Seed Size in Lacustrine and Riverine Populations of Wild Rice (*Zizania palustris)*

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Dedication

Yakoke Hapokni Jean

Abstract

To study the effects of the hydrological regimes of lakes and rivers on seed size of wild rice (*Zizania palustris*), four lakes and four rivers were sampled to measure and model the factors affecting seed size. Based on casual observation by harvesters and biologists it has been hypothesized that seeds produced in riverine habitats are smaller than those produced in lacustrine habitats. We found mean seed mass in lake populations was 15.4 mg (41%) larger than in river populations. When seed mass was partitioned between water body type, regional population pair, and individual population, water body type accounted for 71.3% of the variance. Data collected on seed mass, panicle density, seed scars, root mass, sediment characteristics, and water depths were used to create a statistical model to quantify the effects of each factor on seed size. The two most important environmental factors contributing to seed size were sediment bulk density and water depth at seed collection. Important biological components were seed scar density, proportion of filled seed, and root dry mass.

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INTRODUCTION

Humans have harvested wild rice (*Zizania palustris*) in the Great Lakes region for at least two thousand years (Valppu 2000). During this period, the indigenous peoples have accumulated a traditional ecological knowledge of wild rice. Known to the Ojibwe as Manoomin, it has a strong cultural and spiritual importance to the Tribal peoples sharing its landscape (Ackley 2000, Schlender 2000). This knowledge includes an observation of smaller seeds in riverine populations. Vennum (1988) recounts that seed smaller than 10 mm in length have been referred to as "river rice."

Humans and wildlife have benefitted from the nutritional properties of wild rice. The nutritional quality is as good or better than other cereals (Oelke 1993) and represents a valuable food source to resident and migrant wildlife (McAtee 1917, Hanson 2008). In Minnesota, harvesters collect the fully developed seeds of *Zizania palustris* from shallow lakes and slow moving rivers for sale to specialty processors and for personal consumption. Any state resident has the right to harvest designated populations, but only members of Minnesota tribes may harvest on their reservations and other treaty-negotiated populations. Harvesters prefer the larger seeds in a natural population because larger seed is more desirable to buyers and less effort is required to harvest each kilogram of wild rice.

Wild rice is a member of the aquatic genus *Zizania* (Poaceae, subfamily Zizanieae). The two annual species, *Zizania aquatica* and *Z. palustris*, are found in the north central United States. Although both species are found in Minnesota, *Z. palustris* is of the greatest importance to humans. The range of *Z. palustris* extends from north-central

United States to south-central Canada.

Z. palustris is found in slow riverine and lacustrine environments experiencing some degree of water flow (Jenks 1901, Moyle 1944, Rogosin 1951, Dore 1969), most often in depths between 0.1 and 1.1 m (Thomas and Stewart 1969). It is well known for thriving under seasonal water level fluctuations and disturbance (Chambliss 1940, Moyle 1944, Steeves 1952). Optimal substrate for rooting consists of organic, organic-clay mixture, or organic sediments over clay. Flocculent and organic-flocculent sediment increases the probability of uprooting (Day and Lee 1990).

Annual growth begins in spring when water temperatures reach 6 °C and overwintering seeds germinate (Oelke et al. 1997). Grava and Raisanen (1978) report that the K2 cultivar progresses past the floating leaf stage and has two aerial leaves four weeks after emergence, and that flowering occurs between nine and twelve weeks after emergence. This developmental timeline differs in natural populations. In a study in the Kakagon Sloughs of northern Wisconsin, developmental events occur roughly two weeks later than that reported for the K2 cultivar (Meeker 2000). After flowering, female florets are wind pollinated by pollen traveling up to 3.2 km (Cregan 2004). Six to seven weeks after pollination, seeds are mature (Grava and Raisanen 1978, Meeker 2000).

Counts and Lee (1987, 1990, 1991) have authored several papers addressing seed size variability among populations. Environmental conditions were identified as a likely source of variation of seed size (Counts and Lee 1987). They also found no systematic relationship between climatic variation and the size of seed (Counts and Lee 1990). Local environmental factors (sediment nutrients, water depth, and emergent macrophyte

competition) were responsible for 23.5% of seed mass variation (Counts and Lee 1990). Further, when analyzing the impact of regional climate on seed size, they found no systematic relationship (Counts and Lee1990). Later, they found that of the large variation in seed mass, a considerable amount (57%) is due to differences within populations (Counts and Lee 1991). However, no study has explicitly compared seed biomass in lake and river populations.

Within a single species, seed size may be influenced by environmental variability in the aquatic ecosystem (Fenner 2005). Within wild populations a major and often variable condition is the flow of water through a bed of wild rice (Riemer 1984). Water flow and its associated impacts on water chemistry, sedimentation, and organisms can influence growth and plant forms in both lacustrine and riverine environments (Dawson 1988). The hydrologic regime characteristic of riverine environments provides a benefit of greater sedimentation and an associated influx of nutrients not experienced by lacustrine populations (Meeker 1996). The Kakagon Sloughs are an example of this influx. According to Meeker (1996), different mean sedimentation rates occurs in riverine versus backwater areas of the slough

The central goal of my study was to quantify and compare the biomass of seeds in populations of wild rice in lakes and rivers. The experimental design paired lake and river populations to reduce environmental and genotypic variance. Under these restrictions on the sources of variance, two models were developed to explain differences observed in the biomass of seeds. A random effects model was used to partition variance by type of water body, regional population pairs, and individual population. Then, a fixed effects

model was developed to explain differences in biomass of seeds using physical and biological environmental variables.

METHODS

Site Description

Four pairs of populations were selected (Table 1) as study sites. Each pair consisted of one river and one lake. Three pairs were directly connected by water and one pair, located in Douglas County, Wisconsin, was 1.6 km apart (Lower Ox Lake and the St Croix River). Sites in each pair were well within the 3.2 km distance known for pollen travel (Cregan 2004).

Study sites were selected based on several factors. It was important that sites only had populations of *Zizania palustris* (not *Z. aquatica*). The grouping of sites into pairs also allowed for minimal environmental effects by landscape-scale factors such as climate and watershed. Also, the selection of hydrologically connected or closely

Table 1. Locations of eight study sites arranged by regional population pair.

Population	County, State	Lat N, Long W
Lower Ox Lake	Douglas, WI	46° 18', 91° 46'
St Croix River	Douglas, WI	46° 18', 91° 47'
Moose Lake	Aitkin, MN	46°52', 93°38'
Moose River	Aitkin, MN	46°52', 93°36'
Lake Itasca	Clearwater, MN	47° 13', 95° 11'
Mississippi River	Clearwater, MN	47° 15', 95° 13'
Rice Lake	Aitkin, MN	46° 30', 93° 23'
Rice River	Aitkin, MN	46° 32', 93° 18'

adjacent lake and river populations reduced the potential for strongly differentiated genetic populations. Sites expected to ripen at different times were preferred for logistic practicality. The geographic locations of the four site pairs (population pairs) represent a range of 172 km between the Clearwater County (MN) sites and the Douglas County (WI) sites. Potential sites were also selected to ensure stem densities were high enough to provide adequate availability of seeds. This assessment resulted in the selection of the four site pairs (Table 1).

Field Methods

Plots and Subplots

Sites were revisited two to three weeks prior to expected ripening. Within each site, sampling plots were randomly chosen from suitable water depths (less than 1.6 m) where a minimum density of ten stems per 0.25 m^2 occurred. If a plot did not have the minimum density, another plot was chosen. At each site ten plots were selected. A Garmin Map 76 GPS was used to navigate to and store plot locations.

Binding, Collection of Sediment and Vouchers

Due to the characteristic gradual dropping of ripe seed and stochastic sources of seed loss, a traditional Ojibwe harvesting technique known as "binding" was applied to one 0.25m^2 subplot of each plot. According to Jenks (1901) and Vennum (1988) the practice of binding reduces seed loss to herbivory and wind. At each of the ten plots the sediment was sampled, a voucher specimen was collected and the stems within a 0.25m^2 subplot were bound. A 0.25m^2 PVC sampling square was used to partition stems into a subplot for binding. Using methods described by Vennum (1988), these stems were

bound with jute cord from 25 to 30 cm above the water line, up and around the seed heads, and then the cord was tied to the main bundle, forming a rough 'p' shape (Figure 1). The number of stems bound together in each subplot were counted and recorded. A sampling tag was affixed to the bound stems bearing the plot identification and researcher contact information.

Single plant specimens were collected from directly outside the square of bound stems for species verification. Sediment was also collected next to the bound stems, but from the side opposite from where the voucher was collected. Sediment was obtained using an eight cm diameter aluminum post-hole sampler to a depth of approximately 20 cm (Carson 2002). Both sediment and vouchers were secured in separate plastic bags and refrigerated immediately upon return from the field.

Figure 1. Bound stems of wild rice at Lower Ox Lake

Seed and Plant Collection from Plots

Two to three weeks following binding and collection of sediment samples, ripe seed and plants were collected (September 2009). In addition to the bound subplot, an adjacent 0.25m^2 subplot of unbound stems was sampled. At each subplot, water depth was recorded with a wooden meter stick.

Bound stems were freed by carefully cutting the jute cord over a cloth positioned to catch falling seed. After the cord was removed, stems were spread and swept using a wild

rice harvesting "knocker" (a traditionally used round, smooth wooden rod). Seeds were then collected from the cloth; insects and large non-seed particles were discarded. Seeds were placed in labeled paper sacks and stored in a plastic bin at 4 °C.

The unbound subplot of the plot was located adjacent to the bound subplot (not where the vouchers or sediment was collected) in an area with ≥ 10 stems per 0.25 m^2. As with the bound subplot, a 0.25 m^2 sampling square was used to partition the stems. The stems were bent over the canoe and swept with the ricing knocker onto a cloth. The seeds were put into a labeled paper sack and the other debris was discarded. Following seed collection, all wild rice plants within each subplot were carefully uprooted and placed in plastic garbage bags. All specimens were stored at 4° C in a walk-in cooler until they were processed.

Laboratory Methods

Seed

Seed samples collected from each subplot were sorted into three groups and counted. The first group consisted of seeds that had completed development and were free of any signs of herbivory. Seeds in the second group were undeveloped. The third group was composed of seeds that had been at least partially developed but were either damaged by herbivory or failed to occupy at least two-thirds of their seed coat. The grouping segregated mature, fully developed seeds from damaged or undeveloped seeds.

Awns were truncated from complete seed by snapping between awn tip and the fruit body. The complete seeds from each subplot were counted, placed into envelopes, and dried for ≥ 48 hours at 105°C. The enumerated seeds were then removed from the

envelopes and weighed to 0.1 mg. Ten seeds per subplot were randomly selected and individually weighed to 0.1 mg (Appendix 1).

Plant Morphological Measurements

For each 0.25 m^2 subplot, stem length, average number of seed scars on the panicle, and dry weight of roots were determined. Total panicle count for each subplot was estimated using the greater of either the number of complete stems or the number of complete panicles. Each complete stem including a complete panicle was measured to the nearest 0.1 cm from directly above the top-most adventitious root to the tip of the panicle. Once this measurement was taken, the panicle was removed from the stem. Complete panicles from partial stems were also collected. In each subplot panicles were counted and up to 10 were randomly selected for counting of seed scars. Mean seed scar count per subplot was determined as the product of the average number of seed scars per seed head and the estimated panicle count. Roots were removed from the stems below the upper-most adventitious root. They were thoroughly washed and all loose detritus was removed. Roots were then dried for ≥ 48 hours at 105 °C and weighed to 0.1 g.

Population Attributes

For each plot, a single value was created for each variable by averaging the two subplots. Values for stem lengths, panicle count, seed scars, and the proportion of complete seed were multiplied by the average seed mass in each population to characterize the potential yield of seeds per m^2 for each population.

Sediment

Sediment from each plot was homogenized and 10 ml of sediment was put into

prepared crucibles, weighed to the nearest 0.1 mg and then dried at 105 °C for ≥ 48 hrs, and weighed to obtain dry mass. Sediment bulk density was determined as grams of dry mass per ml. The samples were then placed in a 550°C muffle furnace for two hours, and subsequently weighed to determine the percent organic matter (Greenberg et al. 1992).

Statistical Analysis

Analysis was completed using R language for statistical computing (R Development Core Team, 2009). A paired t-test was used to test for a seed mass difference between lake and river populations. Complementary to the primary goal of the study, data were used to fit a fixed-effects explanatory model for the purpose of identifying important factors contributing to seed size. The explanatory variables included physical characters of the environment, and biological features of the plant. Values obtained for all biological measurements lacked a normal distribution. These were transformed using a natural logarithm. An initial model considered the three major categorical variables (population type, regional population-pair, population) and five quantitative variables that showed at least a moderate degree of correlation to seed mass but were not correlated to another variable. This was determined by examination of the scatter plot regression lines of each independent variable to seed mass and each independent variable to the other independent variables. The initial best-fit model was then determined through step–wise selection using Akaike's An Information Criterion (AIC). The final best-fit model consisted of all terms from the second model that were significant at the 95% confidence level; terms not satisfying this requirement were discarded from the model. The percent of seed mass variation due to type of water body,

region, and individual population was partitioned using a random-effects model (Bates and Maechler 2009).

RESULTS

Binding

Bound and unbound samples were examined for effects of the binding treatment on the seed mass using a data set restricted to plots with seed mass measured on both subplot categories. Although seeds from bound subplots were smaller than unbound subplots (P = 0.02), the effect of stem binding on seed mass was small (a difference of 6.5%). Partitioned by lake and river population type, mean seed mass from non-bound stems in lakes was 12% heavier than seed from bound stems and in rivers the difference was 1%. The difference in lake populations was predominantly due to Rice Lake. Compared to the overall difference between the mean mass of seeds in lakes and rivers (41%) the difference between mass of seeds from bound and not-bound stems was considered negligible and both samples were combined in further analyses.

Seed Mass Analysis

Seeds in lake populations were 15.4 mg heavier than in river populations (paired t-tests; P = 0.005), a difference of 41% (Table 2). There was no overlap among population means of the two types (Figure 2).

Consistent with the comparison among population types, the differences between each of the four lake-river pairs were also significant (Table 3). Mean population values ranged from 17.1 mg at Rice River to 42.4 mg at Lower Ox Lake (Table 3). Seeds in lakes were from 11.9 to 18.2 mg larger than their respective riverine populations.

Individual river populations generally had a smaller range of seed mass than lake populations (Figure 3).

Table 2: Mean mass of seeds by population type, percent difference, and 95% Confidence Interval (CI). CI was calculated using a back transformed (from natural log) margin of error. Lakes n=4: Lower Ox Lake, Moose Lake, Lake Itasca, and Rice Lake. Rivers n=4: St Croix River, Moose River, Mississippi River, and Rice River.

Population Type	Mean (mg)	Difference	Confidence Interval (mg)	
Lake	37.36	41%	35.39	37.81
River	21.98		20.63	23.77

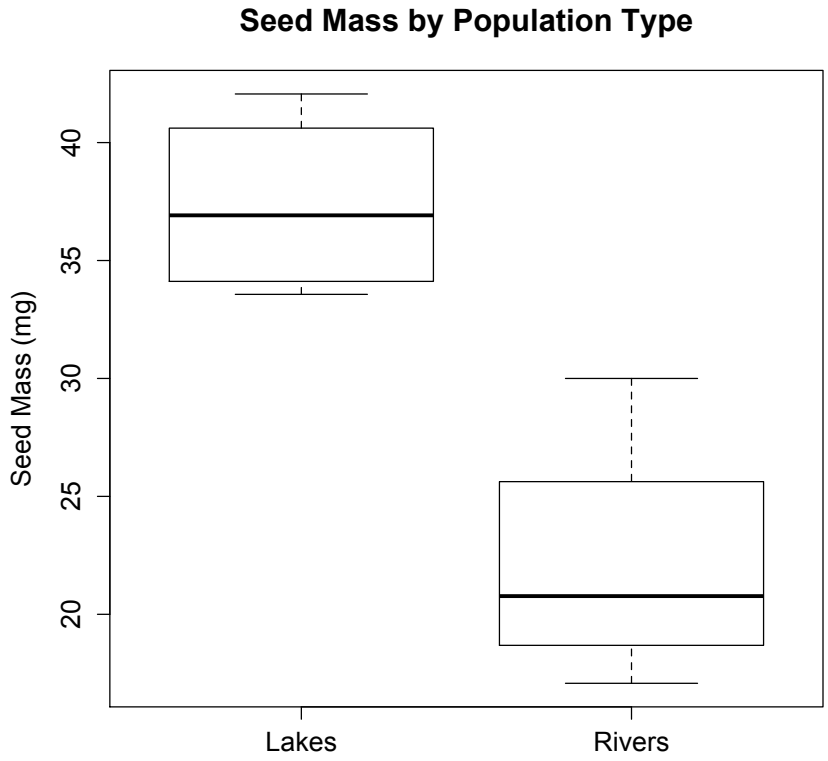

Figure 2. Box plot of population mean mass of seeds for both population types (lakes n=4, rivers n=4). Horizontal lines represent the median values of four population means of each type. Upper and lower box limits are approximate first and third quartiles. Whiskers represent minimum and maximum values.

Table 3: Dates of seed mass collection at each site. Individual mean mass of seed for populations grouped by region. The number of plots at each site (n). Standard error of seed mass means (SE). *P*-value is for a t-test for the means (ln) of each population pair. 95% confidence interval (CI) of the population means using a back transformed margin of error applied to the population mean.

Population	n	Collected	Mean (mg)	SE	CI (mg)		P-value
Lower Ox Lake	9	9/20/09	42.43	2.62	41.27	43.58	< 0.001
St Croix River	10	9/2/09	30.49	1.16	29.40	31.59	
Moose Lake	10	9/6-7/09	39.17	1.43	38.02	40.31	< 0.001
Moose River	8	9/7/09	20.98	2.14	19.81	22.15	
Lake Itasca	3	9/12/09	34.67	3.15	32.80	35.99	0.018
Mississippi River	10	9/11/09	21.25	0.93	20.11	22.39	
Rice Lake	10	9/18/09	34.39	1.88	33.25	35.54	< 0.001
Rice River	10	9/21/09	17.12	0.78	15.97	18.26	

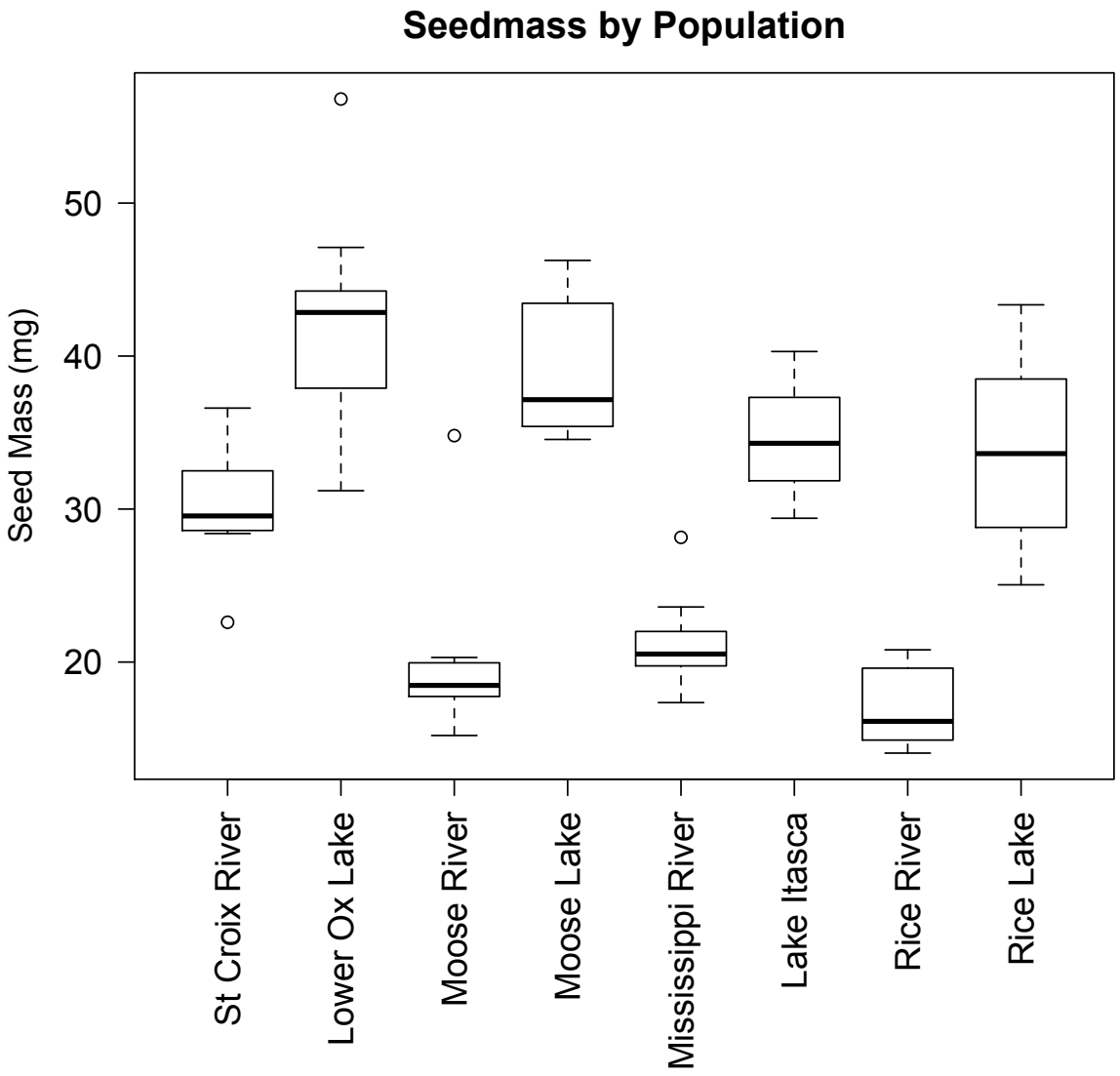

Figure 3. Box plot of mean individual mass of seeds in each population. The number of plots per population is given in Table 3. Horizontal lines represent the median seed mass value of each population. Upper and lower box limits are approximate first and third quartiles. Whiskers represent minimum and maximum values. Circles are plot samples that lie outside of the total range times inter-quartile range.

Site and Population Attributes

Mean water depth at the time of seed collection ranged from 0.05 to 0.80 m (Table 4). At the time of seed collection, several Moose River subplots had no water but were submerged to 0.6 m three weeks prior when stems were bound. Bulk density of sediment was lowest in Moose Lake (0.07 g/ml) and highest in the Mississippi River (0.45 g/ml). A t-test comparison of sediment bulk density means between population types was significant (P <0.001); bulk density of sediment was higher in river than in lake sites. The percent of organic matter in the sediment at each site ranged from 12.6% in Lake Itasca to 39.2% in Lower Ox Lake. Sediment bulk density and percent organic matter were negatively correlated (r = -0.79).

Table 4. Physical attributes, mean water depth, sediment bulk density, and sediment percent organic at each site (population).

Population	Collecting Depth (m)	Bulk Density g/ml	% Organic
Lower Ox Lake	0.58	0.10	28.6%
St Croix River	0.69	0.17	39.2%
Moose Lake	0.46	0.07	34.6%
Moose River	0.05	0.31	27.4%
Lake Itasca	0.80	0.22	12.6%
Mississippi River	0.70	0.45	16.9%
Rice Lake	0.34	0.14	24.4%
Rice River	0.56	0.24	24.1%

Root biomass ranged from 42.2 to 109.6 g/m^2 (Table 5). No difference in mean root mass between lake and river populations was observed. Panicle density was higher in all rivers ($p \leq 0.02$). Mean panicle density was lowest in Rice Lake (92.2/m^2) and highest in

the St Croix River (160.3/m^2). No difference in the proportion of complete seed was observed between lake and river populations (Table 5). However, in comparisons of individual lake and river pairs, differences were significant or nearly so (all $P<0.07$).

The maximum potential for production of seed biomass was estimated in grams per m^2 for each population as the product of seed scars per panicle, panicles per m^2, and mean individual seed mass (Table 5). Corrected potential for production was estimated by multiplying maximum potential seed biomass by the proportion of complete seed. Rice Lake had the largest maximum possible potential production at 417.3 g/m^2 and the largest actual, corrected production at 114.9 g/m^2. The smallest maximum production potential per unit area was in Lake Itasca, but after correction for the proportion of

Table 5. Biological attributes (root mass, panicle count, and seed scars) with mean seed biomass, maximum (Max) and actual seed mass production per m^2 for each population (Pop). Calculations are defined in the text. LX = Lower Ox Lake, SC = St Croix River, ML = Moose Lake, MR = Moose River, IT = Lake Itasca, MISS = Mississippi River, RL = Rice Lake, RR = Rice River.

Pop.	Root Mass g/m^2	Panicles / m^2	Seed Scars/ Panicle	Mean Mass/ seed (mg)	Max. Production g/m^2	Proportion Complete Seed	Actual Production g/m^2
LX	69.2	132.9	26.3	42.1	147.0	0.27	40.1
SC	107.6	160.3	42.6	30.0	204.9	0.60	122.9
ML	81.6	96.4	50.6	39.2	191.1	0.74	141.4
MR	48.4	122.8	49.7	20.3	123.8	0.60	74.3
IT	47.2	110.0	23.9	34.7	91.0	0.62	56.4
MISS	109.6	159.0	73.3	21.3	247.8	0.44	109.0
RL	88.0	92.2	134.5	33.7	417.3	0.77	321.3
RR	68.0	103.4	132.8	17.1	234.5	0.49	114.9

complete seed the population with the lowest production was at Lower Ox Lake with 40.1 g/m^2 (Table 5).

Explanatory Model: Biological and Physical

Selection of a model to explain the individual mass of seed was made from an initial model composed of the categorical and quantitative variables seen in Table 6. Stem length per 0.25 m^2 was not included due to an insignificant correlation with the biomass of seeds. Due to its strong correlation with sediment bulk density and comparatively weak relationship to seed mass, sediment percent organic matter was not included in the model. Starting with the initial model, Akaike's Selection Criteria (AIC) was used in the backwards, step-wise selection of a fixed effects model. A model allowing for interaction

Table 6. Variables considered in the initial model to explain seed mass. Population abbreviations are the same as defined in Table 5.

Catagorical Variables
Population Type: Lake, River
Regional Population Pair: (LX-SC), (ML-MR), (IT-MISS), (RL-RR)
Population: SC, LX, ML, MR, IT, MISS, RL, RR

Quantitative Variables
Water Depth at Seed Maturity
Proportion of Complete Seed
Seed Scars per 0.25m^2 (ln)
Sediment Bulk Density (ln)
Root Biomass per 0.25m^2 (ln)

between terms was rejected because interaction terms were not significant at a 95% confidence level. The stepwise selection process was rerun allowing only for additive terms. This resulted in the selected model containing six main effects and it accounted for 81.5% of the variation in seed mass (Table 7 and Table 8).

The environmental contributors to variation in biomass of seeds were sediment bulk density and collecting depth. The largest contributing plant parameter was seed scars per subplot, followed by root biomass and the proportion of complete seed. Populations accounted for the largest portion of seed mass variance. Quantitative model coefficients had a negative relationship with increasing seed mass, with the exception of water depth at collection, which had a positive relationship. Individual populations had either positive or negative model coefficient values depending on type of water body. Individual lakes had model coefficients from 0.20 to 0.25 and rivers varied from -0.04 to -0.49 (Table A2-1, Appendix 2).

Table 7. Best-fit model terms for seed mass on 12 and 51 df.

Adjusted R^2	F-statistic	P-value
0.8153	24.18	<0.001

Table 8. Summary table of third and final model for seed mass.

Source	Df	Coefficient Est	Sum Sq	F value	Pr(>F)
Collecting Depth	1	0.143	0.206	8.5	0.005
Proportion Complete Seed	1	-0.149	0.173	7.1	0.010
Seed Scars per Subplot (ln)	1	-0.068	1.983	81.9	< 0.001
Sediment Bulk Density (ln)	1	-0.063	2.035	84.1	< 0.001
Root Biomass (ln)	1	-0.034	0.203	8.6	0.005
Population	7		2.418	14.3	< 0.001
Residuals	51		1.234		

Variance of the Mean Mass of Seeds

Variance was partitioned among regional population pair, population type (lake or river), and individual population to find proportionate contributions to the variance in

seed mass. Percent variance was determined by the proportionate variance of each group. The difference between lake and river population types was responsible for the majority of seed mass variation (71.3%) (Table 9). Variation in regional population pairs accounted for 9.9%. Among population variation explained 5.8%, and within populations 13.0%.

Percent correlation was calculated by the proportionate standard deviation of each group to the total standard deviation. The largest correlation was found between population types at nearly 47.9% (Table 9). The eight populations were correlated 13.6% to each other, and 20.5% within each population. Similarly, correlation between regional pairs was 17.9%.

Table 9. A random-fit model characterizing variation in seed biomass for population, regional pair, and population type.

Group	Variance	Std. Dev.	% Variation	Correlation
Population (between)	0.01	0.11	5.8%	13.6%
Regional Population Pair	0.02	0.14	9.9%	17.9%
Population Type	0.14	0.38	71.3%	47.9%
Residual (within populations)	0.03	0.16	13.0%	20.5%

DISCUSSION

Analysis of Seed Mass

Data from the four populations of each type supported the anecdotal observation that lacustrine populations produced larger seeds than riverine populations (P=0.004). The significant difference between the population types carried through to the regional population pairs (all P<0.02). Confidence intervals were remarkably distinct between types of water body and between each water body of a regional pair (Table 2, Table 3).

The tendency of greater biomass of seeds in lacustrine environments is clear even when considering the substantial variability within and among populations (Counts and Lee 1991).

Partitioning of variance seed mass of: type of water body, regional population pair, and population

Just as the hydrologic characteristics of a water body influence the physical environment, the physical environment affects seed size. Counts and Lee (1990, 1991) found substantial variation in the size of seeds among populations and within populations. However, they did not account for type of water body (lake, river) in their calculations. A portion of the variation among populations could be due to differences attributable to the type water body.

We found that variation between types of water bodies accounted for the greatest proportion of variation in seed mass (71.3%) when partitioned between type of water body, region, and population. This differs from Counts and Lee (1990). They found variation among-populations was the greatest contributor at 47.5%. We found only 5.8% of variation attributable to the among-populations group.

Some of the variation Counts and Lee (1990) attributed to their environmental principal component analysis (23.5%) could be explained by type of water body. While our analysis has no directly comparable values, the variables comprising this principal component analysis (sediment nutrients, water depth, emergent macrophyte competition) are all affected by the hydrologic regime of a water body.

Although relative contributions of the among-population variance differ between

these two studies, agreement regarding the contribution of regional variation was observed. Their six regions were found responsible for 10.6% of variation in biomass of seeds. The present study found 9.9% of variation due to regional differences.

Explanatory Model Parameters

Type of water body directly and indirectly impacts biotic and abiotic factors through their differing hydrologic regimes. The use of paired lake and river sites accounted for gross variation, allowed for gene flow, and permitted the influence of differing hydrology to be detected. With these variables held relatively constant, the plastic nature of wild rice reflected the effect of two different hydrologic regimes. The two most important effects explained by the model aside from population effects were sediment bulk density and seed scars per 0.25 m^2 (Table 8). Both panicle density and sediment bulk density were significantly higher in riverine populations.

While lacustrine habitats do generally have some degree of flow (Jenks 1901, Moyle 1944, Rogosin 1951, Dore 1969), it is less than in riverine habitats. Greater flow velocity found in riverine environments picks up the lighter organic and flocculent sediment particles that can reduce sediment bulk density. Although a degree of flow is advantageous for wild rice in competition from perennial macrophytes, too much velocity can result in uprooting. While sufficiently dense sediment may provide a good rooting substrate, too much density can hamper root growth. According to a classification of sediments published by Day and Lee (1989), sediment bulk density in most sites in the present study may be classified as organic, flocculent, or organic-flocculent. The Mississippi River, Lake Itasca, Moose River, and the Rice River sites had higher

sediment bulk densities closer to the organic-clay group. These data, along with the collecting depths at Lake Itasca and the Mississippi River suggest that greater depth in concert with higher bulk density may be related to either lower seed mass or lower biomass of seeds per m^2 (productivity).

Density of seed scars was calculated from the product of panicle (stem) density and the density of seed scars per panicle. Whereas panicle density varied by type of water body, seed scars per panicle appeared to be a population or regional factor. Rice Lake and Rice River both had much higher seed scar density per panicle than any other population. Variation in plant morphology, mass of seed, and response to environmental conditions may be due to the presence or absence of selective pressures particular to a population.

Lake and river seed mass: heaviest and lightest in context of the explanatory model

Lower Ox Lake and the St Croix River had the largest seed for a lake and a river. There appeared to be a tradeoff between mean individual mass and per m^2 biomass of seeds. Lower Ox Lake having the highest sampling variation is consistent with a positive relationship between high mean mass of seed and high variability (Counts and Lee 1991). Lower Ox Lake and the St Croix River had the two lowest values for sediment bulk density. In light of the importance of this parameter as seen in the model, this suggests a relationship between low sediment bulk density and high seed mass. While increased water velocity experienced in rivers may increase the chances for uprooting, especially in the looser sediment of the St Croix, this is compensated by greater biomass of roots for anchorage. Another riverine trait of the St Croix and other rivers of this study is a significantly higher panicle (stem) density. In his study on the Kakagon Sloughs (1996),

Meeker suggested that increased density of stems slowed water velocity and increased sedimentation. Greater panicle density and biomass of roots require a higher input of plant resources; this likely contributes to a corresponding decrease in seed biomass in the St Croix River than in its regional partner Lower Ox Lake. However, the similarities between Lower Ox Lake and the St Croix River such as sediment traits and water depth likely contribute to the Douglas County, Wisconsin sites having the greatest mean seed biomass of their types of water body in our study.

The smallest lake and smallest river seeds were both found in north-central Aitkin County, Minnesota at Rice Lake and Rice River. The strongest environmental influences on the biomass of seeds as seen in the model do not appear to be strongly at work in these populations. According the importance of seed scar density, the small seed mass of Rice Lake and Rice River are strongly related to the particular seed scar density of these two populations. The tradeoff between seed size and seed number can be seen in these two populations as well as in the St Croix River and Lower Ox Lake. The high density of seed scars in the Rice River compensate for lighter seed in terms of biomass of seed produced per m^2 (Table 5).

Stem Binding and Seed Retention

The gradual dropping of ripe seed (shattering) has challenged wild rice field researchers to devise methods for efficient sampling. In addition to shattering, seed can be lost from a plot by wind or herbivory. Methods used previously to address this phenomenon have included buckets to catch falling seed (Haramis and Kearns 2007), paper pollen bags (Cregan 2004), tulle sleeves (Cregan 2004), and tulle sleeves over

panicles sprayed with pesticide (Cregan 2004). The bucket method collected a portion of seed over a one m^2 area (Haramis and Kearns 2007). The bag and sleeve methods retained seed but resulted in submerged panicles and acute seed damage by caterpillars (*Apamea apapmiformis*) sheltered from predating black birds (*Agelaius phoeniceus)*, While both of these methods had some degree of success, an alternative method was investigated that would simultaneously conserve seed and protect it from herbivory. The stem binding practice of the Ojibwe and other Great Lakes Tribes was a promising solution. Although the principal reason binding was used historically was to assert ownership of ripening stems (Vennum 1988), additional advantages to binding have been noted. These include protection from wind, precipitation, and black birds (*Agelaius phoeniceus)*, while the stouter stem bundles also provide scarecrow-like raptor perches (Jenks 1901, Vennum 1988).

We found that stem binding was successful in retaining seed as predicted by Jenks (1901) and Vennum (1988). Undesirable side effects were relatively minor compared to losses described by Cregan (2004) when using bags or sleeves or when using no method for retention. Principal among these was a slight reduction in biomass of seeds in bound subplots in lacustrine populations. It is difficult to ascertain the reason this affect was not found in riverine populations. It may be that the specific phenotypic plasticity found in riverine populations is more adaptable to the fluctuating hydrological conditions found in that habitat.

Binding effects in lakes was principally due to binding differences noted for Rice Lake. Other lake populations had a two to three mg difference between bound and

unbound subplots, but Rice Lake had a 9.9 mg difference. While the sampling procedure described for the methods of this study were rigorously adhered to at all sites, the mechanism of this phenomenon at this lake is unclear. One possible explanation could be a lack of experience and skill required to perform the binding process correctly. Ignorance regarding the precise amount of tension and technique required to properly bind the stems may have resulted in the compression of individual seeds that prevented their full development. Despite this sampling issue, the magnitude of the binding effect was not substantial in comparison to the significant difference of seed biomass between types of water body.

Restoration, Harvesters and Managers

Restoration of wild rice populations is ongoing due to wide declines in abundance. In this work, harvesters frequently express a concern that managers consider seed size issues when sourcing seed for restoration. Unfortunately, due to the lack of data on the size of seeds, managers are uncertain how to adequately address these points (Peter David, Great Lakes Indian Fish and Wildlife Commission, personal communication, August 2010).

Future research contributing to an understanding of the factors responsible for the size of seeds would assist managers to make best possible seed source choices when restoring a population. The biological and physical variables of the final explanatory model need further study to expand on their effects in the context of specific geographic and population characteristics. The influences of regional variation found by Counts and Lee (1990) concur with those found in the present study. The appropriate seed source for

a restoration project may be the nearest population, in the same region, or it may be one sharing a handful of physical or biological parameters. A common garden study would contribute to an understanding of the way rice habitat interacts with the specific genotypic attributes of a wild rice population.

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Appendix 1.

Random seed mass by subplot and population

Individual mass of randomly selected seeds for each harvested subplot at each population, organized by regional population pairs.

Table A1-1. Regional population pair from Douglas County, Wisconsin. Means of all seed from gathered from each subplot and means of up to ten (n) randomly selected individual seeds (RSM) and RSM variance. B = a bound subplot, U = an unbound subplot.

Douglas County, Wisconsin	Subplot	n	Subplot Mean (mg)	RSM Mean (mg)	RSM Variance
Lower Ox Lake	LX1B	10	42.9	43.6	0.0001
	LX1U	10	45.6	46.6	0.0002
	LX2B	10	32.3	36.3	0.0001
	LX2U	7	43.5	43.6	0.0000
	LX3B	10	36.6	40.9	0.0001
	LX3U	10	49.1	48.0	0.0001
	LX4B	10	38.0	35.9	0.0002
	LX5B	10	36.7	32.7	0.0000
	LX5U	10	26.8	25.8	0.0000
	LX7B	10	64.1	34.8	0.0002
	LX7U	3	49.5	49.5	0.0000
	LX8B	10	41.5	38.0	0.0000
	LX8U	10	44.2	42.9	0.0000
St Croix River	SC1U	10	28.6	28.6	0.0001
	SC3U	10	33.2	33.2	0.0000
	SC4U	10	32.5	32.5	0.0000
	SC6U	10	27.2	27.2	0.0000
	SC6B	10	31.4	31.4	0.0112
	SC8U	10	28.6	28.6	0.0001
	SC9U	10	30.4	30.4	0.0001
	SC10U	10	22.6	22.6	0.0000

Table A1-2. Regional population pair from northwestern Aitkin County, Minnesota. Means of all seed from gathered from each subplot and means of up to ten (n) randomly selected individual seeds (RSM) and RSM variance. B = a bound subplot, U = an unbound subplot.

Aitkin County, Minnsota	Subplot	n	Subplot Mean (mg)	RSM Mean (mg)	RSM Var.
Moose Lake	ML1B	10	32.8	37.2	0.0001
	ML1U	10	40.7	38.6	0.0000
	ML2B	10	34.4	35.7	0.0000
	ML2U	10	38.2	37.2	0.0000
	ML3B	10	43.2	40.6	0.0000
	ML3U	10	49.3	46.0	0.0001
	ML4B	10	39.7	41.8	0.0000
	ML4U	10	47.2	47.8	0.0001
	ML5B	10	44.7	44.3	0.0001
	ML5U	10	46.2	49.1	0.0001
	ML6B	10	32.6	38.2	0.0000
	ML6U	10	36.5	39.9	0.0000
	ML7B	10	36.9	36.6	0.0000
	ML7U	10	45.7	41.1	0.0001
	ML8B	10	39.3	37.8	0.0001
	ML8U	10	35.8	38.3	0.0000
	ML9B	10	36.3	33.7	0.0001
	ML10B	10	35.2	38.5	0.0001
Moose River	MR1B	10	18.2	17.7	0.0000
	MR2B	10	15.2	16.0	0.0000
	MR3B	10	18.1	19.4	0.0000
	MR3U	10	21.1	23.1	0.0000
	MR4B	10	18.6	15.7	0.0000
	MR4U	10	18.1	22.0	0.0000
	MR5B	10	18.6	19.8	0.0000
	MR6B	10	17.3	17.9	0.0000
	MR6U	10	23.3	24.6	0.0000
	MR9B	10	46.2	30.2	0.0000
	MR9U	10	23.4	23.4	0.0000
	MR10B	10	15.4	17.7	0.0000
	MR10U	10	19.2	18.8	0.0000

Table A1-3. Regional population pair from Clearwater County, Minnesota. Means of all seed from gathered from each subplot and means of up to ten (n) randomly selected individual seeds (RSM) and RSM variance. B = a bound subplot, U = an unbound subplot.

Clearwater County, Minnesota	Subplot	n	Subplot Mean (mg)	RSM Mean (mg)	RSMvar
Lake Itasca	IT3	10	29.4	29.1	0.0000
	IT4	10	34.3	31.0	0.0000
Mississippi River	MISS1B	10	18.9	15.4	0.0000
	MISS1U	10	19.7	20.2	0.0000
	MISS2B	10	20.6	21.8	0.0000
	MISS2U	10	18.9	19.0	0.0000
	MISS3B	10	16.5	17.1	0.0000
	MISS3U	10	18.2	18.3	0.0000
	MISS4B	10	24.0	24.3	0.0001
	MISS4U	10	32.3	16.5	0.0000
	MISS5B	10	17.7	19.7	0.0001
	MISS5U	10	22.8	19.8	0.0000
	MISS6B	10	20.4	20.3	0.0000
	MISS6U	10	21.7	18.4	0.0000
	MISS7B	10	22.8	22.5	0.0001
	MISS7U	10	21.2	20.6	0.0000
	MISS8B	10	24.6	20.1	0.0000
	MISS8U	10	22.6	24.8	0.0000
	MISS9B	10	19.6	20.3	0.0000
	MISS10B	10	23.5	22.9	0.0000
	MISS10U	7	17.0	16.2	0.0000

Table A1-4. Regional population pair from north central Aitkin County, Minnesota. Means of all seed from gathered from each subplot and means of up to ten (n) individual randomly selected seeds (RSM) and RSM variance. B = a bound subplot, U = an unbound subplot.

Aitkin County, Minnesota	Subplot	n	Subplot Mean (mg)	RSM Mean (mg)	RSMvar
Rice Lake	RL1U	10	25.8	25.3	0.0000
	RL2B	10	30.1	28.7	0.0000
	RL2U	10	56.6	27.4	0.0000
	RL3B	10	28.6	28.1	0.0000
	RL3U	10	48.4	47.3	0.0000
	RL4B	10	32.4	29.1	0.0001
	RL4U	10	46.5	50.3	0.0000
	RL5B	10	39.7	39.0	0.0000
	RL5U	10	31.2	32.8	0.0000
	RL6B	10	32.9	31.6	0.0001
	RL6U	10	37.0	32.3	0.0000
	RL7U	10	32.0	32.2	0.0000
	RL8U	10	28.8	27.4	0.0000
	RL9B	10	21.1	25.4	0.0000
	RL9U	10	29.0	30.4	0.0000
	RL10B	10	29.6	26.9	0.0001
	RL10U	10	35.0	31.6	0.0000
Rice River	RR1B	10	16.8	16.2	0.0000
	RR1U	3	14.3	14.3	0.0000
	RR2B	10	14.0	14.7	0.0000
	RR3B	10	12.8	11.1	0.0000
	RR3U	3	15.3	15.3	0.0000
	RR4B	10	18.6	17.4	0.0000
	RR5B	10	20.8	18.0	0.0000
	RR7B	10	17.2	16.7	0.0000
	RR7U	10	14.6	14.8	0.0000
	RR8B	10	19.1	15.3	0.0000
	RR8U	10	20.1	22.8	0.0000
	RR9B	10	13.7	14.4	0.0000
	RR9U	10	24.1	24.0	0.0000
	RR10B	10	14.9	14.8	0.0000

Appendix 2.

Model terms for fixed effects model of seed mass including terms for individual populations.

Table A2-1. Summary table for final model with model coefficients for each population

Source	Df	Coefficient Est	Sum Sq	F value	Pr(>F)
Collecting Depth	1	0.143	0.206	8.5	0.005
Proportion Complete Seed	1	-0.149	0.173	7.1	0.010
Seed Scars per Subplot (ln)	1	-0.068	1.983	81.9	< 0.001
Sediment Bulk Density (ln)	1	-0.063	2.035	84.1	< 0.001
Root Biomass (ln)	1	-0.034	0.203	8.6	0.005
Population	7		2.418	14.3	< 0.001
Lower Ox Lake		0.204			
Moose Lake		0.247			
Lake Itasca		NA			
Rice Lake		0.221			
St Croix River		-0.040			
Moose River		-0.331			
Mississippi River		-0.278			
Rice River		-0.490			
Residuals	51		1.234		

Appendix 3.

Seed mass as related to selected explanatory variables with regression lines for each population type (lake and river). The figures included in this appendix feature only the data sets for the variables; they are not in context of the model from Table A2-1 or Tables 7 and 8.

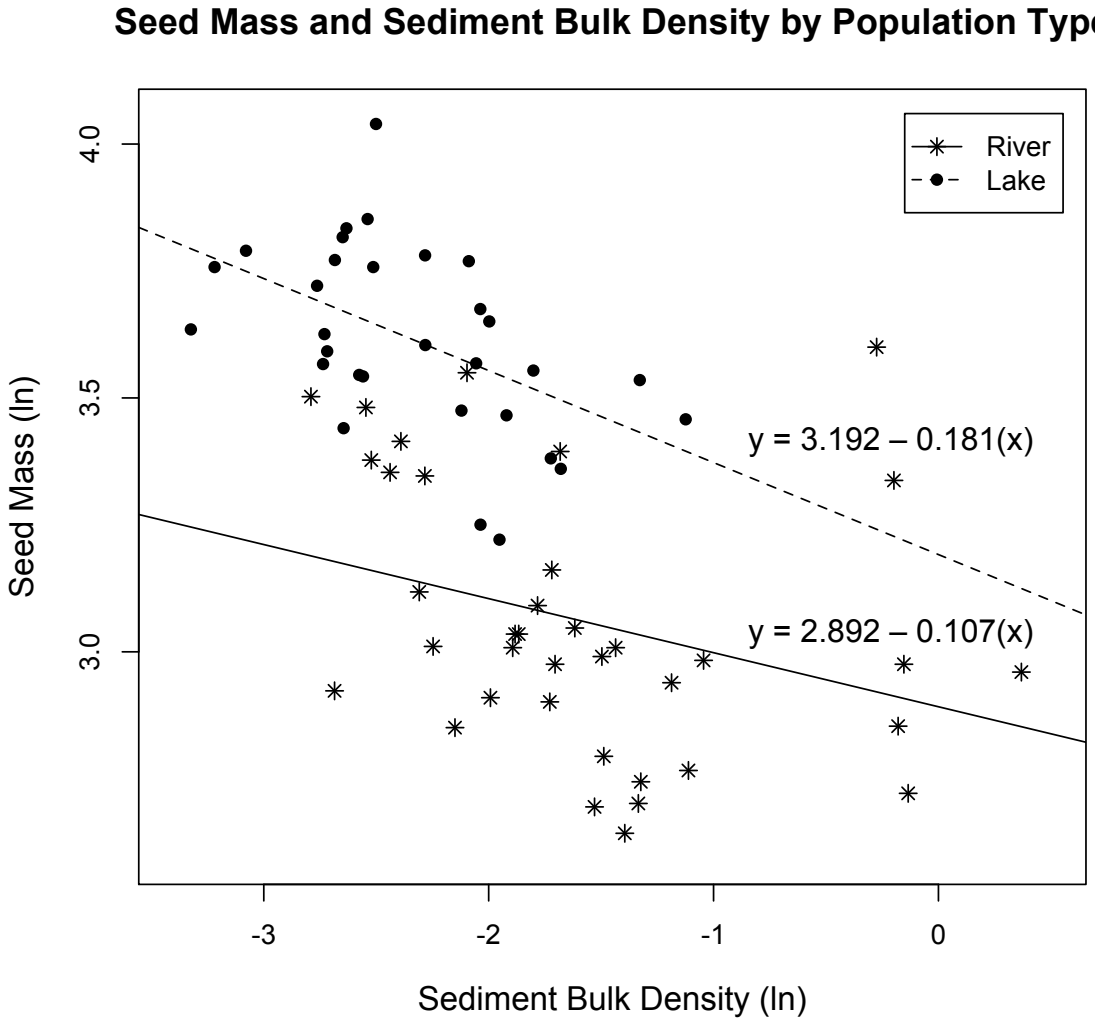

Figure A3-1. The relationship between seed mass (ln(mg)) and sediment bulk density (ln(g/ml)) with regression lines for each population type (lake and river).

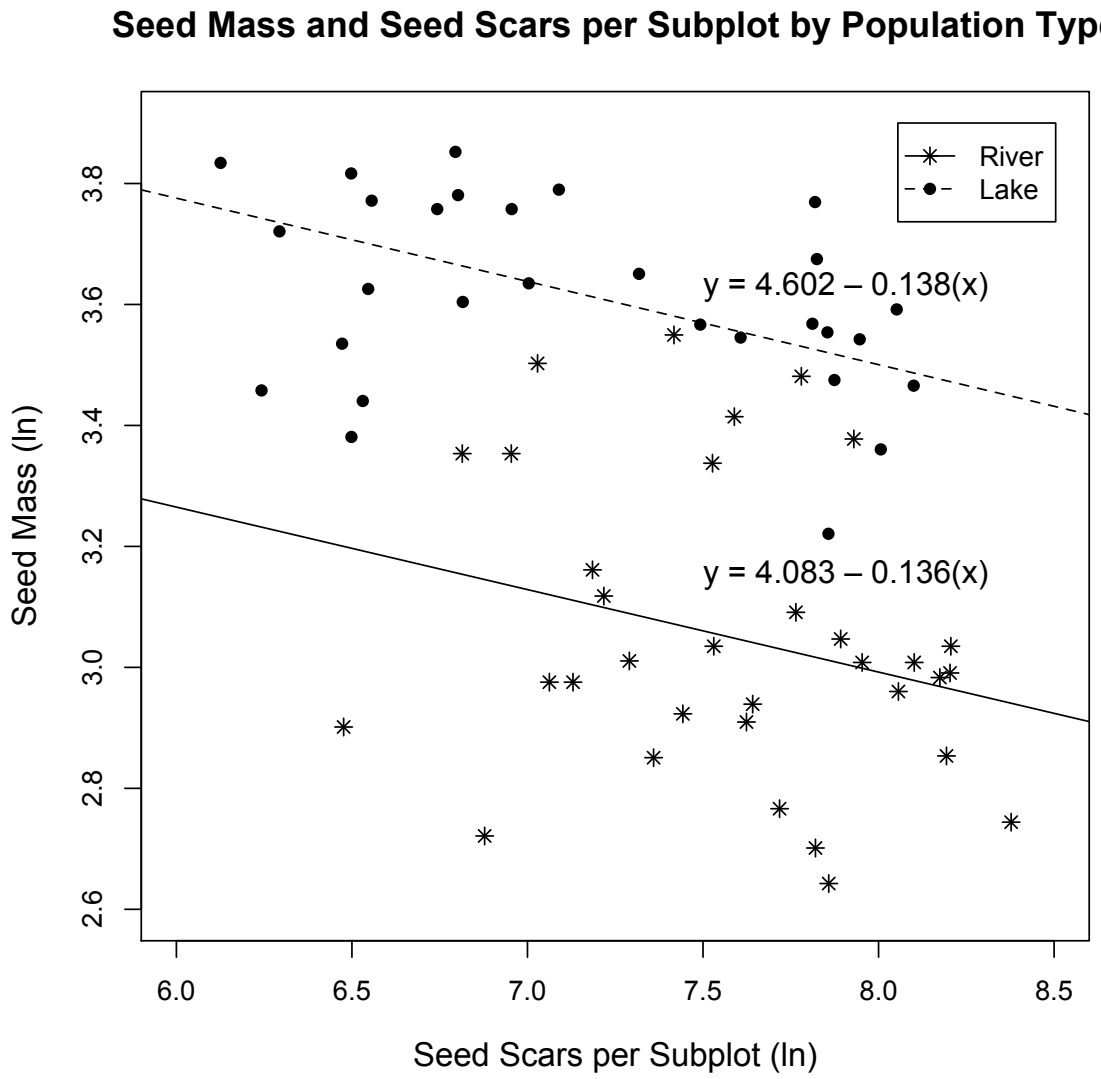

Figure A3-2. The relationship between seed mass (ln(mg)) and seed scars per subplot (ln) with regression lines for each population type (lake and river).

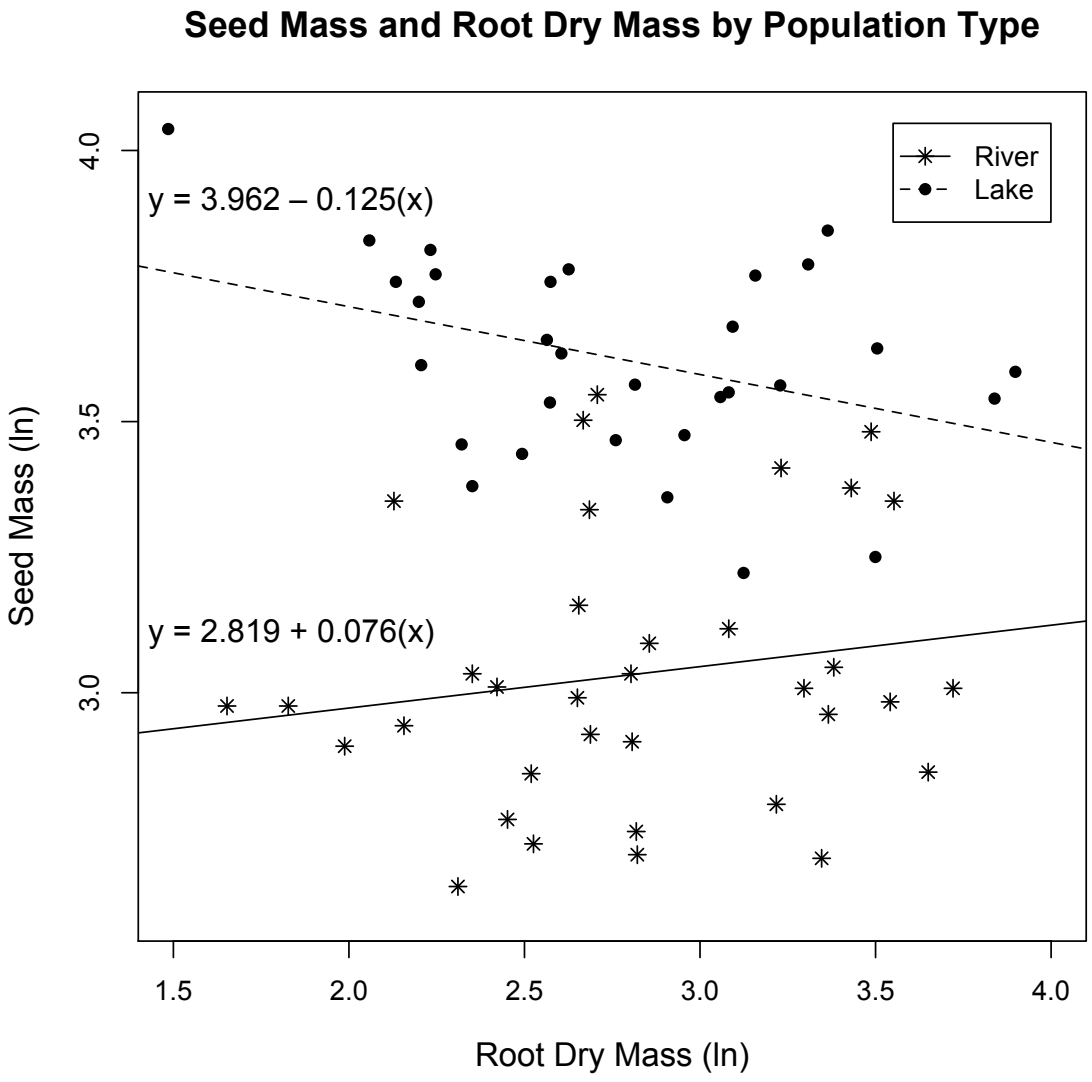

Figure A3-3. The relationship between seed mass (ln(mg)) and root dry mass (ln(g)) with regression lines for each population type.

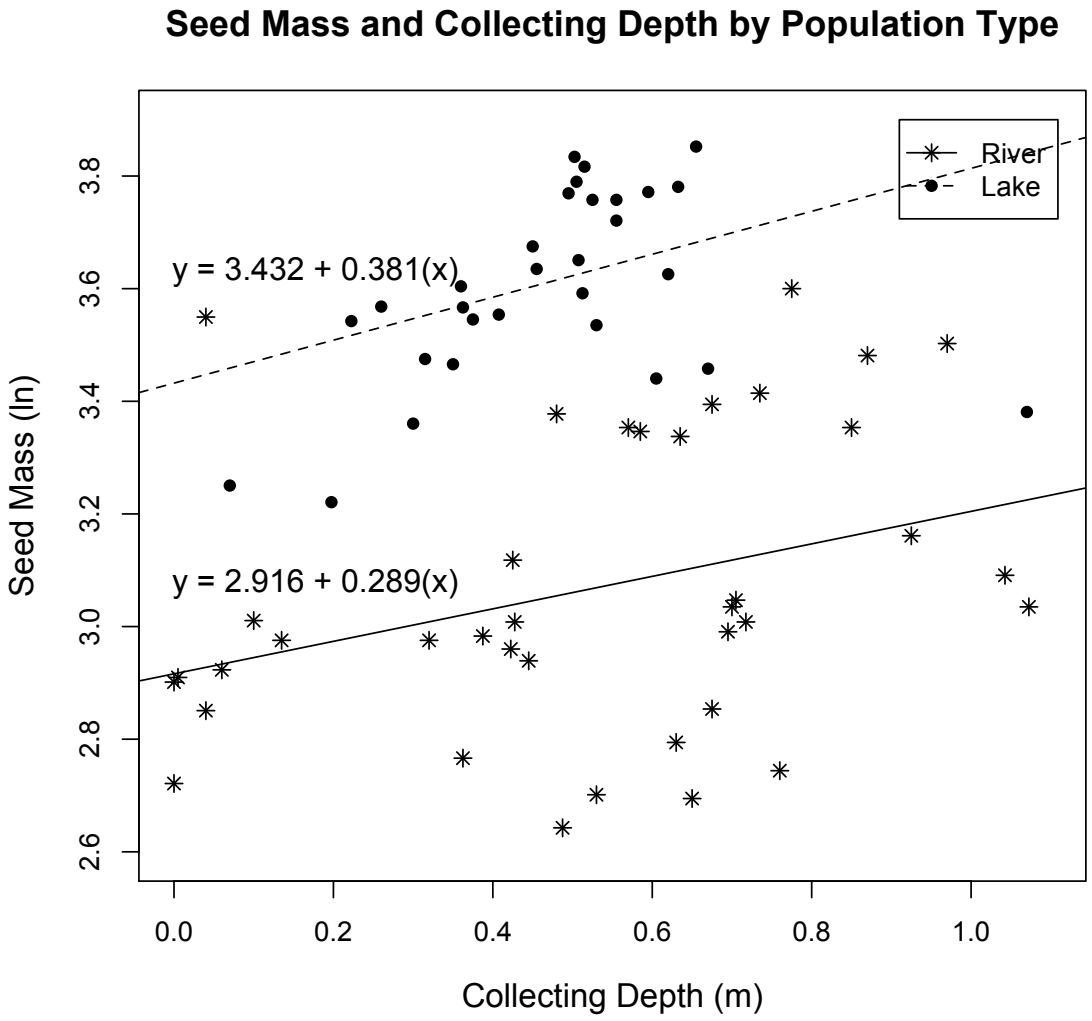

Figure A3-4. The relationship between seed mass (ln(mg)) and collecting depth (m) with regression lines for each population type (lake and river).

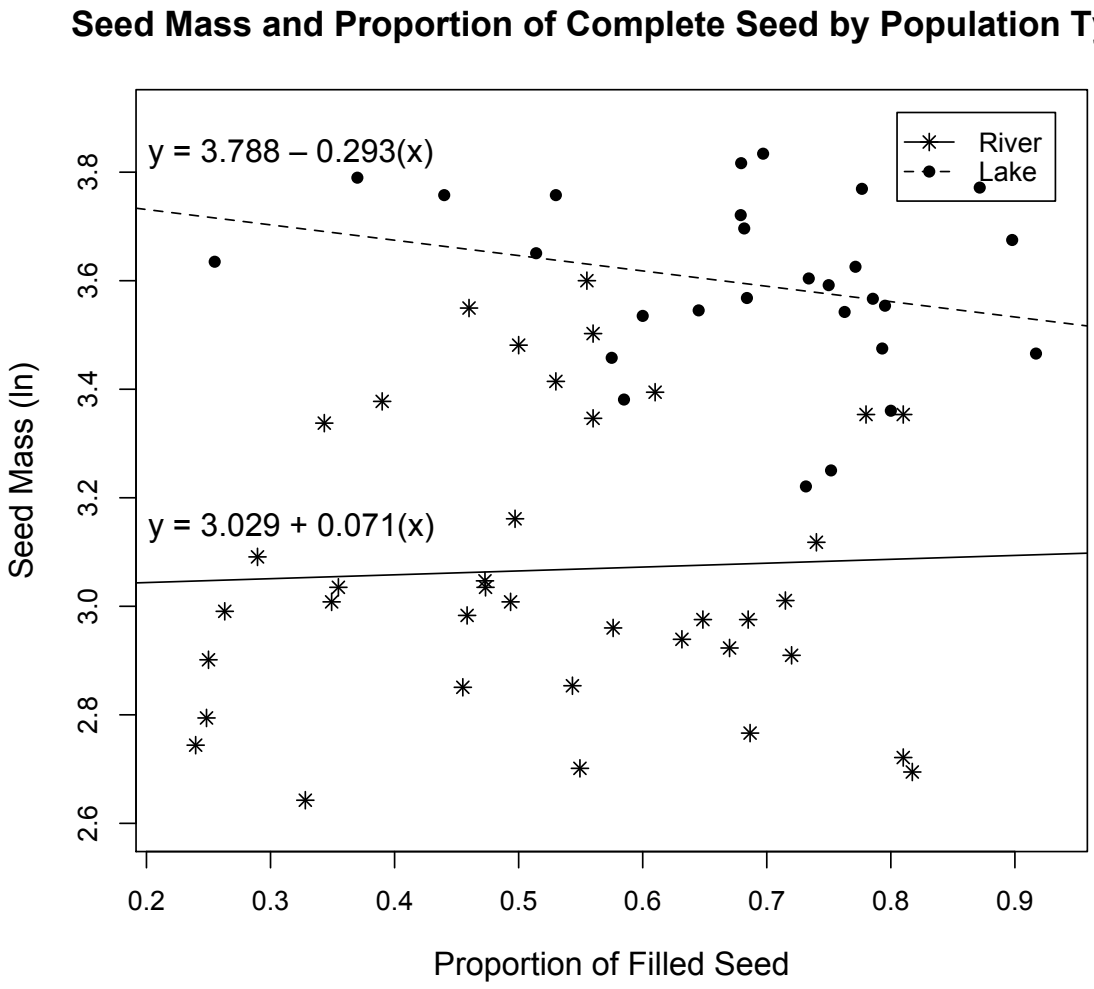

Figure A3-5. The relationship between seed mass (ln(mg)) and the proportion of complete seed with regression lines for each population type (lake and river).