker.
2. One plant will go into each bag, which will be labeled with same information as on the plastic bag used to collect plants in the field.
3. Label the paper bag

   Waterbody name
   Site ID#
   Date
   Technician initials

4. Carefully remove a plant from its plastic bag into a large sink with a screen stopper.
5. Cut off the seed head including the male and female pedicels. Place the seed head into the small brown paper bag, being careful to include any seeds that get knocked off. Another option is to collect all seeds into a separately labeled paper envelope and place this in the bag.



Helpful Tip: The female pedicels are larger and sturdier and are located above the male structures on the stem. Because seeds fall off continuously, counting pedicels is the best way to estimate total seed production. When counting, it is important to count only the female pedicels.

Figure 10. Photo showing the difference between female and male pedicels

6. Wash the roots thoroughly but gently. If any roots break off, retain them with the original plant for weighing. Retain all plant materials for each plant; the plant doesn't have to be intact to be weighed.

7. Cut off the roots of the plant at the node directly above the prop roots, if any exist. If no prop roots, then cut above the sediment roots. Place roots in the same labeled paper bag.
8. Refold the remaining stem of the plant accordion style and place into the paper bag.
9. Keep the bag upright and the top open for air circulation.
10. Repeat until all plants have been processed.
11. Store bags open side up in a dry room until drying can be completed in the incubator or drying oven. Try to complete the drying as soon as possible, within at least 1-2 weeks.
12. Dry plants in an incubator or drying oven at 60° C for 24 hours prior to weighing. The idea is for the plant material to have reached a constant, stable weight.
13. Weigh plants as soon as they are removed from the drying oven, if possible. If plants sit out more than overnight they will need to be re-dried in the oven because the plants will absorb moisture from the air.

About Seed Viability: Viability usually refers to the ability of a seed to germinate, and so the "half-empty hull" test is only a rough estimate. It is important to take care when collecting the plants to collect as many seeds as possible, and also to collect plants at the harvest-ready state, if possible. At that point, seeds left on the plant will be the most representative of the ratio of viable to non-viable seeds produced. Viable seeds should weigh considerably more. If you find they don't, check again. For computing total plant weight, viable and non-viable seeds will be weighted for the % collected per plant.

Weighing Plants

1. Weigh plants immediately after removing from drying oven.
2. Remove each plant from the small paper bag onto a large tray.
3. Record the collection location site and plant height on the Plant Weight Lab Data Sheet, see Appendix A.
4. Separate the 3 parts of the plant: 1) roots, 2) stems/leaves, and 3) seeds.
5. Remove the seeds from the plant and store in a pile.
6. Tare the weigh boat on the scale.
7. Weigh the roots of the plant and record weight in grams to the nearest highest level of accuracy the scale allows, ideally to 0.001 mg.
8. Weigh the stems/leaves of the plant together, including the seed head (minus seeds).
9. Separate the seeds into viable, non-viable, and ergot-infested piles.
    a. To test for viable seeds, press on the seed with your index finger and if over half of the hull is filled, this is considered a proxy for "viable." Viability usually refers to the ability of a seed to germinate, and so this test is only an estimate. When determining an approximate weight for seeds during data analysis, viable and non-viable seeds will be

weighted for their percent left on the plant. This is why it is important to take care when collecting the plants to collect as many seeds as possible, and also to collect plants at the harvest-ready state, if possible.

   b. Seeds with ergots should be noted and counted but <u>not included as part of the wild rice plant weight</u> because the fungal growth is not part of the plant and adds weight.
   c. Record number of seeds with worm holes.

10. Count the viable and non-viable seeds and record these separately on the data sheet.
11. Weigh the viable and non-viable seeds, and record these separately on the data sheet.
12. Count and record the number of female pedicels on the seed heads. Use a tweezers and magnifying glass to help see these small plant parts. Be sure you are only counting the larger pedicels from the top (female) part of the seed head because these are the ones that produce seeds. Male pedicels are smaller, less sturdy, and located on the lower portion of the seed head.
13. Return all plant parts to original paper bag and save them until you are certain that you have all of the data and it is accurately recorded.

SOP #3: IDENTIFYING AQUATIC VEGETATION

Preparations Prior to Field Work

- Look through the list of species often found growing with wild rice (Table 4).
- Determine if there are additional species of concern for your area and add them to the list.
- Obtain a selection of plant identification guides.
- If you don't know how to identify plants or plant taxonomy, reading the field guides will help. Look up words you don't know in the glossary or online. Knowing botanical terms is needed for using plant identification keys.
- Do preliminary training for plant identification by collecting a sampling of plants found in your area and identify them using plant keys. Check with an expert to verify the identifications, if possible.
- For aquatic plant identification training, check with biological stations or colleges in your state; many offer one-day classes for natural resources personnel that range from basic to advanced. In Minnesota, the Water Resources Center at the University of Minnesota offers plant identification courses every summer as part of their wetland delineation certification program.

Equipment Needed

<u>Field (also included in equipment list for SOP #1)</u>

- ☐ Large (~2-gallon) plastic zippered bags
- ☐ Permanent marker
- ☐ Mechanical Pencils
- ☐ Field guides for plant identification, e.g. Wild Rice Monitoring Field Guide
- ☐ Cooler with ice

<u>Lab</u>

- ☐ 2-3 plant identification guides and keys (appropriate for region)
- ☐ Computer for using web-based resources
- ☐ Magnifying glass

How to collect plants

See Step 5 in SOP #1 and the Field Guide for instructions on how collect plants if it is not possible to identify them in the field.

Identifying Plants

The *Wild Rice Monitoring Field Guide* includes photographs of plants commonly found growing with wild rice. The list of plants included in the Field Guide is shown in Table 4.

Plant identification tip: The plants shown in this Handbook are the most common ones found growing with wild rice, but it is likely you will find other species that look similar because they are closely related. When in doubt, collect the whole plant for later identification.

Rare or endangered plants

If possible, identify plants in the field without removing them from the sediment in order to keep the community as intact as possible and because many aquatic plants are relatively rare. In some cases, removing a small part of the plant for closer inspection, such as a leaf or flower, will allow for identification. If it is not possible to identify the plant in the field and you are concerned that it may be rare or endangered, you may wish to photograph it rather than collecting it.

Plants of special concern

Reasons to collect data about other plants growing with wild rice include identifying and locating plants of special concern. These plants may out-compete wild rice or cause other issues, such as recreational water use problems. The resource manager should identify any species of special concern. Plants that are categorized by the Minnesota Department of Natural Resources as "invasive" or "introduced" are noted below.

Field crews should note plants of special concern within the water body where they are sampling. Record the plant's name in column 3 of the field data sheet when found within the quadrat. If found growing outside the quadrat, also make note of its presence in a separate area, such as in the field notes on second page of the field data sheet. Photograph the plant and collect a sample plant for identification in the lab. In order to be able in order to be able to relocate the site where plants are growing, identify the site by recording a GPS point or indicate the location on a map.[8]

[8] For more about threats from plant competition, see "Natural Wild Rice in Minnesota." A wild rice study document submitted to the Minnesota Legislature by the Minnesota Department of Natural Resources, February 15, 2008.

Table 4. Plant species often found growing with wild rice

Common name	Scientific Name	Invasive	Introduced
Arrowhead	*Sagittaria latifolia*	N	N
Bulrush, Hard-stem	*Schoenoplectus acutus*	N	N
Bulrush, Soft-stem	*Schoenoplectus validus*	N	N
Bur-reed, Giant	*Sparganium eurycarpum*	N	N
Cattail, Narrow-leaved	*Typha angustifolia*	N	Y
Cattail, Broad-leaved	*Typha latifolia*	N	N
Cattail, Hybrid	*Typha x glauca*	N	Y
Coontail	*Ceratophyllum demersum*	N	N
Duckweed, Lesser	*Lemna minor*	N	N
Grass, Manna	*Glyceria* species[9]	na	na
Grass, Reed Canary	*Phalaris arundinacea*	Y	N
Horsetail, Water	*Equisetum fluviatile*	N	N
Loosestrife, Purple	*Lythrum salicaria*	Y	N
Lotus	*Nelumbo lutea*	N	N
Pickerelweed	*Pontederia cordata*	N	N
Pondweed, Large-leaved[10]	*Potamogeton amplifolius*	N	N
Pondweed, Curly	*Potamogeton crispus*	Y	N
Pondweed, Floating-Leaved	*Potamogeton natans*	N	N
Pondweed, Leafy	*Potamogeton foliosus*	N	N
Reed, Common	*Phragmites australis*	Y	N
Rush, Flowering	*Butomus umbellatus*	Y	N
Smartweed, Water	*Persicaria amphibia*	N	N
Water-milfoil, Common	*Myriophyllum sibiricum*	N	N
Water-milfoil, Eurasian	*Myriophyllum spicatum* L.	Y	N
Watershield	*Brassenia schreberi*	N	N
Water lily, Common White	*Nymphaea odorata*	N	N
Water lily, Common Yellow	*Nuphar variegate*	N	N

[9] There are many species within the genus *Glyceria* that are commonly referred to as "manna grass". Some are native and some are not. Record "manna grass" for all similar-looking species due to the difficulty in telling them apart without botanical training.

[10] There are many species within the genus *Potamogeton* that are commonly referred to as "pondweeds." Due to the difficulty in telling the species apart without botanical training, record "pondweeds" for these similar-looking species while monitoring wild rice.

References for Identifying Aquatic Plants

Bell Museum Herbarium
http://www.bellmuseum.umn.edu/ResearchandTeaching/Collections/ScientificCollection/PlantCollection/InfoonMinnesotasFlora/index.htm

Board of Water and Soil Resources (BWSR), Minnesota Wetland Delineation
http://www.bwsr.state.mn.us/wetlands/delineation/index.html

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Minnesota DNR, Invasive Plants
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Newmaster, S.G., A.G. Harris, and L.J. Kershaw. (1997) Wetland Plants of Ontario. Lone Pine Publishing, Edmonton, Alberta, Canada. 240 pp.

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http://wisplants.uwsp.edu/search.html

Skawinski, P.M. (2011) Aquatic Plants of the Upper Midwest: A Photographic Field Guide to Our Underwater Forests.

USDA Plants Database, Natural Resources Conservation Service (scope – United States)
http://plants.usda.gov/

Voss, E.G. (1972) Michigan Flora. Part I. Gymnosperms and monocots. Cranbrook Inst. Sci. Bull. 55, Bloomfield Hills, Michigan.

Voss, E.G. (1985) Michigan Flora. Part II. Dicots (Saururaceae-Cornaceae), Cranbrook Inst. Sci. Bull. 59, Bloomfield Hills, Michigan.

Wisflora: Wisconsin vascular plant families
http://www.botany.wisc.edu/wisflora/

Techniques for creating a collection of species

Haynes, R. R. (1984) Techniques for collecting aquatic and marsh plants. Annals of Missouri Botanical Garden 71:229-231.

Wood, R.D. (1970) Hydrobotanical methods. University Park Press, Baltimore, Maryland.

SOP #4: USING GENERIC BIOMASS EQUATIONS

The following two equations define relationships between wild rice biomass (weight) and variables that are easy to measure, such as plant height and potential number of seeds (# female pedicels). These equations provide a short-cut way to estimate biomass without collecting plants. Which one you use will depend upon the input variable you choose; either plant height or number of potential seeds. The decision is based on which variable you prefer to measure or are able to measure most accurately. The following two equations were developed from wild rice plants collected in Minnesota and Wisconsin. See Appendix C for the raw data and summary statistics for all lakes that went into computing these equations.

Generic Wild Rice Biomass Equations
1) Plant weight/stalk = $(9.03 \times 10^{-6}) \times (\text{total plant height in cm})^{2.55}$
2) Plant weight/stalk = $(0.137) \times (\text{number of female pedicels per stalk})^{0.917}$

- **FAQ: Wild rice plant height and seed number change from year-to-year, so how can only one equation capture this change?**
  By using the same biomass equation each year on a water body, quantifiable trends can be recognized (i.e. biomass is increasing, decreasing, or staying the same). The goal of using this method is to obtain an estimate of biomass; not to measure biomass exactly. In order to measure biomass exactly, it would be necessary to collect all plants in a quadrat, dry, and weigh them.

Calculate biomass per unit area by multiplying the weight of the sample stalk by the stalk density:

$$\text{Biomass (g/m}^2) = \text{Weight per stalk} \times \text{Density (\# stalks/m}^2)$$

In order to scale this statistic up for an entire water body (Total Biomass), multiply by the area in square meters. If wild rice grows only in certain areas of the water body and you want a more accurate measurement, divide the water body into zones and calculate the biomass separately for each zone, then sum the results.

GLOSSARY
Biomass is another name for "weight." This Handbook uses "plant weight per stalk" and "grams per square meter" as the units for biomass.

Biomass Equation 1: Plant height - Weight per stalk

This equation may be used to compute plant weight **per stalk** (grams) using total plant height in centimeters as the input variable.

Equation 1 in words: Plant weight per stalk (in grams) = (9.03 x 10^{-6}) times [total plant height in centimeters] raised to the 2.55 power

Equation 1 $\mathbf{y = (9.03 \times 10^{-6})x^{2.55}}$

Where x = total plant height in centimeters
y = plant weight per stalk (units in grams)

Statistics: n=132; p<<0.001

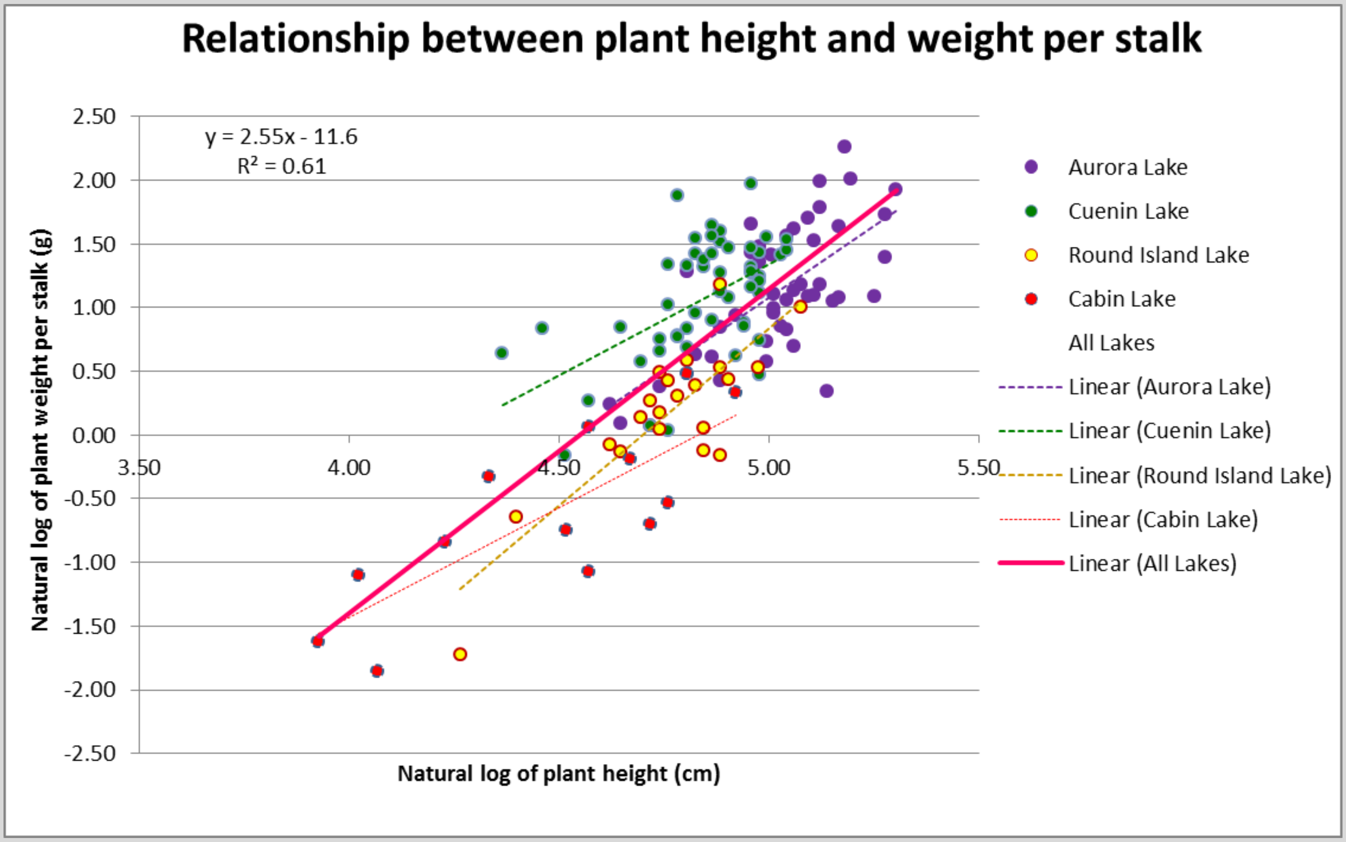


Figure 11. Relationship between plant height and weight for Equation 1

Total plant height is measured in centimeters from the sediment-water interface, or top of highest roots, to height of tallest stalk. Plant weight per stalk (y) is given in grams (T. Kjerland analysis of data collected by Vogt, 2011 and Lewis, 2014).
Note: Data from Campers and Stone Lakes were not included in this equation because the linear regressions showed a lack of significance for these two lakes.

Biomass Equation 2: Number of potential seeds per stalk - Weight per stalk

The following equation may be used to compute **plant weight per stalk** using number of **potential seeds** (#pedicels) **per stalk** as the input (x) variable. Weight is given in grams.

Equation 2 in words: Plant weight per stalk (in grams) = 0.137 times [potential seed number per stalk (pedicels)] raised to the 0.917 power.

Equation 2 $\mathbf{y = 0.137x^{0.917}}$

Where x = Number of pedicels per stalk
y = Plant weight per stalk (units in grams)

For Equation 2: n=162; p<<0.001

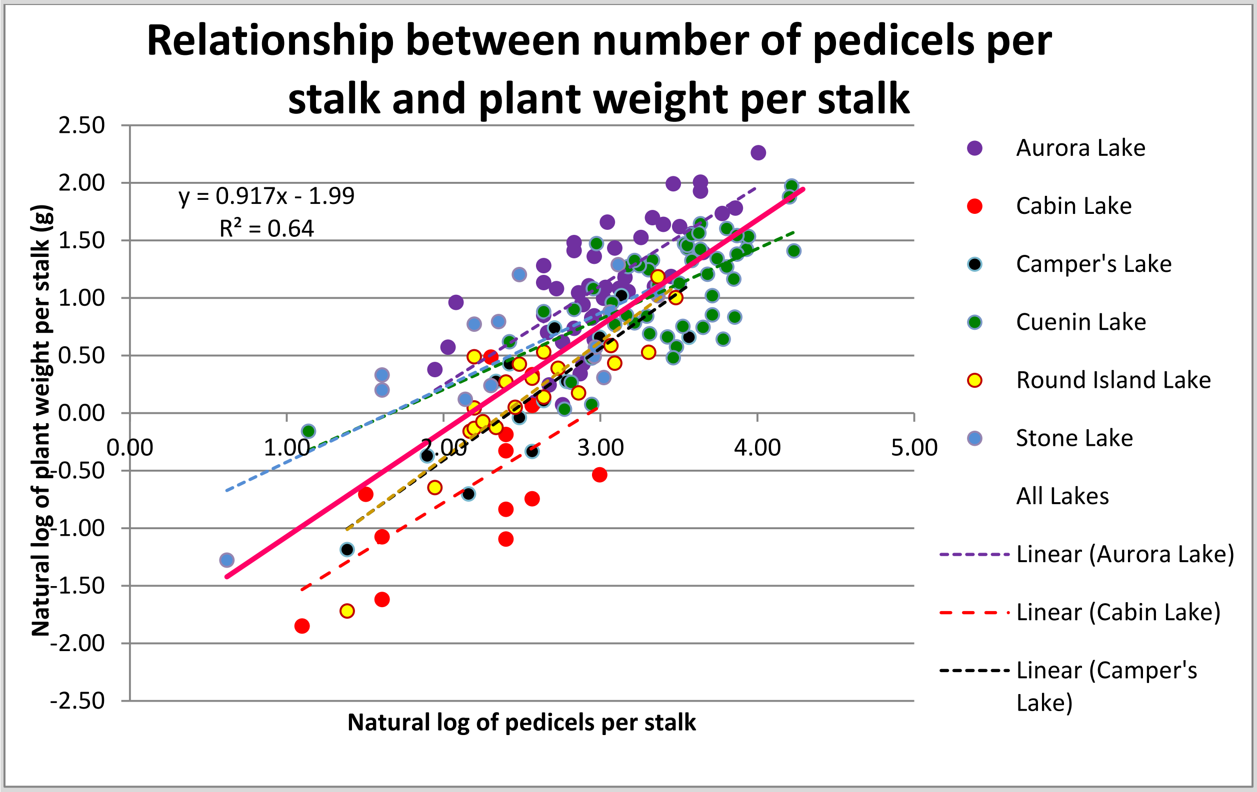


Figure 12. Relationship between pedicel number per stalk and weight per stalk for Equation 2

Step-by-Step Instructions for using <u>Equation 1</u> in Microsoft Excel

STEP 1. Set up an Excel spreadsheet and enter the data or use the file provided for download from the Minnesota Sea Grant Program web page: **www.seagrant.umn.edu/coastal_cities/wildrice**.
See SOP #5 for instructions on how to enter field and lab data into the spreadsheet. Use the example below to learn how to add columns for the computing biomass.

Enter total plant heights (units are in cm) in the appropriate column. In this example, we use column A.

STEP 2. In column B, add a heading, "plant weight per stalk in grams." Type the following function into Cell B2. Note that the caret ^ symbol in Excel means, "raise to the power of." Remember the equal sign before the function.

	A	B
1	Total plant height in cm	Plant weight per stalk in grams
2	122	1.89
3	117	1.70
4	91	0.89
5	97	1.05
6	137	2.54

= 0.00000903*A2^2.55

<u>Verify you did it correctly:</u>
If the plant height is 122 cm, the plant weight in grams per stalk would be 1.89 (grams). The actual number of digits shown will depend on your cell formatting.

Common problems:

- Did you forget the "=" sign before the function?
- Did you paste everything exactly as it reads from the right side of Equation 1 into the cell in column B? **= 0.00000903*A2^2.55**
- Did you use the correct number of zero's (5) to the left of the "9"?

STEP 3. Copy and paste the function in cell B2 down the column.

STEP 4. Convert the number of stalks measured in the field with a 0.5 m^2 quadrat to units of stalks per square meter (1.0 m^2) by multiplying by 2. Enter the density of stalks per square meter in Column C.

STEP 5. Compute **biomass per square meter**

Enter "=B2*C2" in Column D to compute grams of wild rice per square meter.

	A	B	C	D
1	Total plant height in cm	Plant weight per stalk in grams	Density_stalks per meter	Biomass_grams per meter
2	122	1.89	2	3.78
3	117	1.70	20	33.93
4	91	0.89	86	76.87
5	97	1.05	2	2.10
6	137	2.54	24	60.89

= B2*C2

STEP 6. Find the **average biomass per square meter** (g/m^2). This statistic can be used to compare annual trends on a given water body. Enter the following formula to compute the average:

	A	B	C	D
1	Total plant height in cm	Plant weight per stalk in grams	Density_stalks per meter	Biomass_grams per meter
2	122	1.89	2	3.78
3	117	1.70	20	33.93
4	91	0.89	86	76.87
5	97	1.05	2	2.10
6	137	2.54	24	60.89
7				35.5

=AVERAGE(D2:D6)

STEP 7. Compute **biomass for an entire water body**. Multiply average biomass per square meter computed in Step 6 by the wild rice area measured by the field crew. The result will be the biomass of wild rice in a given wild rice lake or stream, expressed as grams per square meter. If using zones to delineate areas, weight the average biomass by the proportion of the area represented by each zone.

Step-by-Step Instructions for Using Equation 2 in Microsoft Excel

STEP 1. Set up an Excel spreadsheet or use the file provided for download from the Minnesota Sea Grant Program web page: **www.seagrant.umn.edu/coastal_cities/wildrice**
See SOP #5 for instructions on how to enter field and lab data into the spreadsheet. Use the example below to learn how to add columns for the computing biomass.

Enter number of pedicels in the appropriate column. In this example, we use column A.

STEP 2. In column B, add a heading, "plant weight per stalk in grams." Type the following function into Cell B2. Note that the caret ^ symbol in Excel means, "raise to the power of."

Remember the equal sign before the function.

	A	B
1	Number of pedicels per STALK	Plant weight per stalk in grams
2	13	1.46
3	13	1.46
4	21	2.19
5	7	0.82
6	8	0.92

= 0.137*A2^0.917

Verify you did it correctly:

If the number of pedicels is 13, the plant weight in grams per stalk would be 1.46 (grams). The actual number of digits shown will depend on your cell formatting.

Common problems:

- Did you forget the "=" sign before the function?
- Did you paste everything exactly as it reads from the right side of Equation 2 into the cell in column B? **= $0.137x^{0.917}$**
- Did you use the correct number of zero's (5) to the left of the "9"?

STEP 3. Copy and paste the function in cell B2 down the column.

STEP 4. Convert the number of stalks measured in the field with a 0.5 m^2 quadrat to units of stalks per square meter (1.0 m^2) by multiplying by 2. Enter the density of stalks per square meter in Column C.

STEP 5. Compute **biomass per square meter**

Enter "=B2*C2" in Column D to compute grams of wild rice per square meter.

	A	B	C	D
1	Number of pedicels per STALK	Plant weight per stalk in grams	Density_stalks per meter	Biomass_grams per meter
2	13	1.46	248	363
3	13	1.46	46	67
4	21	2.19	98	214
5	7	0.82	22	18
6	8	0.92	172	159

= B2*C2

STEP 6. Find the **average biomass per square meter** (g/m^2). This statistic can be used to compare annual trends on a given water body. Enter the following formula to compute the average:

	A	B	C	D
1	Number of pedicels per STALK	Plant weight per stalk in grams	Density_stalks per meter	Biomass_grams per meter
2	13	1.46	248	363
3	13	1.46	46	67
4	21	2.19	98	214
5	7	0.82	22	18
6	8	0.92	172	159
7				164.3

=AVERAGE(D2:D6)

STEP 7. Compute **biomass for an entire water body**. Multiply average biomass per square meter computed in Step 6 by the wild rice area measured by the field crew. The result will be the biomass of wild rice in a given wild rice lake or stream, expressed as grams per square meter. If using zones to delineate areas, weight the average biomass by the proportion of the area represented by each zone.

SOP #5: DEVELOPING AREA-SPECIFIC BIOMASS EQUATIONS

Any software program that can do linear regression may be used to compute a biomass equation. The steps below are for Microsoft Excel because this is the most widely available.

If you do not have the Data Analysis Toolpak installed for your version of Excel, you will need to install it. Instructions for installing the Data Analysis Toolpak are available online from Microsoft.
It only takes about one minute to install and it is included with Microsoft Office Essentials.

Instructions for How to Load the Microsoft Excel Data Analysis ToolPak
http://office.microsoft.com/en-us/excel-help/load-the-analysis-toolpak-HP001127724.aspx

Spreadsheet design. Be sure to clearly label the columns on your spreadsheets so that someone looking at this spreadsheet (maybe you!) in future years can tell exactly what data each column refers to. It is important to indicate units of measurement the formulas require. Also note if PLANT HEIGHT was measured as "above water" or "total".

Be sure to save your data often!

Data entry and analysis instructions

1. Set up spreadsheet to house data from the Field Data Sheet and Lab Data Sheet. The easiest way to do this is to use the pre-designed spreadsheet available for download at www.mnseagrant.umn.edu/coastal_cities/wildrice.[11] The filename is "Spreadsheet for Field and Lab Data" (See Figure 13 and Figure 14; also see Appendix A). Alternatively, set up the spreadsheet using instructions shown in Table 5.

2. Set up a "METADATA" tab (unless you are using the downloadable spreadsheet mentioned in 1, which already includes metadata). Metadata are descriptions of the data that define the meaning and units of measure of each column heading, or variable (See Figure 15). This information can be extremely helpful to someone else looking at the data, thus making the data more broadly useful and increasing its longevity. By storing the metadata within the spreadsheet, there won't be an additional file to keep track of.

3. Enter data from Field Data Sheet

4. Enter data from the Lab Data Sheet

[11] The spreadsheet available for download from the Minnesota Sea Grant website, "Spreadsheet for Field and Lab Data" was designed to double as an import configuration for the Ambient Water Quality Monitoring System (AWQMS). The import configuration, "Wild Rice Field and Lab Data" (available in AWQMS) is compatible with this spreadsheet for submitting data to the U.S. Environmental Protection Agency's STORET/WQX data management and storage system.

5. QA/QC the data by checking for outliers, missing data and data entry errors (See page 68).

6. Convert units of variables if needed. Convert plant height and water depth to centimeters. Plant weight should be recorded in grams. Multiply by 2 to convert stalk density measured in a 0.5 m^2 area quadrat (0.71 m x 0.71 m frame) to stalks/1.0 m^2.

7. Protect the data (this is to prevent problems with accidentally changing the field or lab data)
   a. Copy the worksheet with data onto a new tab and use this separate tab for computing biomass; or
   b. Lock the cells that have original data in them (using Excel's "lock cell" feature; do not use a password—leave the password blank so that it's easier to unlock the cells).

8. For each sample point, compute the biomass per square meter area using Generic Biomass Equation #1 (page 60) or Equation #2 (page 61).[12]
   a. If using a generic biomass equation, you're done!
   b. If calculating a site- or area-specific biomass equation, continue to 8.

9. Verify that all of the formulas are entered correctly in your spreadsheet. The variables to be used in the linear regression should be log-transformed (ie. natural log of plant height, natural log of plant weight per stalk, and natural log of pedicels per stalk).

10. Calculate biomass equation by performing a linear regression as described on page 69.

[12] For AWQMS/WQX users, keep all columns and maintain their order when using the spreadsheet with the import configuration, "Wild Rice Field and Lab Data". Do not include extra columns that you may have added, such as to convert units or compute biomass. The AWQMS system will not recognize extra columns and may generate an error message.

Set up spreadsheet

Enter the column headings shown in Table 5 horizontally in a new spreadsheet.[13]

Table 5. Column headings and formulas for combined field and lab data

Legend: grey = from both data sheets; green = from Field Data Sheet; orange = from Lab Data Sheet; white = formula; g = grams

Column Headings	Data or Formula?	Formula and Notes
Date	Data	MM/DD/YYY
Water Body	Data	"Monitoring Location ID" is the AWQMS/WQX term associated with a waterbody name or sample site.
Sample ID#	Data	"Sampling Component Name" is the AWQMS/WQX term associated with a sample point or plot.
Activity ID	Formula[14]	=CONCATENATE(B2,":",C2,":",TEXT(A2,"yyyymmdd"))
Number of rice stalks **per 0.5 m^2**	Data	Quadrat area is one-half square meter (0.71 m x 0.71 m)
Taxon Present 1 (Y/N)?	Formula	=IF(ISTEXT(G2),"Y","N")
Other vegetation 1	Data	Other plants in quadrat, one name per column
Taxon Present 2 (Y/N)?	Formula	=IF(ISTEXT(I2),"Y","N")
Other vegetation 2	Data	Other plants in quadrat, one name per column
Taxon Present 3 (Y/N)?	Formula	=IF(ISTEXT(K2),"Y","N")
Other vegetation 3	Data	Other plants in quadrat, one name per column
Taxon Present 4 (Y/N)?	Formula	=IF(ISTEXT(L2),"Y","N")
Other vegetation 4	Data	Other plants in quadrat, one name per column
Plant Height-TOTAL (cm)	Data	Enter data in the column corresponding to how plant height was measured—either as total or above water. *If using this spreadsheet as an import configuration for WQX/AWQMS, include both column headings for plant height (TOTAL and ABOVE).*

[13] Notes pertaining to AWQMS/WQX users refer to the Ambient Water Quality Monitoring System (AWQMS) and WQX systems for managing and transferring data into the U.S. EPA STORET database.
[14] Unique identifier for a sample or measurement as consistent with AWQMS/WQX terminology

Column Headings	Data or Formula?	Formula and Notes
Plant height-ABOVE (cm)	Data	Enter data in column corresponding to how plant height was measured—either as total or above water. *If using this spreadsheet as an import configuration for WQX/AWQMS, include both column headings for plant height (TOTAL and ABOVE).*
Water depth (cm)	Data	Units are in cm
Number of stalks on sample plant	Data	Only needed if computing site- or area-specific biomass equation
Brown spot fungal disease	Data	(0, low, high)
Shoot_weight (Units in grams)	Data	
Root_weight_g	Data	
Viable seed weight_g	Data	
Viable seed number	Data	
Non-viable seed weight_g	Data	
Non-viable seed number	Data	
Number pedicels per PLANT	Data	
Number seeds with ergots	Data	
Number seeds with worm holes	Data	
Number of Total seeds found	Formula	Viable seed number + Non-viable seed number
Number of pedicels per STALK	Formula	Number pedicels per plant/# stalks per sample plant
Ratio Viable seeds	Formula	(Viable seed number)/ (#Total seeds found)
Ratio Non-viable seeds	Formula	(Non-Viable seed number)/ (#Total seeds found)
Viable seed weight average_g	Formula	(Viable seed wt_g)/ (Viable seed number)
Nonviable seed weight average_g	Formula	(Non-Viable seed wt_g)/ (NonViable seed number)
Average seed weight_g	Formula	[(Viable seed wt ave_g)*(Ratio viable seeds)] + [(NonViable seed wt ave_g)*(Ratio NonViable_seeds)]
Total seed weight_g	Formula	Number pedicels per PLANT * Average seed wt_g
Number of rice stalks **per 1.0 m^2**	Formula	**2 x** (Number of rice stalks per 0.5 m^2) *Note: This formula assumes using a quadrat with area equal to one-half square meter, as per*

Column Headings	Data or Formula?	Formula and Notes
		instructions in this Handbook. Dimensions would be 0.71 m x 0.71 m for a 0.5 m^2 quadrat.
actual plant weight PER STALK_g	Formula	(actual_plant_weight TOTAL_g)/ Number of stalks per sample plant
Actual plant weight TOTAL_g	Formula	(Shoot wt_g) + (Root wt_g) + (Total seed wt_g)
natural log plant weight per stalk_g	Formula	LN(actual plant weight PER STALK_g)
natural log total plant height_cm	Formula	LN(TOTAL plant height_cm)
natural log pedicels per stalk	Formula	LN(Number of pedicels per STALK)

	S	T	U	V	W	X	Y
1	Shoot weight (Units in grams)	Root weight_g	Viable seed weight_g	Viable seed number	Non-viable seed weight_g	Non-viable seed number	Number pedicels per PLANT
2	4.301	1.944	0.456	10	0.122	14	106

Figure 13. Example of data entered in the Lab Data portion of spreadsheet

	A	B	C	D	E	F
1	Date (MM/DD/YYYY)	Water body	Sample ID#	**Activity ID**	Number of rice stalks **per 0.5 m^2**	**Taxon Present 1 (Y/N)?**
2	10/15/2014	Name of Lake	RL08	Name of Lake:RL08:20141015	124	**Y**

Figure 14. Example of data entered in the Field Data portion of spreadsheet

	A	B	C	
1	**Variable**	**Description**	**General Notes**	**AWQMS - WQX User No**
29	Number of Total seeds found	Sum of all seeds found when plant when plant was collected	Viable seed number plus non-viable seed number	The remaining columns a biomass. For AWQMS - will be ignored when usi the existing import confi Region 5 Wild Rice Data
30	Number of pedicels per STALK	Number of pedicels per plant divided by the number of stalks on the plant being measured		
31	Ratio Viable seeds	Ratio of seeds considered viable according to proxy measure of amount of hull filled	Viable seeds were those with **half or greater** of the seed casing filled with a solid seed	
32	Ratio Non-Viable seeds	Ratio of seeds considered non-viable according to proxy measure of amount of hull filled	Nonviable seeds were those with **less than half** of the seed casing filled with a solid seed	
33	Viable seed weight average_g	Average weight of viable seeds		
34	Non-Viable seed weight average_g	Average weight of non-viable seeds		
35	Average seed weight_g	Average weight of seeds found adjusted for ratio that were viable vs. non-viable		
36	Total seed weight_g	Number pedicels per PLANT * Average seed weight_g		
37	Number of rice stalks **per 1.0 m^2**	**2 x** (Number of rice stalks per 0.5 m^2)	This formula assumes using a quadrat with area equal to one-half square meter, as per instructions in the Wild Rice Monitoring Handbook. Dimensions would be 0.71 m x 0.71 m for a 0.5 square meter quadrat.	
38	Actual plant weight per STALK_g	"Actual plant weight TOTAL_g" divided by "Number of stalks per sample plant"		
39	Actual plant weight TOTAL_g	Sum of shoots, roots, and seeds on sample plant		
40	Natural log plant weight per stalk_g	Natural log of plant weight per stalk	Log-transformed variable for creating site- or area-specific biomass equation	
	Natural log tot[illegible]eight_cm	Natural log of total plant height in centimeters	Log-transformed variable for creating site- or area-	

Main **Metadata** Sheet1

Ready 100%

Figure 15. Example of metadata

QA/QC (data quality control)

Always quality-check the data prior to beginning any analysis. Reasons why this is important:

- Because in every data set there are almost always errors. The sources may be human errors (such as in data entry) or sampling errors (such as instrument calibration or misuse).
- It saves time later. Data analysis takes time; you do not want to have to redo it. You want to be sure the dataset you are working from is not going to change. Even one decimal point out of place or too many zeros in a number can throw off statistics significantly and distort conclusions.
- Data checking helps identify outliers in your data set. Even 1-2 outliers can strongly influence statistics computed from your data.

STEP 1. Verify that the data were entered accurately. If you cannot read a handwritten number properly and don't have any way to check, then you should throw out that data point. If possible, have a different person from the one who entered the data, check every entry to make sure all values are entered correctly. One way to do this is to have the first person read off the numbers from the field and lab data sheets while the second person looks at the computer screen to verify the numbers. Alternatively, use a double-entry method, which will generally catch the most mistakes. However, it is more time-consuming that merely checking re-entered data. For the double-entry method, have the second person enter the data in a different spreadsheet. Use a "comparison" function to compare the two spreadsheets, which will automatically highlight discrepancies. Research the discrepancies and input correct numbers.

STEP 2. Calculate summary statistics: mean, median, standard deviation, and ranges.

STEP 3. Graph your data.

a. Research any suspicious data points, such as outliers. Outliers are usually defined as points that are more than 3 standard deviations from the mean. Some statistical packages will calculate and identify outliers. Even one high or low number can affect the computations (See Figure 16). If you do have outliers, go to 3b. If not, go to 3c.
b. Decide how to handle outliers. Whether or not to remove outliers depends on the statistical analysis. If they are valid data points, they should be kept. You should know why you're eliminating a data point and have a good reason, such as you suspect it to be an error or you think there was some interference with collecting the data point properly. One reason for eliminating a data points would be if you suspect operator error or an equipment malfunction. Always document removing any outliers and why you removed them. Adding a column to the metadata tab would be one way to record removal of data points. Go to 3c.
c. Look for unexpected relationships in the graphs which may indicate a problem with the data.

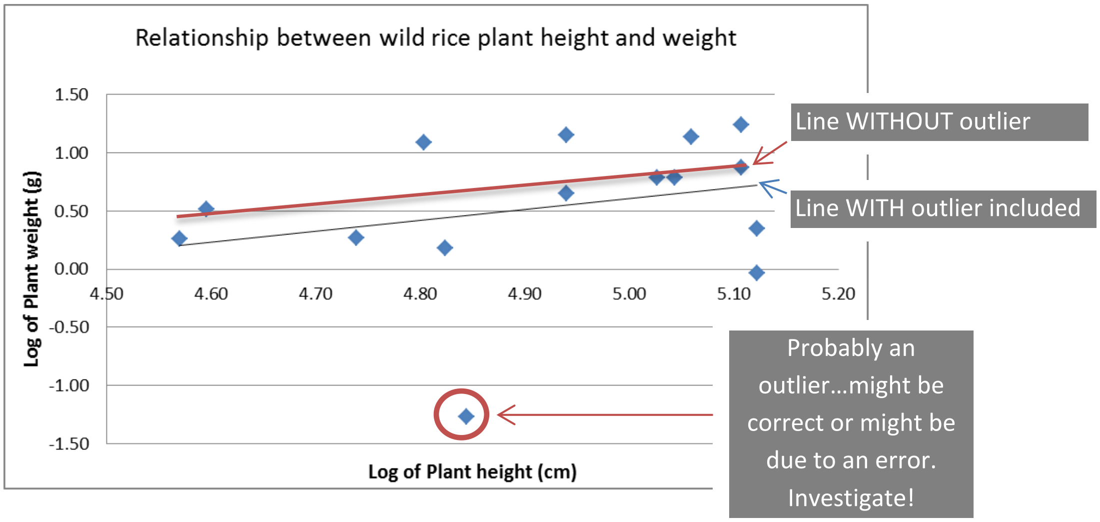


Figure 16. Illustration of outlier effects

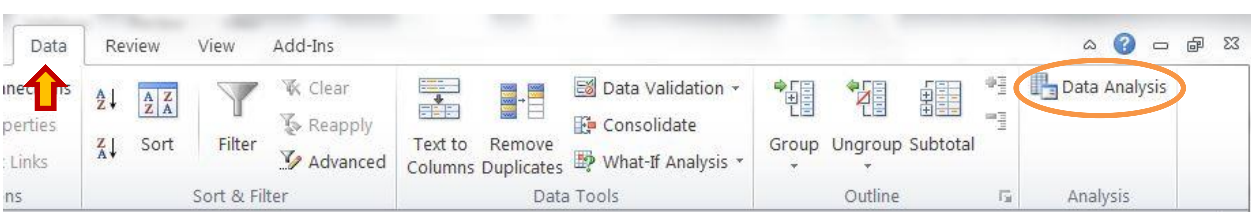
STEP 4. Another way to check for suspicious data points is to sort your data in each column from largest to smallest and check the high and low ends (being very careful to sort every column simultaneously so you don't disassociate the data across rows).

Calculate Biomass Equations

Verify that you have set up the spreadsheet properly. The list in Table 5 (page 65) shows the proper column headings and cell contents. The easiest way to do this is to use the spreadsheet available for download from the Minnesota Sea Grant website (www.mnseagrant.umn.edu/coastal_cities/wildrice).

Next, perform a linear regression with **natural log of plant height** as the x-axis (input variable) and **natural log of plant weight** on the y-axis (outcome variable). The steps below walk you through this process.

STEP 1. Click the "Data" tab in Excel and select "Data Analysis." This requires the free and downloadable Microsoft Data Analysis Toolpak if you are using Excel.

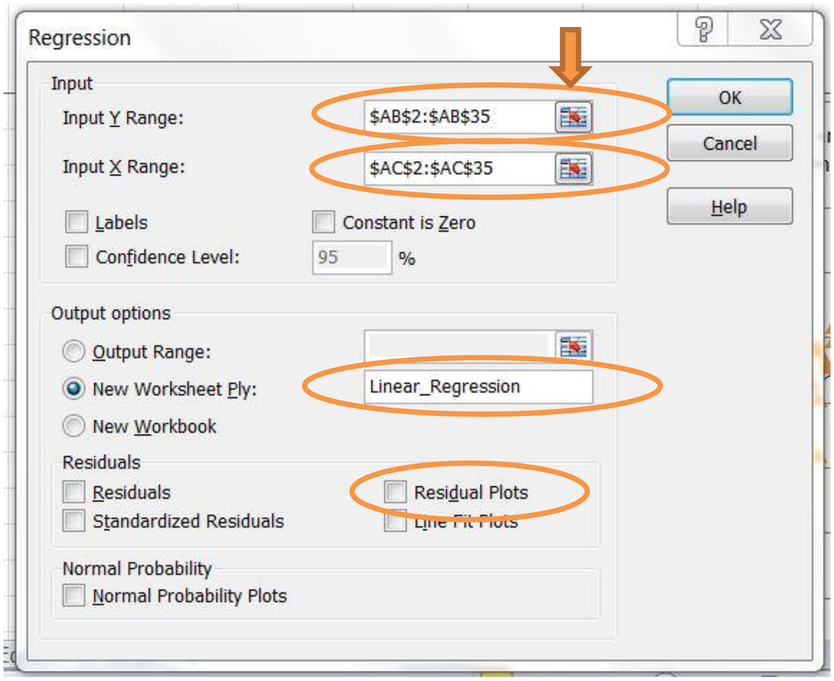


STEP 2. Select "Regression" from within the Data Analysis window. A new window pops up with options for setting up a linear regression (see below). Select the icon with the tiny red arrow and

highlight the "Input Y Range" which will be "natural log of plant weight". Hit "Enter" to reselect the same icon to close the input window.

Repeat for entering "Input X Range." The X data will be the "natural log of plant height."

(Note: Alternatively, you can type range values directly in to the box. In the example below, the text to enter would be, $AB$2:$AB$35 for the Y Range).



Select "New Worksheet Ply:" and type in a meaningful title, such as "Linear Regression." This will create a new tab in your spreadsheet to store the results of the regression.

STEP 3. Examine the results of the regression (see Figure 17 for an example).

Find the R-Square value. The R-Square (R^2) value represents the percentage of the change in the y direction (plant weight) that can be explained by changes in the x direction (plant height). If plant height were a perfect predictor of plant weight, the R^2 value would be 1. If the plant height predicted none of the variability in plant weight, the R^2 value would be 0. The higher the R^2 value, the stronger the relationship is between your "x" and "y" variables.

How high should the R^2 be? There are no hard and fast rules for how high an R^2 value needs to be, because it depends on how much predictability you need and on the type of data being compared. By looking at the R^2 values in the equations in this Handbook, you can get an idea of the range of values to expect using different types of plant data. Appendix C shows all of the linear regression equations for each of the lakes used to create the biomass equations, along with their R^2 values.

Next find the y-intercept and slope values. These will be used to create your final equation.

Find the P-value for the slope of the regression line (listed in the row labeled "X Variable 1"). The P-value and R-squared indicate whether the slope of the regression line differs from zero at the given level of significance. P-value should be 0.05 or less. If it's larger than 0.5, you may wish to collect more plants because you don't have enough statistical significance.

Remember, the "y" and "x" input values were natural logs of the plant height and weight, therefore to use this equation for directly computing plant weight from height in cm, you need do some algebra, as explained in Step 4.

Example of linear regression output

The figure below shows the result of a linear regression performed on data collected from the 5 lakes used to create Equation 1 (T. Kjerland analysis of data collected by Vogt, 2011 and Lewis, 2014.) The "R-Squared" value is circled, as are the intercept, slope, and p-value of the slope.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.7795387
R Square	0.6076805
Adjusted R Square	0.6046627
Standard Error	0.4911812
Observations	132

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	48.58053	48.58053	201.3626	3.44182E-28
Residual	130	31.36366	0.241259		
Total	131	79.94419			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-11.615254	0.874831	-13.2771	5.89E-26	-13.34600293	-9.88451	-13.346	-9.88451
X Variable 1	2.5536677	0.17996	14.19023	3.44E-28	2.197639163	2.909696	2.197639	2.909696

Slope of the line

y-intercept

p-values << 0.001

Figure 17. Linear regression results for relationship shown in Equation 1: natural log of plant height vs. natural log of plant weight

STEP 4. Create an equation based on the output of the linear regression. Your equation in the format y = mx + b, where m = slope and b = y-intercept. Example equation from linear regression above:

$$y = 2.55x - 11.6$$

STEP 5. Transform the equation you found in Step 4 so that the "y" input is simply plant weight in grams and the "x" is plant height in cm. This is necessary because the equation at this point is still using log-transformed variables.

You want to convert your equation from Step 4 to the following form:

Plant weight (g) = a(plant height cm)^{m}

The exponent "m" of this equation is exactly the same as the slope "m" of the linear regression. The coefficient "a" is e (2.7182), the base of natural logarithms, raised to the power "b" from the linear regression: $a = e^{b}$

To Transform Your Equation
START HERE → Example equation from linear regression: y = 2.55x – 11.6

Note: At this point, keep all digits for these calculations. Later, round the numbers to account for significant figures.

$$m = 2.55x$$

$$a = e^{-11.615} = 0.00000903 = 9.03x10^{-6}$$

Final Equation

Plant weight (g) = 0.00000903*(plant height, cm)$^{2.55}$

CONGRATULATIONS! YOU'RE DONE! From now on, you will be able measure only your "x" variable, such as "plant height in cm", and use your new equation to compute plant weight (biomass). Go to SOP#4 if you wish to learn more about using biomass equations.

To create biomass equation for pedicel number-weight: These same steps may be used for creating a biomass equation to relate pedicel number to plant weight. Use "natural log of number pedicels per stalk" (i.e. number of potential seeds) as the "x" variable when performing the linear regression. Use "natural log of plant weight per stalk_g" as the "y" variable.

Work an example problem for practice

Working through this problem will allow you to test out the linear regression methods and make sure you're doing the process correctly. To do the problem, open an Excel spreadsheet and follow the steps below:

STEP 1. Enter the following data as shown below.

Data for sample problem from Round Island Lake
(T. Kjerland analysis of data collected by Vogt, 2011.)

	A	B	C	AI	AJ
1	Date	Water_body	Sample_ID#	nat_log_plant _weight	nat_log_plant height
100	8/25/2011	Round Island Lake	RI01	0.27	4.72
101	8/25/2011	Round Island Lake	RI02	-0.16	4.88
102	8/25/2011	Round Island Lake	RI03	1.00	5.08
103	8/25/2011	Round Island Lake	RI04	-0.65	4.40
104	8/25/2011	Round Island Lake	RI05	-0.13	4.65
105	8/25/2011	Round Island Lake	RI06	0.53	4.98
106	8/25/2011	Round Island Lake	RI07	0.53	4.88
107	8/25/2011	Round Island Lake	RI08	-1.72	4.26
108	8/25/2011	Round Island Lake	RI09	0.42	4.76
109	8/25/2011	Round Island Lake	RI10	0.04	4.74
110	8/25/2011	Round Island Lake	RI11	-0.07	4.62
111	8/25/2011	Round Island Lake	RI12	0.30	4.78
112	8/25/2011	Round Island Lake	RI13	0.18	4.74
113	8/25/2011	Round Island Lake	RI14	0.43	4.90
114	8/25/2011	Round Island Lake	RI16	1.18	4.88
115	8/25/2011	Round Island Lake	RI17	0.05	4.84
116	8/25/2011	Round Island Lake	RI18	0.39	4.82
117	8/25/2011	Round Island Lake	RI19	0.49	4.74
118	8/25/2011	Round Island Lake	RI20	0.59	4.80
119	8/25/2011	Round Island Lake	RI21	0.14	4.69
120	8/25/2011	Round Island Lake	RI22	-0.12	4.84

STEP 2. Starting with STEP 1 above under the heading, "Calculate Biomass Equation," run through the steps using the data you entered.

STEP 3. The linear regression results are shown below. Check to make sure they match yours. Troubleshooting: If the results don't match, first check that you entered the data properly. Next, make sure you selected the correct columns on your spreadsheet when running the regression.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.84019892
R Square	0.70593422
Adjusted R Square	0.69045708
Standard Error	0.33102324
Observations	21

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	4.997932	4.997932	45.6114	1.88119E-06
Residual	19	2.081951	0.109576		
Total	20	7.079883			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-13.041295	1.958297	-6.65951	2.28E-06	-17.14005639	-8.94253	-17.1401	-8.94253
X Variable 1	2.77500479	0.410891	6.753621	1.88E-06	1.914999191	3.63501	1.914999	3.63501

STEP 4. Transform the equation because the variables are in a log-transformed format at this point.

START HERE → Equation from linear regression: $y = 2.775x – 13.041$

m = 2.77 (Slope =“X Variable 1”)

Intercept = -13.041

$a = e^{-13.041} = 0.00000217 = 2.17 \times 10^{-6}$

Final Equation for Sample Problem – FOR ILLUSTRATION PURPOSES ONLY

Plant weight (g) = (2.17×10^{-6})*(plant height, cm)$^{2.77}$

Biology of Wild Rice

This section provides a brief introduction to the biology of wild rice. Particularly useful references include: Aiken, et al. (1988) “Wild Rice in Canada”; Vennum, (1988) “Wild Rice and the Ojibway People,” and Dore, (1969) “Wild Rice.”

LIFE CYCLE

Wild rice is an annual plant. Wild rice (*Zizania palustris*[15]) seeds sprout and grow an entirely new plant each year. Some wild rice plants have been known to grow up to 10 feet tall! And this astonishing feat happens without roots from the prior year to “jump start” growth in the spring.
Once mature, wild rice seeds fall from the parent plant into the water and sink quickly down into the sediment. Their aquadynamic shape and weight aids them in moving easily through the water. Wild rice seeds have sharp barbs on one end called “awns” that act like rudders and help drill the seeds down into the muck by keeping them vertical as they fall through the water.


Tonya Kjerland

Because they are heavy, seeds usually don’t fall too far from the parent plant, which helps insure that they land in a spot where they can grow. The exception is when currents are swift, such as in rivers, or in high winds, or when seeds are carried by birds or animals.

The average height and weight of rice plants on Big Rice Lake in St. Louis County, Minnesota was 1.5 m (~5 feet, from sediment to height of tallest stalk) and ~6 grams (0.01 lbs). over the past 16 years[16]. For more examples and information about variability, see “Case Study: 1854 Treaty Authority – Results of Long Term Monitoring of Wild Rice.”

Overwintering. Wild rice seeds only germinate under conditions that mimic being buried in aquatic sediments over a winter or with scarification. Normally, seeds must be kept cold and wet for a period of about 3 months in order to germinate. Desiccation of seeds reduces germination rates considerably. Another way to break seed dormancy is to scrape away the pericarp by hand or mechanically, which is called “scarification.”

The emerging seedling phase (~late April, early May in northern Minnesota). In the early spring, wild rice seeds germinate, probably triggered by temperature, chemical, and light cues in their surroundings. The seed sends a shoot upward at the same time that it sends other shoots downward into

[15] A note on taxonomy. The taxonomy has not always been clear within the literature. For one thing, northern wild rice (*Zizania palustris*) and southern wild rice (*Zizania aquatica*) are frequently confused. Refer to Aiken, et.al. (1988, pp. 21-38) for more on this subject.
[16] The average height and weight is based on data collected between 1998 and 2014 by the 1854 Treaty Authority (Vogt, 2014.) Weight was computed from height using the “Number of Sample Points Equation”. The sizes of these plants may not reflect their natural historic sizes due to possible human impacts, including numerous mining operations in the area.

the sediment. The upward growth of the stem growing towards the surface of the water is prioritized energetically over root elongation. This is because the plant must reach the surface of the water and produce aerial shoots in order to be able to reproduce.

The shoot growing upward towards the light relies on nutrients transferred from the sediment by the early small root system and the seed's own stored energy to grow new cells. If the water is too deep, the plant might take too long to reach the surface, and become dormant, die or not be able to generate enough energy to reproduce before the season ends.

Floating leaf phase (~May to early June).

As soon as the first and only stem (at this point) reaches the surface of the water, the plant sends out two or more leaves along the surface. These leaves develop a waxy cuticle (covering) on one side and stomata (openings to allow for gas exchange of O_2 and CO_2) on both sides, primarily on the top surface (John Pastor, personal observation). Once the floating leaves begin to develop, the plant can use them to photosynthesize more efficiently than is possible under water. Photosynthesis is the means by which plants convert energy in the form of light into biologically-usable energy. At this point, the plant puts more energy into root development to create a foundation for producing aerial (above water) shoots.

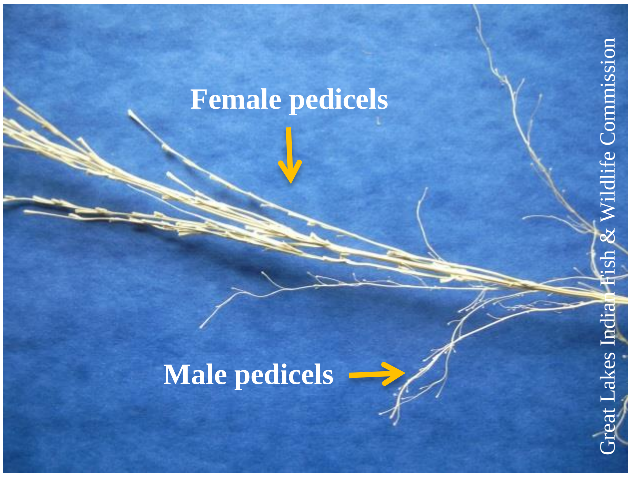
This is a critical phase for wild rice survival. The floating leaves are like buoys attached to the roots. If the water level rises suddenly, or there is choppy water as from a storm or wakes from passing motorboats, the young plant may be easily uprooted because the root system is still not fully developed, and waves create a force against the floating leaf which can uproot the whole plant. Rapidly-rising water levels that remain high may also damage the plant due to the increased difficulty of photosynthesizing under water. If the water level rises gradually, plants may be able to recover by growing taller.

Aerial leaf phase (~ mid June to July). Once root development is sufficient, the plants begin sending shoots up out of the water. Nutrients and sugars are retracted from the floating leaves and used to build shoots whereupon the floating leaves die.

At this point, the plant is able to produce sufficient resources from the energy generated by photosynthesis and nutrients in the sediment. The plant sends up one or more stems that have reproductive structures at the top. The main stem will likely have the most seeds, but there may be many additional stems with seed heads. Factors affecting the number of stems include water depth, nutrient availability, and space to grow.

Reproductive phase (~early to mid August). The female flowers mature earlier than the male flowers, ensuring that the female flowers will be ready for pollination when pollen is available. Male flowers are located below the female flowers on the stem. This helps decrease self-pollination and encourages cross-pollination with other plants because the pollen is shed below the female flowers. Pollen is generally dispersed by wind, although flies, bees, and other insects gather wild rice pollen and may secondarily fertilize female flowers, according to unpublished observations. However, little is known about insects gathering wild rice pollen.

Once female flowers are pollinated, they immediately start forming seeds. The seeds will be tightly held against the seed head at first, and will begin as empty seed hulls. As the seed grows, it fills in the hull and becomes firm. A viable seed is one which will germinate. For the purposes of this Handbook, viable seeds are considered to be those with half or more of the seed hull filled/solid. Each seed grows on a stalk called a pedicel. The male flowers also grow on pedicels, but these are smaller and more delicate compared to the female flowers.



Helpful Tip: The female pedicels are larger and sturdier and are located above the male structures on the stem (see photo, left). Because seeds fall off continuously, counting pedicels is the best way to estimate total seed production. When counting, it is important to count only the female pedicels.

Milk phase (~mid to late August). During the milk phase, the seeds become solid inside and appear plump, but when broken open will be filled with a milky white substance.

Mature phase (~early to mid September). Like berries, the seeds ripen gradually in sections. Due to the gradual ripening of seeds, it is possible to harvest multiple times from the same plants over 2-3 weeks. Seeds are considered mature and viable when the hull is at least halfway filled with solid seed. At this point, the seeds will be easily removed from the stem. High winds at this phase can destroy a harvest in a matter of hours by knocking the seeds from their pedicels. The color of the plants turns from bright green to a lighter, more subdued green, and then to golden amber.

Senescent phase (starts late September to early October). Once plants have lost nearly all of their seeds, stems begin to dry out, rot, and bend over, sinking back into the water. In some places where there are slow currents and dense production for many years, the sediment becomes covered with a thick mat of decaying wild rice. This does not seem to hinder the development of seeds in the coming spring, and instead tends to correlate with highly productive areas, according to anecdotal observations. Wild rice plants take a year or longer to decay and release nutrients for the next generation, which research has shown contributes to annual population variability.

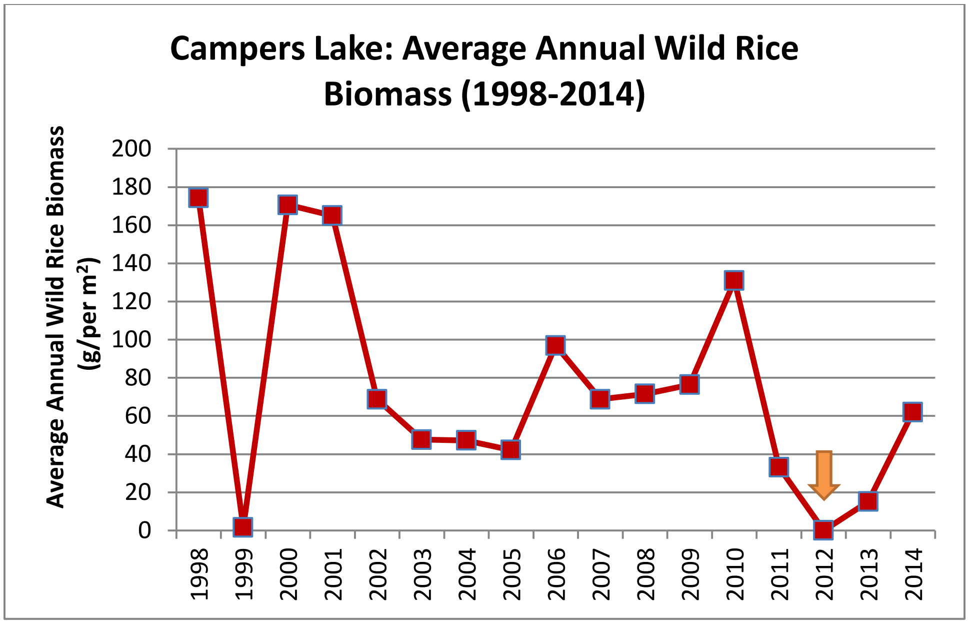
VARIABILITY

Wild rice growth varies greatly from year-to-year in amount and spatial distribution. Many sources have reported a 3 to 5 year cycle in amount of wild rice produced. Analysis of 15 years of data collected by the 1854 Treaty Authority in northeastern Minnesota showed that these lakes often had years of high production followed by a crash and several years of recovery, as well as other, less defined patterns (T. Kjerland analysis of data collected by Vogt, 2014.) These results suggest that abundance and distribution commonly vary in natural wild rice stands.

The documented variability in productivity across years is a clear indication that measurement of a wild rice population needs to be based on several years of data. Due to the lack of knowledge about wild rice populations, more research on these patterns is needed to gain a better understanding of the mechanisms that control variability in wild rice productivity.

Wild rice seeds can remain dormant over a growing season, and probably for 5 years or more. No one knows how long wild rice seeds remain viable in the sediment in natural settings. Evidence for survival of seeds in sediments is demonstrated by cases in which an entire water body becomes barren of wild rice for one season, and then the following year makes a recovery to former, or near former, production levels.

A case in point is Campers Lake in Lake County (MN DNR ID# 38-0679 00), which had no wild rice growing on in it 2012. The following year, the wild rice returned to a greater-than-average cover of 96% and an average biomass of 15 grams per square meter. A similar event happened in 1999 on this same lake, with a full recovery the following year. (See Figure 18)



T. Kjerland analysis of data collected by Vogt, 2014

Figure 18. Wild rice biomass on Campers Lake over 16-years illustrates the variability that can be seen in wild rice populations and the recovery possible from buried seed.

Note: On average, 20 quadrats were measured for all years except 1999, when only 6 were measured due to a low percentage of lake coverage (~6%) by wild rice (Vogt, 2014).

Many water bodies that historically supported vast stands of wild rice have become less productive or even totally lost their wild rice due to human impacts and natural disturbances such as beaver dams. More research is needed to understand conditions and mechanisms that lead to successful wild rice restoration. The successes that have been achieved demonstrate that, when suitable wild rice habitat is restored, these areas can become productive wild rice stands again.

WILD RICE HABITAT

Aspects to consider:

- Water Quality
- Water Depth
- Water Flow
- Sediment
- Plant Community Interactions

Water Quality

Wild rice is considered to be a bio-sentinel for water quality due to its tendency to thrive under specific conditions. If you are interested in measuring effects of water quality on wild rice, you should consider the whole system. Differing conditions of water chemistry have been recorded within wild rice stands compared to open water areas, but these fluctuate considerably with the seasons. In general, water

quality is highly variable across time and location, so it is important to measure it over the entire growing season and in conjunction with quantifiable measurements of wild rice growth. Methods described in this Handbook may be adapted for use throughout the growing season.
Surface water chemistry influences wild rice growth through mechanisms taking place largely in bottom sediments, so sediment characteristics and chemistry should be studied concurrently with water quality. Water flows should also be measured due to the effects of hydrology on sediment transport and transport of associated water-borne particles or elements.

In theory, because it rapidly takes up large amounts of nutrients, wild rice might also *affect* water quality in ways that are beneficial for the ecosystem as a whole. While it is beyond the scope of this Handbook to review the research on water quality and wild rice, this section considers two cases that illustrate complex interactions.

The case of sulfate/sulfide serves to illustrate an important set of interactions between water chemistry, sediments, and wild rice growth. The case of nutrients—phosphorus and nitrogen— explains the most likely mechanism for population oscillations and demonstrates interactions between nutrient availability, decomposition of wild rice detritus, and wild rice productivity. Both cases show the importance of considering the big picture of how the various parts of the system interact through space and time to better understand how water quality affects the condition and extent of wild rice beds.

Sulfate/Sulfide. Recent research regarding the effects of sulfate on wild rice shows that when plants are grown in water with elevated levels of sulfate, each successive generation produces fewer seeds, and a smaller proportion of viable seeds[17]. The same study found other negative effects of increased sulfate, including a reduced germination rate and decreased survival of seedlings. In other words, each successive year of exposure to high sulfate in surface or ground water levels leads to further decreases in the plants' ability to thrive and reproduce. In natural stands, the effect of elevated levels of sulfate is expected to be a continual reduction in the amount of wild rice plants and their reproductive ability.

Although the mechanisms for how elevated sulfate in surface water affects wild rice are still being studied, recent research supports the hypothesis that the conversion of sulfate to sulfide in anoxic bottom sediments is the cause of these detrimental effects (Summary report of the meeting to peer review MPCA's *Draft analysis of the wild rice sulfate standard study* by Eastern Research Group, 2014).

GLOSSARY
Anoxic means a lack of available oxygen for biological processes such as respiration. Anoxic sediments are common in wetlands and many other aquatic environments. An anoxic environment develops due to the normal functioning of bacteria in the process of decomposing biological materials.

Sulfate, which is the common form of sulfur in surface and ground waters, is converted to sulfide in anoxic environments by sulfate-reducing bacteria. This occurs in a region of the sediment where reduction of sulfate to sulfide is the favorable form of respiration for bacteria, which has been referred to as the sulfidic zone (Canfield and Thamdrup, 2009). In an environment without oxygen, these bacteria in sediments convert sulfate to sulfide as part of their natural life cycle of decomposition and respiration.

[17] This section on water quality draws from Pastor (2013) and Moyle (1944).

Sulfate occurs naturally in rocks. It is also discharged and regulated in various industrial processes, such as domestic waste water treatment plants. When rocks high in sulfur are brought to the surface, as in taconite or copper-nickel mining, this brings with it the likelihood that water from the mining operation or leaching from overland runoff will carry high amounts of sulfate into streams and lakes.

Minnesota has a sulfate standard for wild rice waters of 10 mg/L. This standard was established in 1973 based on a scientific survey conducted in 1944 by John Moyle, a respected Minnesota DNR biologist. Moyle sampled waters across the state of Minnesota and showed that while wild rice thrived in waters with low sulfate, no large productive stands existed in waters with sulfate levels higher than 10 mg/L, or 10 ppm. Recent research commissioned by the State of Minnesota and conducted by several research teams from the University of Minnesota strongly supported the science behind this standard ((Summary report of the meeting to peer review MPCA's *Draft analysis of the wild rice sulfate standard study* by Eastern Research Group, 2014).

At the writing of this Handbook (Spring 2015), the Minnesota Pollution Control Agency has put forth a draft proposal recommending a new sulfate standard for wild rice waters. Analysis of the proposal is outside the scope of this Handbook.

Nitrogen and Phosphorus. Nitrogen is the most likely limiting nutrient for the production of wild rice, and phosphorus is likely the second-most limiting nutrient.

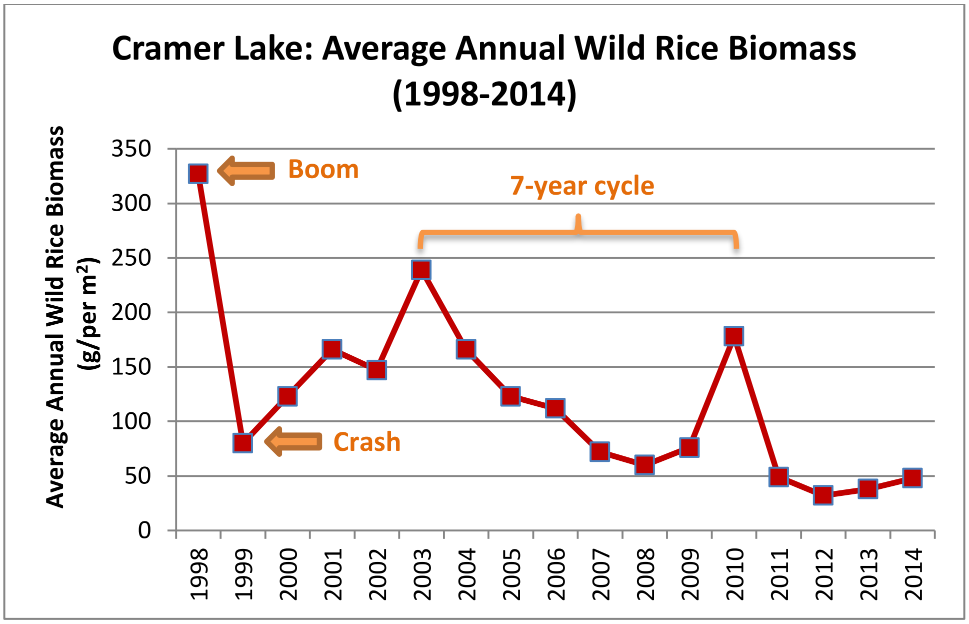
What this means is that even if all other conditions are right for growth, without sufficient nitrogen, the plant's growth will be limited.

Besides recycling existing materials in the system, the primary naturally-occurring sources for nutrients in wild rice waters are surface runoff from the land and ground water inflows. Many land use factors affect the amount of nutrients that will be carried into the water from the land such as amount of erosion, amount of agriculture, use of suburban fertilizers, wastewater treatment plants, etc. Other examples of factors affecting nutrient transport are topography (height and slope of land), morphology (shape of the lake or stream), number of inlets, flow rates, and amount/rate of precipitation.

Wild rice tends to grow best when there is an adequate but not over-supply of nutrients. Too much phosphorus or nitrogen in the water column may lead to increased competition from plants that are able to draw nutrients directly out of the surface water, such as floating-leaved plants. Wild rice gleans its nutrients from the root-sediment zone.

Population patterns.[18] Wild rice harvesters and biologists have long observed that wild rice populations show patterns that resemble cycles. These cycles are not always observed in natural wild rice stands, and vary across sites, but, in general, the cycle consists of a "boom" year of great production followed by a population "crash" to very low levels, then two to three years of recovery, and another boom year (Figure 19). These patterns are sometimes called population oscillations, and are common in natural populations of many plants and animals.

[18] This section on population oscillations draws upon research by Grava and Raisanen (1978), Sain (1984), Walker et al. (2006) and Walker et al. (2010.)



T. Kjerland analysis of data collected by Vogt, 2014

Figure 19. Population variability pattern on Cramer Lake in Northern Minnesota illustrates cycles that have long been observed by wild rice harvesters and biologists.

Why does this happen? In the absence of other mitigating factors, population oscillations may be regulated by nitrogen availability and plant decomposition in the root zone of bottom sediment (Walker, et al. 2010).

To understand what this means, it is important to know how decomposition works. Decomposing vegetation is often referred to as "straw" or "litter." Bacteria are the primary decomposers of wild rice straw, although small invertebrates and fungi also likely play a role in the process. The rate at which they decompose the straw is affected by its chemical composition, such as the ratio of carbon to nitrogen. As bacteria decompose wild rice, they essentially "feed" on the straw and incorporate nitrogen from the straw as well as from the water and sediment into their cells. Thus, less nitrogen remains available for the plants to take up via their roots because it is tied up in bacteria.

Eventually, the straw is mostly decomposed. Gradually, the nitrogen gets released back into the sediment in a usable form (mostly as ammonium) which is again available for plants to use.

The timing of all this is important. If the litter nitrogen isn't recycled back into the root zone environment in a form usable by plants when they need it, then plant production may be reduced.

Decomposition rates vary for different types of plants and, consequently, so does the timing of the release of "available" nitrogen. Wild rice has been shown to decay slowly over the course of about one year, with dead roots taking even longer due to their higher concentrations of lignin, which is harder to decompose.

To understand this process, it is important to understand the timing of when nitrogen is needed in the wild rice growth cycle. Wild rice has the highest nitrogen needs about 1.5 to 2.0 months after seedling emergence, and again during seed production. The amount of nitrogen available at that time will have a strong effect on the amount of wild rice produced. Since wild rice takes a year or more to decompose, the nitrogen from the previous year's straw is still "immobilized" in the bacterial biomass during the following year of growth.

When large amounts of wild rice straw are produced, most of the available nitrogen in the system remains in the plant litter until after the growth spurt for the following year is over. Without enough nitrogen to grow, the following year's crop is strongly nitrogen limited and a "crash" in production occurs. However, by the following year, the nitrogen bound in bacteria is released as the bacteria die as they exhaust their food resources. The wild rice populations begin to recover gradually until the next highly productive year, whereupon the cycle starts again.

Note that this description of wild rice growth and nitrogen recycling in the bottom sediments is somewhat idealized since nitrogen dynamics are one of many factors that affect wild rice productivity. Examples of other factors include water levels, water quality, storms, temperature, wind, wave action, and more. More research is needed to better understand the main causes of variability in the distribution and abundance of wild rice.

Water Depth

Wild rice grows across a limited range of water depths. It is important for the wild rice to be able to get high enough out of the water soon enough after ice-out to produce seeds before the season ends. According to analysis of points sampled for six lakes in Minnesota and Wisconsin, up to four feet (~1.2 m) in August-October is the maximum depth for most stands of wild rice[19]. Michigan may have a larger range of desirable depths due to the ranges and hybridization of two species of wild rice of varying sizes: *Zizania palustris* and *Zizania aquatica*, which predominate in northern and southern Michigan, respectively. No studies designed to analyze maximum rooting depth of wild rice were found at the time of writing of this Handbook.

Water depths that are either too high or too low during the critical growing periods, especially during the floating leaf stage, will hamper wild rice growth. However, plants are quite adaptive to water depths, and respond to water depth changes by allocating more or fewer resources to adding height. Observers report that wild rice seeds will also remain dormant when the water depths are too high, indicating some mechanism (such as pressure) may dampen germination in poor growth conditions. Wild rice seeds require water deep enough to allow them to grow to the emergent state, but once the stalks are strong enough the plants are likely to be able to sustain themselves in fairly low depths.

[19] This sample set included 4 lakes in northeastern Minnesota and two in Wisconsin as described in the section on wild rice biomass equations.

Water depth averaged only ~0.37 cm (14.4 inches) in the most productive part of the Vermilion River (Table 6). An interplay between water depth, current, nutrient supply, and variability throughout the year is likely important. Also, water depth affects the makeup of the community of plants and thus the level of competition.

Table 6. Water depth comparison chart of wild rice stands and water depths

During the top 10 most productive years, water depth ranged between 37 and 95 cm in a set of water bodies from northern Minnesota*

Lake Name	Year	Average Annual Wild Rice Biomass (g/m^2)	Average water depth at time of wild rice sampling (cm)	Number of quadrats sampled
Vermilion River	2006	749	37	19
Vermilion River	2002	586	69	22
Vermilion River	2008	468	66	20
Stone Lake	2001	467	66	20
Stone Lake	1998	440	59	20
Breda Lake	2001	385	77	20
Vermilion River	2004	384	50	22
Vermilion River	2013	379	43	20
Breda Lake	1998	343	70	20
Stone Lake	2002	331	95	22

*Data collected by the 1854 Treaty Authority on 9 lakes and 1 river in Minnesota (1998-2014). Water depth was collected at the same time as wild rice data in August/September.

Helpful Tip: Water depth should be measured over the entire growing season and at points coinciding with wild rice stands.

Water Flow

Research and common wisdom suggests that wild rice requires some water flow to do well, possibly due to input of nutrients and oxygen provided by currents[20]. Wild rice tends to grow best near inlets and outlets. Stagnant waters do not support wild rice populations.

Water flow rates and spatial patterns generally have a large impact on the amount of sediment transported and where it gets deposited. Sedimentation rates may be an important determining factor in the availability of nutrients and minerals that wild rice needs to grow. The transport of sediment is affected by many factors such as shape of the lake or stream. While some current is helpful, too much can lead to “sediment scouring”, in which softer, more organic materials are flushed away so that the area no longer supports wild rice.

[20] This section on water flow draws from research by Meeker (1996).

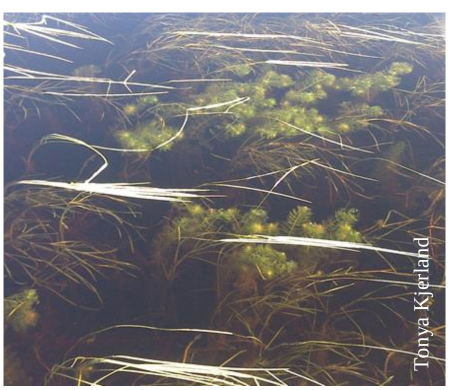
Sediment

Research has shown that wild rice grows over a wide range of sediment types, but there is disagreement over the conditions in which wild rice does best.[21] The characteristics of the sediment that seem to matter most include:

- **Texture**—the sediment must be soft enough for roots to penetrate, but not too soft. Hard substrates may be unsuitable mainly due to a lack of nutrients rather than to the inability of roots to take hold. Soft sediment is generally better, and wild rice seems to thrive in some sites that are too soft for other species.
- **Amount of organic matter**—wild rice generally does better in organic sediments
- **Amount of available nutrients**— wild rice is both influenced by nutrient availability while in turn affecting nutrients due to plant uptake and litter decomposition. The supply rate is what matters most, but this is difficult to measure—and not the same as measuring standing pools of nutrients. For these reasons, while nutrients in sediment are important, it is difficult to list optimal levels.
- **Oxygen levels/Redox potential**—lower growth in anoxic sediments (see Sulfate/Sulfide).

In determining where unsuitable sediment conditions may be affecting wild rice habitat, consider historical records as well as current uses of the waterway. For example, certain types of boating activity such as duck hunting in the fall, churn up the sediment. Some level of this activity may be helpful to wild rice growth if it distributes wild rice seeds more broadly. During other times of the year, boating activity is likely to be harmful, such as from high wakes uprooting young plants, removal of wild rice around docks, or chopping up the plants with motors. Research in many U.S. lake areas has pointed to the significance of boat wakes in degrading nearshore habitats.

Effects of sedimentation, i.e. the deposition of sediment over time, on wild rice have not been studied extensively. Evidence suggests that wild rice prefers flowing water and may alter local sedimentation patterns as it grows. Sediment would be expected to have a positive effect due to the transport of nutrients from land and upstream. However, sediment deposition may have a negative effect if it causes the burial of seeds too deeply for germination. The ability of wild rice to survive in the sediment for mulitple years may be a natural protection against seed burial, and also may explain why it has been reported that churning up sediment (i.e. a moose running through a wetland) may result in fresh growth where previously there was none. More research is needed to understand the effects of sedimentation on wild rice growth.



PLANT COMMUNITY INTERACTIONS

This Handbook recommends identifying other plants as an important parameter for wild rice monitoring plans because, while other plants are suspected to have effects on wild rice, not much is known about how this happens or which species are most influential. Observations suggest that certain types of vegetation

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[21] This section on sediment draws from research by Lee and Stewart (1984), Lee (1986), Aiken et al. (1988), Day and Lee (1989), Painchard and Archibald (1990), Lee and McNaughton (2004).

have negative effects on wild rice, creating areas of lower density or no wild rice. On the other hand, wild rice is frequently found growing productively with other plants. More research is needed to understand the species that have positive, neutral, or negative impacts on natural stands of wild rice.

Wild rice plants must compete for space, light, and nutrients with other plants. In some situations, wild rice may be disadvantaged by being an annual which must grow from a new seed each year. When wild rice populations crash or have a bad year, this opens up the space for perennials to take over the space.

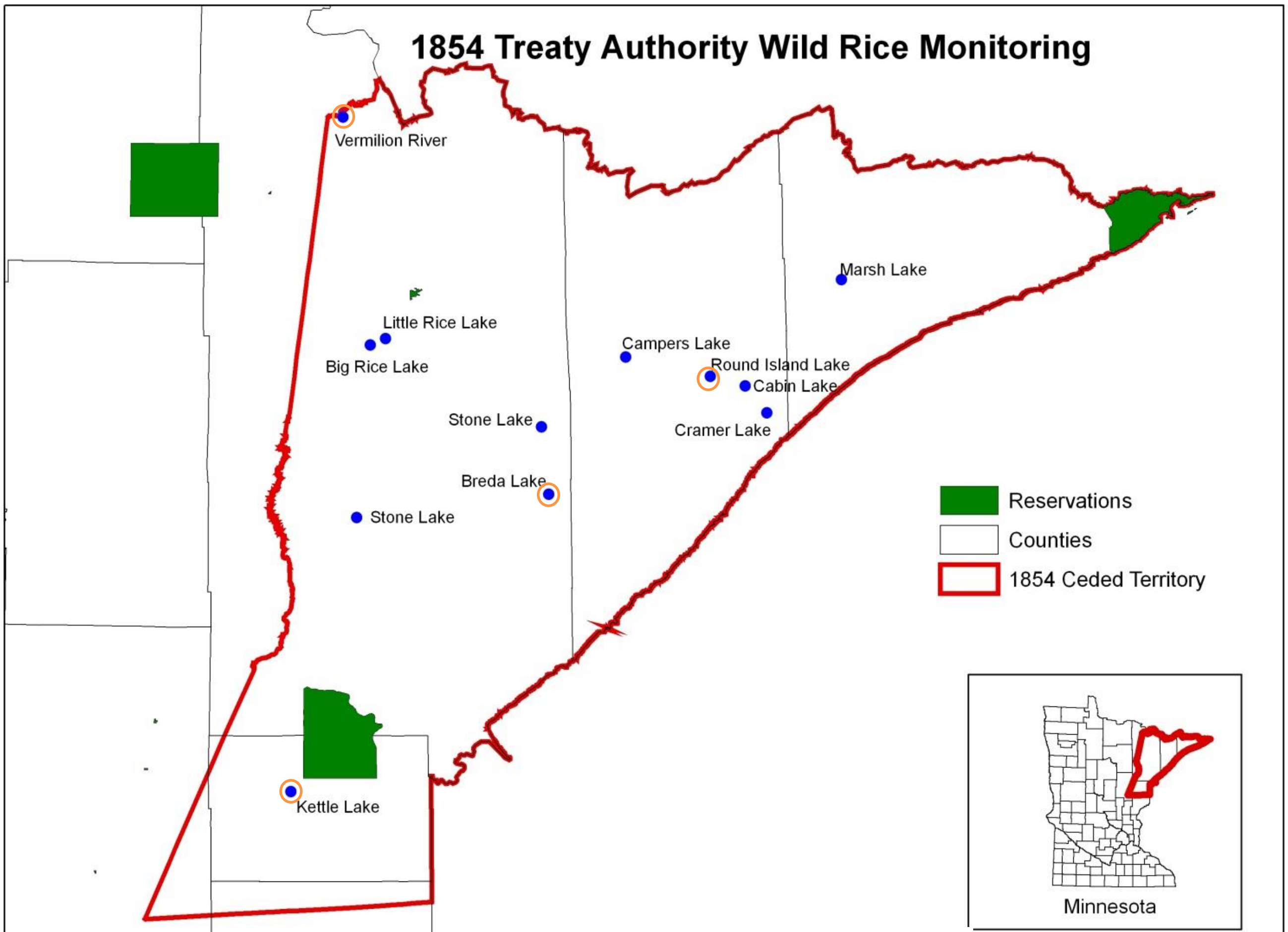
Perennial plants have roots left over from the previous season, which gives them an advantage in being able to grow more quickly in the early season, sometimes shading or crowding out wild rice seedlings and reducing survival.

Besides space and light, plants compete for limited nutrients from the sediment. Plants that are most efficient at "harvesting" nutrients from the sediment due to their root structures or other efficiencies will have a better chance to thrive.

From a management perspective, it is important to keep ecological systems intact and avoid drastic actions (i.e. winter drawdowns) when it is unclear what impact these actions will have on the ecosystem. Little is known about wild rice interactions with other plants, and even less is known about interactions with animals such as aquatic insects, bacteria, frogs, turtles, or muskrats. Wild rice naturally thrives within highly a diverse population of other plants and animals.

Case Study: 1854 Treaty Authority in Minnesota – Results of Long Term Monitoring of Wild Rice

This section demonstrates ways to analyze data collected using methods described in this Handbook. Results are presented from a set of four wild rice waters: Breda Lake, Kettle Lake, Round Island Lake, and Vermilion River. Since 1998, the 1854 Treaty Authority has monitored wild rice waters using methods that are nearly identical to those described in this Handbook (Vogt, 2014).



Map modified from 1854 Treaty Authority

Figure 20. Map of wild rice water bodies where detailed monitoring is conducted annually by the 1854 Treaty Authority

BREDA LAKE

DNR # 69-0037 00

Context. Located in St. Louis County, Breda Lake is a 137 acre (55 hectare) lake. Petrel Creek flows into and out of Breda Lake, and is fairly large. For this reason, the lake is subjected to highly variable water level fluctuations. Breda Lake is shallow; typically less than 3 feet deep across the whole lake. Most or all of the lake can produce rice, but there is often sparse rice or other vegetation (such as water lily) dominating the south end. Although the access is by a 30 minute paddle down Petrel Creek to the lake, it can have heavy use by wild rice harvesters, and some use by duck hunters. There is no public access or development around the lake. Management efforts have included wild rice seeding in the past by the U.S. Forest Service. There has also been some cutting and prescribed burning on a small island area on the north end in an effort to improve waterfowl habitat.

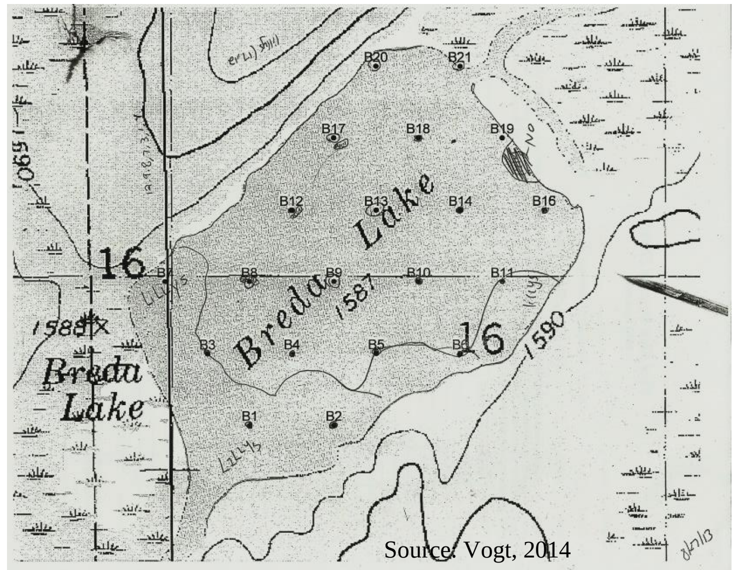
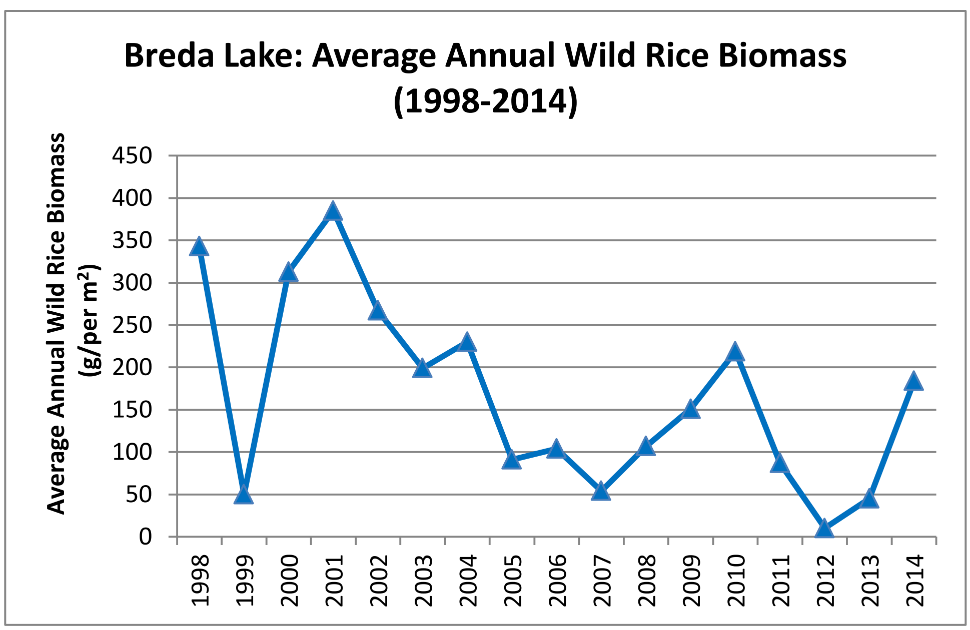


Figure 21. Topographic map showing wild rice sampling points on Breda Lake

Computing biomass. For each water body in this case study, the average annual wild rice biomass amounts were calculated using the "Biomass Equation 1" from this Handbook. Biomass equations are explained in the Sampling Design section. Biomass values represent grams per square meter as measured using 0.5 m^2 quadrats (photo, right). The same sample plots were measured every year, as shown on the map above in

Figure **21**. Quadrats with areas of 0.5 m^2 were used in this study, but to make the data easier to talk about these values were multiplied by 2 to be shown as biomass per 1.0 m^2.



Biomass. Population cycles of 3-6 years are evident in the Breda Lake system. A crash in production in 2015 or 2016 to below 50 g/m^2 would be expected based on this pattern. However, other factors such as weather or flooding might change the actual outcome.

Figure 22. Trends in average wild rice biomass on Breda Lake show population cycles of 3-6 years.

Spatial analysis is useful for relating biomass to other spatial factors such as plant competition, land use or stream inflows. Maps below show the highest biomass areas in red and the lowest areas in green. These maps were created with ArcMap using the inverse distance-weighted (IDW) interpolation method. This means that biomass between quadrats was estimated using a mathematical calculation.

To incorporate spatial analysis into your work, it is recommended that each year wild rice beds be delineated using a GPS. While mapping wild rice beds with a GPS is highly subjective (and takes time), it is needed for doing interpolations in spatial analysis. The accuracy level does not need to be any greater than the distance between sample points.

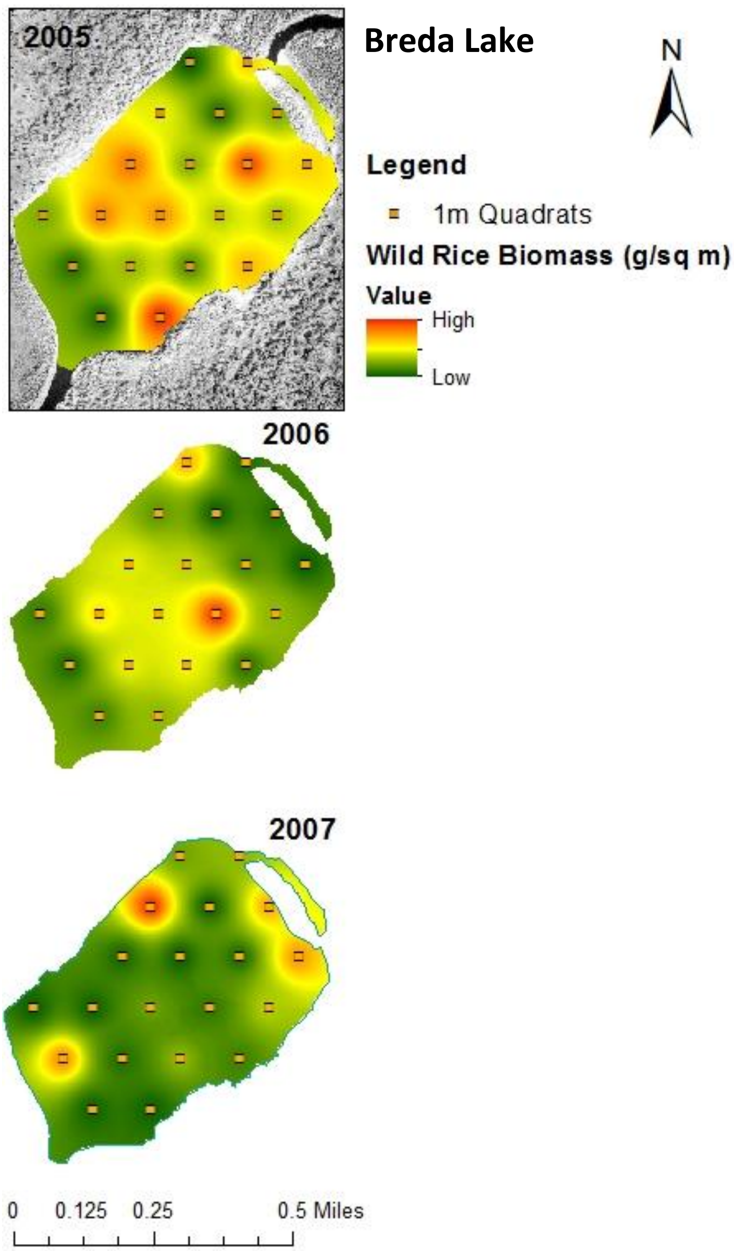
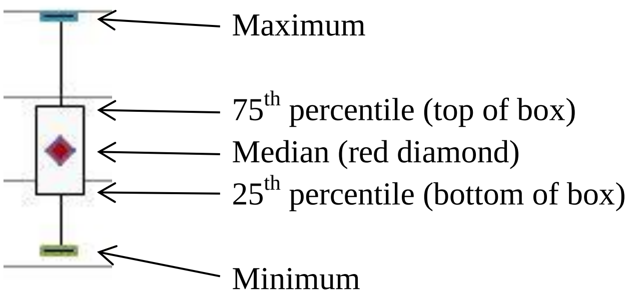


Figure 23. Heat maps of wild rice biomass on Breda Lake between 2005-2010 show that the spatial distribution of areas of highest and lowest biomass vary across time.

Density. The natural variability in density structure of the population is clear from the box and whisker plots below. These plots show changes in average wild rice density (# stalks/m^2) since 1998.

How to read the box and whisker plots



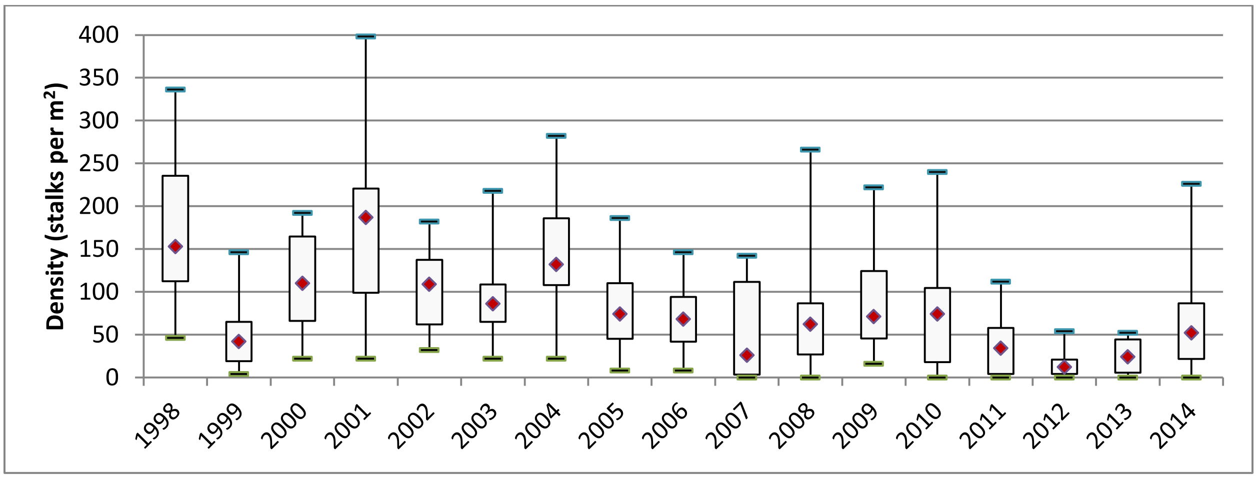


Figure 24. Breda Lake: Wild rice density (1998-2014)

Range of plant characteristics. In the most productive year, median wild rice stalk density was 187 stalks per square meter. Plant height ranged from 33 to 77 inches (0.84 to 2.0 m). Biomass of the most productive plot was 780 g/m^2, and this sample point had a water depth of 30 inches (0.76 m) on August 15, 2001, the date of sampling.

Table 7. Breda Lake: Range of values in most productive year (2001) since 1998

Variable	Min	Median	Max
Total Plant Height (inches)[meters]	33 [0.84]	52 [1.3]	77 [2.0]
Density (Stalks per m^2)	22	187	398
Wild Rice Biomass (g/m^2)	91	354	780
Water Depth at Sampling Date (inches)[meters]	18 [0.46]	30 [0.76]	37 [0.94]
Water Depth at most productive plot = 30 in. [0.76]			

Source: T. Kjerland analysis of data collected by Vogt, 2011

Other plants. 28% of plots contained at least one other species of plant besides wild rice over the entire monitoring period, for a total of 9 different species. The most prevalent species identified was water lily, *Nymphaea or Nuphar spp*. (16% of all plots). Next most prevalent were bladderwort, *Utricularia spp.* (3%), pondweed, *Potamogeton spp.* (3%), and bur-reed, *Sparganium spp.* (2%).

Summary. As expected, after the "down" years of 2012 and 2013, Breda Lake showed a rise in productivity in 2014. Nonetheless, density box plots show that there is a trend over the past ten years (2005-2014) of reduced median density (below 100 g/m^2) compared to the previous seven years. This may indicate a persistent dampening of productivity relative to past conditions. Collection of "related environmental variables", as described in this Handbook, would help identify possible causes.

KETTLE LAKE

DNR #09-0049 00

Context. Located in Carlton County, Kettle Lake is a 611-acre (247-ha) lake with no well-defined inlet, but a large outlet to Kettle River. Inflows are from wetland seepage and drainage from a peat operation. Water levels can fluctuate and be fairly high at times. Flooding in 2012 led to total wild rice failure. Public access is by carrying watercraft down to the lake from a parking area. Harvesters make use of the lake in years when the crop is good. The eastern end—about 25% of the lake—is covered by bog, but rice can be produced across the rest of the lake. Wild rice is often sparse near the center. There is no development on the lake.

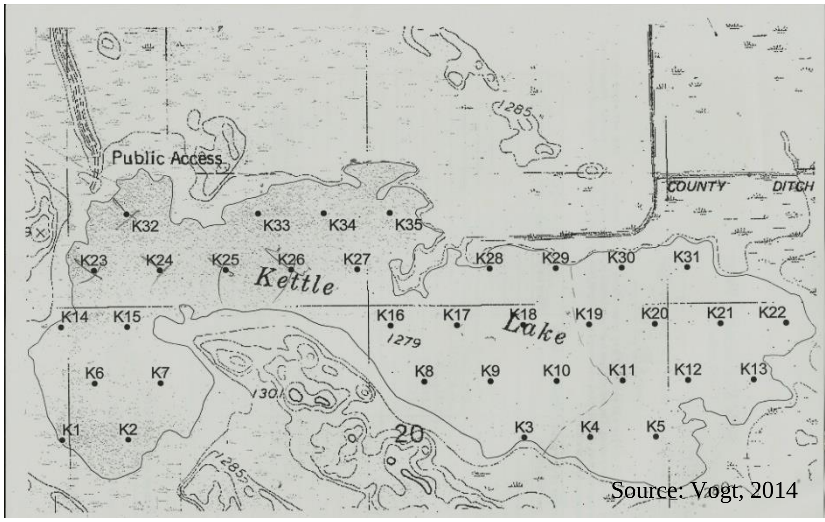


Figure 25. Topographic map showing wild rice sampling points on Kettle Lake

Biomass. Kettle Lake wild rice productivity crashed for the entire lake in 2000, 2005 and 2012. Each time the lake recovered within one to two years. Kettle Lake is a good example of the natural variability and resilience of wild rice beds, and how a lack of plants in one year does not indicate the ability of a sufficient seed bank to produce wild rice in following years. Seed banks are seeds that lie dormant in the sediment.

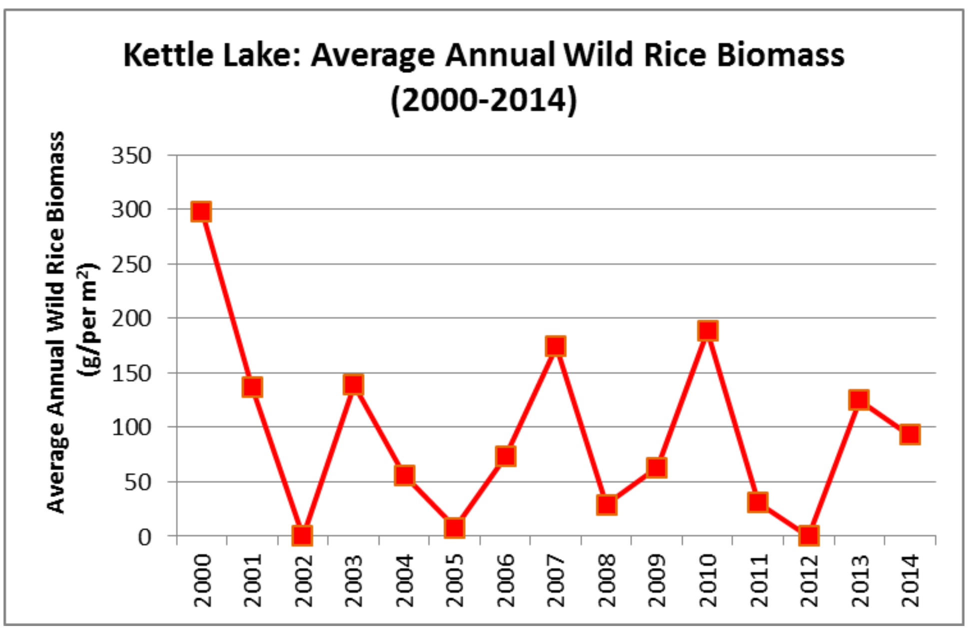


Figure 26. Trends in wild rice biomass on Kettle Lake

Table 8. Kettle Lake: Range of values in most productive year (2000) since 2000

Variable	Min	Median	Max
Total Plant Height (inches)[meters]	56 [1.4]	69 [1.8]	78 [2.0]
Density (Stalks per m^2)	34	55	166
Wild Rice Biomass (g/m^2)	113	265	576
Water Depth at Sampling Date (inches)[meters]	30 [0.76]	41 [1.0]	57 [1.5]
Water Depth at most productive plot = 30 in[0.76 cm]			

Source: T. Kjerland analysis of data collected by Vogt, 2014

Range of plant characteristics. In the most productive year, median wild rice stalk density was 55 stalks per square meter. Plant height ranged from 56 to 78 inches (1.4 to 2.0 m). Biomass of the most productive plot was 576 g/m^2, and had a water depth of 30 inches (0.76 m) on the date when monitoring occurred, August 18, 2000.

Density. The box and whisker plots show that the spread of wild rice density varies greatly from year-to-year on Kettle Lake. It also shows that spatial distribution of density across the lake varies within a given year. Therefore, the amount of biomass also varies across the lake. These plots show changes in wild rice density (# stalks/m^2) since 2000.

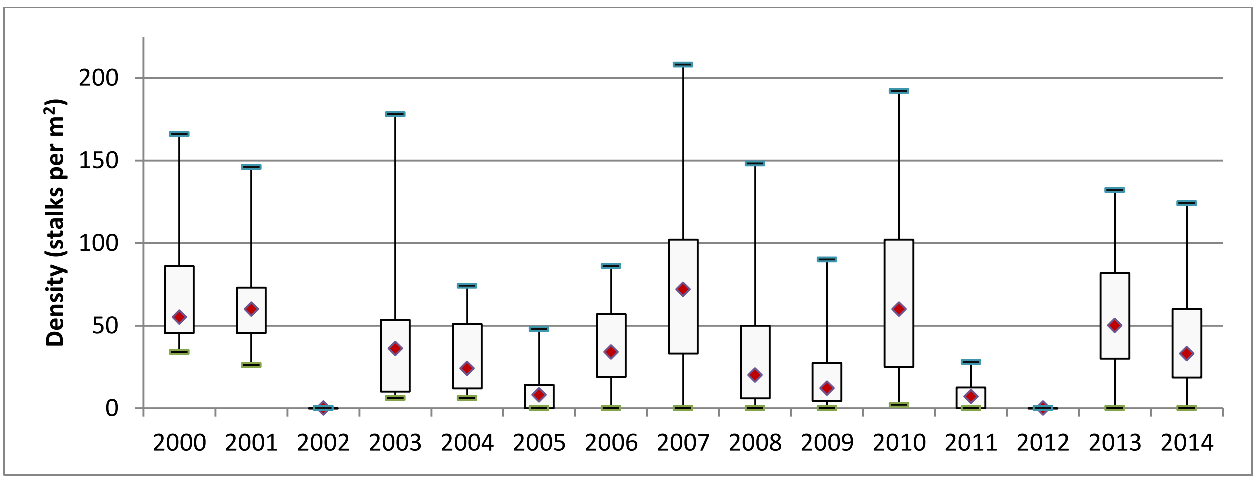


Figure 27. Kettle Lake: Wild rice density (2000-2014)

Other plants. 40% of plots contained at least one other species besides wild rice over the 13 years monitored, for a total of 12 different species. The most prevalent species identified was watershield, *Brassenia schreberi* (19% of all plots). Next most prevalent were pondweed, *Potomageton spp.* (14%), bur-reed, *Sparganium spp.* (5%), and coontail, *Ceratophyllum demersum* (3%).

Summary. Kettle Lake is a resilient, healthy wild rice lake. The population showed recovery after a total crash in production in 2012, which was a year of extreme flooding. The density box and whisker plots demonstrate why more sample points are needed to measure biomass in years of low productivity. Lack of wild rice in a point means there is no measure of density at that point except "zero." Therefore, more sample sites should be added in order to measure density in years when wild rice is sparse.

ROUND ISLAND LAKE

DNR #38-0417 00

Context. Located in Lake County, 54-acre [22-ha] Round Island Lake is shallow and produces wild rice across most or all of its area. There is no defined inlet, and a small creek on the south is the only outlet. The lake has a history of beaver activity, which has been managed by the Minnesota Department of Natural Resources, Ducks Unlimited, and the 1854 Treaty Authority. Public access is by a narrow, rough road that provides only carry-down access to the lake. The access road is on private land, but there is a permanent conservation easement in place to allow for public access for ricing, hunting, and fishing on the public lands surrounding most of the lake. There is no development on the lake. The lake contains a fairly unique flora of small white water lily and small yellow water lily, as identified by the Minnesota Department of Natural Resources.

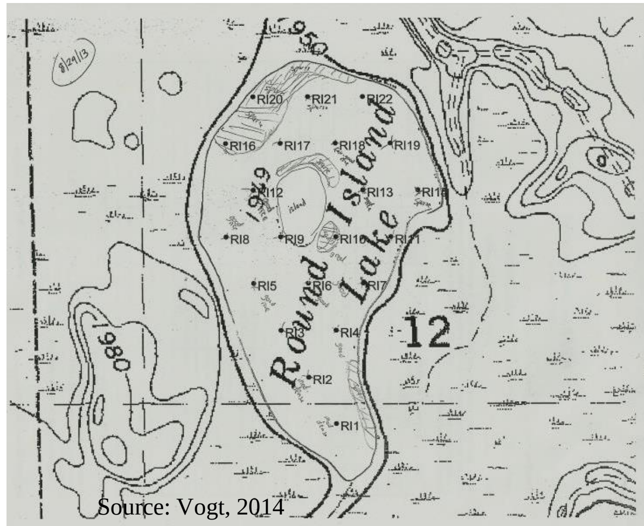


Figure 28. Topographic map of Round Island Lake shows location of wild rice sampling points

Biomass. Population cycles of 3-6 years are evident in the graph below showing average annual biomass. Maximum biomass produced in "boom" years appears to be holding steady at about 250-300 grams per square meter (g/m^2). Note the "crash" in 2008 when biomass fell to record lows, followed 3 years later by a total recovery to maximum levels of production. Graphical estimates would predict productivity for 2015 to increase.

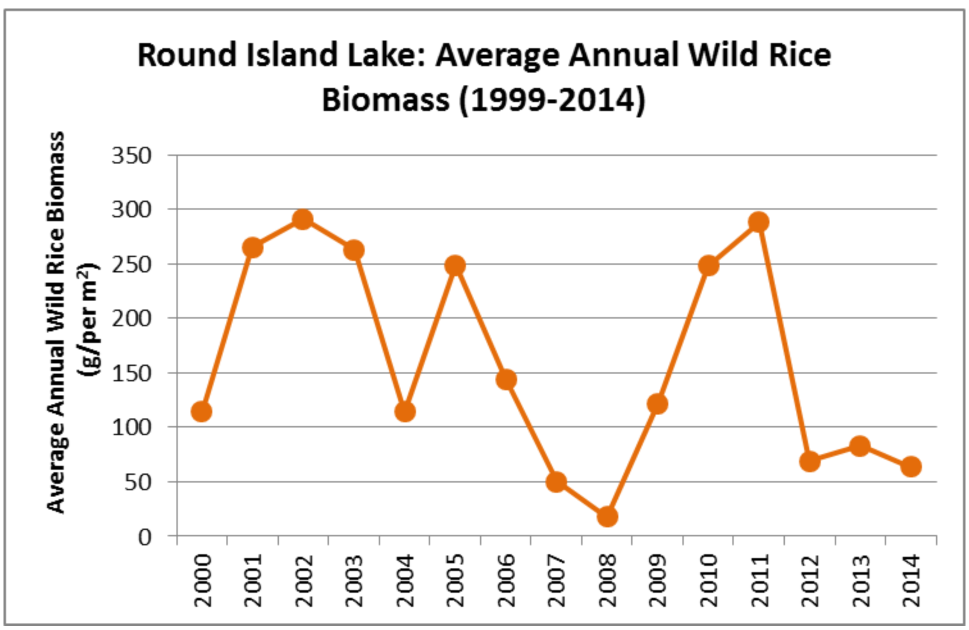


Figure 29. Wild rice biomass on Round Island lakes demonstrates a crash in 2008 followed by gradual recovery in subsequent years

Density of wild rice was highest in the northern half of the lake, but there were two hot spots south of the island with high amounts of biomass, probably due to larger (but fewer) plants. The similarities between the maps of number of pedicels per plant (potential seeds) and individual plant weight are not surprising, given that the number of potential seeds is positively related to plant weight, or biomass.

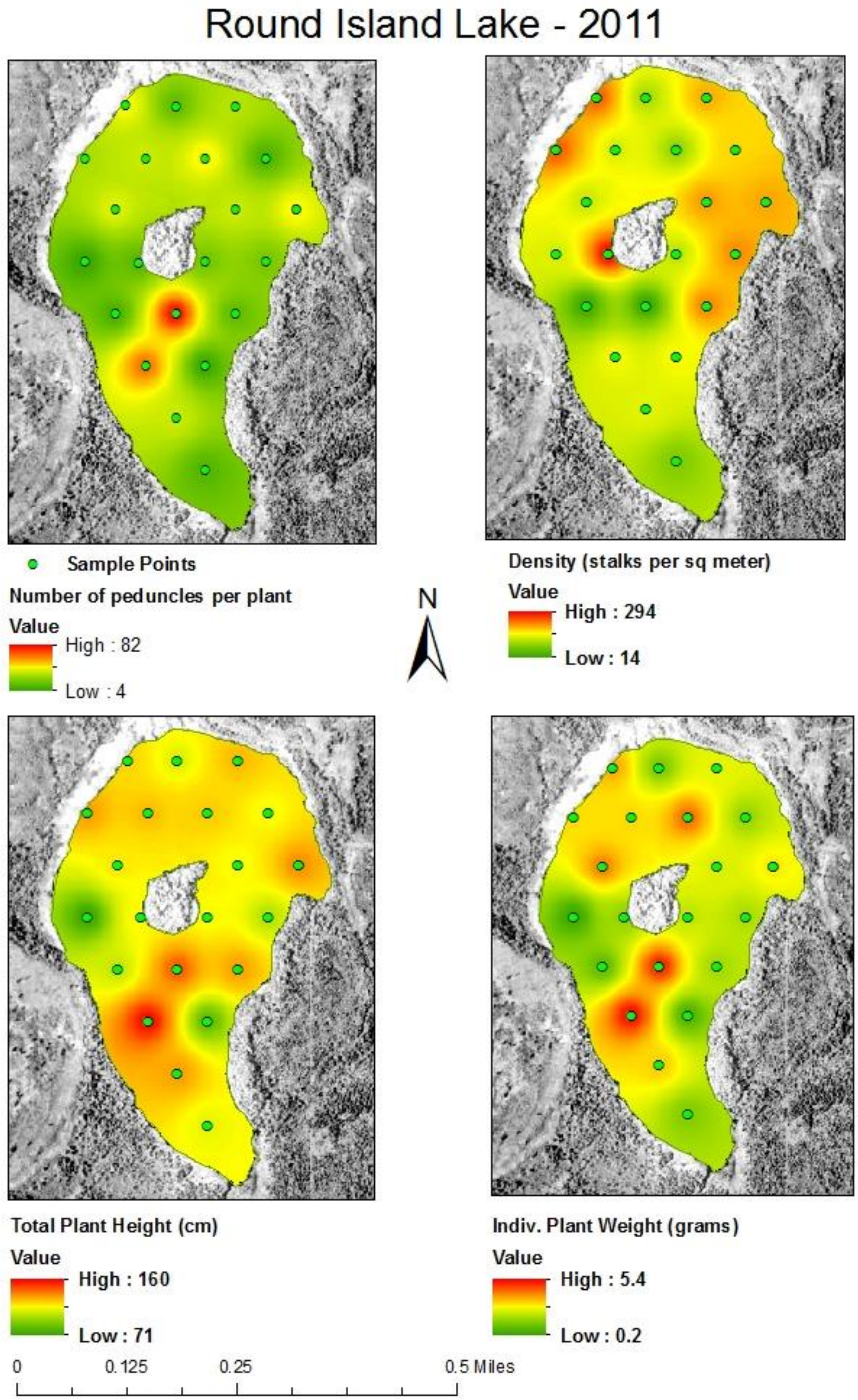


Figure 30. Heat maps of Round Island Lake depict four different parameters of the wild rice population in 2011: number of pedicels, density, plant height, and individual plant weight

Range of plant characteristics. In the most productive year, median wild rice stalk density was 146 stalks per square meter. Plant height ranged from 14 to 68 inches (0.36 to 1.72 m). Biomass of the most productive plot was 661 g/m^2, and it had a water depth of 10 inches [0.25m] on the date when monitoring occurred, August 20, 2002.

Table 9. Round Island Lake: Range of values in most productive year (2002) since 1999

Variable	Min	Median	Max
Total Plant Height (inches)[meters]	14 [0.36]	42 [1.07]	68 [1.72]
Density (Stalks per m^2)	62	146	626
Wild Rice Biomass (g/m^2)	12	308	661
Water Depth at Sampling Date (inches)[meters]	2 [0.051]	11 [0.228]	24 [0.61]
Water Depth at most productive plot = 10 in. [0.25m]			

Source: T. Kjerland analysis of data collected by Vogt, 2014

Other plants. 61% of plots contained at least one other species of plant besides wild rice between 2001 and 2013, for a total of at least 7 different species, not including unknowns. The most prevalent species identified was water lily, *Nymphaea or Nuphar spp.* (46% of all plots). Next most prevalent were bladderwort, *Utricularia spp* (11%), spatterdock, *Nuphar polysepala* (6%), pondweed, *Potamogeton spp.* (6%), and watershield, *Brassenia schreberi* (5%).

Table 10. Weight of wild rice seeds, roots, and shoots based on plants collected in 2011 on Round Island Lake

Variable	Min	Median	Max
Individual Total Plant Weight (grams)	0.179	2.38	5.45
# Potential Seeds	4	29	82
Root Weight (grams)	0.013	0.232	0.986
Shoot Weight (grams)	0.150	1.87	4.21
Viable Seed Weight (grams)	0.016	0.291	0.813

Source: T. Kjerland analysis of data collected by Vogt, 2011

Plant weight data. Round Island Lake was one of the lakes used to create the biomass equations shown in this Handbook. Table 10 shows summaries of the plant weight data from this lake.

Helpful Tip: The values shown above are reasonable ranges for lakes of *Zizania palustris* with similar growing conditions. However, due to genetic and environmental differences, other wild rice populations may show different values. For example, another lake in northern Minnesota showed values on the order of 5-10 times greater than the values shown here (Kjerland, unpublished data).

Summary. It is somewhat surprising that Round Island Lake sustains such a large population of water lily (46% of all plots measured) while still maintaining a strong production of wild rice. Round Island Lake is another example of how a wild rice water body can experience a year of nearly zero production (2008) followed by a full recovery.

VERMILION RIVER

Context. Vermilion River is located in northern St. Louis County. The monitored river reach spans 303 acres [123 ha]. There is also wild rice in other parts of the river. There is no development around this section of the river with the exception of the Goldmine Resort, which has a few cabins and docks on the west end of the reach. Fishing boats use the river channel. The land ownership around the area is primarily state and federal. Public access is afforded by carry-down entry, and is a short paddle down a creek into the river. The area can have significant use by harvesters, and it has been an area of consistently good production along the open/deep river channel. Water levels tend to fluctuate, as is common in a river system, but this doesn't seem to damage the wild rice. In some years, wild rice worms have had a large impact on the crop.

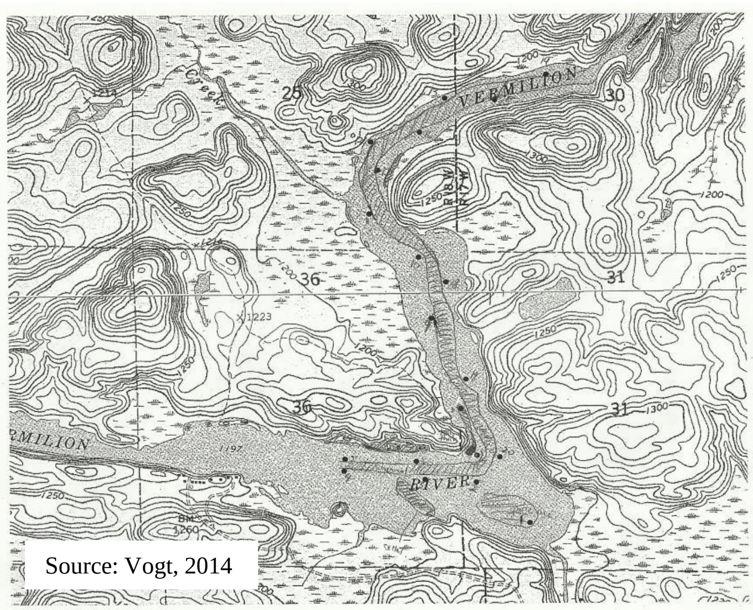


Figure 31. Topographic map of Vermilion River reach showing wild rice sampling points

Biomass. The river produces wild rice consistently well year-to-year. Population oscillations are less evident in the wild rice data from Vermilion River compared to the lakes studied. One explanation could be that flowing water provides consistent nutrient supplies and removes the previous year's wild rice stalks. These conditions would dampen the productivity-nutrient dynamics described in the "Biology of Wild Rice" section.

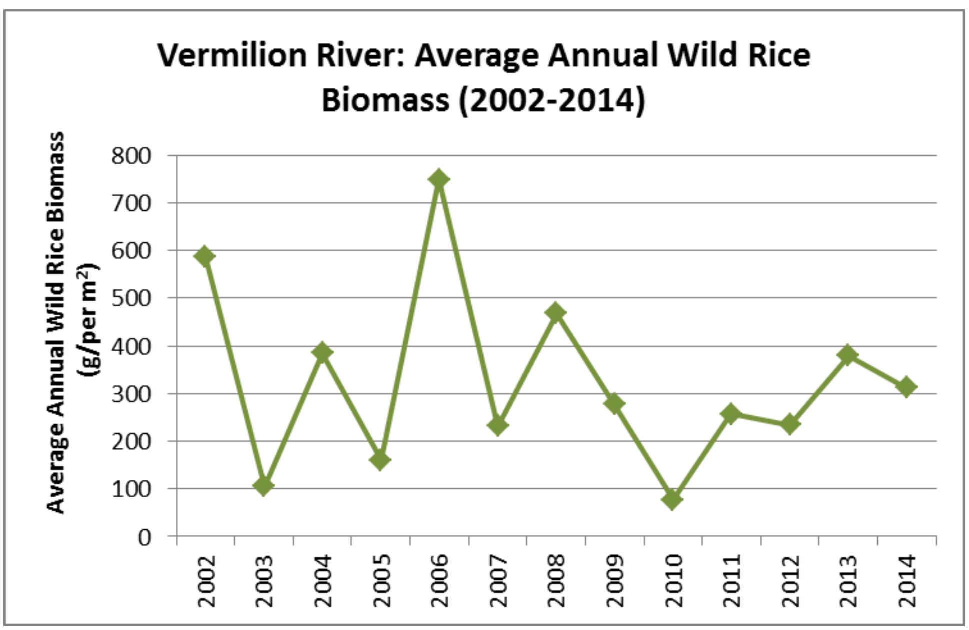


Figure 32. The amount of wild rice biomass growing on the Vermilion River has frequently been the highest among the wild rice waters monitored by the 1854 Treaty Authority

Range of plant characteristics. In the most productive year, median wild rice stalk density was 186 stalks per square meter. Plant height ranged from 47 to 79 inches (1.2 to 2.0 m). Biomass of the most productive plot was 1445 g/m^2 (the highest among the 10 water bodies monitored). The water depth was 13 inches (0.33 m) on the date when monitoring occurred—August 23, 2006.

Table 11. Vermilion River: Range of values in most productive year (2006) since 2002

Variable	Min	Median	Max
Total Plant Height (inches)[meters]	47 [1.2]	61 [1.5]	79 [2.0]
Density (Stalks per m^2)	52	186	410
Wild Rice Biomass (g/m^2)	99	727	1435
Water Depth at Sampling Date (inches)[meters]	0.25 [0.0064]	13 [0.33]	33 [0.84]
Water Depth at most productive plot = 13 in.[0.33 m]			

Source: T. Kjerland analysis of data collected by Vogt, 2014

Other plants. 65% of plots contained at least one other species of plant besides wild rice over the entire monitoring period, for a total of 16 different species. The most prevalent species identified was duckweed, *Lemna spp.* (42% of all plots). Next most prevalent were arrowhead, *Sagittaria latifolia* (15%), coontail, *Ceratophyllum demersum* (9%), and pickerel weed, *Pontederia cordata* (8%).

Summary. The Vermilion River has consistently produced good harvests of wild rice and was frequently the best performing water body among those monitored over the past 16 years. As mentioned above under the "Biomass" section, one explanation for this pattern may be the river flow. The Vermilion River had the highest percentage of plots with other plants besides wild rice at 65%, and 42% of these were duckweed, which was also unusual. However, the presence of these other plant species does not appear to hamper wild rice growth in this highly productive river.

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Resources

Standard operating procedures for Related Environmental Variables:

American Public Health Association. (2011) Standard methods for the examination of water and wastewater. 25th Ed., Washington, DC.
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Wisconsin Volunter Stream Monitoring Program. (2015) http://watermonitoring.uwex.edu/wav/monitoring/index.html
See also, Appendix D

For creating a reference collection of plants:

Haynes, R. R. (1984) Techniques for collecting aquatic and marsh plants. Annals of Missouri Botanical Garden 71:229-231.
Wood, R.D. (1970) Hydrobotanical methods. University Park Press, Baltimore, MD.

For identifying aquatic plants

See list in SOP#3: Identifying Aquatic Vegetation
See *Wild Rice Monitoring Field Guide* for Plant Identification Key

Appendix A: Field and Lab Data Sheets

Go to next page →

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Wild Rice Field Data Sheet

Water body name:___________________________

County:________________ Township: ______________ Range: ________________ Sections(s): _______________
Date: _______________ Crew: ________________________ Sheet is #____ of ____ (# of sheets for water body)

Be sure to record the units of measurement you are using!

			SAMPLE PLANT		
Sample ID#	**# of rice stalks within 0.5 m^2 quadrat**	**Other vegetation present**	**Height ☐ Above water ☐ Total cm / in**	**Water depth cm / in**	**# of stalks on plant (if collecting whole plants)**

(Source: Kjerland, T. 2015. *Wild Rice Monitoring Field Guide*. The University of Minnesota Sea Grant Program, Publication #SH15. ISBN 978-0-9965959-0-2. Field data sheet modified from 1854 Treaty Authority "Wild Rice Density Sheet," 2010.)

Wild Rice Field Notes

Water body name________________________________

Do not forget to map area occupied by wild rice.

Indicate Sample Point ID #'s where appropriate.

Weather conditions (current and past 2-3 days):____

Plots skipped (record Sample Point ID#'s and reason for skipping)

____

____

Observed **Shoreline use** (docks, roads, parking lots, houses, buildings, access points)

____

____

Observed **Water use** (boat traffic, other recreational use)

____

____

Potential concerns for wild rice growth (i.e. pollutants, leaking septic systems, runoff or erosion areas, dredging, physical damage, etc.)

____

____

Brown spot fungal disease - Record severity level *3-5 times per water body* as "0" if wild rice leaf is free of disease, "low" (less than $^{1}/_{3}$ of leaf covered) or "high" (more than $^{1}/_{3}$). See photos in Field Guide or SOP #1.

ID#:	Leaf coverage: ☐ 0 (none) ☐ Low (less than $^{1}/_{3}$) ☐ High (more than $^{1}/_{3}$)
ID#:	Leaf coverage: ☐ 0 (none) ☐ Low (less than $^{1}/_{3}$) ☐ High (more than $^{1}/_{3}$)
ID#:	Leaf coverage: ☐ 0 (none) ☐ Low (less than $^{1}/_{3}$) ☐ High (more than $^{1}/_{3}$)
ID#:	Leaf coverage: ☐ 0 (none) ☐ Low (less than $^{1}/_{3}$) ☐ High (more than $^{1}/_{3}$)
ID#:	Leaf coverage: ☐ 0 (none) ☐ Low (less than $^{1}/_{3}$) ☐ High (more than $^{1}/_{3}$)

Presence of animals, birds, pathogens, or pests

Type	**Presence (check if present)**	**Comments**
Beaver	☐	
Muskrat	☐	
Rusty Crawfish	☐	
Swans	☐	
Ducks	☐	
Geese	☐	
Rice worms	☐	
Ergots	☐	
Leaf sheath & stem rot	☐	
Unusual seed head shape	☐	
Other	☐	
Unknown	☐	

Instructions for Collecting Wild Rice Field Data

1. **Locate sample points using GPS unit.**
2. **Collect water quality and sediment samples, if part of sampling plan.**
3. **Lower the 0.5 m^2 quadrat straight down over the wild rice plants.**
   When placing the quadrat, if there are any stalks leaning in or out, they should be pulled in or out accordingly. If the sample point doesn't contain wild rice, then measure water depth, document presence of other vegetation, write "0" in the other columns, and move on.

4. **Record number of rice stalks within quadrat.**
   Count stalks, not plants.
5. **Identify other plants in the quadrat.**
   Consider creating abbreviations for names of other vegetation to save space.

6. **Select a sample plant that is nearest a designated corner of the quadrat.**
7. **Measure plant height.**
   Decide whether you will measure above water plant height or total plant height, and check the box to indicate your choice. (Note: At this point, you should also take into account whether you will eventually only collect seed heads or the entire plant, Step 9.) If measuring above water plant height, measure from the water line to the top of the tallest stem. If measuring total plant height, pull the plant and record measurement from the top of the roots (if 2+ sets, top of the prop root) to the top of the tallest stem (stems have seeds). Circle the unit of measurement.

8. **Measure water depth.**
   For this measurement, you can either use a Secchi disk or other tool OR, if you pulled the plant, you can measure from the top of the sediment roots or prop roots (if they exist) to the water line. Circle the unit of measurement.

9. **Collect a sample to take back to the lab for analysis.**
   See Step 9 on page 16 of the *Wild Rice Monitoring Field Guide* for instructions on collecting wild rice plants. Decide whether you will collect seed heads only or the entire sample plant. If only collecting seed heads, collect seed heads from every stem on the sample plant. If collecting the entire plant, count and note the number of stalks on the sample plant. Store seed heads or plants on ice until returning to the lab. Be sure to label the bag properly.

10. **Record Field Notes.**
11. **Record brown spot fungal disease severity (randomly at 3-5 points across the waterbody).**
12. **Estimate wild rice stand area.**

Note: Upon returning to the lab, process samples as soon as possible.

(Source: Kjerland, T. 2015. *Wild Rice Monitoring Field Guide*. The University of Minnesota Sea Grant Program, Publication #SH15. ISBN 978-0-9965959-0-2.)

Wild Rice Lab Data Sheet Water body name:_________________________

Date: ___________ Staff initials: ____________________Sheet #___ of ___ (# of sheets for water body)

Plant materials dried for _____ hours at _____ degrees Celsius

Date plant materials were dried: ____________________ Date plant materials were weighed: ____________________

Record weight to the nearest 0.001 grams

Sample ID#	Shoot weight (g)	Root weight (g)	Viable seed weight (g)	Viable seed number	Non-viable seed weight (g)	Non-viable seed number	Number of pedicels per PLANT	Number of seeds with ergots	Number of seeds with worm holes

(Source: Kjerland, T. 2015. *Wild Rice Monitoring Field Guide*. The University of Minnesota Sea Grant Program, Publication #SH15. ISBN 978-0-9965959-0-2.)

(Sample field and lab data sheets filled out)

Wild Rice Field Data Sheet

Water body name: Round Island Lake

County: Lake Township: 59N Range: 8W Sections(s): 12

Date: 8/25/11 Crew: AL, MB Sheet is # 1 of 1 (# of sheets for water body)

Be sure to record the units of measurement you are using!

Sample ID#	# of rice stalks within 0.5 m^2 quadrat	Other vegetation present	SAMPLE PLANT: Height ☒ Above water ☐ Total cm / in	SAMPLE PLANT: Water depth cm / in	SAMPLE PLANT: # of stalks on plant (if collecting whole plants)
RI14	101	AH = arrowhead	31	22	2
RI13	104	AH	24	21	2
RI19	88	WS = watershield	28	17	1
22	98	pondweed, AH	24	26	3
18	44	∅	23	26	3
21	51	∅	19	24	1
17	60	pondweed	24	26	3

Wild Rice Field Notes Water body name Round Island Lake

Do not forget to map area occupied by wild rice.

Indicate Sample Point ID #'s where appropriate.

Weather conditions (current and past 2-3 days): Sunny & calm, rained hard past 2 days

Plots skipped (record Sample Point ID#'s and reason for skipping)

RI23 - on shore, RI40 = dock

Observed **Shoreline use** (docks, roads, parking lots, houses, buildings, access points)

New house being built on southern shore, near R20

Observed **Water use** (boat traffic, other recreational use)

Vegetation (including rice) cleared for dock & beach area for new home (near R20)

Potential concerns for wild rice growth (i.e. pollutants, leaking septic systems, runoff or erosion areas, dredging, physical damage, etc.)

Rainbow-colored, oily film on water near bridge over Turtle Creek - possibly from road; timber being harvested upstream

Brown spot fungal disease - Record severity level *3-5 times per water body* as "0" if wild rice leaf is free of disease, "low" (less than $^1/_3$ of leaf covered) or "high" (more than $^1/_3$). See photos in Field Guide or SOP #1.

ID#: 19	Leaf coverage:	☐ 0 (none)	☒ Low (less than $^1/_3$)	☐ High (more than $^1/_3$)
ID#: 13	Leaf coverage:	☐ 0 (none)	☒ Low (less than $^1/_3$)	☐ High (more than $^1/_3$)
ID#: 11	Leaf coverage:	☐ 0 (none)	☒ Low (less than $^1/_3$)	☐ High (more than $^1/_3$)
ID#: 7	Leaf coverage:	☒ 0 (none)	☐ Low (less than $^1/_3$)	☐ High (more than $^1/_3$)
ID#: 1	Leaf coverage:	☒ 0 (none)	☐ Low (less than $^1/_3$)	☐ High (more than $^1/_3$)

Presence of animals, birds, pathogens, or pests

Type	**Presence (check if present)**	**Comments**
Beaver	☐	
Muskrat	☒	1 lodge = RI14
Rusty Crawfish	☐	
Swans	☒	flock of 4
Ducks	☐	
Geese	☐	
Rice worms	☐	
Ergots	☒	RI 31, 42
Leaf sheath & stem rot	☐	
Unusual seed head shape	☒	crow's foot = RI03
Other	☐	
Unknown	☐	

Wild Rice Lab Data Sheet Water body name: Round Island Lake

Date: 8/30/15 Staff initials: MB Sheet # 1 of 1 (# of sheets for water body)

Plant materials dried for 24 hours at 60 degrees Celsius

Date plant materials were dried: 8/30/15 Date plant materials were weighed: 8/30/15

Record weight to the nearest 0.001 grams

Sample ID#	Shoot weight (g)	Root weight (g)	Viable seed weight (g)	Viable seed number	Non-viable seed weight (g)	Non-viable seed number	Number of pedicels per PLANT	Number of seeds with ergots	Number of seeds with worm holes
RI01	1.063	0.187	0.016	2	0.044	9	11	0	0
RI02	2.574	0.214	0.445	19	0.090	11	35	0	2
RI03	4.205	0.436	0.331	16	0.219	28	65	0	0
04	0.436	0.048	0.008	1	0.031	6	5	0	0
05	0.623	0.076	0.171	8	0.005	1	8	0	0
06	3.981	0.515	0.106	8	0.359	57	82	0	0

Appendix B: Estimating Wild Rice Stand Area

Background
Each method below includes a description, contact person, organization and experience using the method. In any given year, knowing the area where wild rice grew is essential for computing overall biomass and for mapping, such as interpolating values between sample points. Therefore, it is useful to create a rough approximation of the outline of areas where wild rice is found growing each year. Because outlining wild rice beds with a GPS unit is subjective, the accuracy of area measurements may vary between surveyors. Furthermore, wild rice stands often move considerably from year to year due to the variability of annual growth. In order to standardize these rough approximations, it is recommended that whoever does the work be given clear instructions, make notes on what criteria they use to determine where to map, and if possible, the same crew assesses each area each year. Because of GPS inaccuracy and field technician subjectivity associated with collecting this type of data, it should only be used as an estimate for comparing year-to-year variability *within* a specific wild rice waterbody. It is not intended to provide a mechanism for assessing relative condition or productivity *between* (or *across*) wild rice waterbodies.

The most accurate method for creating wild rice maps is to use a hand-held GPS unit and a boat as described in Method A. Radomski et al. (2011) found that using a hand-held GPS unit to delineate bulrush stands in lakes where a surveyor can boat or wade around an area was a reliable method for estimating stand area. Surveyor instructions should be consistent for how to perform the delineation, such as how to handle areas with mixed wild rice and other vegetation. Other factors influencing mapping in the Radomski et al. (2011) study were plant density, patch size and fragmentation, water depth, weather conditions, and lakeshore development. Another consideration is the type of GPS unit, GPS settings, and GIS data processing. For example, surveyors processed the data by extending their nearshore bulrush track lines to the land-lake boundary layer and connected track lines of offshore stands. Radomski et al. (2011) recommend using a 5-sec. interval for the tracking function, depending on desired level of precision.

Method A: Canoe or walk around the wild rice stand using a GPS with a tracking function to record points and produce an outline (bare minimum points needed = 4; 5-sec. or shorter setting for tracking function recommended). The edge of the stand may be identified by moving to the open water where there is no wild rice and then defining the edge according to the most outlying stem. Even one stem is considered part of the wild rice stand. This is a relatively time-consuming method. If there are areas without wild rice, or areas in which wild rice is of differing densities, these areas may need to be treated separately.
Source: Valerie Brady, Natural Resources Research Institute, 5013 Miller Trunk Highway, Duluth, Minnesota 55881; Contact info: http://www.nrri.umn.edu/staff/vbrady.asp; How used: estimating areas of various stands of aquatic vegetation.

Method B: While completing sampling, the field crew uses a map of the water body printed on waterproof paper with a grid of GPS points. Throughout the day, the crew draws areas of
1) wild rice, 2) sparse rice, 3) open water, or 4) other vegetation. Later, using a transparent grid overlaid

on the lake map, estimate area of wild rice in relation to total lake area. These polygons may also be digitized for use with mapping software. For purposes of making within lake comparisons, "Sparse wild rice" may be defined as areas with greater than one canoe length between rice stalks.
Source: Darren Vogt, 1854 Treaty Authority, 4428 Haines Road, Duluth, Minnesota 55811-1524; Contact info: www.1854treatyauthority.org/contactus.htm ; How used: for annual wild rice inventories.

Variation on Method B: Print a color photo of each site from Google Earth for the field crew rather than using the map of GPS points. In the field, the crew uses a marking pen to draw the outlines of the wild rice beds on the photo. Later, back in the office, an analyst brings up the Google Earth image again. Looking at what the field crew drew and the measuring tool in Google Earth, an area estimate for the wild rice stand is determined. This variation would be expected to have a lower accuracy compared to the method used by the 1854 Treaty Authority.
Source: Valerie Brady, Natural Resources Research Institute, 5013 Miller Trunk Highway, Duluth, Minnesota 55881; Contact info: http://www.nrri.umn.edu/staff/vbrady.asp; How used: estimating areas of various stands of aquatic vegetation.

Method C: Use laser range-finders to estimate stand size. This method has been successfully used for other types of aquatic vegetation such as cattails. Accuracy depends upon the field crew being able to see clearly the edge of the bed from where they are AND have a good vertical target at that edge to "shoot" the laser against. This is a time-saving method, but accuracy with wild rice remains uncertain.
Source: Valerie Brady, Natural Resources Research Institute, 5013 Miller Trunk Highway, Duluth, Minnesota 55881; Contact info: http://www.nrri.umn.edu/staff/vbrady.asp; How used: estimating areas of various stands of aquatic vegetation.

Appendix C: Data and Summary Statistics for Lakes Included in Biomass Equations

How the Generic Biomass Equations were Determined

First, a word of caution: the biomass equations presented in this Handbook are not meant to exactly determine the weight or biomass. Rather, they are the best nondestructive approximation available that can be applied broadly to show trends over time within a site. Ways to use computed biomass are illustrated in the Case Study section. Comparisons between two water bodies in absolute amounts should be used with caution. More research is needed to further develop the statistical relationships between wild rice height, seed number, and biomass across different water bodies.

Minnesota: Wild rice plants were collected between August 22 and 25, 2011, from four lakes in the 1854 Ceded Territory in northeastern Minnesota: Cabin, Campers, Round Island and Stone lakes. The number of plants collected ranged from 13 and 21 per lake, respectively, for a total of 64 plants.

Wisconsin: Wild rice plants were collected between August 18 and September 17, 2014, from two lakes in northeastern Wisconsin, within 30 miles of Rhinelander: Aurora Lake (n=45), Cuenin Lake (n=53).

River wild rice was not represented in either Minnesota or Wisconsin, so it may be especially desirable to create new biomass equations if you are monitoring a river rice site.

Methods: Each plant was carefully uprooted from the sediment to retain all root material and measured for height. Plant roots were washed carefully in the lake water and then folded accordion-style and stored on ice in labeled plastic zippered bags. Plants were handled so as to preserve as many seeds as possible. Plants were thoroughly washed in the lab to remove all sediment and allowed to air dry in a drying room. Prior to weighing, plants were dried at 60°C for 24 hours.


Photo: GLIFWC

Plants were separated into 3 portions—roots, shoots, and seeds. Seeds were characterized as either viable or non-viable based on visual and physical inspection. Seed weight included both viable and non-viable seeds according to their proportions found in the sample. Total plant weight included roots, shoots, and seeds.

Biomass equations were computed using statistical software. Separate equations were developed for plant height-biomass and seed number-biomass. Lakes that were included in the equations showed statistical significance for the relationship represented by the equation. See SOP #5 for further explanation about how the biomass equations were computed.

Table 12. Characteristics for lakes used to create biomass equations

Lake Name	Aurora	Cabin	Campers	Cuenin	Round Island	Stone
ID	1592700 (WBIC)	38026000 (MNDNR)	38067900 (MNDNR)	1568800 (WBIC)	38041700 (MNDNR)	69004600 (MNDNR)
County State	Vilas WI	Lake MN	Lake MN	Oneida WI	Lake MN	St. Louis MN
Year wild rice plants were collected for biomass equations	2014	2011	2011	2014	2011	2011
Area (acres) [hectares]	94 [38]	67 [27]	56 [23]	28 [11]	54 [22]	230 [93]
Max depth (ft) [m]	4 [1.2]	3 [0.91]	3 [0.91]	4 [1.2]	4 [1.2]	3 [0.91]
Bottom	30% sand, 30% gravel, 0% rock, 40% muck	N/A	N/A	0% sand, 0% gravel, 0% rock, 99% muck	N/A	N/A
% Littoral area	100%	100%	100%	100%	100%	100%
Sulfate, as SO_4	N/A	2007-2012 range: 1.5 to 3.3 mg/L (MPCA)	N/A	N/A	2011-2012 range: 0.5 to 0.6 mg/L (MPCA)	2007-2012 range: 2.5 to 4.7 mg/L (MPCA)

WBIC = Water body ID; MNDNR = Minnesota DNR Lake ID

Sources: Minnesota Pollution Control Agency (MPCA) lake profile web pages; MN DNR Lake finder web pages; WI DNR lake pages

Resources for lakes included in the biomass equations

Aurora Lake

- Wisconsin Department of Natural Resources, Wisconsin State Natural Areas Program: Aurora Lake (no. 127) http://dnr.wi.gov/topic/lands/naturalareas/index.asp?sna=127
- Wisconsin Department of Natural Resources. (1999) Biotic inventory and analysis of the Northern Highlands-American Legion State Forest: A baseline inventory (1992-96) and analysis of natural communities, rare plants and animals, aquatic invertebrates, and other features in preparation for State Forest Master Planning, http://dnr.wi.gov/files/PDF/pubs/er/ER0093.pdf

Cabin Lake

- Minnesota Department of Natural Resources Lake Finder profile http://www.dnr.state.mn.us/lakefind/lake.html?id=38026000
- Minnesota Pollution Control Agency lake profile http://cf.pca.state.mn.us/water/watershedweb/wdip/waterunit.cfm?wid=38-0260-00

Campers Lake

- Minnesota Department of Natural Resources Lake Finder profile http://www.dnr.state.mn.us/lakefind/lake.html?id=38067900
- Minnesota Pollution Control Agency lake profile http://cf.pca.state.mn.us/water/watershedweb/wdip/waterunit.cfm?wid=38-0679-00

Cuenin Lake

- Wisconsin Department of Natural Resources, Cuenin Lake
  http://dnr.wi.gov/lakes/lakepages/LakeDetail.aspx?wbic=1568800

Round Island Lake

- Minnesota Department of Natural Resources Lake Finder profile
  http://www.dnr.state.mn.us/lakefind/lake.html?id=38041700
- Minnesota Pollution Control Agency lake profile
  http://cf.pca.state.mn.us/water/watershedweb/wdip/waterunit.cfm?wid=38-0417-00

Stone Lake (Lake ID 69004600)

- Minnesota Department of Natural Resources Lake Finder profile
  http://www.dnr.state.mn.us/lakefind/lake.html?id=69004600
- Minnesota Pollution Control Agency lake profile
  http://cf.pca.state.mn.us/water/watershedweb/wdip/waterunit.cfm?wid=69-0046-00

Table 13. Wild rice plant characteristics and water depths of lakes used for biomass equations

(T. Kjerland analysis of data collected by Vogt, 2011)

	Range of Values, 2011					
	Cabin Lake, MN			Campers Lake, MN		
Variable	Min	Median	Max	Min	Median	Max
Total Plant Height (inches)[m]	20 [0.51]	38 [0.97]	54 [1.4]	22 [0.56]	38 [0.97]	74 [1.9]
Shoot weight (g)	0.102	0.573	1.33	0.238	2.62	5.18
Root weight (g)	0.010	0.104	0.188	0.060	0.640	1.94
Seed weight (g)	0.000	0.036	0.155	0.007	0.203	0.808
Individual Plant Weight (g)	0.157	0.720	1.63	0.305	3.86	7.04
Density (Stalks per m^2)	2	6	86	2	32	110
# Potential Seeds per Plant	3	11	39	4	39	106
Water Depth at Sampling Date (inches)[m]	12 [0.30]	19 [0.48]	34 [0.86]	8 [0.20]	18 [0.46]	35 [0.89]
% Wild Rice Coverage, 1998-2013	47	89	100	0	86	100

	Range of Values, 2011					
	Round Island Lake			Stone Lake		
Variable	Min	Median	Max	Min	Median	Max
Total Plant Height (inches)[m]	28 [0.71]	47 [1.2]	63 [1.6]	38 [0.97]	55 [1.4]	66 [1.7]
Shoot weight (g)	0.150	1.87	4.21	1.03	2.45	12.7
Root weight (g)	0.013	0.232	0.986	0.060	0.490	1.82
Seed weight (g)	0.016	0.291	0.813	0.050	0.342	3.16
Individual Plant Weight (grams)	0.179	2.38	5.45	1.22	3.54	17.70
Density (Stalks per m^2)	14	152	294	2	26	162
# Potential Seeds per Plant	4	29	82	5	21	267
Water Depth at Sampling Date (inches)[m]	13 [0.33]	26 [0.66]	34 [0.86]	18 [0.46]	29 [0.74]	43 [1.1]
% Wild Rice Coverage, 1998-2013	31	84	100	21	52	75

	Range of Values, 2014					
	Aurora Lake, WI			Cuenin Lake, WI		
Variable	Min	Median	Max	Min	Median	Max
Total Plant Height (inches)[m]	40 [1.0]	61 [1.5]	79 [2.0]	31 [0.8]	51 [1.3]	61 [1.5]
Shoot weight (g)	1.38	4.30	26.2	0.692	2.93	13.4
Root weight (g)	0.357	1.04	8.11	0.169	1.57	7.84
Seed weight (g)	0.341	1.04	6.88	0.0249	0.260	1.21
Individual Plant Weight (grams)	2.55	7.43	37.3	1.03	4.67	21.8
Density (Stalks per m^2)	18	98	186	6	80	240
# Potential Seeds per Plant	8	53	312	14	48	216

	Range of Values, 2014					
	Aurora Lake, WI			Cuenin Lake, WI		
Variable	Min	Median	Max	Min	Median	Max
Water Depth at Sampling Date (inches)[m]	20 [0.51]	34 [0.86]	48 [1.2]	11 [0.28]	23 [0.58]	42 [1.1]
% Wild Rice Coverage, 1998-2013	na	na	na	na	na	na

GLOSSARY

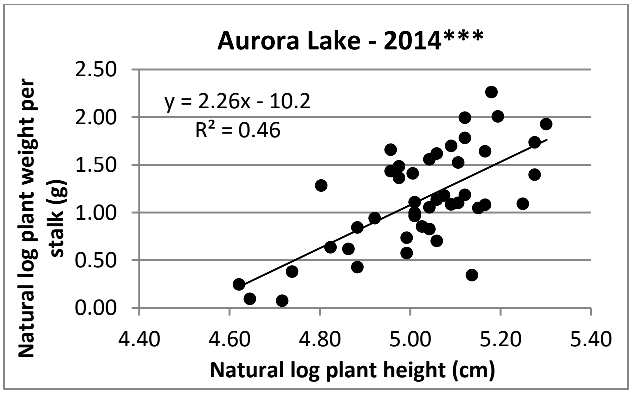
Median is the middle value of a set of numbers. The median and mean/average will be very similar when a set of numbers is normally distributed, but the median will be different, and more representative, when there is a great deal of "skewness" to the data. Wild rice density data tend to be skewed towards more plots of low density with only a few plots having high density.

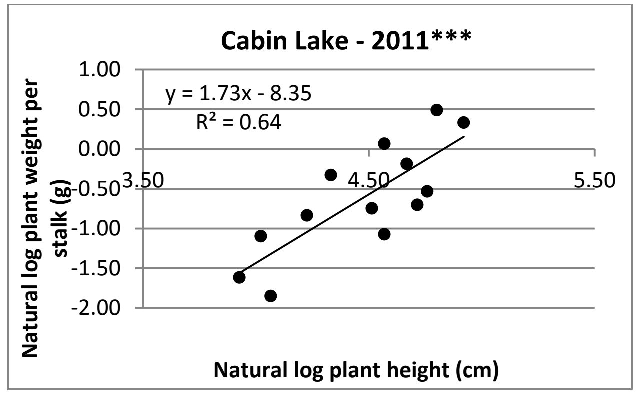
Helpful Tip: While these values provide a good ballpark estimate, it is possible that the population of wild rice plants you measure will show significantly different characteristics from those listed here. For example, other lakes may have higher values that differ by 10 times or more, on average (Kjerland, unpublished data).

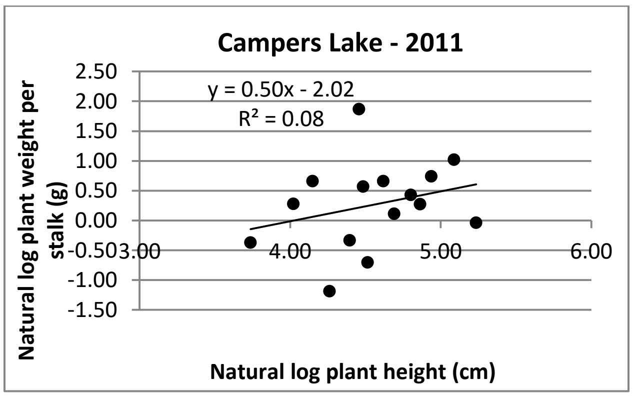
Equation 1: Plant Height – Weight

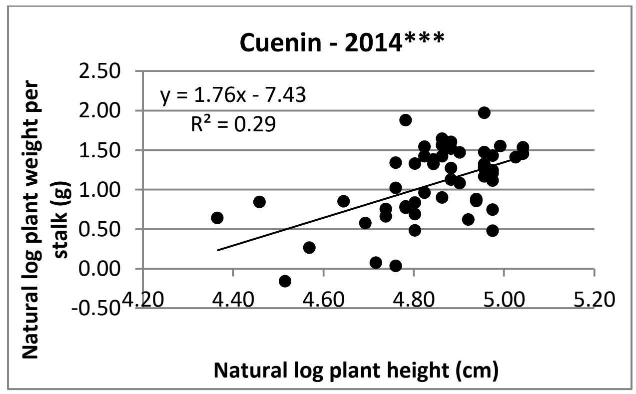
Note: Variables were log transformed

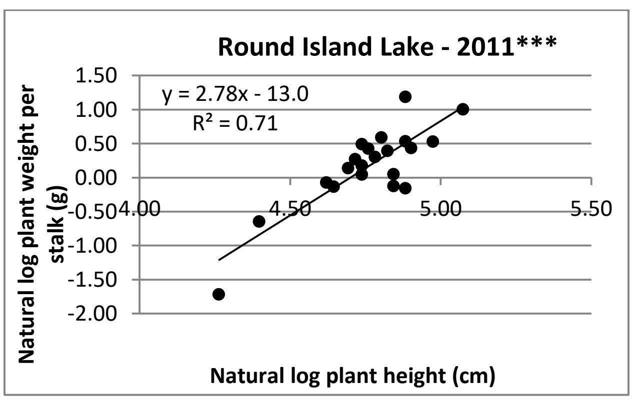
Statistical significance: * = p ≤ 0.05   ** = p ≤ 0.01   *** = p ≤ 0.001

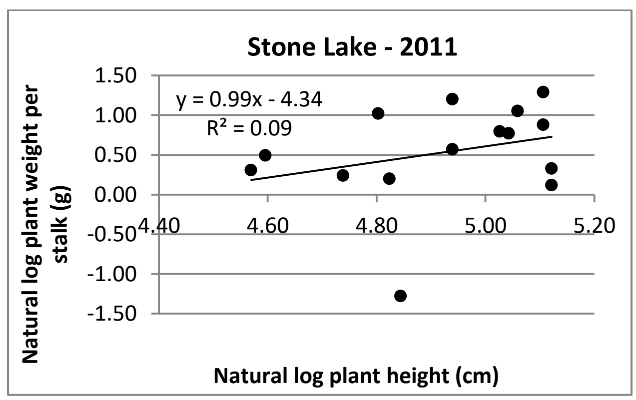








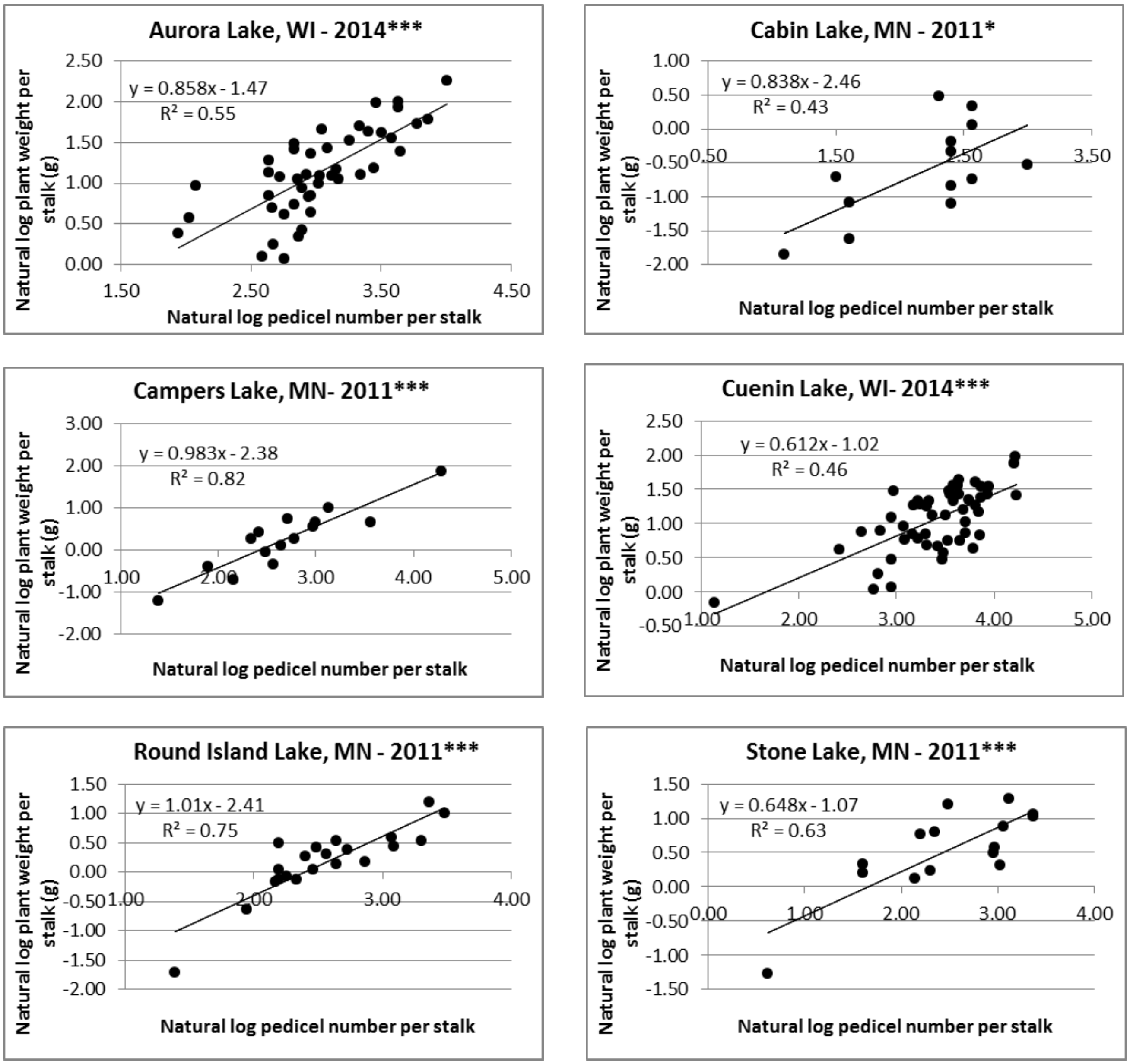




Equation 2: Number of potential seeds (pedicels) per stalk - Weight per stalk (g)

Note: Variables were log transformed

Statistical significance: * = $p \leq 0.05$ ** = $p \leq 0.01$ *** = $p \leq 0.001$



Appendix D: Water Quality and Sediment Sampling Methods

Modified from methods provided by Nancy Schuldt, Fond Du Lac Band of Lake Superior Chippewa

For more information on recommended parameters to measure and sampling frequency, see the section in this Handbook, "Related Environmental Variables,"

SURFACE WATER

Field measurements for surface water <u>every site visit</u>*:

- Electrical conductance (EC25)
- Dissolved oxygen (mg/L and % saturation)
- pH
- Temperature
- Turbidity (using field sensor or lab meter)
- Secchi transparency in lakes
  - In lakes with shallow, clear waters, use a secchi tube (transparency tube)
  - In deeper areas of lakes, use a secchi disk

**These are standard multi-sensor probe parameters, i.e. Hydrolab, YSI, etc.*

Laboratory analyses for surface water <u>every site visit</u> (surface grab sample):

- Alkalinity
- Total hardness
- Color (true and apparent) and Dissolved Organic Carbon if resources allow (color is low-cost, but less accurate proxy for dissolved carbon)
- Nitrogen
  - Ammonium [nitrate + nitrite],
  - Total Kjeldahl Nitrogen (TKN)
  - Total-N (has lower detection limit, therefore preferred over TKN)
- Phosphorus (Total, Ortho-P)
- Total suspended solids
- Chlorophyll *a*
- Sulfate

Laboratory analysis of <u>surface water</u> performed once annually per water body: One sample per year from each lake, stream, and river site should be analyzed for the following suite of toxic chemicals and heavy metals: unionized ammonia (only if NH_4-N is relatively high [≥0.10 mgN/L], i.e. when pH>8.5, as a rule of thumb), chloride, aluminum, arsenic, cadmium, chromium, copper, lead, nickel, selenium, and zinc.

SEDIMENT

Laboratory analysis of <u>sediments</u> performed once annually per sample site (top 5 cm grab, using petite Ponar or Eckman dredge)

- Nitrogen (TN)
- Phosphorus (TP)
- % water (Total solids)
- Total volatile solids (a measure of organic matter, same as ash-free dry weight [AFDW])
- Iron (essential micronutrient)

SAMPLING PARAMETERS AND JUSTIFICATION

Chemical Parameters		Justification
***Field Measurements** - Water*		
Electrical Conductance (EC25)		General characteristic; indicator of overall mineral content
Oxygen, Dissolved & % Saturation		General characteristic; indicator of organic loading
pH		General characteristic
Secchi Transparency		General characteristic; trophic state indicator
Temperature		General characteristic
Turbidity*		Indicator of sedimentation/erosion, primary productivity

*Turbidity (may be measured either with a multi-sensor probe or with a lab instrument)

Chemical Parameters		Justification
Laboratory Measurements - Water		
Alkalinity, Total		General characteristic; measure of acid buffering capacity
Chlorophyll a		A measure of algal density; trophic state indicator
Color, true and apparent		Measure of substances suspended and in solution
Dissolved Organic Carbon		Measure of refractory organic compounds (resistant to rapid microbial degradation) in surface runoff, seepage
Hardness, Total		A measure of mineral concentration
Nitrogen, Ammonium	**Most likely limiting nutrient (Walker, et al, 2010)**	Nutrient; potentially toxic to aquatic organisms
Nitrogen, Nitrate+Nitrite		Nutrient
Nitrogen, Kjeldahl, Total (TKN)		Nutrient (organic-N + ammonium-N, most is organic-N in natural, or unpolluted, waters)
Nitrogen, Total		Nutrient
Phosphorus, Ortho	**2nd most likely limiting nutrient**	Nutrient; trophic state indicator
Phosphorus, Total		Nutrient; trophic state indicator

Suspended Solids, Total	Indicator of sedimentation/erosion
Sulfate	Can be inhibitory to wild rice
Laboratory Measurements – Sediment	
Nitrogen, Total Kjeldahl	Nutrient
Phosphorus, Total	Nutrient
Total Solids/% Water	Required for dry-weight calculations
Total volatile solids	A measure of organic matter
Iron, Total	Essential micronutrient
Toxic Chemicals	
Ammonia, unionized	Potentially toxic to aquatic organisms
Chloride	Same
Arsenic, Total	Same
Cadmium, Total	Same
Chromium, Total	Same
Copper, Total	Same
Lead, Total	Same
Nickel, Total	Same
Selenium, Total	Same
Zinc, Total	Same

ANALYTICAL METHODS

Analytical methods change over time as science progresses and a variety of scientifically acceptable methods for measuring water quality and sediment exist (Elias et al., 2008). These methods have different detection limits and procedures for handling samples. The Resources section of this Handbook provides a list of reliable sources to use for determining the analytical methods to use.

SAMPLE CONTAINERS AND PREPARATION

The following is an example from Fond du Lac Band of Lake Superior Chippewa. Consult with your organization's Quality Assurance Project Plan (QAPP) for surface water to determine the appropriate procedures to use for preparing sample containers and handling samples.

All sample containers may be provided by the laboratory performing the analysis. Pre-cleaned containers may be purchased from commercial sources, or the containers may be cleaned and re-used. Unless the containers are pre-cleaned with a manufacturers certificate, the laboratory must verify the cleaning procedure by randomly selecting at least one of each type of container per month, filling it with deionized water and an appropriate preservative, waiting at least 24 hours, and analyzing the water for all analytes of interest. A record of these checks is to be maintained by the Laboratory Director. When containers are re-used, the following cleaning procedures are used:

General Chemistry:	1 liter wide-mouth plastic bottles; washed with detergent and rinsed three times with warm tap water, then at least three times with deionized water.
Chlorophyll:	1 liter amber glass or plastic bottles; prepared same as General Chemistry.
Metals:	250 or 500 mL wide-mouth plastic bottles; prepared same as General Chemistry, then rinsed with dilute HNO_3, tap water, dilute HCl, tap water, and finally at least three times with deionized water.
Nutrients:	250 or 500 mL wide-mouth plastic bottles; prepared same as General Chemistry, then rinsed with dilute HCl, tap water, and finally at least three times with deionized water.
Ortho Phosphorus:	Same as Nutrients
Dissolved Organic Carbon:	50 ml amber glass bottles with TFE lined caps; soak 24 hours in 10% HCl acid bath, rinse with deionized water. Seal bottles with aluminum foil then combust at 400 ^{o}C for 1 hour.

Sediments collected by Ekman or Ponar dredge for nutrient/sediment characteristics analysis should be transferred immediately to labeled zippered plastic bags, and stored in a cooler until delivery to the contract lab for analysis.

MEASURING SUBSTRATE CLASS

To bring up substrate, use some sort of device to grab a small sample of the sediment from the shore side of the boat (Minnesota DNR, 2012). Record the code of the substrate class and Sample ID# on a data sheet designed to include the related environmental variables sampled.

The following table is from the Minnesota Sensitive Lakeshore Manual by the Minnesota Department of Natural Resources (2012, pp. 14). For more information on determining substrate classes, see the Minnesota DNR Lake Survey Manual (Minnesota DNR, 1993).

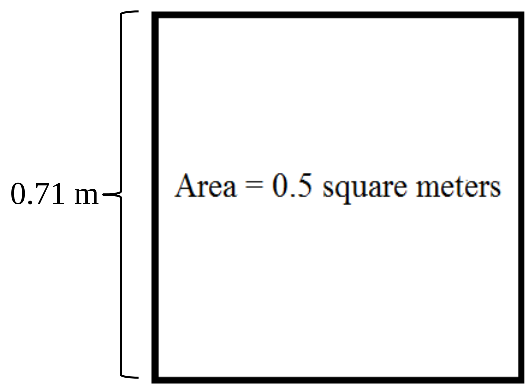
Table 14. Substrate class

Substrate Group	Type	Code	Description
Hard Bottom	Boulder	BO	Diameter over 10 inches
	Rubble	RU	Diameter 3 to 10 inches
	Gravel	GR	Diameter 1/8 to 3 inches
	Sand	SA	Diameter less than 1/8 inch
	Sand/Silt	SS	Sand bottom overlaid with thin layer of silt
Soft Bottom	Silt	SI	Fine material with little grittiness
	Marl	MR	Calcareous material
	Muck	MU	Decomposed organic material

Appendix E: Instructions for Making a Square Quadrat Frame

How to make a quadrat frame with area equal to 0.5 m^2

Materials needed:
- 10’ (foot) Plastic PVC pipe, ~1” in diameter
- 4 right-angle elbows (90 degree angle), fit to diameter of PVC pipe (if available, one elbow should be a different color for marking one corner of the quadrat)
- PVC solvent cement (glue)
- Saw capable of cutting PVC (ideally a fine-toothed saw, blade 3” – 4” in width)
- Measuring tape or yard stick
- Colored tape (optional, for marking one corner of the quadrat if colored PVC elbow unavailable)



Directions:
- Cut four (4) lengths of PVC pipe to 0.71 m (~28”).
- Assemble the quadrat taking care to ensure that the interior dimensions exactly measure 0.71 m for the inside measurement of each side (0.71 m x 0.71 m ≈ 0.5 m^2).
- Apply solvent-cement/glue evenly to outside end of pipe and inside of right-angle elbow
- Insert pipe into elbow and turn ¼ turn to spread glue.
- Hold the pipe and fitting together for about 15 seconds (note: the glue dries very quickly)
- Lay out the frame on a flat surface, & continue attaching right-angle elbows to pipes until a square is formed.
- Mark one corner of the quadrat using colored tape, using a colored PVC elbow, or by making a small notch with the saw. This mark is needed for selecting the sample plant (the one nearest to this corner).
- Allow to dry flat.

Appendix F: Statistical Basis for Determining the Number of Sample Points Required

This section explains the statistical foundation for recommending 40 points as the minimum number to sample. The "Number of Sample Points Equation" is based on a study to clarify the most efficient techniques for estimating the biomass of aquatic plants (Downing and Anderson, 1985) across a range of temperate lakes and ponds.

The sample point number recommendations in this Handbook are also based on research showing size of a water body is not a factor in determining the frequency of aquatic plant species occurrence (Newman et al, 1998; MN DNR, 2012). In sampling for other plants that co-occur with wild rice, the sample point numbers recommended will also be valid.

Downing and Anderson tested five sizes of quadrats ranging from 100 cm^2 to 1 m^2.[22] The authors analyzed patterns of spatial distribution of biomass to determine the optimum number of sample plots. They looked at 22 aquatic plant studies from around the world with a total of 1200 sample plots in order to develop an equation for the number of samples required.

Number of Sample Points Equation

Please note: You don't need to know how to use this equation.

Number of sample points = $5.75\bar{x}^{-0.433}A^{-0.157}p^{-2}$

Where $\bar{x}$ = Mean standing biomass in g/m^2
A = area of the quadrat used in square meters
p = statistical precision desired

Source: Downing and Anderson (1985), p. 1866.

According to this study, the number of sample points required depends on the current year's (standing) biomass. Other information needed is the quadrat size and desired level of statistical precision. A quadrat size of 0.5 m^2 and a statistical precision of 20% of the mean are recommended in the Handbook methods. Figure 33 illustrates the rationale behind recommending a statistical precision of 20% of the mean, which is a widely accepted measure and results in a reasonable sampling effort.

Figure 33 shows average annual wild rice biomass on 10 water bodies in northeastern Minnesota (1998-2014) and the corresponding number of sample points required to measure biomass at two different levels of precision (Downing and Anderson, 1985; T. Kjerland analysis of data collected by Vogt,

[22] These are area measurements. The length of each side of a 0.5 m^2 quadrat is equal to 0.71 m.

2014). The blue dots represent the recommended level of precision—standard error at 20% of the mean. The red dots represent a more stringent standard error at 15% of the mean. The orange line represents 40 sample points.

Based on analysis of the natural growth patterns on these 10 water bodies, sampling 40 points per year will result in achieving a 20% standard error of the mean in most years (4 out of 5). More sample points will be needed in years when the rice is less abundant to achieve the same level of accuracy as in productive years. Less sample points will be needed in years when the rice is more abundant. See also, Table 2 on page 26.

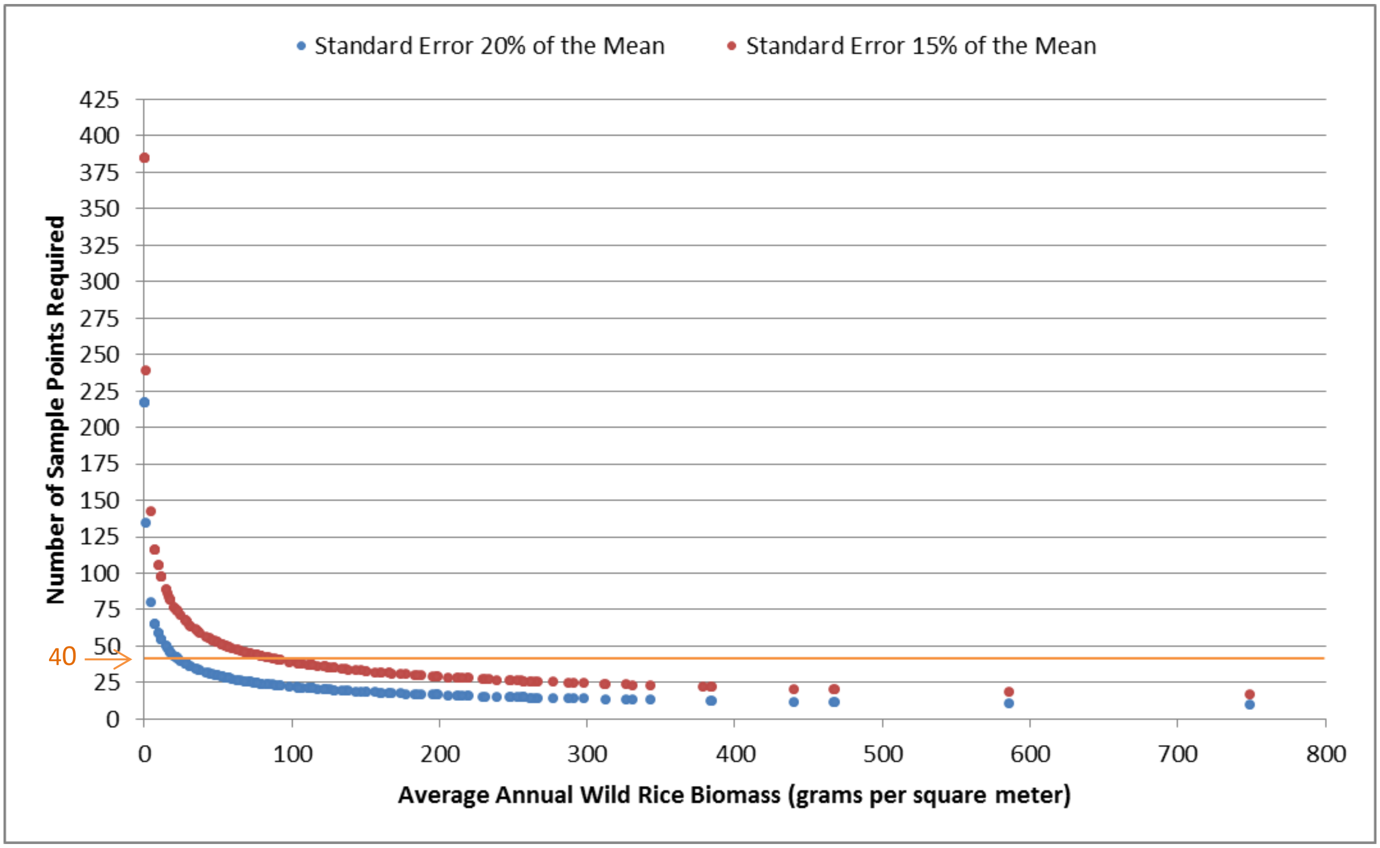


Figure 33. Illustration of the increasing number of sample points required as the level of statistical precision desired is raised from a standard error of 20% of the mean to a standard error of 15% of the mean