APPENDIX F. REPORT OF LAKE WINNEBAGO PALEOECOLOGICAL STUDY

1.0 METHODS

Two sediment cores were collected in the northern and southern areas of Lake Winnebago on November 14, 2016. The cores were collected with a gravity corer with a 6.5 cm diameter plastic liner. The cores were sectioned into 2 cm slices on the day of collection. Samples were stored in plastic bags in a refrigerator. Table 1.0-1 lists the location, water depth, and length of each core.

Samples were retained for analysis of water content and loss on ignition. Ten samples from one core from each basin will used to determine if the lowest most sample (section) analyzed was deposited at least 100 years ago. In the same cores, the diatom community in the top sample and the bottom most sample where suitable diatom preservation occurs was used to estimate open water phosphorus concentrations. In the North Basin the core from Site 1 was analyzed and in the South Basin the core from Site 2 was analyzed.



Table 1.0-1. Location, sampling depth, and core length.

Basin		Location	Water Depth (ft)	Core Length (cm)
North Posin	Site 1	44.10886° -88.39657°	20	52
NOITH DASIN	Site 2	44.10887° -88.39440°	20	52
South Basin	Site 1	43.90610° -88.40113°	17	44
	Site 2	43.90607° -88.39887°	16	54

2.0 RESULTS

Geochemistry

The percentage of water and loss on ignition (LOI) was determined in the sediment cores for use in the dating analysis. This information is used to calculate the bulk density of the sediments.

In both basins, the percentage of water and LOI increase from the bottom to the top of the cores Figure 2.0-1). At least part of the reason this occurs with water is the result of sediment compaction. The increase in organic matter (LOI) indicates that more organic matter is retained in the sediments, most likely as a result of increased productivity of the lake.





Diatom preservation

In both locations, there was some diatom degradation throughout each core, even in the most recently deposited samples. Even though there was some degradation in the surface samples, it did not appear severe enough to adversely affect the results. Degradation did not seem to be greater for any particular type of diatom, whether they were small or large, or lightly or more heavily silicified. In the North Basin, diatom degradation was judged to be too severe below 36 cm; therefore, the bottom most sample analyzed in this core was 34-36 cm. Diatom taxa that were identifiable in the 36 cm and greater sections were similar to the important taxa in the 34-36 cm sample, so it is unlikely that the phosphorus estimation from this site would be very different if it were possible to have analyzed deeper sections. In the South Basin diatom preservation was good enough to analyze the bottom most sample (52-54 cm).

In 1993 a number of sediment cores were collected and dated by a graduate student, Marie-Pierre Gustin, at UW-Milwaukee. Her analysis indicated that cores needed to be 35-40 cm in length to reach a time period of 130 years ago. Undoubtedly, more sediment has been deposited in the lake over the 23 years since she collected her cores. Still, it is likely that the bottom sample where the diatom community was analyzed from the North Basin was not deposited more than 130 years ago. However, as is discussed below, the diatom community in the bottom samples of both cores was similar. It is more likely that the bottom most sample in the South Basin was deposited prior to European settlement. The actual date of deposition will not be known until the dating analysis is completed.

Multivariate Statistical Analysis

Various statistical methods were used to compare environmental conditions at the top and bottom of the cores. An exploratory detrended correspondence analysis showed that the gradients of species responses were relatively long and so a unimodal, detrended model was used for constrained (detrended correspondence analysis; DCA) ordinations (CANOCO 5 software, ter Braak and Smilauer, 2012). The analysis has been done on many WI lakes including the Lake



Winnebago sediment cores. This analysis was used for a comparison of environmental conditions in the top and bottom samples of each lake. To determine the main directions of variation in the limnological variables, a canonical correspondence analysis (CCA), using inter-sample distances on a correlation matrix of log-transformed values. Species data was square root transformed with downweighting of rare species.

The DCA analysis was performed to examine the similarities of the diatom communities between a number of stratified Wisconsin lakes but most importantly between the top and bottom samples of the Lake Winnebago cores. The results revealed two clear axis of variation in the diatom data, with 39% and 26% of the variance explained by axis 1 and axis 2, respectively (Figure 2.0-2). Sites with similar sample scores occur in close proximity reflecting similar diatom composition. The arrows symbolize the trend from the bottom to the top samples.



The bottom samples for the North and South cores plot together but the surface samples are farther apart. This indicates that historically the lake's water quality was spatially more homogeneous. The distance between the bottom and top samples is greater than for some other lakes indicating present day conditions are significantly different from historical times.



Diatom Communities of North and South Basins

A comparison of the diatom communities in the bottom and top samples for each core are shown in Figures 2.0-3 and 4. At both sites the planktonic diatom *Aulacoseira ambigua* and the group benthic *Fragilaria* were the most common. The latter diatoms grow on the bottom sediment and their presence indicates that the water is clear enough for light to reach the bottom for a significant part of the growing season. *Gyrosigma* was also more abundant in the bottom samples and this taxa also grows on the sediments. This large diatom is rare in the open water of most Wisconsin lakes and its abundance is probably a response to decent water clarity and wind induces water currents which allows this diatom to grow enough to compete with other benthic diatoms.

In the top sample *A. ambigua* abundance was replaced by *Aulacoseira granulata* which is an indication of higher phosphorus concentrations. There was also a reduction of benthic diatoms in the surface sample which likely signals a reduction in water clarity. A common diatom in the cores was Aulacoseira crassipunctata. This diatom is rarely found in lakes in the Upper Midwest. This diatom may be present in Lake Winnebago because there is enough wind generated currents to keep this relatively heavy diatom afloat. Another diatom found in the cores that is uncommon is *Nitzschia supralitorea*. In the Lake Winnebago cores is was most common in the surface sample from the North Basin. In the 2007 National Lake Assessment dataset this diatom was only found in lakes with elevated phosphorus levels. Its presence in the surface samples but not the bottom samples is further evidence that phosphorus concentrations are higher at the present time compared with pre-Euroamerican times.





the bottom sample indicates the water clarity was better historically.







Calibration Dataset

The calibration dataset was developed from 106 deep Wisconsin lakes. Although Lake Winnebago is polymictic, the diatom community was dominated by planktonic taxa which are more indicative deeper lakes. This likely is because the lake is turbid enough that light generally does not penetrate deep enough for a macrophyte community to become established in the area where the sediment cores were collected.

The diatom community, especially at the bottom of the cores, was uncommon for Wisconsin lakes. There were some taxa present that rarely are seen in other Wisconsin lakes. The three most common were *Aulacoseira crassipunctata*, *Nitzschia supralitorea*, and *Gyrosigma* spp. In order to improve the phosphorus estimation, the training set was augmented with estimated abundances in lakes where the phosphorus concentrations were typical of these taxa. The national dataset for the 2007 U.S. EPA was used to estimate phosphorus concentrations where these taxa were most common. *Gyrosigma* is interesting since it is typically found growing on sediments and is common in rivers and shallow areas of lakes. Its presence in the open waters of Lake Winnebago in historical samples likely indicates that water clarity was better historically in the lake than it is at

the present time. The near absence of this taxa from other Wisconsin lakes likely reflects the fact that Lake Winnebago is a large, relatively shallow lake which experiences significant mixing from wind events. Only a large, heavy, highly motile diatom such as *Gyrosigma* is able to grow in this environment.

A canonical correspondence analysis of the dataset indicated that the most important environmental variable on axis 1 was alkalinity and the most important variable on axis 2 was phosphorus (Figure 2.0-5). The range of phosphorus concentrations in the WI dataset was 3 to $114 \mu g/L$.



Inference Models

Weighted averaging calibration and reconstruction (Birks et al. 1990) was used to estimate phosphorus concentrations from the diatom community. Software used for this analysis was C2 ver. 1.7.6 (Juggins 2014). The best model for estimating phosphorus was weighted averaging with classical deshrinking. Classical deshrinking typically works best when inferred values are near the ends of the mean of the training set. This is the case with Lake Winnebago. The performance of the model is listed in Table 1.0-2. The prediction error of the model is calculated as the root mean square error (RMSE) which is defined as the square root of the mean squared differences between the observed and inferred phosphorus values. Estimating the model performance this way is likely an underestimate because it is an estimate of the model error and not the prediction error



(Næs et al. 2002). A more realistic estimate of the prediction error is provided by bootstrap cross validation. With bootstrapping, random samples are selected from the original calibration dataset and replaced in the dataset (Juggins and Birks 2012). This was repeated 1000 times and the squared errors for the core samples are accumulated across all bootstrap cycles into the root mean square error of prediction (RMSEP). This is a more realistic estimate of the model's performance in predicting phosphorus concentrations.

 Table 1.0-2. Performance of two different models for phosphorus.
 RMSE is root mean square error

 and RMSEP is the root mean square error of the prediction.
 RMSE

Model	r ²	r² _{boot}	RMSE	RMSEP
WI Deep Lakes	0.64	0.40	0.217	0.255

The average summer phosphorus concentration in Lake Winnebago for 2013-2016 was 110 μ g/L although concentrations were lower in 2015 and 2016. This was determined by averaging values measured in the open lake at 4 sites. The sites were Station 713245 (3 miles off Oshkosh), Station 713243 (2 miles off Neenah), Station 714005 (Deep Hole – North), and Station 713302 (Deep Hole – South). This time period was used because it is likely the period covered by the surface sediment sample.

The diatom inferred summer phosphorus concentrations are presented in Table 1.0-3. The model predicts the present day surface summer phosphorus concentrations to be 108 μ g/L in the North Basin and 94 μ g/L in the South Basin. The model predicts the historical summer phosphorus concentration to be 40-47 μ g/L.

Table 1.0-3. Inferred ph	osphorus values in to	p and bottom samples	for both models.	Units are µ/L.
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		Тор	Bottom
WI Model	North Basin	108	40
	South Basin	94	47

In the North Basin, even though the sample used for the historical phosphorus inference was shallower than the South Basin (34-36 cm vs 52-54 cm) the diatom communities were very similar and it is likely the North Basin sample represents historical phosphorus concentrations. It would be accurate to estimate that presettlement phosphorus concentrations are in the range of 40-47 μ g/L assuming that the dating confirms that the bottom samples were deposited at least 100 years ago.

Dating and Sedimentation Rate

In order to determine when the various sediment layers were deposited, 10 samples from the North and South Basin cores were analyzed for lead-210 (²¹⁰Pb) and radium-226 (²²⁶Ra). Lead-210 is a naturally occurring radionuclide. It is the result of natural decay of uranium-238 to radium-226 to radon-222. Since radon-222 is a gas it moves into the atmosphere where it decays to lead-210. The ²¹⁰Pb is deposited on the lake during precipitation and with dust particles. After it enters the lake and is in the lake sediments, it slowly decays until it reaches a radioactive equilibrium with ²²⁶Ra. The half-life of ²¹⁰Pb is 22.26 years (time it takes to lose one half of the concentration of ²¹⁰Pb) which means that it can be detected for about 130-150 years. This makes ²¹⁰Pb a good choice to determine the age of the sediment since Euro-American settlement began in the 1800s. Sediment



age for the various depths of sediment were determined by constant rate of supply (CRS) model (Appleby and Oldfield 1978). Bulk sediment accumulation rates $(g/cm^2/yr)$ were calculated from the output of the CRS model. The sedimentation rate was much lower than expected which meant that depths older 150 years were submitted to the lab. This resulted in only 6 of the 10 depths being suitable for this analysis.

The radiochemical analysis revealed that the cores from the North and South basins were undisturbed enough that reasonable dates could be obtained in the cores. The sedimentation rates could also be determined in each core. The profile of ²¹⁰Pb activity clearly shows that the samples analyzed for diatoms deep in the cores was deposited prior to the onset of Euro-American settlement as ²¹⁰Pb activity was at background levels below 20 cm in both cores (Figure 2.0-6). Radon 226 is the breakdown product of lead-210 and it is subtracted from the ²¹⁰Pb activity to estimate dates and sedimentation rates. The "bottom" sample in the North Basin was 34-36 cm and 52-54 in the South Basin.



The profile of the sedimentation rates in both cores were similar with sedimentation beginning to to increase around 1990 (Figure 2.0-7). The historical sedimentation rate is similar in both cores which is expected. It is likely that the sedimentation rate was not constant from the mid-1800s to 1990 but not enough samples were analyzed during this time period to detect changes in the sedimentation rate.

In 1993 Dr. Marie-Pierre Gustin, who was a graduate student at University of Wisconsin-Milwaukee, collected many cores throughout Lake Winnebago. She observed that there was some mixing of the surface sediments (Gustin 1995). It appears that there was some mixing in the cores collected for the present project to about a depth of about 6 cm at both sites. No correction was made for the mixing as Appleby and Oldfield (1992) pointed out that when using the CRS model, ²¹⁰Pb dates with a mixing zone spanning 10 years accumulation gives a maximum error of less than 2 years.



While it is clear from the ²¹⁰Pb activity profiles that the "bottom" diatom samples were deposited at least 150 years ago, we can estimate when the samples were deposited by assuming that the presettlement sedimentation rate was nearly constant. It should be emphasized that there is a large error associated with this estimate. The "bottom" sample (34-36 cm) from the North Basin was deposited at about 1725 and the "bottom" sample (52-54 cm) from the South Basin was deposited about 1310.





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