Wild Rice Monitoring Handbook

By Tonya Kjerland

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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 waterbody. 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

[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).

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 is typically broader (i.e. surveys to identify aquatic plants and other organisms in a lake, measurements of biodiversity, and locations of specific species such as rare/endangered plants or nuisance plants). 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 waterbody (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 waterbody. 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 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 and count number of stalks on sample 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 people still 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*.


Annette Drewes

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.


Fond du Lac Natural Resources

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

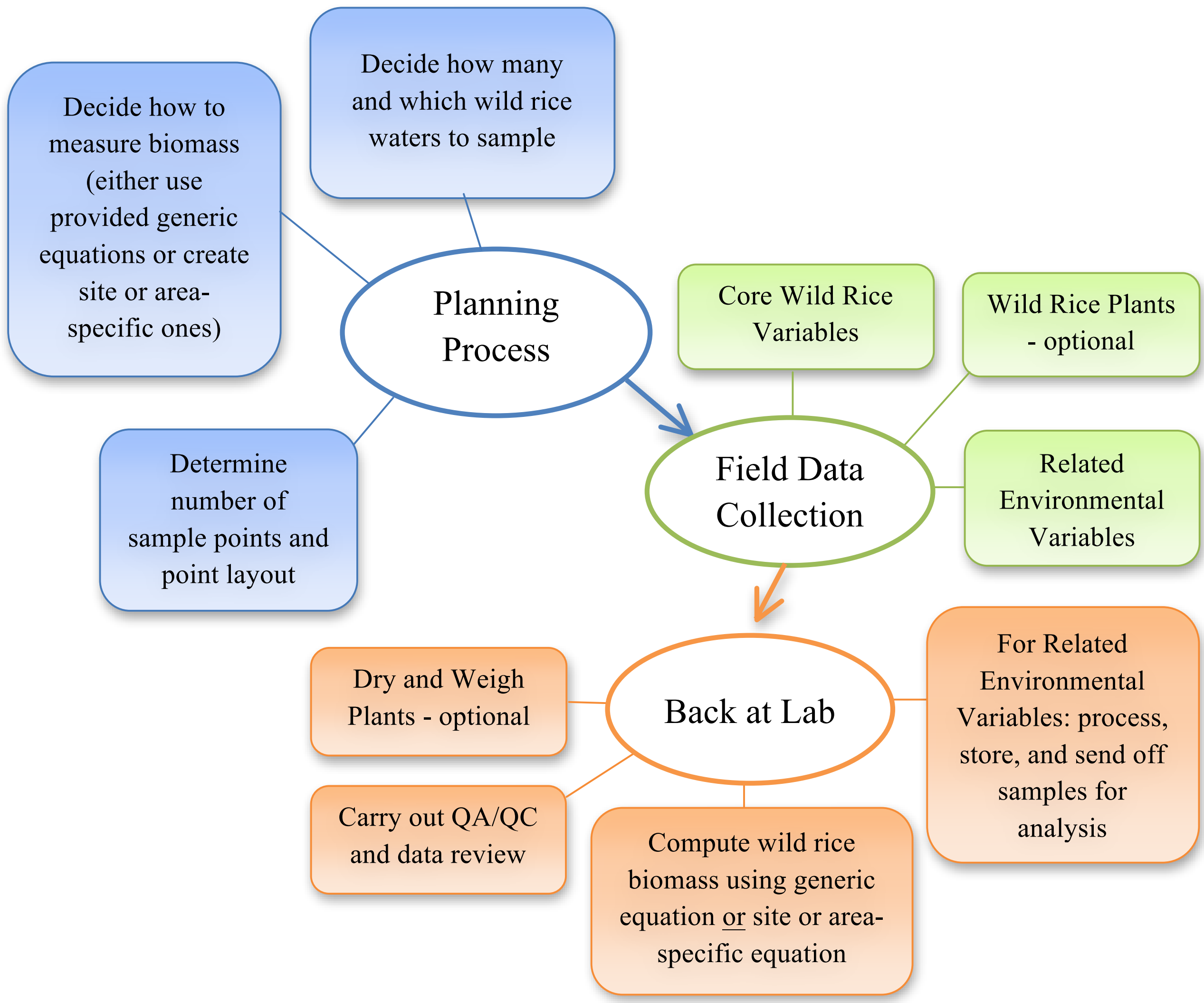
To respect the cultural and spiritual significance of wild rice, follow these important protocols:

- Obtain appropriate permissions and know the cultural boundaries for research with wild rice.
- Put tobacco in the water before taking samples or collecting data.
- Offer a prayer of gratitude and a statement of your good intentions. (The prayer can take many forms. Speak in your own words according to your religious traditions.)

Sampling Design

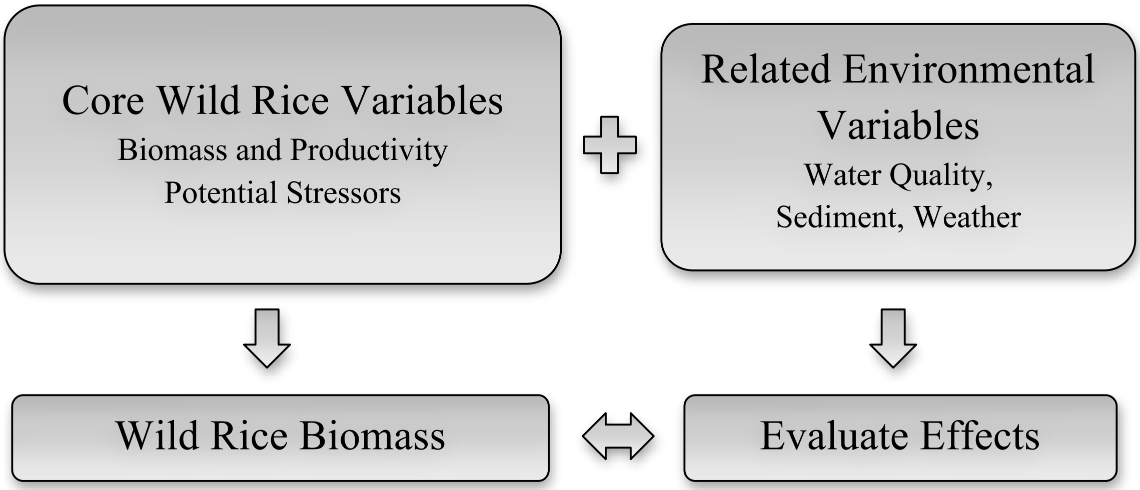
This overview recommends data to collect and provides guidance for designing the sampling plan. The main decisions to be made are: 1) how many and which wild rice waters to sample; 2) how to measure biomass; and 3) the number of sample points and point layout (see Figure 16).

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. It can also be expressed as "plant weight per stalk." Whole lake production can be calculated 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).

Table 1. Core wild rice variables. 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.

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 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 waterbody. 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 wild rice stalk that is nearest to a designated corner of the quadrat. Whichever plant this is growing from is the sample plant. 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.

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[2] See Appendix C for details about how these biomass equations were created.
[3] T. Kjerland analysis of data collected by Darren Vogt (2011) and Melissa Lewis (2014.)

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

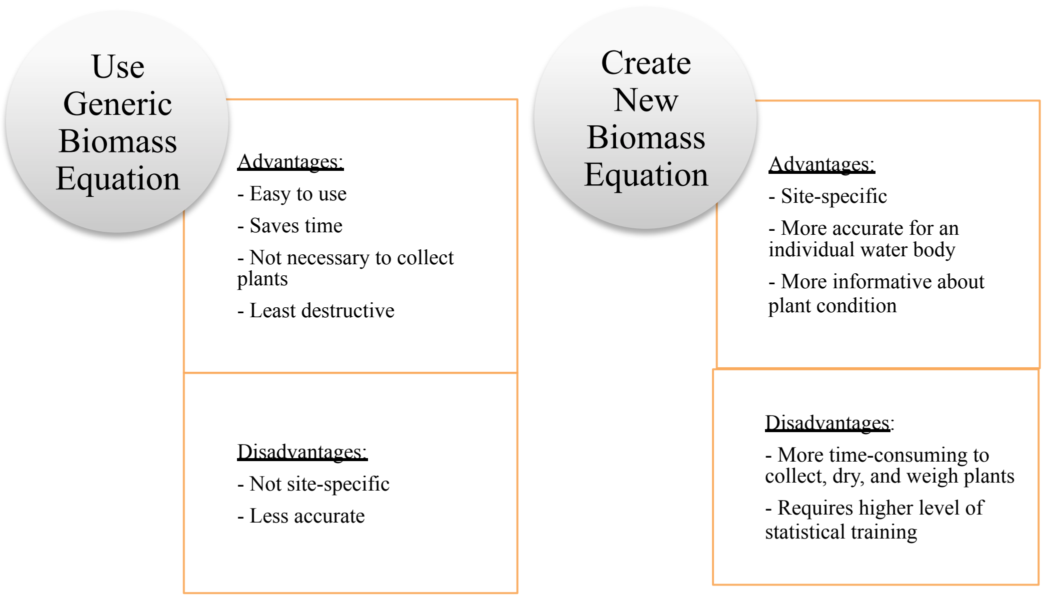


Figure 2. Advantages of creating site-specific biomass equations or using generic ones

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 the 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 waterbody.

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.

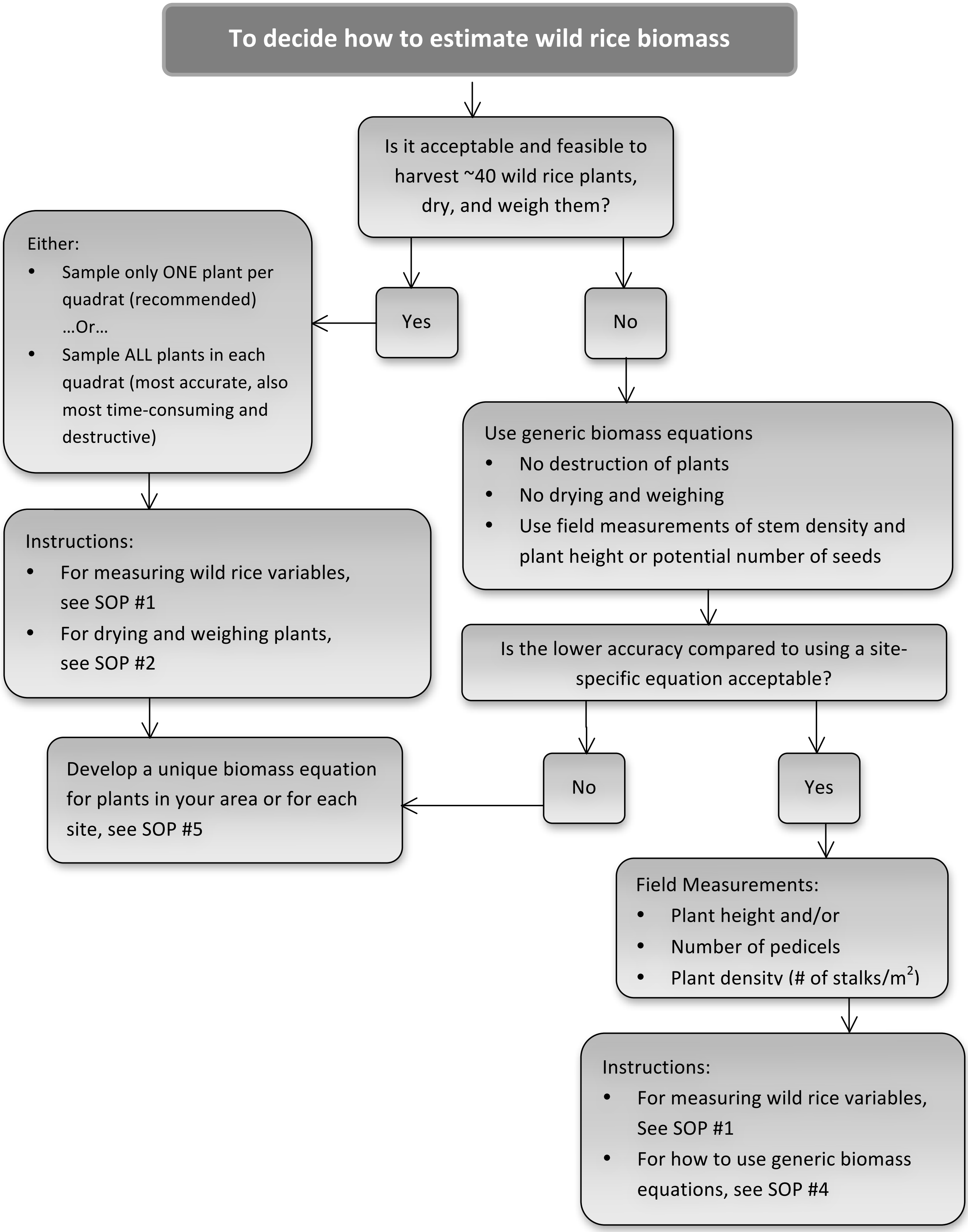
Differences between wild rice stands and water bodies. If it is not clear whether or not you need to develop your own biomass equations, one approach would be to conduct a pilot study of the wild rice in the waterbody you are measuring and compare results to the characteristics of the wild rice and associated 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 sub-sampling 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, like when the whole lake is influenced by a cause for concern (i.e. surrounded by agriculture, residential development, etc), the reference sites may need to be located on a different waterbody.

<u>Suggested sites for measuring related environmental variables:</u>

- 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 waterbody 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.

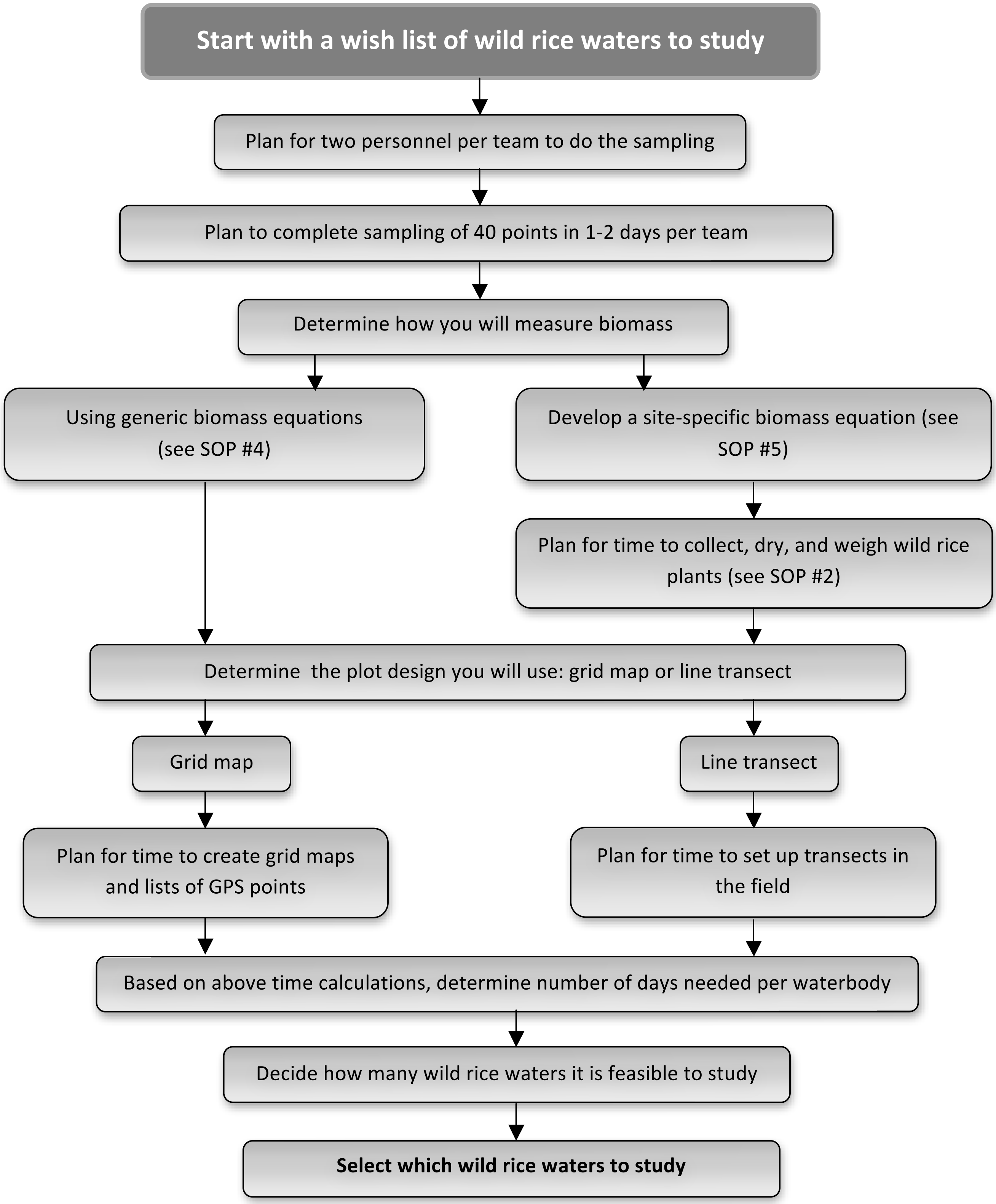
Be sure to be consistent about which side of the boat you take the measurements on (left or right each time.) This will avoid bias based in deciding which side looks "better."

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

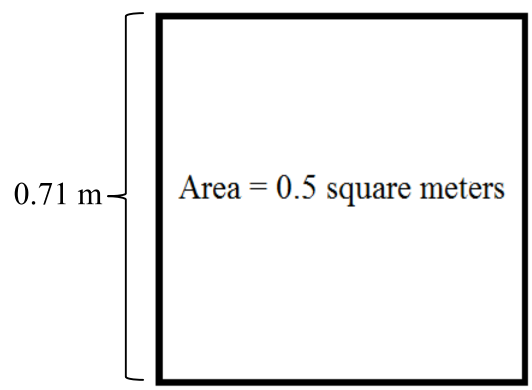
Three primary factors affect the number of sample points:

- ✓ 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**.

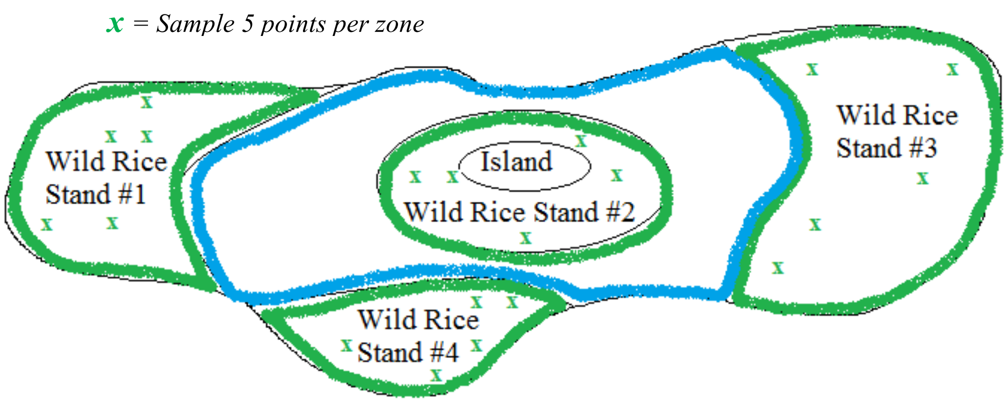
Size of area. The same number of sample points should be used regardless of resource size. This recommendation is based on research showing size of a waterbody is not a factor in determining the number of sample points required for determining the amount of biomass or frequency of species occurrence (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 waters with scattered beds of wild rice or deep lakes, consider using a zone technique for determining the total number of points to sample. Estimate the area of each stand (See **Appendix B**). Divide the waterbody into zones based on wild rice distribution and sub-sample the zones.

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 for the year for each zone (see Table 2). Table 2 shows the number of sample points required for each zone given hypothetical averages for wild rice found during the pilot study.



Wild rice stand (zone)	Estimated average wild rice biomass (g/m^2) based on 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 (SE)** is a measure of how accurately we know the true mean of a population based on the sample measurements taken; it takes into account both standard deviation and the number of samples (SE = standard deviation divided by the square-root of the number of samples).

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 waterbody 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. 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. Number of Sample Points. The required sample size varies with quadrat size, wild rice biomass, and level of statistical precision. The recommendations from this Handbook are shaded grey. The statistical basis for this table is explained in ***Appendix F****.*

	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 waterbody 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. 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: *Using a 0.5 m^2 quadrat, 40 points produces good precision ~80% of the time. If wild rice is sparse, 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

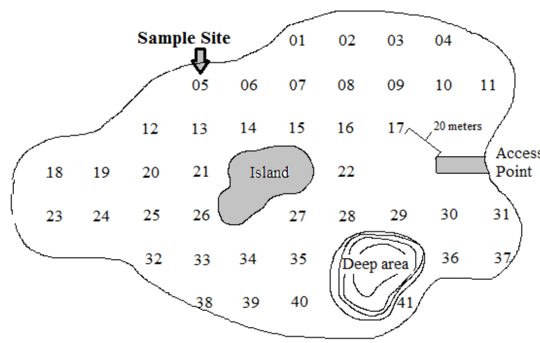
Two options are presented for designing the location of sample points: grid map and line transect. A third possibility is a variation on the grid map, which is to randomly subsample points from the grid. Related environmental variables should be located at the same points as wild rice, and/or in areas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

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.

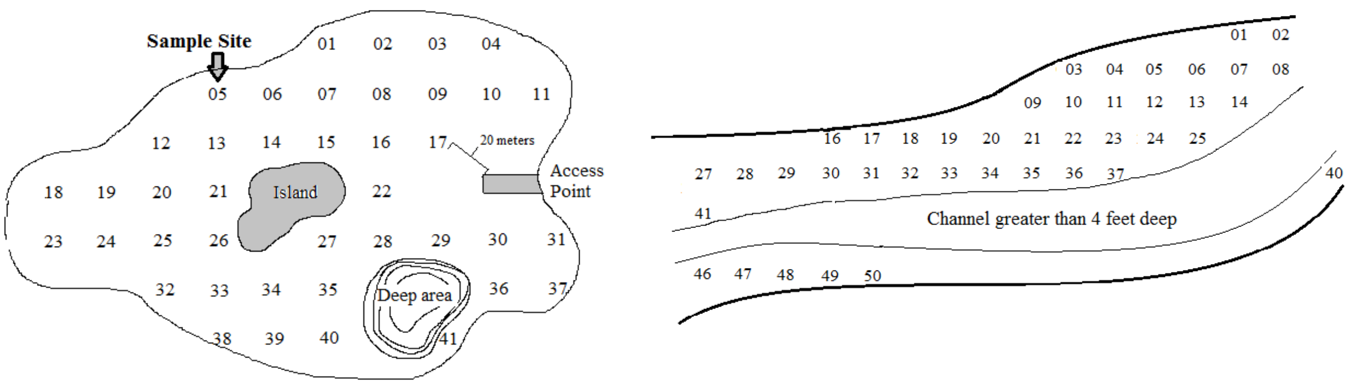
> **Helpful tips:** If not all sample points in the grid are selected, try to cover the entire area of interest rather than clustering sample points. This will help avoid confounding factors that might exist in smaller areas (such as point-source pollutant discharges or nutrient inflows from streams)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

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

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

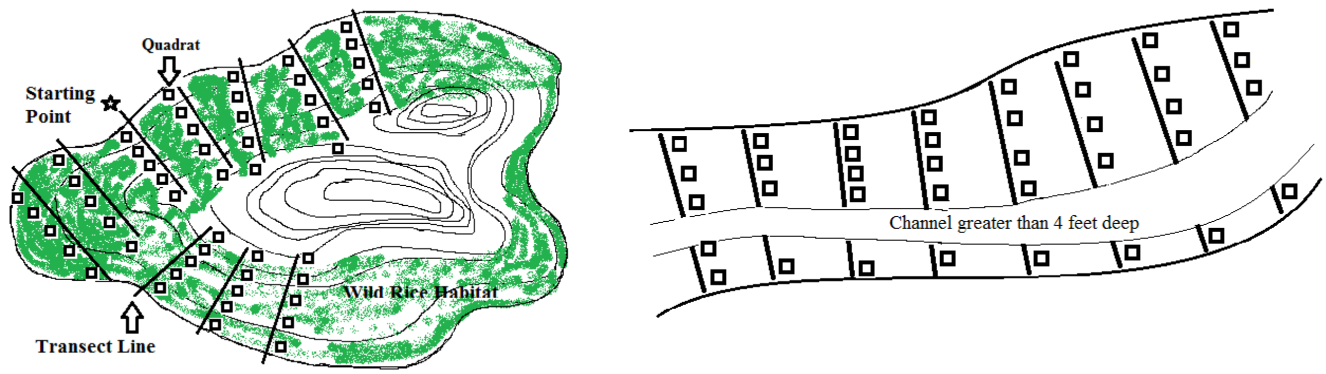
Disadvantages:

- 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 waterbody 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 (≥ 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 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 (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 to still have 40 sites. Sample points should ideally be selected prior to going into the field to avoid the bias of picking the "best" points to sample.

Eliminating sites <u>prior</u> 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 <u>suitable wild rice habitat</u>.

Eliminating sites <u>during</u> 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; even "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.

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.
- **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 waterbody 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 During Monitoring 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.

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.

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.

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

- **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.
  - Remember, 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.

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:

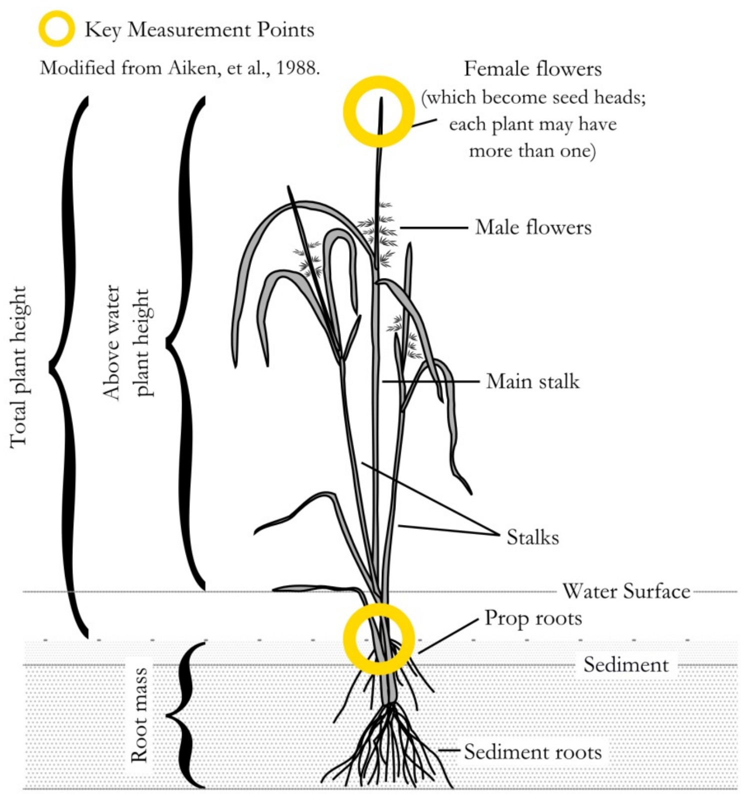
- Stalk density within the quadrat frame
- Water depth within the quadrat frame or as close as possible
- Collect entire sample plant collected so its dry weight can be determined back in the lab
- TOTAL sample plant height
- The number of stalks on the sample plant
- The names of other plant species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

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 waterbody 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

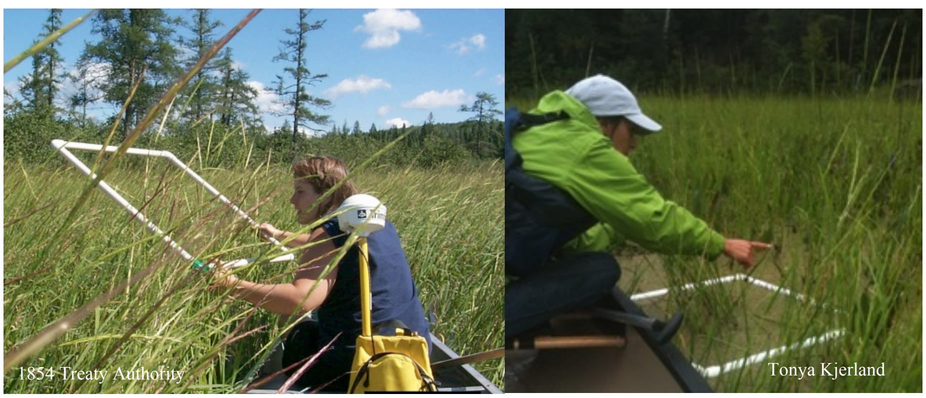
Helpful Tip: Use a copy of the Field Data Sheet to record data.

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 15.

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 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.

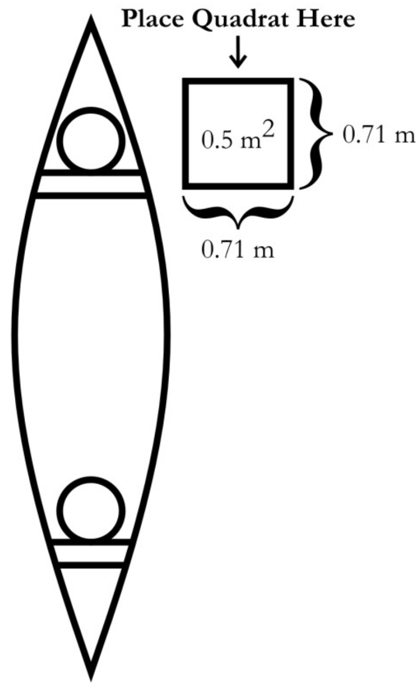


Figure 9. Quadrat placement

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**

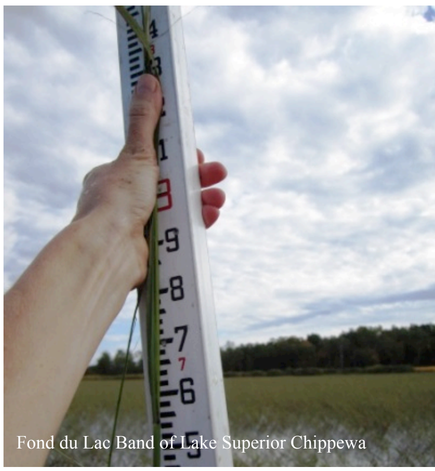
- Find the corner of the quadrat marked with colored tape, notch, or PVC elbow
- Select the wild rice stalk that is nearest to this designated corner. Whichever plant this stalk is growing from is the sample plant.
- This will be the plant you measure and either:
    A) collect seed heads from, OR
 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

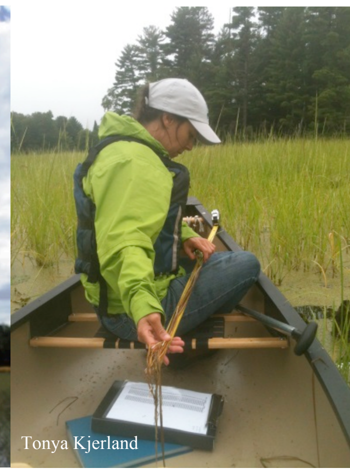
Fond du Lac Band of Lake Superior Chippewa

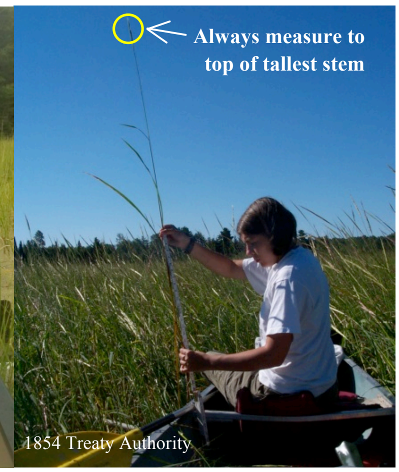
7

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; allow the disk to settle to the bottom so that the stick rests directly on top of it.

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.

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 #, waterbody 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 #, waterbody 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.* 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.

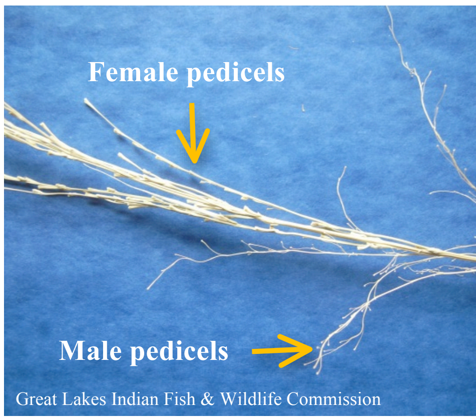
About Collecting Seed Heads

For information on processing the samples (i.e. counting potential seeds/female pedicels) see 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 SOP #4 Using Generic Wild Rice Biomass Equations.

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



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 waterbody.
  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 waterbody 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 were chosen due to their ease of implementation.

Back in the Lab: Dry and Weigh 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, First Nations, 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 needed 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 with 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

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; seeds may account for 10 to 25%.

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 equations.

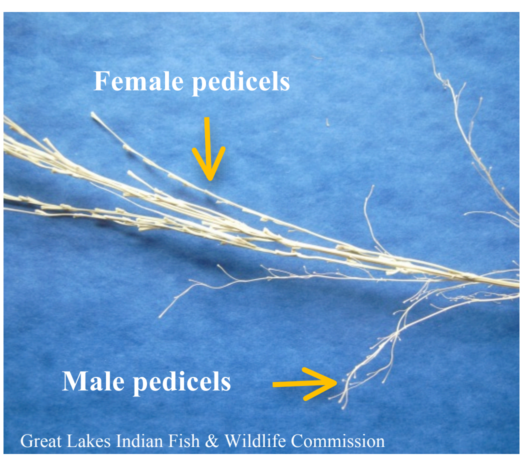
Timing. Ideally, plants will be dried as soon as possible after collecting, but they can be stored for up to several weeks if kept in a very dry location. Rather than keeping plants in a refrigerator where they are likely to decay quickly, wash the plants at once and put them into paper bags to air dry. When plants begin to decay they lose weight, and counting pedicels becomes 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 (<u>minus seeds</u>).
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 not included as part of the wild rice plant weight because the fungal growth is not part of the plant and adds weight.
c. Record number of seeds with wormholes.

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

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

- ☐ 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

Lab

- ☐ 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 or the Field Guide for instructions if it is not possible to identify plants 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 named 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 waterbody 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. www.bellmuseum.umn.edu/ResearchandTeaching/Collections/ScientificCollection/PlantCollection/InfoonMinnesotasFlora/index.htm
Board of Water and Soil Resources (BWSR), Minnesota Wetland Delineation. www.bwsr.state.mn.us/wetlands/delineation/index.html
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Techniques for creating a collection of species

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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 waterbody, 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:

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

To scale this statistic for an entire waterbody (Total Biomass), multiply by the area in square meters. If wild rice grows only in certain areas, to create a more accurate measurement, divide the waterbody into zones, calculate the biomass separately for each zone and sum the results.

Biomass Equation 1: Plant height - Weight per stalk

Use this equation to compute plant weight **per stalk** based on **total plant height**.

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

x = total plant height (input in centimeters)
y = plant weight per stalk (output in grams)

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.

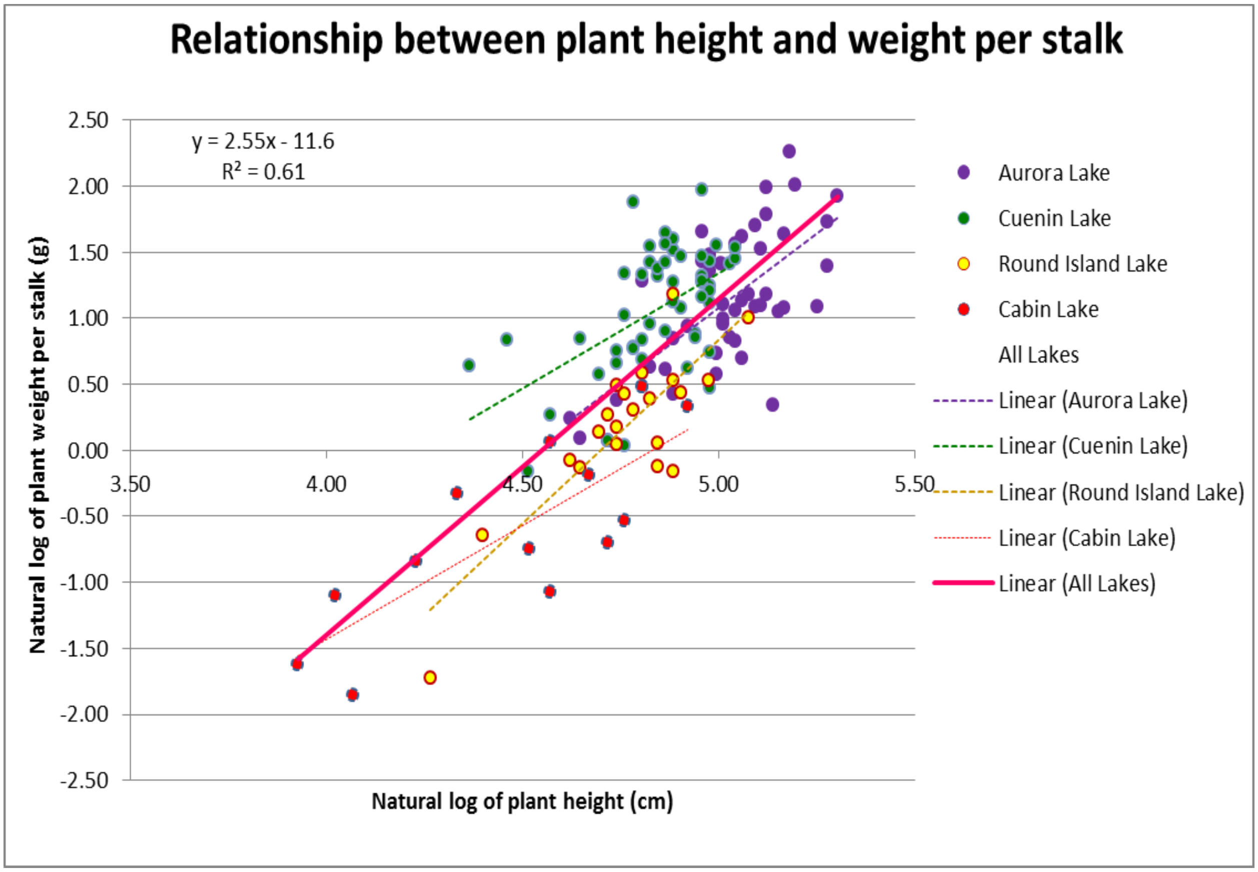


Figure 11. Relationship between plant height and weight for Equation 1 (n=132; p<<0.001).
Total plant height is measured in centimeters from the sediment-water interface, or top of highest roots, to height of tallest stalk. Plant weight per stalk (y) is given in grams (T. Kjerland analysis of data collected by Vogt, 2011 and Lewis, 2014).
Note: Data from Campers and Stone Lakes were not included 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

Use this equation to compute **plant weight per stalk** using number of **potential seeds** (#pedicels) **per stalk:**

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

x = number of pedicels per stalk
y = plant weight per stalk (output 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.

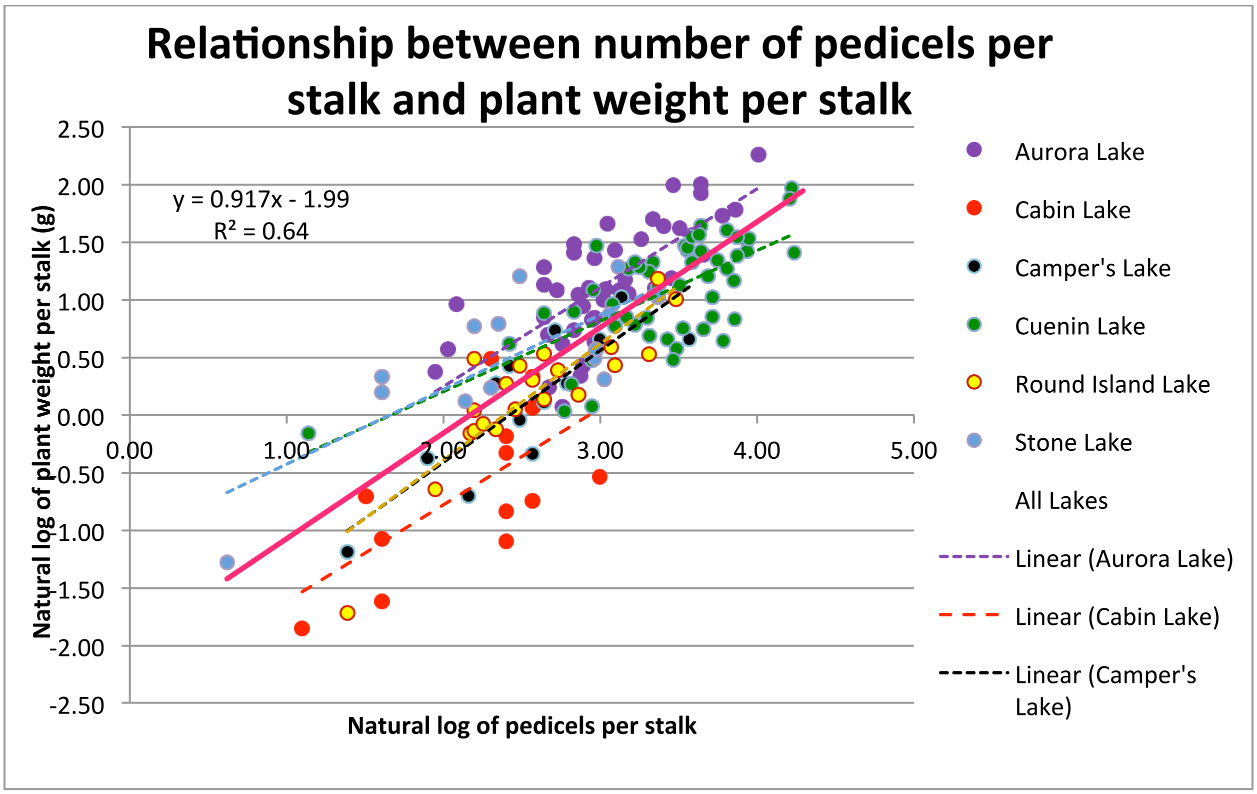


Figure 12. Relationship between pedicel number per stalk and weight per stalk for Equation 2. (n=162; p<<0.001). (T. Kjerland analysis of data collected by Vogt, 2011 and Lewis, 2014.)

Step-by-Step Instructions for using Equation 1 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: www.seagrant.umn.edu/coastal_communities/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^$^{2.55}$

Verify you did it correctly:
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 waterbody. 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 waterbody**. 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. 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: www.seagrant.umn.edu/coastal_communities/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? **= 0.137*A2^0.917**
- 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 waterbody. 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 waterbody**. 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. 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.

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.seagrant.umn.edu/coastal_communities/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

5. QA/QC the data by checking for outliers, missing data and data entry errors.

[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.

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 or Equation #2.[12]
   a. If using a generic biomass equation, you're done!
   b. If calculating a site- or area-specific biomass equation, continue to 9.

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.

[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/YYYY
Waterbody	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).*
Plant height-ABOVE (cm)	Data	Enter data in column corresponding to how plant height was measured—either as total or above water.
Water depth (cm)	Data	Units are in cm

[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
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 (example dimensions = 0.71 m x 0.71 m).*
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... height_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.

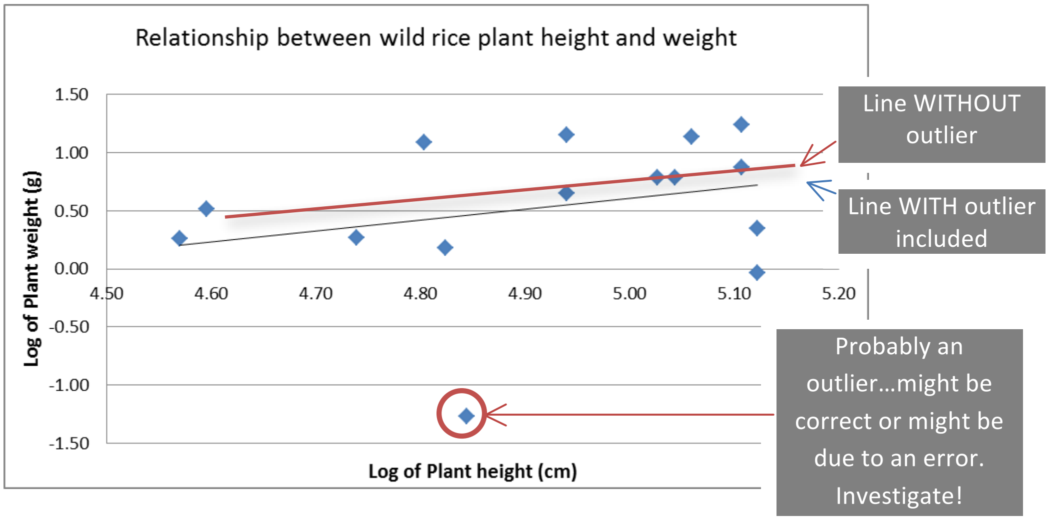


Figure 16. Illustration of outlier effects.

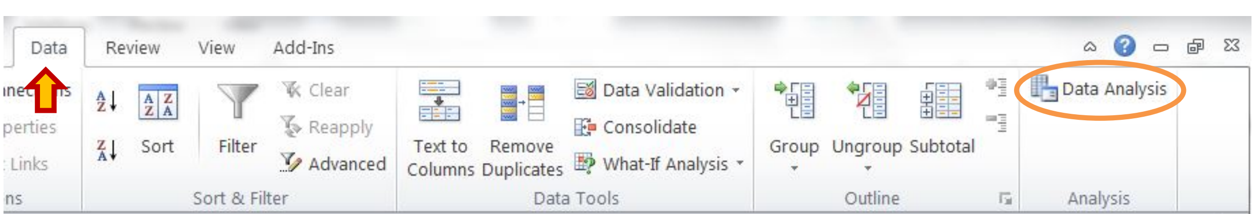
STEP 4. Another way to check for suspicious data points is to sort your data in each column from largest to smallest and check the high and low ends (being very careful to sort every column simultaneously so you don't disassociate the data across rows).

Calculate Biomass Equations

Verify that you have set up the spreadsheet properly. The list in Table 5 shows the proper column headings and cell contents. The easiest way to do this is to use the spreadsheet available for download from Minnesota Sea Grant: www.seagrant.umn.edu/coastal_communities/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.

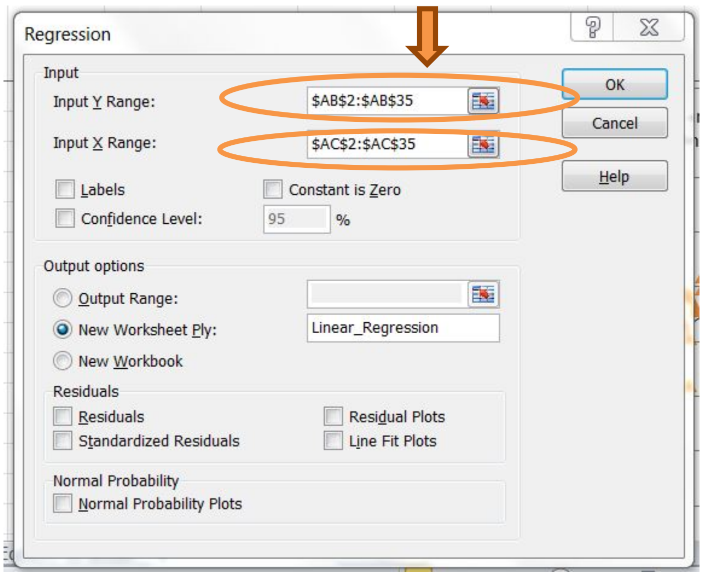


STEP 2. Select "Regression" from within the Data Analysis window. A new window pops up with options for setting up a linear regression (see below). Select the icon with the tiny red arrow

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 0.05, 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 4 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.

$$m = 2.55$$

$$a = e^{-11.615} = 0.00000903 = 9.03\text{x}10^{-6}$$

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).

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: $y = 2.775x - 13.041$

$$m = 2.77 \text{ (Slope =“X Variable 1”)}$$

$$\text{Intercept} = -13.041$$

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

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


Tonya Kjerland

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.

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.

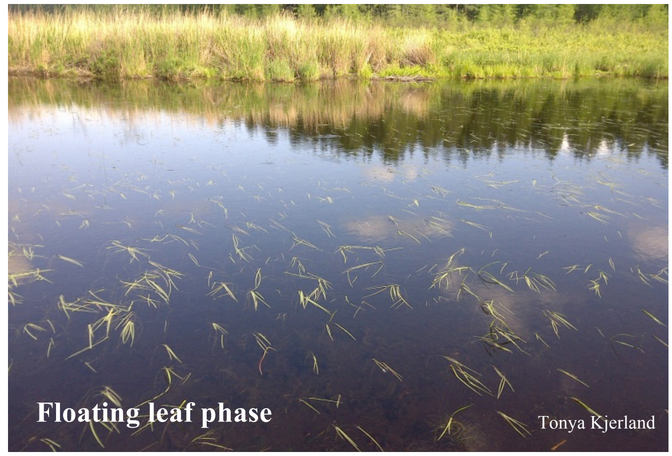
Overwintering. Wild rice seeds only germinate under conditions that mimic being buried in aquatic sediments over a winter or with scarification. Normally, seeds must be kept cold and wet for a period of about 3 months in order to germinate. Desiccation of seeds reduces germination rates considerably. 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 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.

[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.

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.



Floating leaf phase

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.

Aerial leaf phase

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 that 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.

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 (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 waterbody 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***).

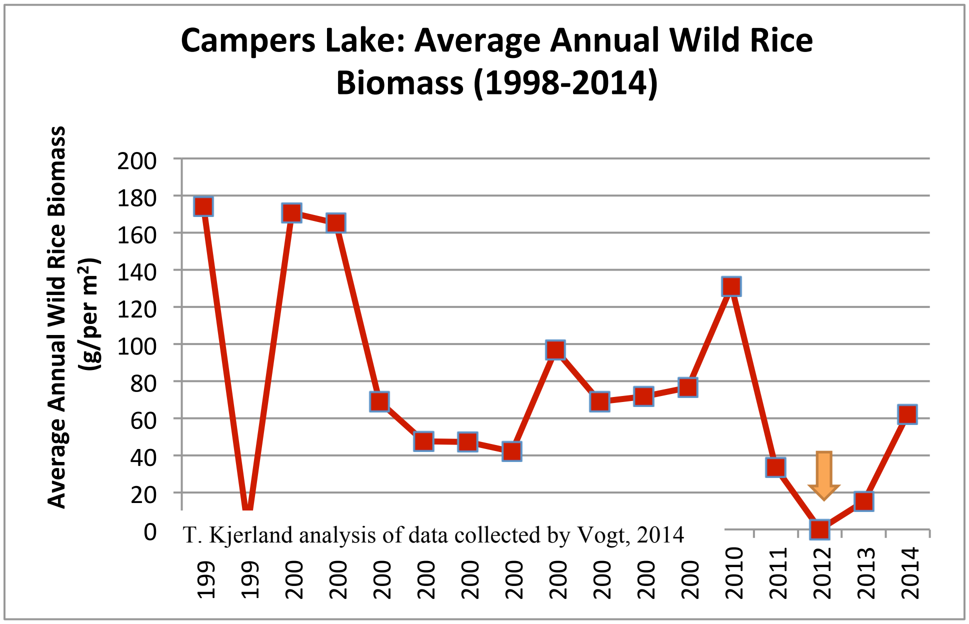


Figure 18. Wild rice biomass on Campers Lake over 16 years illustrates the variability that can be seen in wild rice populations and the recovery possible from buried seed.

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.

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. 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[16]. 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

[16] This section on water quality draws from Pastor (2013) and Moyle (1944).

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.

Sulfate occurs naturally in rocks. It is also discharged and regulated in various industrial processes, such as domestic wastewater 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.

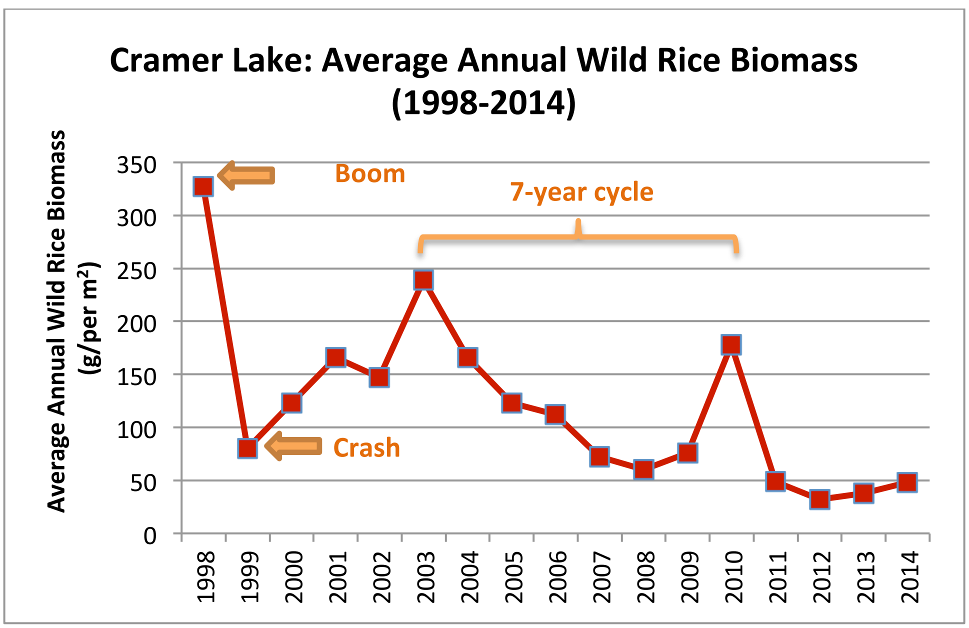
Nitrogen and Phosphorus. Nitrogen is the most likely limiting nutrient for the production of wild rice, and phosphorus is likely the second-most limiting nutrient.

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.[17] 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.



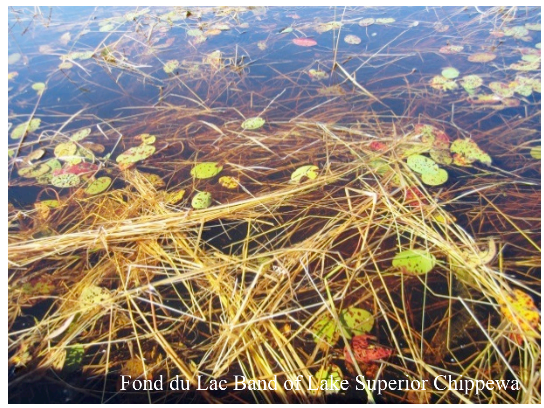
T. Kjerland analysis of data collected by Vogt, 2014

Figure 19. Population variability pattern on Cramer Lake in Northern Minnesota illustrates cycles that have long been observed by wild rice harvesters and biologists.

[17] This section on population oscillations draws upon research by Grava and Raisanen (1978), Sain (1984), Walker et al. (2006) and Walker et al. (2010.)

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.[18] 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.

Water depth averaged only ~0.37 cm (14.4 inches) in the most productive part of the Vermilion River (

[18] This sample set included 4 lakes in northeastern Minnesota and two in Wisconsin as described in the section on wild rice biomass equations.

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[19]. 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.

[19] 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.[20] The characteristics of the sediment that seem to matter most include:

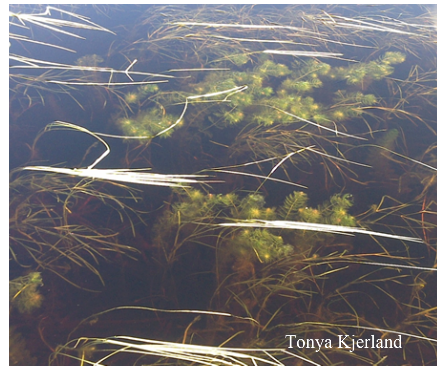
- **Texture**—the sediment must be soft enough for roots to penetrate, but not too soft. Hard substrates may be unsuitable mainly due to a lack of nutrients rather than to the inability of roots to take hold. Soft sediment is generally better, and wild rice seems to thrive in some sites that are too 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 multiple 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.

[20] 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).

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 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 drawdown) 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).

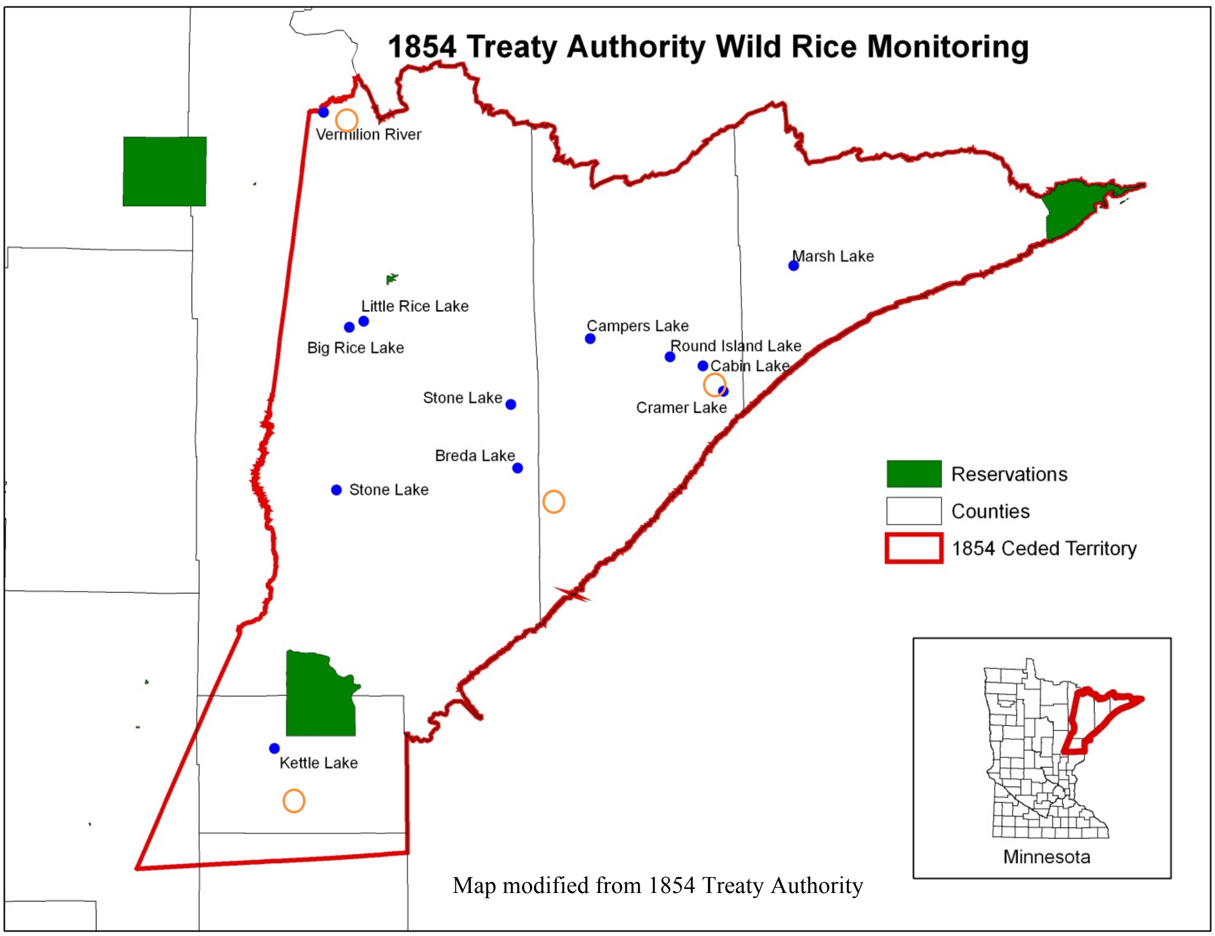


Figure 20. Map of wild rice water bodies where detailed monitoring is conducted annually by the 1854 Treaty Authority

Breda Lake

DNR # 69-0037 00

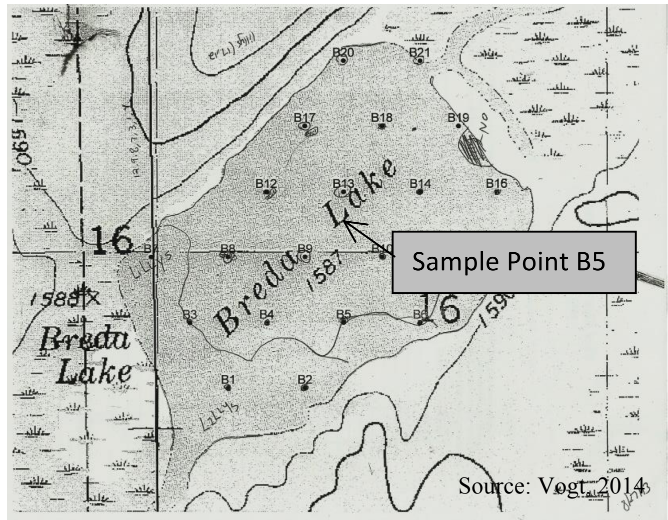


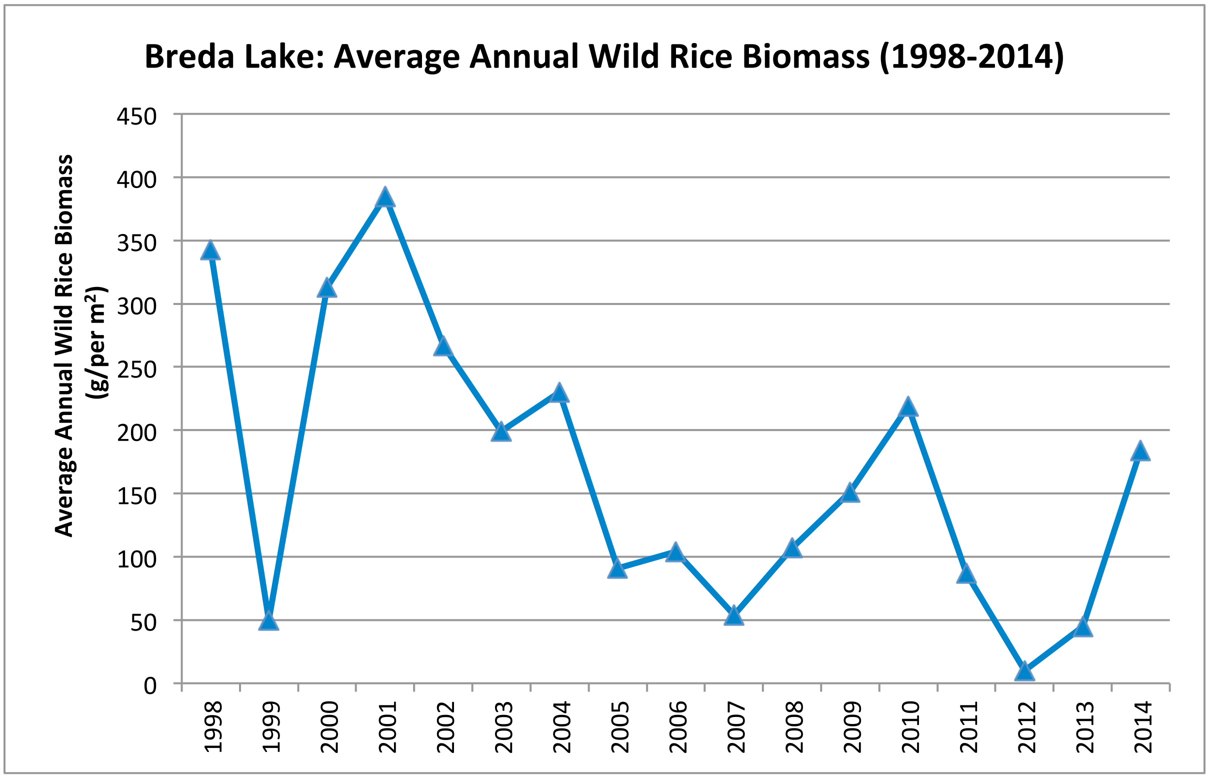
Figure 21. Topographic map showing wild rice sampling points on Breda Lake.

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.

Computing biomass. For each waterbody 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. 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.

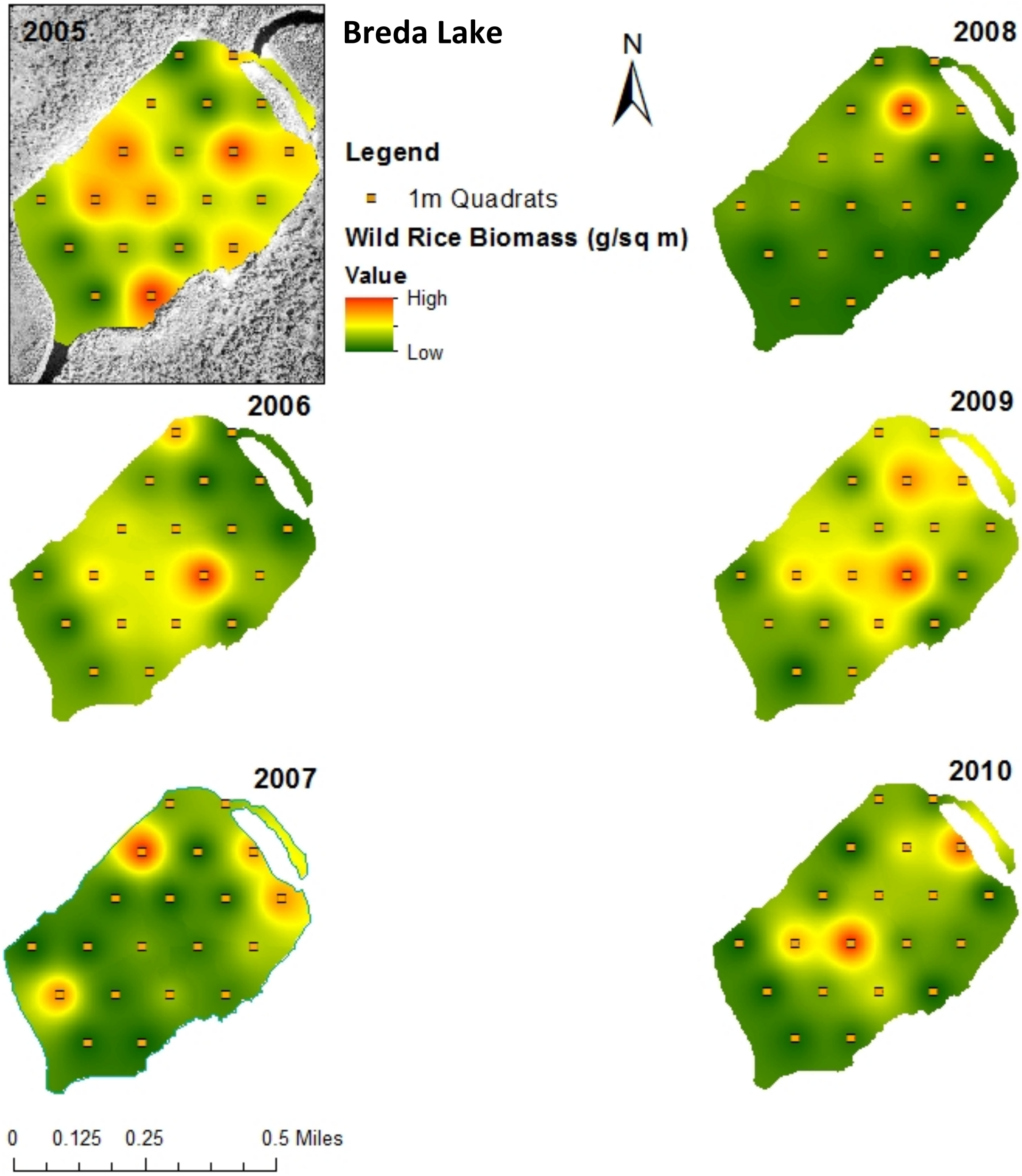


Figure 23. Heat maps of wild rice biomass on Breda Lake between 2005-2010 show that the spatial distribution of areas of highest and lowest biomass vary across time.

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.

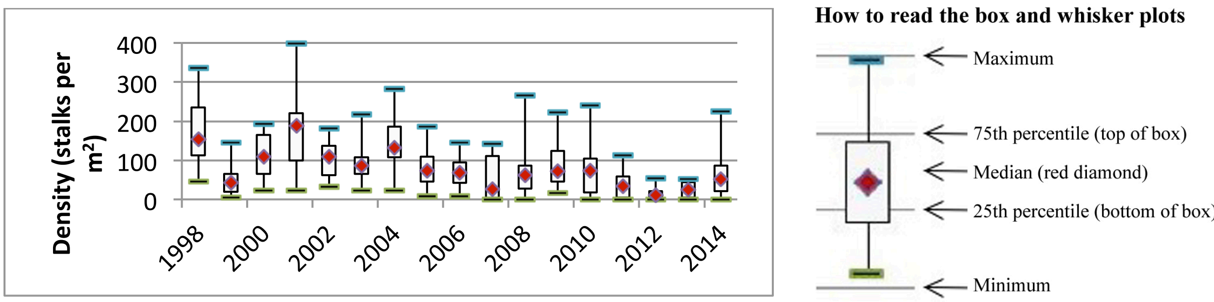


Figure 24. Breda Lake: Wild rice density (1998-2014).

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.

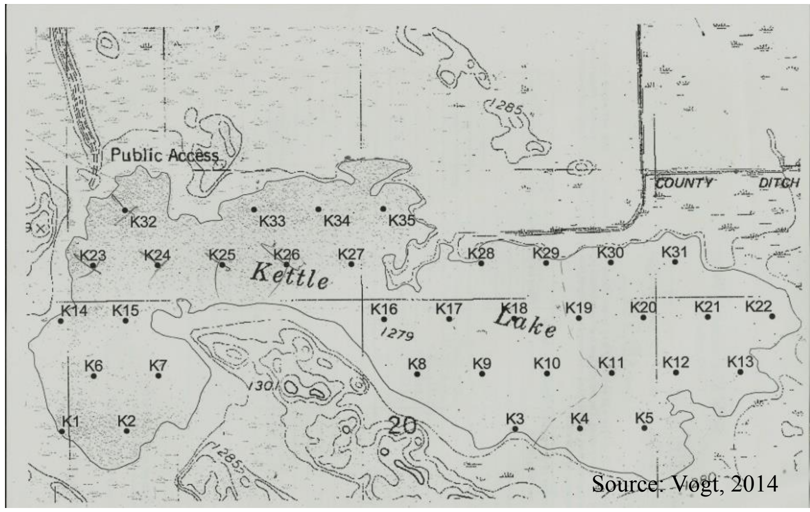


Figure 25. Topographic map showing wild rice sampling points on Kettle Lake.

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.

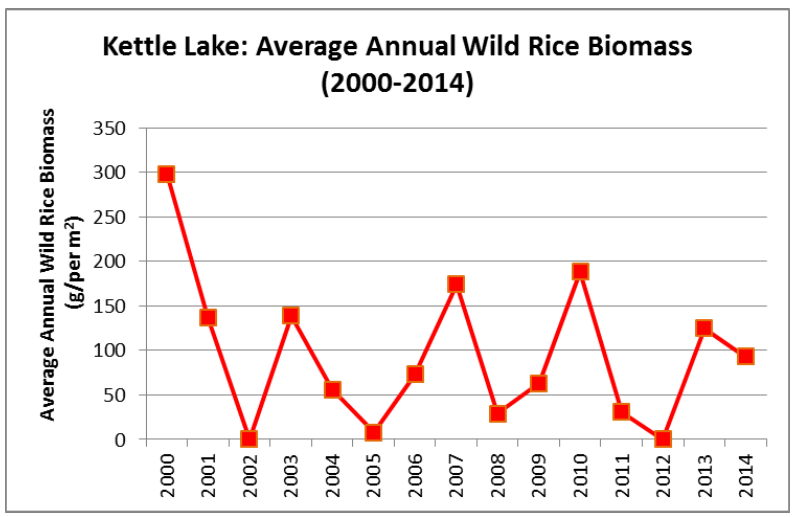


Figure 26. Trends in wild rice biomass on Kettle Lake.

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.

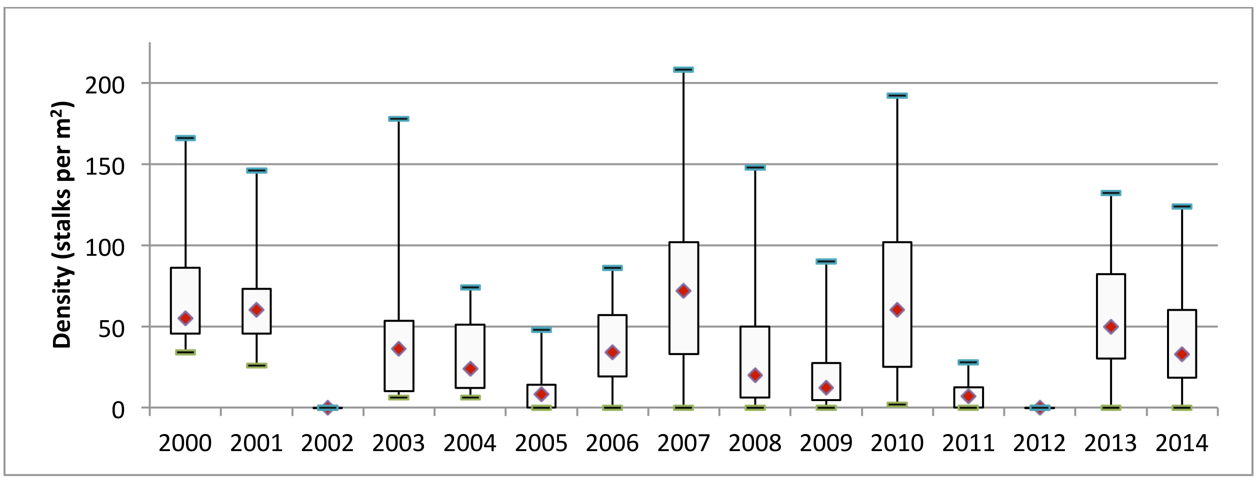


Figure 27. Kettle Lake: Wild rice density (2000-2014).

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

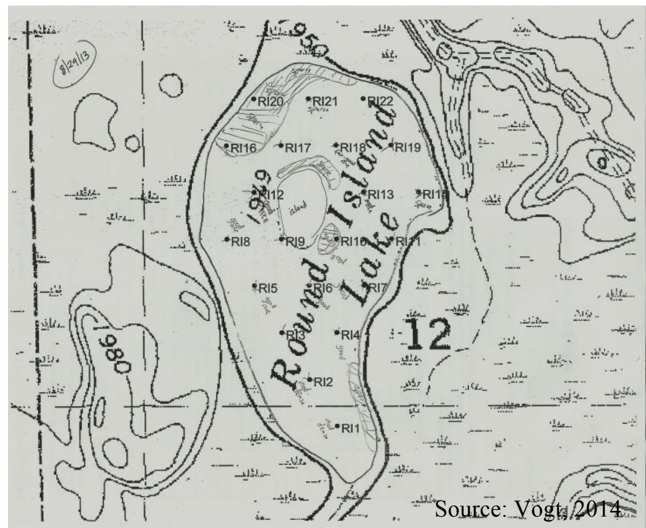


Figure 28. Topographic map of Round Island Lake shows location of wild rice sampling points.

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.

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.

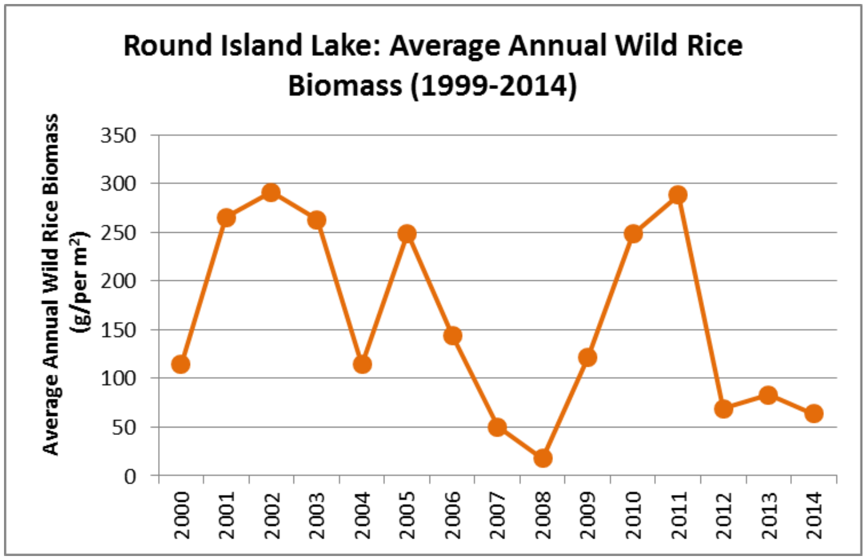


Figure 29. Wild rice biomass on Round Island lakes demonstrates a crash in 2008 followed by gradual recovery in

As shown in the "heat" maps of Round Lake on the next page, 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.

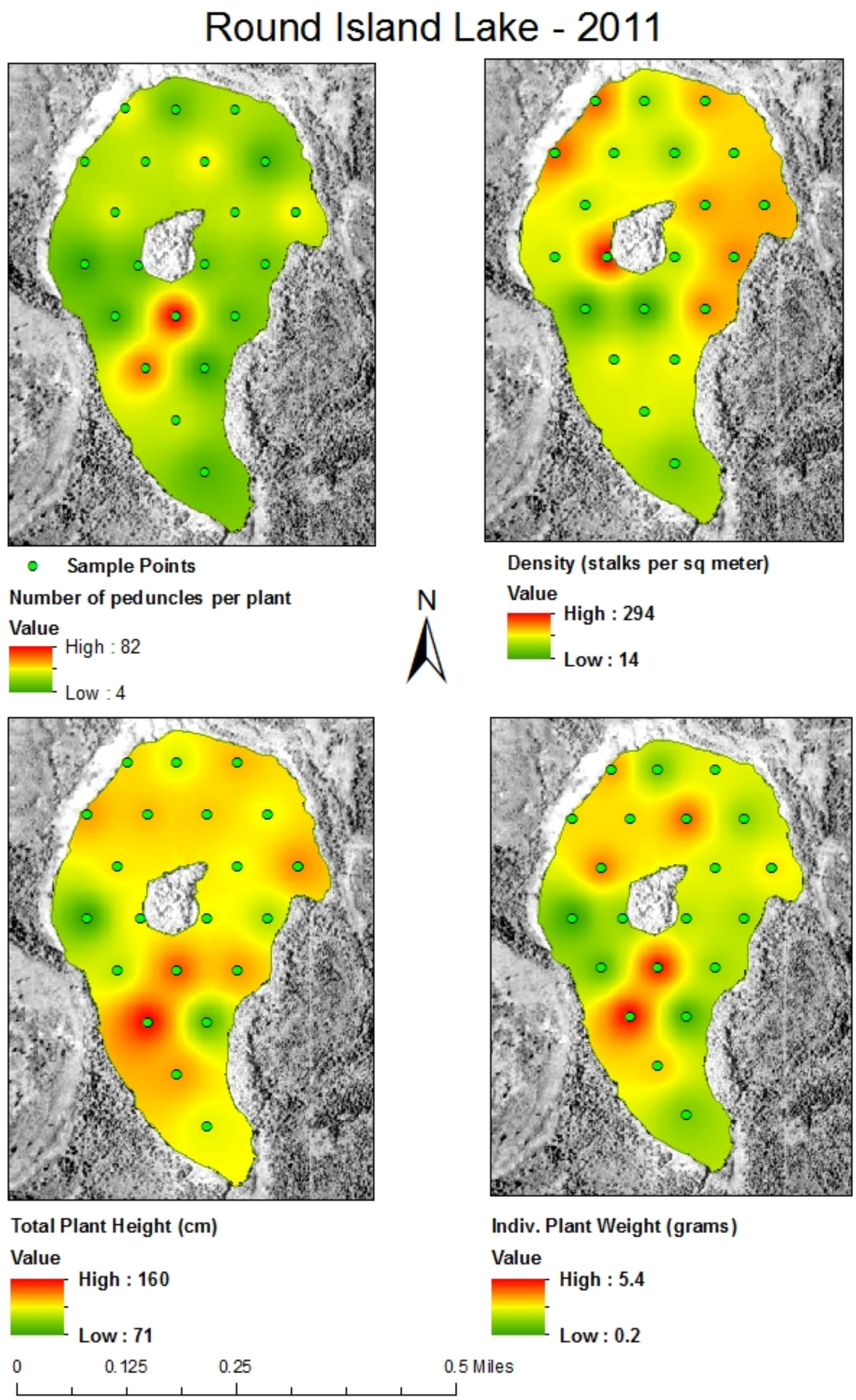


Figure 30. Heat maps of Round Island Lake depict four different parameters of the wild rice population in 2011: number of pedicels, density, plant height, and individual plant weight.

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 waterbody can experience a year of nearly zero production (2008) followed by a full recovery.

VERMILION RIVER

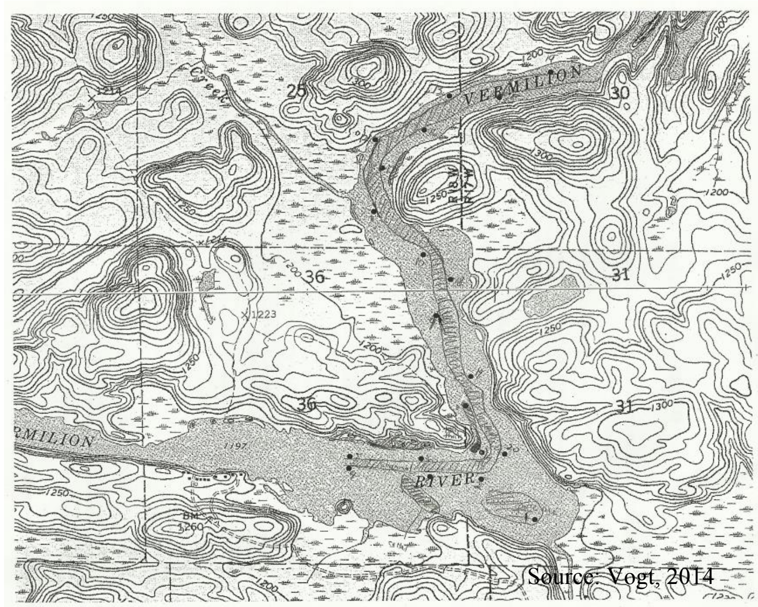


Figure 31. Topographic map of Vermilion River reach showing wild rice sampling points.

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.

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.

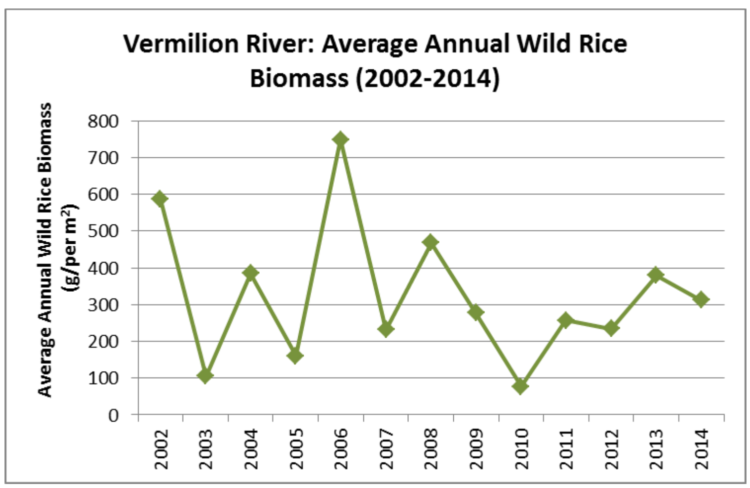


Figure 32. The amount of wild rice biomass growing on the Vermilion River has frequently been the highest among the wild rice waters monitored by the 1854 Treaty Authority.

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 1435 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 waterbody 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

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Elias, J. E, R. Axler, and E. Ruzycki. (2008) Water quality monitoring protocol for inland lakes. Version 1.0. National Park Service, Great Lakes Inventory and Monitoring Network. Natural Resources Technical Report NPS/GLKN/NRTR—2008/109. National Park Service, Fort Collins, Colorado.

Minnesota Pollution Control Agency (MPCA) Citizen Stream Monitoring Program http://www.pca.state.mn.us/index.php/water/water-types-and-programs/surface-water/streams-and-rivers/citizen-stream-monitoring-program/index.html

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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.
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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

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 the Field Guide or Handbook (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	Present	Comments
Beaver	☐	
Muskrat	☐	
Rusty Crawfish	☐	
Swans	☐	
Ducks	☐	
Geese	☐	
Rice worms	☐	
Ergots	☐	
Leaf sheath & stem rot	☐	
Unusual seed head shape	☐	
Other	☐	
Unknown	☐	

(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.)

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 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 ___

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 waterbody 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. Prior to weighing, plants were dried at 60°C for 24 hours.

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 = Waterbody 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
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

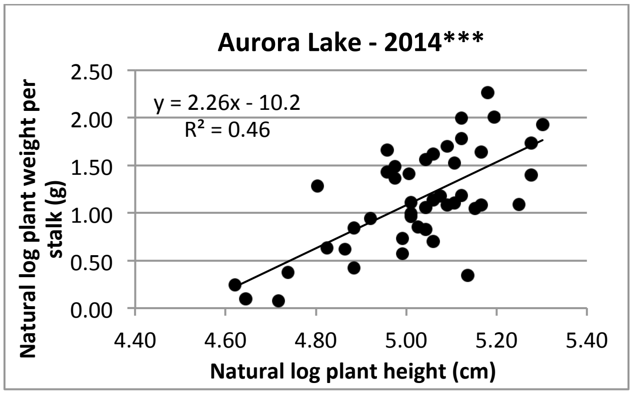
GLOSSARY
Median is the middle value of a set of numbers. The median and mean/average will be very similar when a set of numbers is normally distributed, but the median will be different, and more representative, when there is a great deal of "skewness" to the data. Wild rice density data tend to be skewed towards more plots of low density with only a few plots having high density.

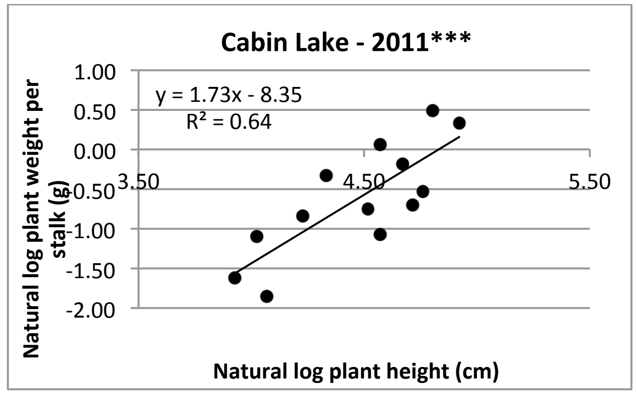
Helpful Tip: While these values provide a good ballpark estimate, it is possible that the population of wild rice plants you measure will show significantly different characteristics from those listed here. For example, other lakes may have higher values that differ by 10 times or more, on average (Kjerland, unpublished data).

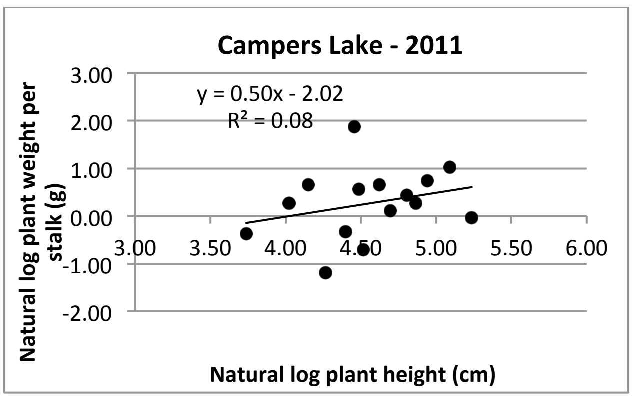
Equation 1: Plant height – weight per stalk

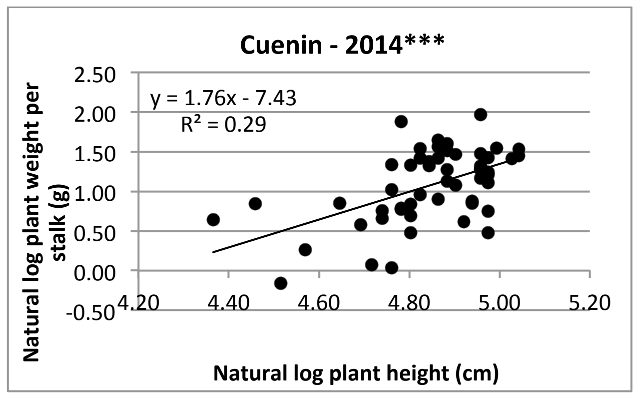
Note: Variables were log transformed

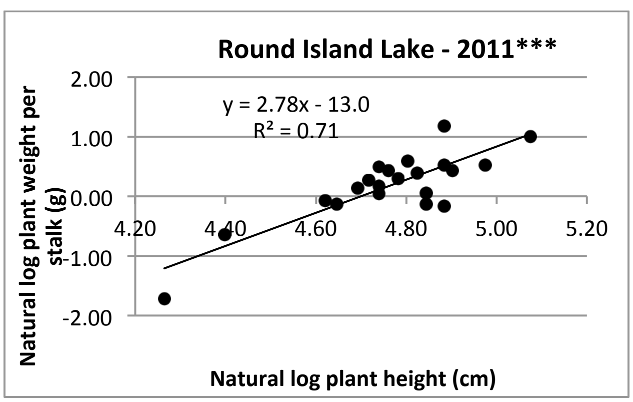
Statistical significance: * = p ≤ 0.05 ** = p ≤ 0.01 *** = p ≤ 0.001

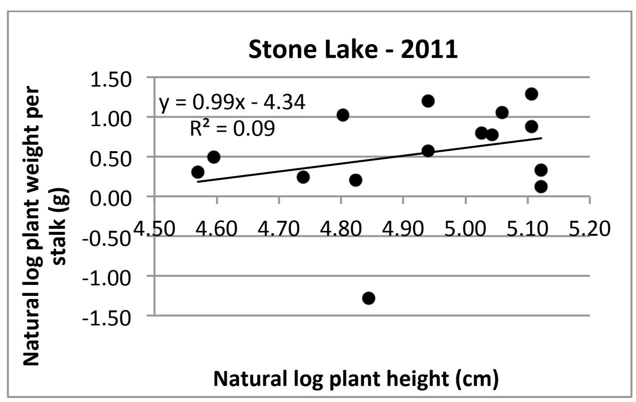








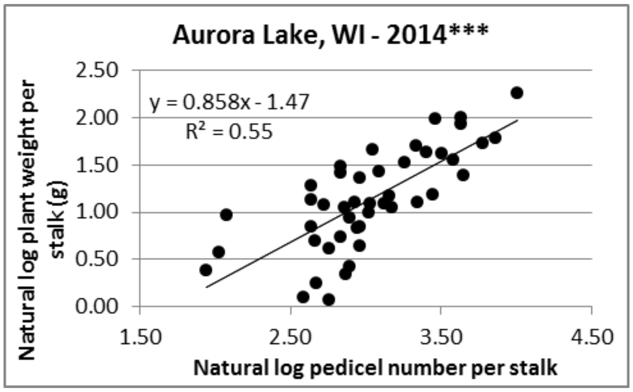


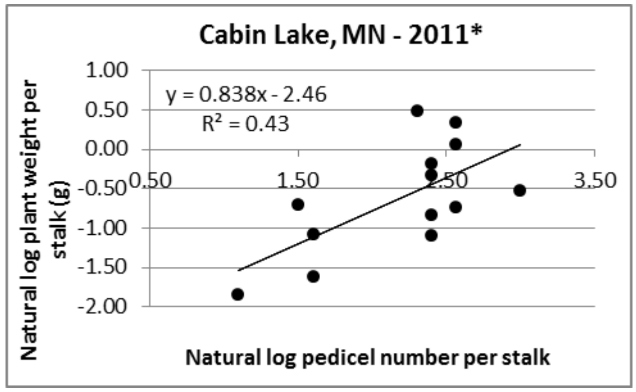


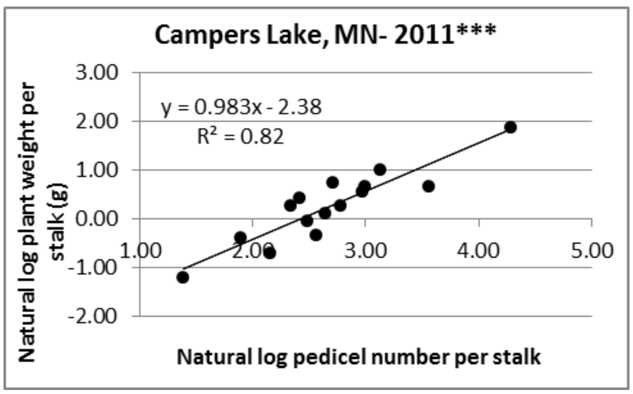
Equation 2: Number of potential seeds (pedicels) per stalk - weight per stalk

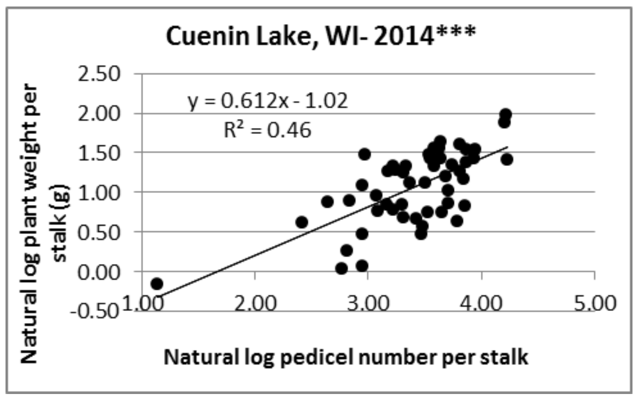
Note: Variables were log transformed

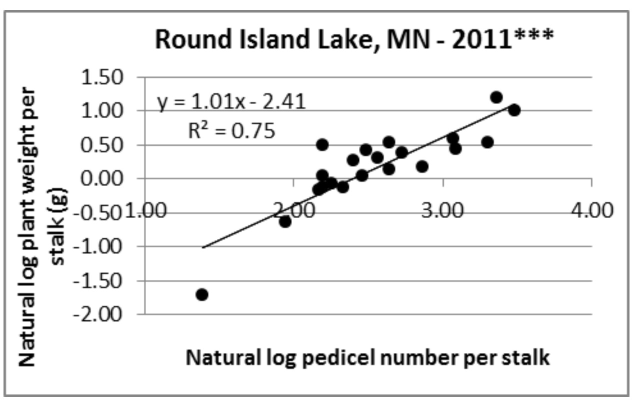
Statistical significance: * = p ≤ 0.05 ** = p ≤ 0.01 *** = p ≤ 0.001

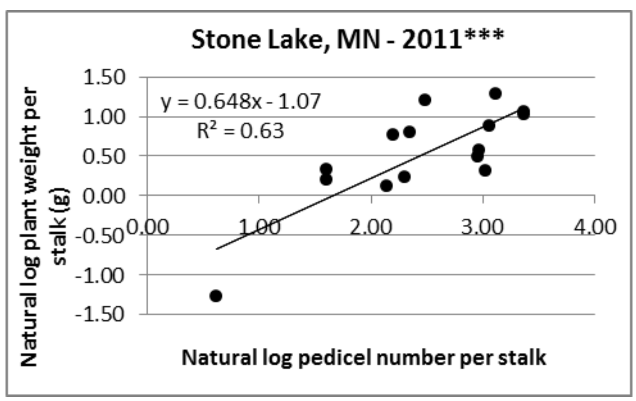












Appendix D

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 waterbody: 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)*

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

<u>Toxic Chemicals</u>

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

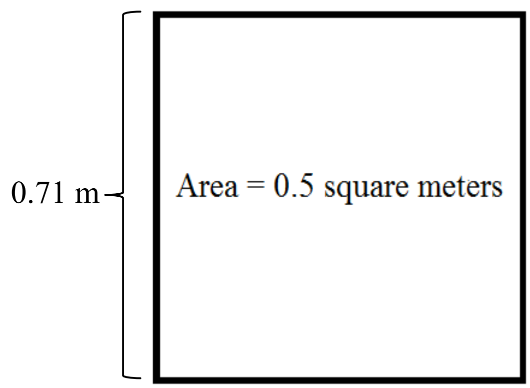
How to make a quadrat frame with area equal to 0.5 m^2

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 waterbody 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.[21] 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 = $5.75x^{-0.433}A^{-0.157}p^{-2}$

Where 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.

[21] These are area measurements. The length of each side of a 0.5 m^2 quadrat is equal to 0.71 m.

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, 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 standard error of the mean no greater than 20% 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.

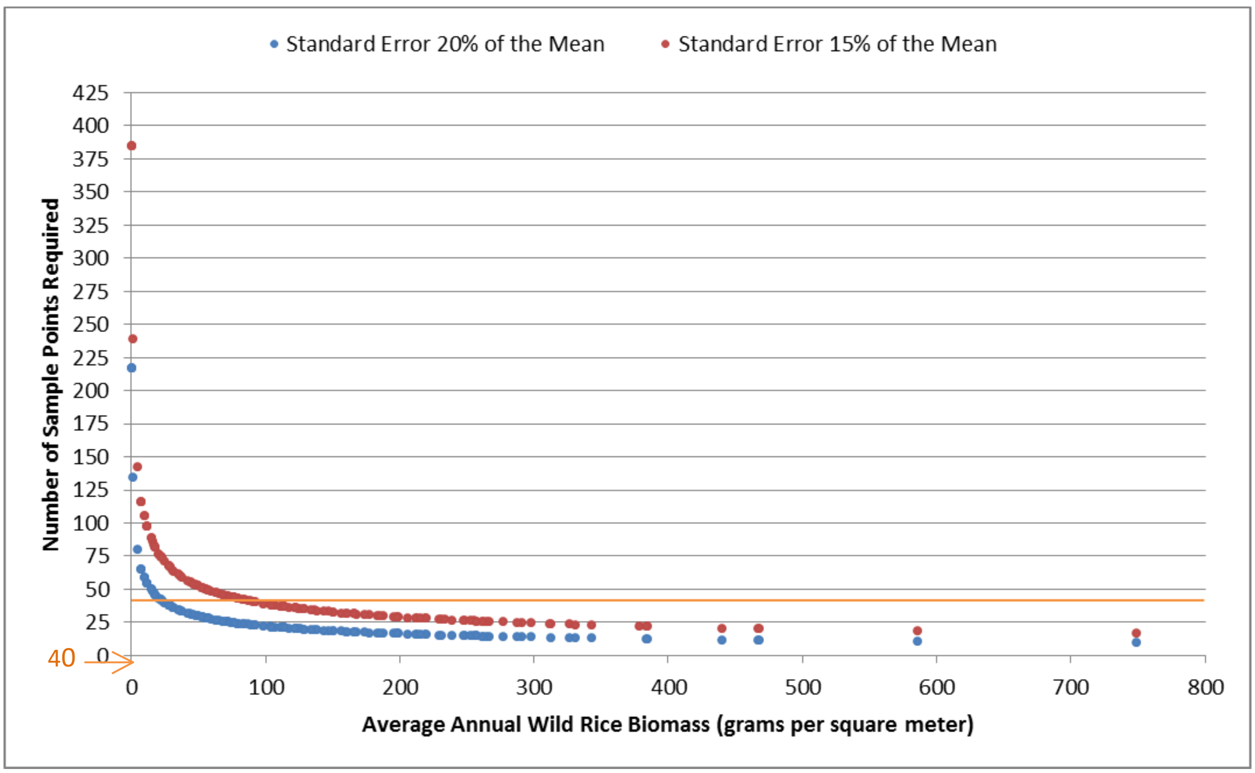


Figure 33. Illustration of the increasing number of sample points required as the level of statistical precision desired is raised from a standard error of 20% of the mean to a standard error of 15% of the mean.