Kentuck Lake

Forest & Vilas Counties, Wisconsin

Advanced Water Quality Study & AIS Monitoring: 2015 Annual Report

March 2016

Sponsored by:

Kentuck Lake Protection & Rehabilitation District

WDNR Grant Program

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Onterra, LLC
Lake Management Planning

Kentuck Lake

Vilas and Forest Counties, Wisconsin

Advanced Water Quality Study & AIS Monitoring: 2015 Annual Report

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A. Additional 2015 water quality data and methods from Kentuck Lake

INTRODUCTION

Kentuck Lake, Forest and Vilas Counties, is a 957-acre meso-eutrophic spring lake with a maximum depth of 40 feet and a mean depth of 13 feet (Figure 1). A headwater lake within the Menominee River Basin, Kentuck Lake is drained via the Kentuck Creek. The lake's surficial watershed encompasses approximately 2,724 acres (Figure 1), the vast majority of which is comprised of forests and wetlands.

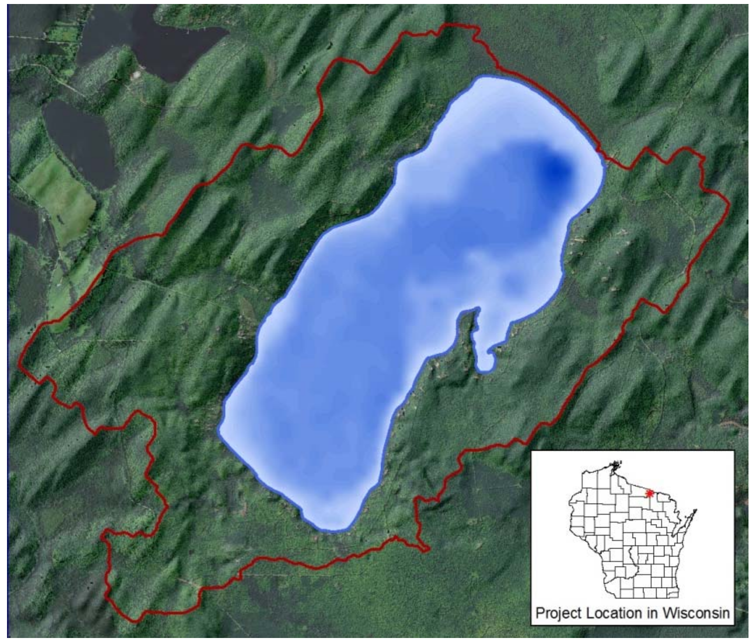

Figure 1. Kentuck Lake and watershed boundary (red outline).

Historical water quality data indicate that Kentuck Lake has experienced periods with higher nutrient and higher phytoplankton concentrations and periods with lower nutrient and lower phytoplankton concentrations. The data also document that the transition between these periods of higher and lower nutrient levels can be rapid, as was observed from 2010 to 2011 when growing season (April-October) near-surface total phosphorus concentrations and chlorophyll-*a* (a measure of phytoplankton biomass) concentrations increased by 184% and 654% respectively when compared to growing season averages from 2001-2010. With the increase in phytoplankton biomass in 2011, Secchi disk transparency (a measure of water clarity) also declined by over 50% when compared to averages from 2001-2010. In addition, the phytoplankton blooms were dominated by potentially harmful cyanobacteria (blue-green algae), which at higher concentrations have the potential to produce compounds known as cyanotoxins that can be hazardous to human and animal health (Sorichetti et al. 2014).

Concerned about the sudden onset of severe phytoplankton blooms in 2011 along with the discovery of the non-native, invasive plant Eurasian water milfoil, members of the Kentuck Lake Protection and Rehabilitation District (KLPRD) elected to move forward with the creation of a comprehensive lake management plan for Kentuck Lake. With the assistance of Onterra, the KLPRD received a Wisconsin Department of Natural Resources (WDNR) Lake Management Planning Grant in 2012, and Onterra implemented baseline studies of the lake's water quality, watershed, shoreline, and aquatic plant community over the growing season of 2013 and winter of 2014. Using current and historical water quality data, modeling confirmed that the concentration of phosphorus within the lake from 2011-2013 was significantly higher than expected given the lake's relatively small and mainly forested watershed. Watershed modeling indicated that approximately 72% (1,100 pounds) of Kentuck Lake's annual phosphorus budget from 2011-2013 was originating from an unknown source that was not accounted for in the model.

The baseline water quality data collected as part of the lake management planning project indicated that this large amount of unaccounted phosphorus was likely originating from within the lake itself, a phenomenon termed *internal nutrient loading*. Internal nutrient loading involves the release of phosphorus once bound within bottom sediments into the overlying water column. The conditions that cause internal nutrient loading to occur are discussed in the following section. As stated within the Kentuck Lake Comprehensive Management Plan (Onterra 2015), analysis of water quality data indicated that internal nutrient loading likely occurs to some degree every year in Kentuck Lake; however, these data were not conclusive. It also was not clear what was driving changes in water quality in terms of higher and lower nutrient concentrations over longer periods of time within the lake.

The lake management plan concluded that a more detailed analysis of Kentuck Lake's water quality was needed to determine if internal nutrient loading was occurring and to investigate the drivers of water quality change over time. Management Goal 2 within the lake management plan is to "Enhance Kentuck Lake's Water Quality Conditions." One of the actions under this goal was to "initiate a three-year focused water quality assessment to investigate Kentuck Lake's thermal behavior and its influence on internal phosphorus recycling." Understanding that the lake management planning project was a baseline assessment of Kentuck Lake, the KLPRD elected to move forward with an advanced and more detailed study focusing on the lake's water quality.

The advanced water quality study was proposed to occur from the spring of 2015 through the winter of 2017. Prior to the start of the water quality study while the lake management plan was being finalized, KLPRD volunteers collected temperature and dissolved oxygen profiles at various locations throughout the lake every other week during the open water season of 2014. Advanced water chemistry collection was then proposed to take place during the open water season of 2015, the winter and open water season of 2016, and the winter of 2017. The KLPRD successfully collected temperature and dissolved oxygen data in 2014, and these results are briefly discussed in this report. A detailed discussion of the 2014 study results can be found in the Kentuck Lake Comprehensive Management Plan (Onterra 2015).

In 2015, the KLPRD successfully applied for a WDNR Lake Protection Grant to aid in funding an advanced water quality analyses from the spring of 2015 through the winter of 2017. This advanced water quality study was designed to answer the following six questions:

1) Does internal nutrient loading occur in Kentuck Lake and is it a significant contributor to the lake's phosphorus budget?
2) What drives differences in Kentuck Lake's phosphorus concentrations and phytoplankton abundance between years?
3) How has Kentuck Lake's water quality changed over the past approximately 200 years?
4) Are cyanobacteria migrating into the hypolimnion to access sediment-released nutrients?
5) Is groundwater a significant contributor to Kentuck Lake's phosphorus budget?
6) Are there any feasible/realistic management actions that can be implemented to enhance the lake's water quality or to prevent further impairment?

This report serves as an annual report discussing the results from the first year (2015) of this two-year water quality study. As is discussed in detail in the Results and Discussion Section, the 2015 data provide empirical evidence that internal nutrient loading from anoxic bottom sediments occurs in

Kentuck Lake, and the lake's relatively shallow morphology allows these nutrients to be transported to surface waters through entrainment and mid-summer mixing events where they fuel phytoplankton growth. The variations in annual phosphorus concentrations from 2011-2015 are shown to be correlated with the estimated annual duration of anoxia which is determined by annual climatic conditions. In addition, nutrients released from deeper sediments exposed to anoxia are sufficient to create a phytoplankton biomass which elevates water pH (>9.0), which likely results in subsequent nutrient release from shallower, oxic sediments.

It is believed that the period from 2001-2010, which saw low and relatively stable nutrient concentrations and phytoplankton biomass, was largely a result of unusually high walleye abundance (>8 fish/acre) and resulting trophic structure with low planktivore (panfish) and high zooplankton abundance. Walleye consume planktivorous fish, planktivorous fish consume zooplankton, and zooplankton consume phytoplankton. This trophic structure is believed to have decreased phytoplankton abundance through two primary mechanisms: 1) increased predation by zooplankton and 2) a reduction in phosphorus availability due to increased sedimentation (zooplankton fecal pellets) and its accumulation within the zooplankton community.

Walleye abundance declined rapidly in 2010 to around 2-3 fish/acre, densities which WDNR fisheries biologists consider to be indicative of a healthy and sustainable walleye population. Coinciding with the reduction in walleye abundance, planktivore abundance increased. With higher planktivore abundance and likely reduced zooplankton abundance and predation upon phytoplankton, nutrient concentrations have been more variable in Kentuck Lake since 2011 and largely dependent upon the duration of anoxia and amount of nutrients released from bottom sediments. This report presents the data and methods that were used to draw these conclusions and also discusses some possible strategies that are currently being investigated to lessen the impacts of internal nutrient loading in Kentuck Lake.

In addition to water quality, the WDNR Lake Protection Grant also provided funding to aid in monitoring the non-native, invasive aquatic plants Eurasian water milfoil (*Myriophyllum spicatum*; EWM) and curly-leaf pondweed (*Potamogeton crispus*; CLP) in Kentuck Lake in 2015 and 2016. Surveys in 2015 indicated that while the lake's CLP population remains very low, the EWM population is larger and more widespread than what was observed in 2014. The 2015 AIS survey results and potential management strategies are discussed in detail in the Results and Discussion Section.

Historical Water Quality & Internal Nutrient Loading Definition

As mentioned previously, Kentuck Lake has experienced periods with relatively low phosphorus and phytoplankton biomass and periods with higher phosphorus and higher phytoplankton biomass (Figure 2). The average growing season near-surface total phosphorus was 36 μg/L from 1988-2000, 22 μg/L from 2001-2010, and 44 μg/L from 2011-2015. Similarly, average growing season chlorophyll-*a* concentrations were 24 μg/L from 1988-2000, 10 μg/L from 2001-2010, and 36 μg/L from 2011-2015.

Phytoplankton production in Kentuck Lake is mainly driven by the availability of phosphorus. Correlation analysis of water quality data shows that summer (June, July, & August) near-surface total phosphorus and summer chlorophyll-*a* concentrations have the strongest positive relationship (R^2 = 0.79) (Figure 3). Like most Wisconsin lakes, phosphorus is the limiting plant nutrient in Kentuck Lake, or the nutrient controlling phytoplankton production. Correlation analysis also illustrated a strong negative relationship between summer chlorophyll-*a* concentration and summer Secchi disk transparency (R^2 = -0.78), indicating Kentuck Lake's water clarity is primarily dependent upon phytoplankton abundance (Figure 3).

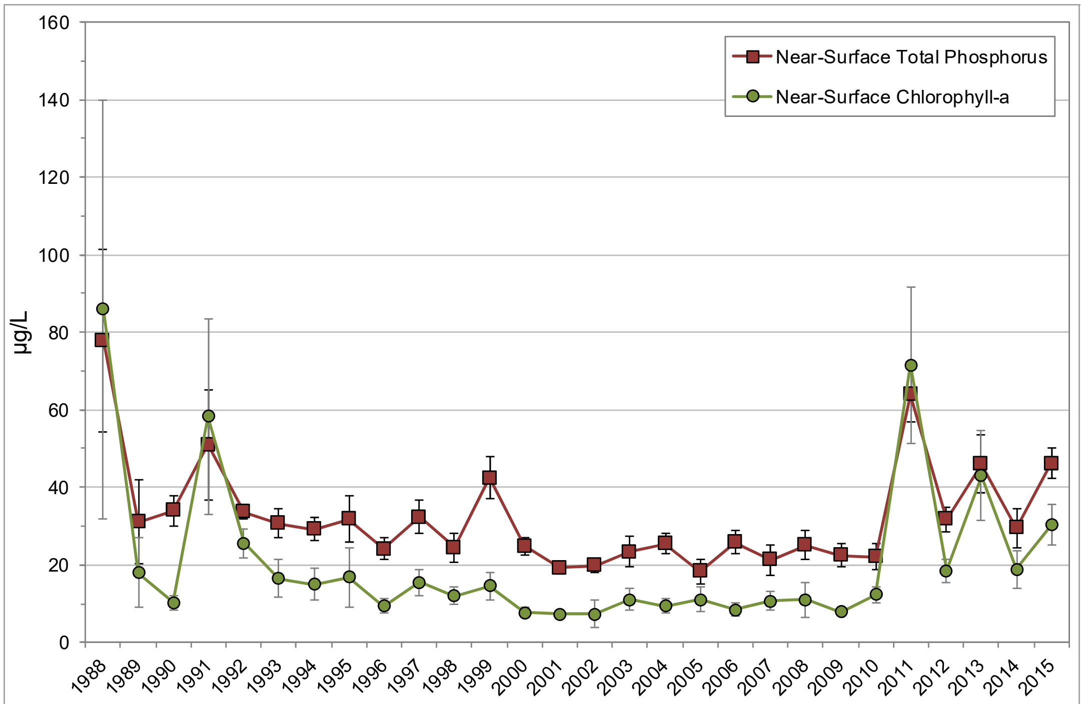

Figure 2. Average annual growing season (April-October) near-surface total phosphorus and chlorophyll-*α* collected from the Kentuck Lake deep hole sampling location (Site 1). Bars indicate one standard error.

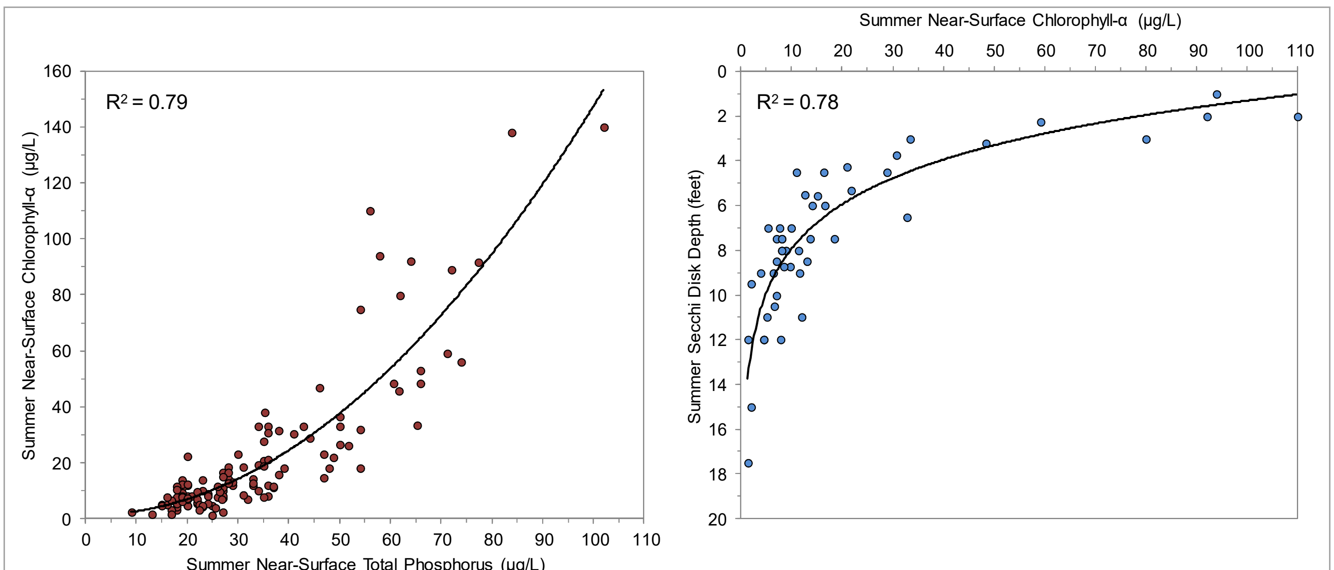

Figure 3. Relationship between summer near-surface total phosphorus and summer near-surface chlorophyll-α concentrations (left) and summer Secchi disk transparency and summer near-surface chlorophyll-α concentrations (right) in Kentuck Lake. Data collected from deep hole sampling location (Site 1) from 1988-2015.

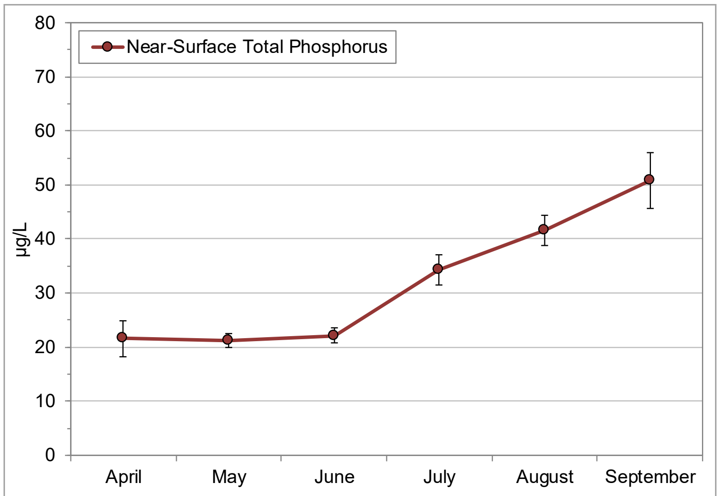

Figure 4. Average 1988-2015 monthly near-surface total phosphorus concentrations from Kentuck Lake illustrating increasing concentrations over the growing season. Data collected at the deep hole sampling location (Site 1). Bars indicate one standard error.

Water quality data available from Kentuck Lake show that near-surface total phosphorus concentrations tend to increase over the course of the open-water season on an annual basis (Figure 4). While the magnitude of increase varies between years, phosphorus concentrations tend to remain relatively low and stable from April through June and begin to increase in July and into September. This atypical pattern of annual phosphorus concentrations was an early signal to Onterra and WDNR scientists that internal nutrient loading was likely occurring in Kentuck Lake. Typically, phosphorus concentrations tend to be higher in the spring when precipitation and runoff are higher, lower in the summer as phytoplankton consume phosphorus, die, and sink to the bottom, and higher again with fall mixing and increased precipitation. One of the objectives of the water quality study is to determine what drives changes in the magnitude of phosphorus increase between years in Kentuck Lake.

Internal Nutrient Loading

Lakes typically act as phosphorus 'sinks', meaning that less phosphorus leaves the lake than the amount that entered from its watershed. Most of the phosphorus that enters a lake tends to eventually settle and become bound within the bottom sediments. Internal nutrient loading, or internal nutrient recycling, involves the release of phosphorus once bound in the lake sediment back into the overlying water column. Release of phosphorus from bottom sediments can occur under the following conditions:

Anoxia

Lake stratification occurs when temperature and density gradients are developed with depth in a lake. During stratification, the lake can be broken into three layers: The *epilimnion* is the surface layer with the lowest density and has the warmest water in the summer months and the coolest water in the winter months. The *hypolimnion* is the bottom layer the highest density and has the coolest water in the summer months and the warmest water in the winter months. The *metalimnion*, often called the thermocline, is the layer between the epilimnion and hypolimnion where temperature changes most rapidly with depth.

In the presence of oxygen, phosphorus remains bound to ferric iron within the sediment. When the overlying water becomes anoxic, or devoid of oxygen, the iron is reduced to ferrous iron and the bond with phosphorus is broken resulting in both iron and phosphorus being released into the water (Pettersson 1998). Anoxia typically develops following stratification, or the formation of distinct layers of water based on temperature and density. The density gradient between the cold, dense layer of water near the bottom, the *hypolimnion*, and the warmer, less dense layer of water at the surface, the *epilimnion*, prevents these layers from mixing together. Consequently, oxygen depleted through sediment oxygen demand, or the removal of oxygen consumption through biological activity within the sediments, is not replaced via atmospheric diffusion and anoxic conditions result.

While internal phosphorus loading due to anoxia occurs on many lakes in Wisconsin, it is often not problematic because the phosphorus released remains confined deep in the hypolimnion in the summer where it is inaccessible to phytoplankton. In *dimictic lakes*, or lakes that undergo a complete mixing event (turnover) twice per year, the phosphorus released from bottom sediments remains in the hypolimnion until surface water temperatures cool and the lake mixes in the fall. While spring and fall turnover events can stimulate diatom and golden-brown algae blooms, because water temperatures are generally cooler these turnover events do not often stimulate cyanobacteria blooms. However, anoxia-induced internal phosphorus loading can become problematic if the hypolimnetic phosphorus becomes available to phytoplankton in the summer when water temperatures are warm, stimulating cyanobacteria blooms.

Hypolimnetic phosphorus can become available to phytoplankton at the surface in *polymictic lakes*, or lakes which have the capacity to break stratification and completely or partially mix multiple times during the open water season. Polymictic lakes tend to be relatively shallow with larger surface areas making them prone to breaking stratification and mixing when wind energy is sufficient. While a continuum exists between dimictic and polymictic lakes, the Osgood Index (Osgood 1988) is used to determine the probability that a lake will remain stratified all summer. This probability is estimated by using the ratio of the lake's mean depth to its surface area. Lakes with an Osgood Index of less than 4.0 are deemed polymictic. As is discussed later in this report, Kentuck Lake is defined as polymictic with an Osgood Index value of 2.0.

Elevated pH

The pH scale ranges from 0 to 14 and is an indicator of the concentration of hydrogen (H+) within the lake's water and is an index of the lake's acidity. Water with a pH value of 7 has equal amounts of hydrogen ions and hydroxide ions (OH-) and is considered neutral. Water with a pH of less than 7 has a higher concentration of hydrogen ions and is acidic, and water with a pH of greater than 7 has lower hydrogen ion concentrations and is considered basic or alkaline. The pH scale is logarithmic, meaning that for every 1 pH unit the hydrogen ion concentration changes tenfold. The normal range for lake water pH in Wisconsin is about 5.2 to 8.4, though values lower than 5.2 can be found in some acid bog lakes and higher than 8.4 in some marl lakes and highly productive softwater likes like Kentuck Lake (Shaw and Nimphius 1985).

Carbon dioxide dissolves in and reacts with lake water to form carbonic acid which lowers the water's pH. However, during the day, photosynthesizing phytoplankton (and *macrophytes*) consume carbon dioxide and can raise water pH. When phytoplankton become overly abundant they have the capacity to raise a lake's pH to 9.0 or greater during the day. When pH reaches these levels, the capacity of phosphorus to remain bound within the sediment is reduced, and phosphorus can be released from sediment under these conditions even in the presence of oxygen (Solim and Wanganeo 2009).

Macrophytes are larger aquatic plants that can be seen with the naked eye, and include flowering plants such as pondweeds and milfoils and macroalgae like muskgrasses among others.

Cyanobacteria Migration

While most species of phytoplankton are unable to obtain phosphorus bound within the sediment, certain species of cyanobacteria (blue-green algae) are able to access phosphorus within the sediment and make it available to other phytoplankton within the water column (Cottingham et al. 2015). These cyanobacteria spend a portion of their life cycle on the lake bottom (Barbiero and Welch 1992). Three genre in particular that are found in Kentuck Lake, *Gloeotrichia, Anabaena*, and *Aphanizomenon* overwinter on the lake bottom and begin to uptake sediment-bound phosphorus in the spring. In fact, studies have shown that *Gloeotrichia* takes up more phosphorus from the sediment than it actually requires for growth and is termed *luxury uptake* (Carey et al. 2014). Once it has acquired sufficient amounts of nutrients, it migrates from the sediment into the water column to reproduce. While the mechanism is yet unknown, *Gloeotrichia* releases phosphorus that it obtained from the sediment into the water column which can stimulate the growth of other phytoplankton (Carey and Rengefors 2010). This uptake and delivery of phosphorus from the sediments into the water column by cyanobacteria has shown to be significant form of internal phosphorus loading in certain lakes (Barbiero and Welch 1992).

Recently, it has also been proposed that cyanobacteria may migrate from the surface down into the hypolimnion to access phosphorus, iron, and other nutrients released from the sediment under anoxic conditions (Molot et al. 2014). Under oxygenated conditions, iron is in a form (ferric) that is generally unavailable for use by most cyanobacteria, but under anoxic conditions iron is reduced to a form (ferrous) that is readily utilized by cyanobacteria. Because ferrous iron will only be found under anoxic conditions, it is believed cyanobacteria migrate into the hypolimnion to access it. Cyanobacteria have a higher iron demand when compared to true-algae, and it is required for *nitrogen fixation*. Nitrogen fixation, a unique ability of cyanobacteria among phytoplankton, is the ability to utilize atmospheric nitrogen and convert it into a usable form. When nitrogen becomes limiting within the water, cyanobacteria can access atmospheric nitrogen which provides them with a competitive advantage over true-algae. In summary, it is hypothesized that ferrous iron made available through anoxia allows cyanobacteria to dominate over other phytoplankton.

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PROJECT METHODS

Water Quality

Surface Water Sampling

Water quality samples were collected by KLPRD volunteers from five locations distributed across the lake (Map 1) every other week (biweekly) from late-April through October 2015 for a total of 14 sampling events. A 3.0 L Van Dorn Sampler was used to collect the water samples. Temperature and dissolved oxygen profiles and Secchi disk transparency were collected from each location during each sampling event. Onterra ecologist Brenton Butterfield met with KLPRD volunteers Tom Carlstedt and Kathy Bra on April 29, 2015 for training and collection of samples for the first sampling event of the year (Photo 1).

Photo 1. KLPRD volunteer Tom Carlstedt collects water quality samples on Kentuck Lake on April 29, 2015.

At sampling sites 1 and 5, water quality samples were collected from the near-surface (3 feet), near-oxycline (1 foot below first dissolved oxygen reading of 2.0 mg/L), and near-bottom (3 feet above the bottom). If dissolved oxygen was above 2.0 mg/L throughout the entire water column, the near-oxycline sample was taken midway between the surface and the bottom. Water samples were collected from the near-surface and near-bottom at sites 2, 3, and 4. The water quality parameters collected from each water quality sampling location can be found in Table 1. In the winter of 2016, samples were collected and analyzed for total phosphorus only, and samples were collected from the near-surface and near-bottom from site 1 and the near-surface of site 5.

Table 1. Water quality parameters collected from five sampling locations in Kentuck Lake in 2015.

	Site 1 & 5			Site 2, 3, & 4	
Water Quality Parameter	NS	NO	NB	NS	NB
Total Phosphorus	●	●	●	●	●
Dissolved Phosphorus	●	●	●		
Nitrate-Nitrite Nitrogen	●	●	●		
Ammonia Nitrogen	●	●	●		
Total Iron	●	●	●		
Chlorophyll-*a*	●			●	

NS = Near-surface; NO = Near-oxycline; NB = Near-bottom

In addition to the parameters listed in Table 1, samples were also collected from sites 1 and 5 from the near-surface, near-oxycline, and near-bottom for phytoplankton taxa identification. These samples were collected with a 3.0 L Van Dorn Sampler and were analyzed by Jim Kreitlow (WDNR). The phytoplankton were identified to at least the genus level, and if possible was identified to species. In addition, the most abundant phytoplankton taxa within each sample were also noted. The goal of this sampling was to determine which phytoplankton taxa were dominant at different points throughout the growing season and to determine if living cyanobacteria were captured near the bottom within anoxic waters.

> **Phytoplankton** are free-floating, microscopic photosynthesizing organisms and include algae and cyanobacteria among others.

Sediment Core Analysis

On September 22, 2015, Onterra ecologists and then WDNR paleoecologist Paul Garrison (now with Onterra) visited Kentuck Lake and collected two sediment cores (Photo 2). One was collected near sampling site 1 and the other from near site 4 (Map 1). These sediment cores are currently undergoing analyses to determine how Kentuck Lake's water quality has changed over the past 200 years. This analysis will show if Kentuck Lake's water quality has changed over time, particularly following European settlement. The results of the sediment core analysis will be included in next year's final report.

On February 24, 2016, Onterra ecologists collected a total of six sediment cores (Photo 2) from two locations (Map 1) which were delivered to Bill James at UW-Stout to undergo nutrient release analysis. These cores will be exposed to oxic and anoxic conditions to determine the amount of phosphorus released under these varying conditions.

Photo 2. Left: Onterra's paleoecologist Paul Garrison (front) and aquatic ecologist Dan Cibulka (back) view one of two sediment cores collected from Kentuck Lake in September 2015. Right: Onterra's paleoecologist Paul Garrison with one of six sediment cores collected for nutrient release analysis in February of 2016.

Groundwater Quality Assessment

On September 22 and 23, 2015, Onterra ecologists Tim Hoyman and Dan Cibulka visited Kentuck Lake to conduct a shallow groundwater assessment. The goal of this study was to determine where groundwater enters and leaves Kentuck Lake and to determine if groundwater entering the lake carries significant concentrations of phosphorus. Groundwater typically is not a significant source of phosphorus to lakes, but this study was included to rule out groundwater as a significant contributor.

A mini-piezometer was constructed to determine the static water head level relative to the surface of the lake at 67 locations spread 500 feet apart around the perimeter of Kentuck Lake (Map 1). Groundwater samples would be collected at locations where groundwater was determined to be flowing into the lake and analyzed for total phosphorus. Unfortunately, the mini-piezometers that were constructed were unable to penetrate through the rock/cobble present in the shallow areas of Kentuck Lake, and the 2015 groundwater assessment was unsuccessful. An attempt to modify the mini-piezometers is being made and this study is going to be reattempted in the summer of 2016.

Climatic Data

Annual climatic data from 2001-2015 were available and obtained from a weather station at the Eagle River Union Airport in Eagle River, approximately 13 miles southwest of Kentuck Lake, while annual climatic data from 1988-2000 were obtained from a weather station in Rhinelander, approximately 30 miles southwest of Kentuck Lake (Weather Underground 2015). Climatic data obtained included average daily air temperature, average daily wind speed, and average daily wind direction.

Aquatic Invasive Species Monitoring

Two visual, meander-based surveys of Kentuck Lake's littoral zone were completed in 2015 aimed at locating and mapping areas of CLP and EWM. The Early-Season Aquatic Invasive Species (ESAIS) Survey was completed on June 30, 2015 and the EWM Peak-Biomass Survey was completed on September 16, 2015. Because CLP reaches its peak growth in June, the primary goal of ESAIS Survey is to locate and map occurrences of CLP. While EWM reaches its peak growth later in the summer, it can often be mapped during the ESAIS Survey as well; however, the areas of EWM mapped during the ESAIS Survey are used to guide mapping during the EWM Peak-Biomass Survey and they are reassessed during this survey.

During these surveys, the CLP and EWM were mapped by using either 1) point-based or 2) area-based mapping methodologies. Large colonies, >40 feet in diameter, were mapped using polygons (areas) and were qualitatively attributed a density rating based upon a five-tiered scale ranging from *highly scattered* to *surface matting*. Point-based mapping techniques were applied to locations that were considered as *small plant colonies* (<40 feet in diameter), *clumps of plants*, or *single or few plants*.

PROJECT RESULTS & DISCUSSION

2014 Data & Evidence for Polymixis

As discussed previously, KLPRD volunteers collected temperature and dissolved oxygen profiles from seven locations around Kentuck Lake biweekly from late-May through early-October in 2014. While Kentuck Lake is defined as polymictic based on the Osgood Index (equation below), the 2014 data were collected to obtain empirical evidence of polymixis.

$$Osgood\ Index\ (2.0) = \frac{Mean\ Depth\ (4.0\ m)}{\sqrt{Lake\ Area\ (3.9\ km^2)}}$$

An **isopleth** is a graph of two variables (e.g. temperature and depth) with lines representing equal values.

The data collected in 2014 confirmed that Kentuck Lake is indeed polymictic as the lake experienced a complete mixing of the water column (turnover event) in late-June/early-July. As is illustrated in the temperature and dissolved oxygen *isopleths* from 2014 (Figure 5), the temperature gradient indicates that Kentuck Lake stratified in late-May of 2014 and remained stratified through most of June. Between late-June and early-July, sustained high winds from a south-southwest direction imparted enough energy to mix the entire water column. The relatively uniform temperature of approximately 21°C throughout the water column in early July points to this mixing event. The water column remained mixed throughout the rest of the open-water season.

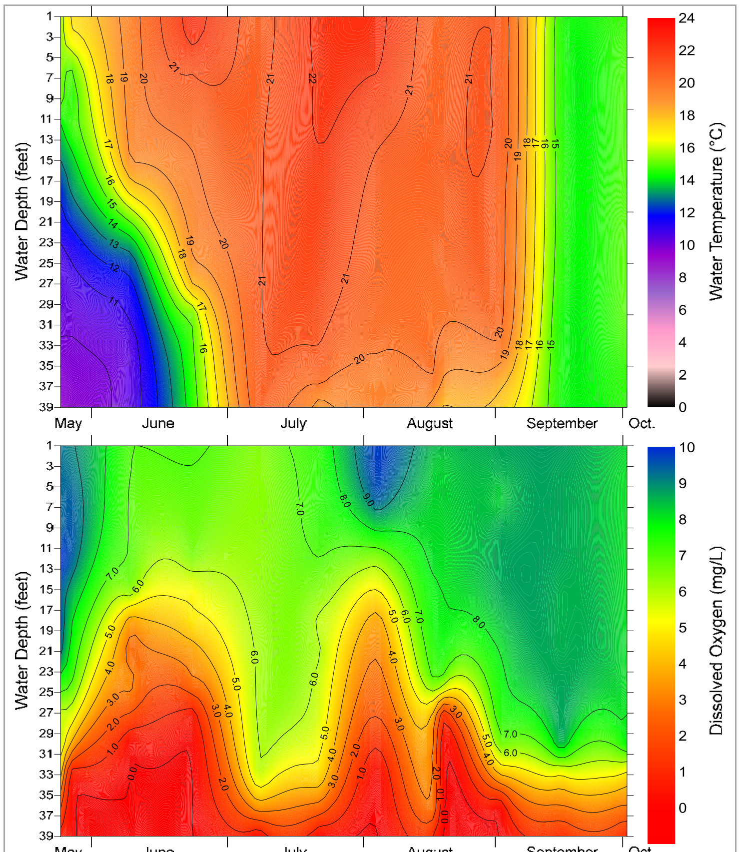

Figure 5. Kentuck Lake 2014 temperature and dissolved oxygen isopleths. Data collected by KLPRD volunteers at the deep hole sampling location (site 1).

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2015 Water Quality Results

Temperature and Dissolved Oxygen

In 2015, KLPRD volunteers collected temperature and dissolved oxygen profiles from five locations around Kentuck Lake (Map 1) biweekly from late-April through mid-October. Like in 2014, the lake began to stratify and anoxic conditions developed in early-June (Figure 6). Unlike 2014 where anoxic conditions persisted for approximately four weeks until the mixing event in late-June/early-July, anoxic conditions persisted approximately 11 weeks until late-August of 2015. In late-August, a period of cooler weather in combination with high winds resulted in a complete mixing event and an end to the 11-week period of anoxia. Two weeks following the late-August turnover event, a period of hot, calm weather in early September allowed anoxic conditions to develop again. However, this second period of anoxia was relatively short-lived as another mixing event occurred in late-September. The lake remained mixed (unstratified) for the remainder of the sampling period. Isopleths from sampling sites 2, 3, 4, and 5 can be found in Appendix A.

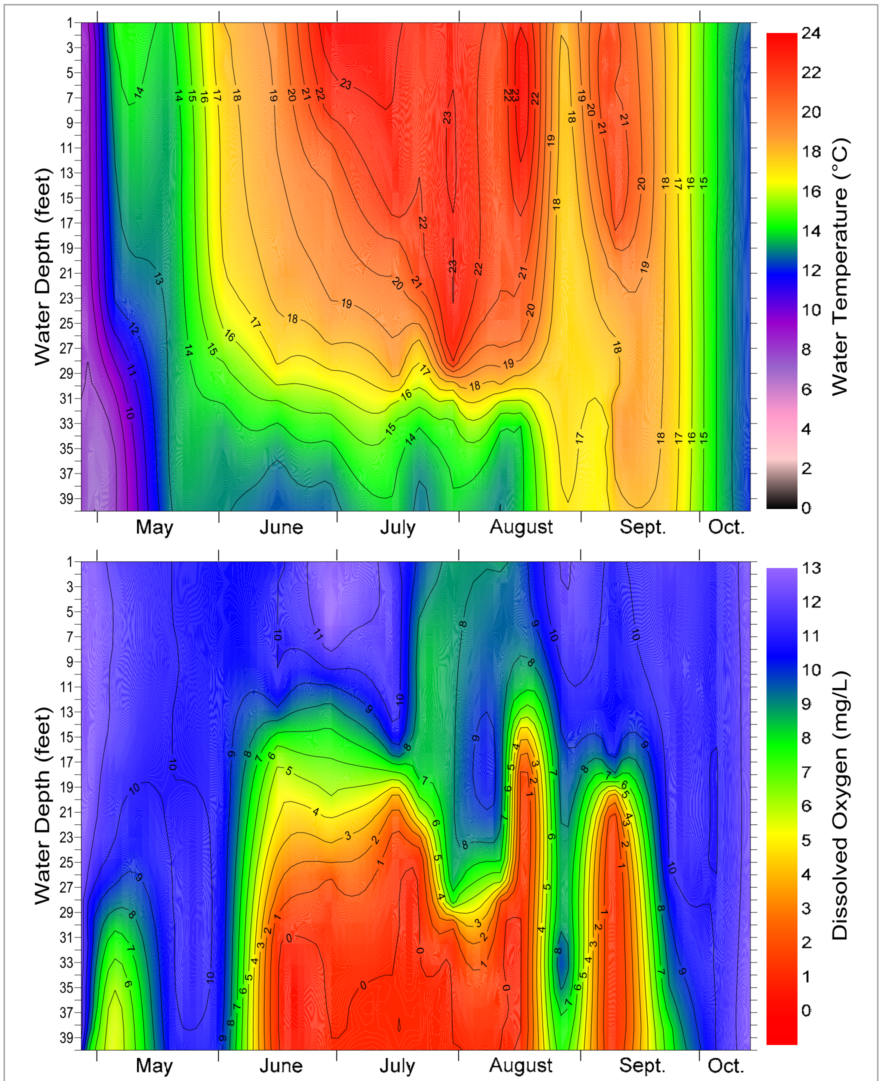

Figure 6. Kentuck Lake 2015 temperature and dissolved oxygen isopleths. Data collected by KLPRD volunteers at the deep hole sampling location (site 1).

Nutrients, Phytoplankton, and Water Clarity

Phosphorus

In 2015 both *soluble reactive phosphorus* (SRP) and *total phosphorus* (TP) were collected. Soluble reactive phosphorus (dissolved phosphorus) is a measure of orthophosphate, the soluble form that is available for uptake by plant cells. Total phosphorus is a measure of all forms of phosphorus, including SRP and phosphorus bound to sediment particles or within plankton cells (particulate phosphorus).

Near-surface TP concentrations were not statistically different between the five sampling locations distributed across Kentuck Lake in 2015 (one-way ANOVA $\alpha = 0.05$). Near-surface TP in 2015 followed the pattern of increasing concentration that has been observed in past years in Kentuck Lake. Near-surface TP concentrations were relatively low from late-April through early-June with an average concentration of 22 µg/L (Figure 7). In mid-June, coinciding with the onset of stratification and anoxia, near-surface TP concentrations increased at a rate of 0.8 µg/L/day before reaching a maximum concentration of 69 µg/L in late-July. From early-August through mid-October, near-surface TP concentrations were relatively stable with an average concentration of 57 µg/L. The 2015 average growing season near-surface TP concentration was 46 µg/L compared to 30 µg/L in 2014.

Because the shallower sampling sites 2, 3, 4, and 5, remained oxic during the sampling events, their near-bottom TP concentrations were similar to those measured near the surface. Site 1, the deep hole, was the only site to exhibit prolonged periods of anoxia and near-bottom TP concentrations differed greatly from those at the surface. Near-bottom TP concentrations from Site 1 are discussed here while figures displaying near-bottom TP concentrations from sites 2, 3, 4, and 5 can be found in Appendix A.

In April and May when the lake was still well mixed, near-bottom TP concentrations collected from approximately 37 feet were relatively low and similar to near-surface concentrations (Figure 7). With the onset of stratification and anoxia in early-June, near-bottom TP concentrations increased rapidly at a rate of 22.0 µg/L/day, reaching a maximum of 660 µg/L in late-June. Following this maximum, near-

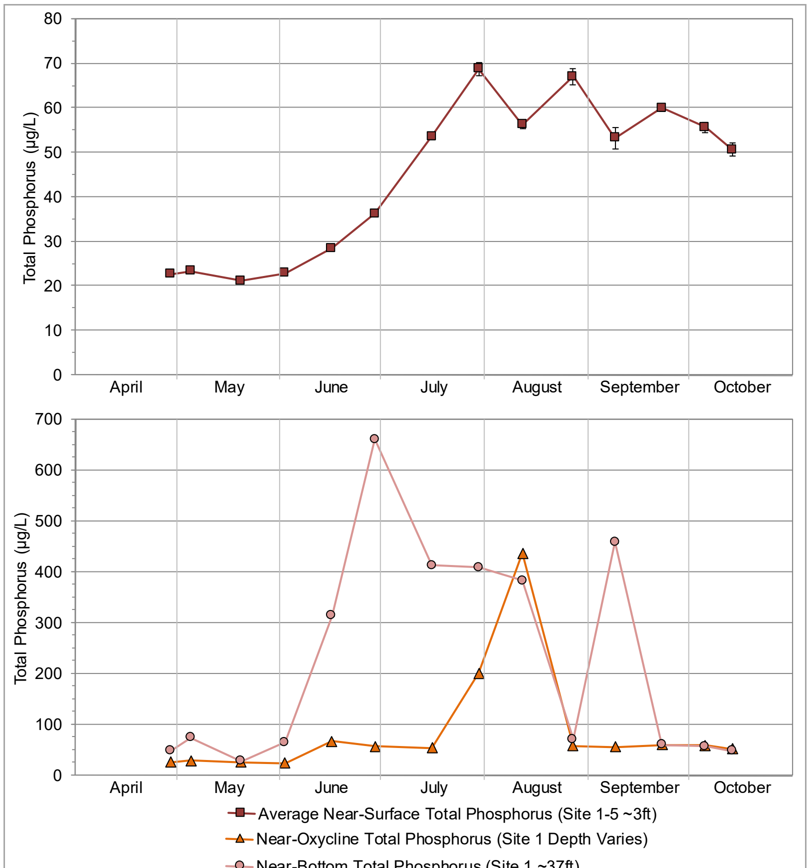

Figure 7. Kentuck Lake 2015 average near-surface total phosphorus concentrations (top) measured from sites 1, 2, 3, 4, and 5 and near-bottom and near-oxycline total phosphorus concentrations (bottom) measured from site 1. Bars on near-surface total phosphorus indicate one standard error.

bottom TP concentrations decreased slightly to around 400 µg/L from mid-July through mid-August. During the complete mixing event in late-August, near-bottom TP concentrations declined 70 µg/L and were similar to concentrations measured at the surface. During the brief, second period of anoxia in early-September, near-bottom TP concentrations increased rapidly to 458 µg/L before falling again to 60 µg/L in late-September. Near-bottom TP concentrations remained similar to surface concentrations into October.

Near-oxycline (1 foot below depth with dissolved oxygen <2.0 mg/L) TP concentrations collected from Kentuck Lake's deep hole also increased with the onset of thermal stratification and anoxia in June. However, concentrations measured through mid-July were lower than those measured from the near-bottom indicating a gradient of increasing phosphorus concentrations moving from the oxycline to the bottom. From mid-July through mid-August, near-oxycline TP increased to similar concentrations that were measured near the bottom. During the late-August mixing event, there was no oxycline present as the lake was oxic from the surface to the bottom. A sample was thus collected at mid-depth, and the TP concentration was similar to that at the surface and near the bottom.

Soluble reactive phosphorus within the epilimnion is rapidly incorporated into phytoplankton, meaning it is generally found in low concentrations within the epilimnion (Wetzel 2001). In Kentuck Lake, near-surface concentrations collected from sites 1 and 5 in 2015 were not statistically different (one-way ANOVA α = 0.05), and the average SRP concentrations from these two sites were relatively low over the course of the sampling period (Figure 8). In April and May, near-surface SRP concentrations fluctuated from below the limit of detection to approximately 2 µg/L. Near-surface SRP concentrations were below the limit of detection from June until the late-August mixing event when the average concentration was 7 µg/L. Near-surface SRP concentrations remained above detectable limits through the remainder of the sampling period, presumably due to decreased assimilation rate by phytoplankton or increased rate of loading to the epilimnion.

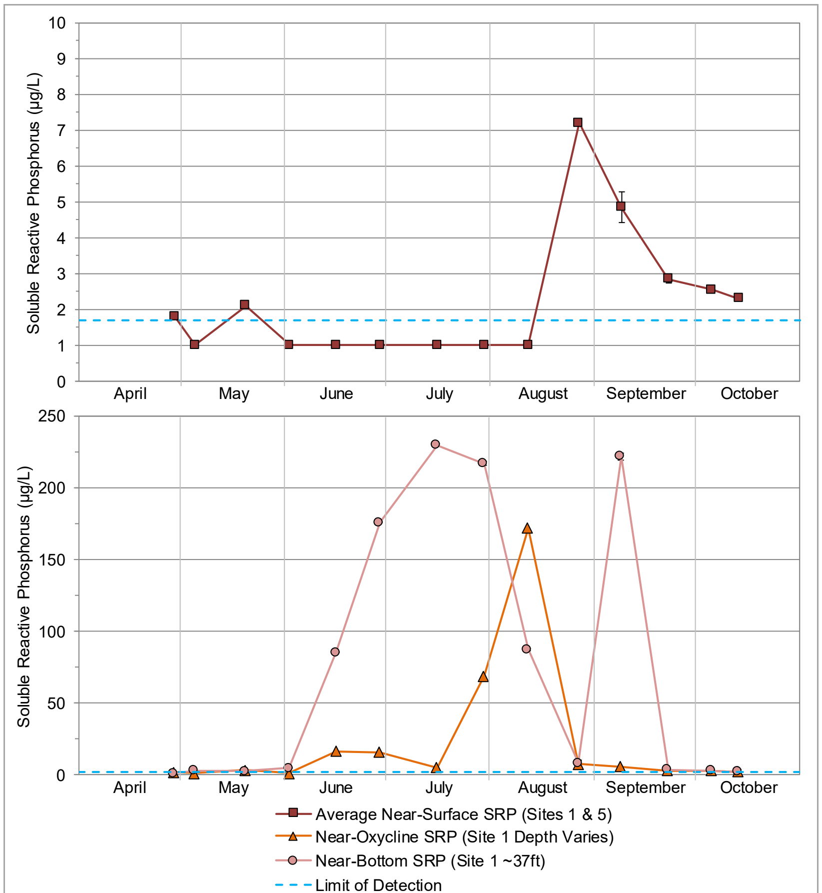

Figure 8. Kentuck Lake 2015 average near-surface soluble reactive phosphorus concentrations (top) measured from sites 1 and 5 and near-bottom and near-oxycline soluble reactive phosphorus concentrations (bottom) measured from site 1. Bars on near-surface soluble reactive phosphorus indicate one standard error.

Near-bottom and near-oxycline SRP concentrations collected from site 1 followed a pattern similar to TP concentrations. Near-bottom SRP concentrations were low in April and May and increased rapidly in June with the onset of anoxia at a rate of approximately 5.0 µg/L/day, reaching a maximum concentration of 230 µg/L on July 16. Near-bottom and near-oxycline SRP concentrations were similar to near-surface concentrations during the late-August mixing event. Near-bottom SRP concentrations increased again rapidly with the brief onset of anoxia in early-September to a concentration of 222 µg/L before falling to match near-surface concentrations two weeks later.

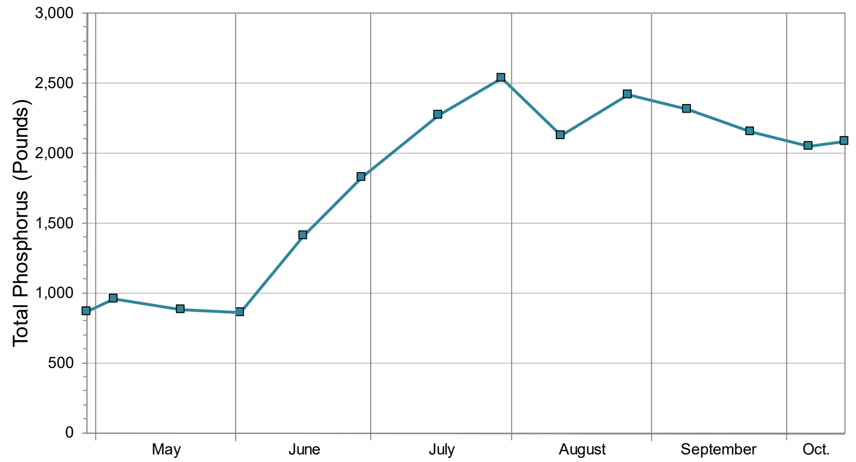

Figure 9. Kentuck Lake 2015 estimated lake-wide pounds of phosphorus.

Using the estimated volume of Kentuck Lake and the phosphorus data collected, it is estimated that approximately 1,500-1,600 pounds of phosphorus were released from bottom sediments into the overlying water in Kentuck Lake in 2015 (Figure 9). Using the estimated external phosphorus load determined from watershed modeling during the lake management planning project, approximately 77-79% of Kentuck Lake's phosphorus in 2015 originated from internal nutrient loading, indicating it is the single-largest contributor of phosphorus to the lake.

Nitrogen

In Kentuck Lake, nitrogen is the second-most important nutrient after phosphorus in terms of phytoplankton production. In 2015, samples were collected to measure nitrate (NO_3^-) plus nitrite (NO_2^-) and ammonium (NH_4^-). These inorganic forms of nitrogen are taken up by phytoplankton, and because nitrate must be converted into ammonium before it can be taken up by phytoplankton, ammonium is the most energy-efficient source of nitrogen (Wetzel 2001). Typically, concentrations of all three of these forms of nitrogen are relatively low in oxygenated water as they are quickly taken up by phytoplankton. Ammonia, like phosphorus, accumulates in the sediment under oxic conditions and can be released from the sediments under anoxic conditions. Studies have shown that cyanobacteria growth rates increased when their source of nitrogen was ammonia (Wetzel 2001 and McCarthy et al. 2013), and phytoplankton generally prefer to use ammonia over other forms of nitrogen (Beutel 2006).

In 2015, nitrate plus nitrite was measured from the near-surface, near-oxycline, and near-bottom from sites 1 and 5 in Kentuck Lake. One sample, collected from site 1 near-bottom, had a concentration above detection limits of 21 µg/L (Figure 10). The remaining samples from both sites and from multiple depths did not have concentrations above the limit of detection.

Concentrations of ammonia remained below the limit of detection in April and May 2015 at both sites 1 and 5 (Figure 10). With the onset of stratification and anoxia in early-June, concentrations of ammonia rapidly increased near the bottom and near the oxycline at site 1, but remained below the limit of detection at the surface, likely due to assimilation by phytoplankton. Near-bottom concentrations at site 1 increased at a rate of 61 µg/L/day before reaching a maximum of 1,790 µg/L in

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late-June. Near-bottom concentrations then declined to between 1,000-1,500 µg/L through early-August and declined further to 267 µg/L during the late-August mixing event. With the onset of anoxia again in early-September, near- bottom concentrations rapidly increased to 2,860 µg/L before falling again two weeks later to 118 µg/L. Concentrations of ammonia from the near-surface of site 1 and site 5 did not rise above the limit of detection until late-July, and were detectable into early September. It is presumed that phytoplankton at this time had reduced their assimilation rate of ammonia or more ammonia was being internally loaded to the epilimnion increasing concentrations above the limit of detection.

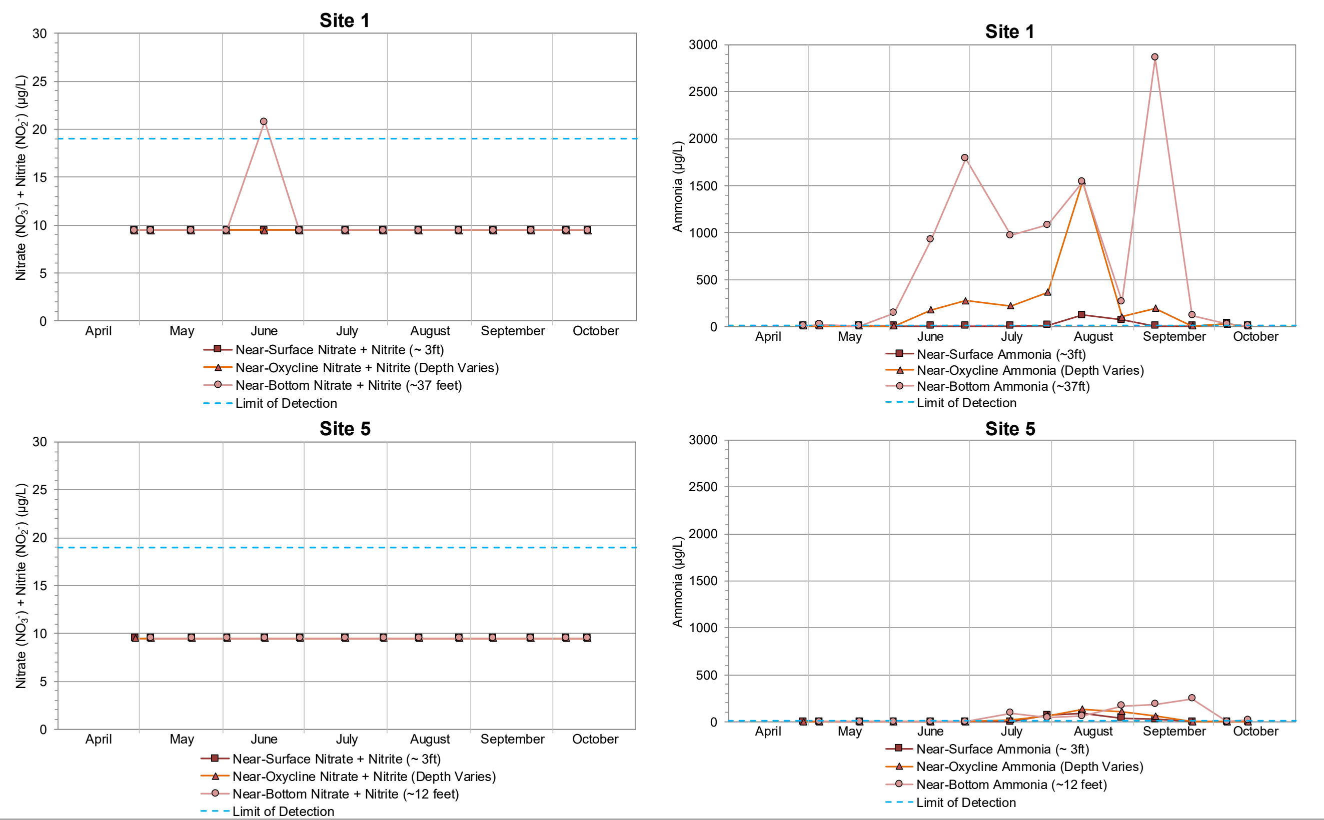

Figure 10. Kentuck Lake 2015 nitrate + nitrite nitrogen (left) and ammonia nitrogen concentrations (right) collected from sites 1 and 5.

Iron

In 2015, total iron was measured from the near-surface, near-oxycline, and near-bottom of sites 1 and 5 (Map 1). As mentioned earlier, under anoxic conditions iron (ferrous iron) is also released from bottom sediments into the overlying water after dissociating from phosphorus. While it has been known that phosphorus (and nitrogen) drive the biomass or amount of phytoplankton produced, it is not well understood why cyanobacteria dominate some phytoplankton communities and not others.

It has recently been hypothesized that the availability of ferrous iron will provide cyanobacteria a competitive advantage over other phytoplankton and allow them to dominate (Molot et al. 2014). Cyanobacteria require a higher amount of iron when compared to other phytoplankton as it increases their *nitrogen fixing* capacity,

Nitrogen fixation is the process in which nitrogen gas (N_2) in the atmosphere is converted into a usable form such as ammonium (NH_4^+) or nitrogen dioxide (NO_2).

and laboratory studies have shown that additions of iron significantly increase the growth and production of cyanobacteria (Molot et al. 2010 and Sorichetti et al. 2014). It is believed ferrous iron (and other nutrients) released from bottom sediments that becomes available to cyanobacteria provides them with a competitive advantage over other phytoplankton that do not have the ability to fix nitrogen (Cottingham et al. 2015).

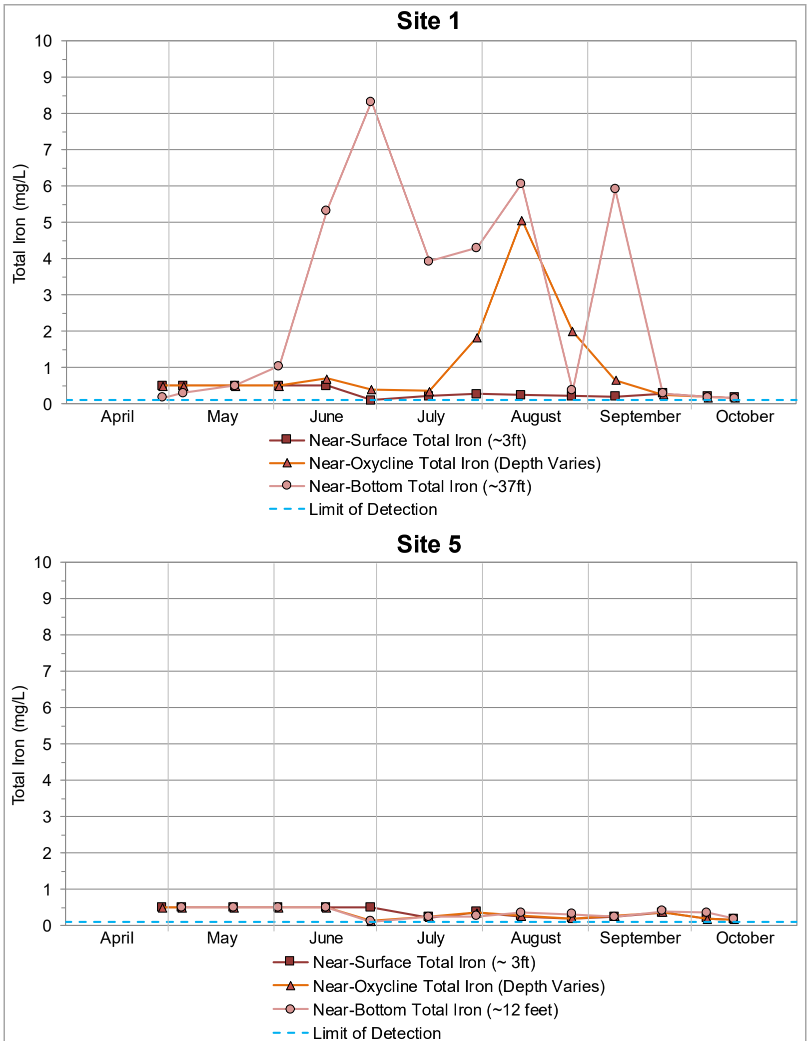

Figure 11. Kentuck Lake 2015 total iron concentrations measured from sites 1 and 5.

At site 1 in 2015, near-bottom total iron concentrations followed a similar pattern to those of total phosphorus, soluble reactive phosphorus, and ammonia (Figure 11). Concentrations in April and May ranged from below the limit of detection to less than 0.5 mg/L. With the onset of stratification and anoxia in early-June, near-bottom total iron concentrations increased rapidly by 0.3 mg/L/day, reaching a maximum concentration of 8.3 mg/L in late-June. From mid-July to mid-August, near-bottom total iron concentrations declined to between 3.9 and 6.1 mg/L and declined further during the late-August mixing event to 0.4 mg/L. During the brief onset of anoxia in early-September, near-bottom iron concentrations increased again to 5.9 mg/L before declining to 0.3 mg/L in late-September. Near-bottom concentrations were approximately 0.2 mg/L during the two October sampling events.

Near-oxycline/mid-depth total iron concentrations from site 1 remained below the limit of detection until mid-June (Figure 11). From mid-June through mid-July, near-oxycline total iron concentrations remained around 0.5 mg/L and then increased rapidly from late-July to early-August where concentrations were similar to those measured near the bottom. Near-oxycline concentrations declined during the late-August mixing event and continued to decline to approximately 0.2 mg/L into October.

Near-surface total iron concentrations measured from site 1 and concentrations measured from all depths at site 5 were not statistically different (Figure 11). As mentioned previously, site 5 was relatively shallow and did not experience anoxia during any of the sampling period and concentrations measured across the three depths at this location can be considered epilimnetic, or near-surface, concentrations. Near-surface total iron concentrations remained below the limit of detection until late-

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June. From late-June through mid-October, near-surface concentrations remained relatively consistent at around 0.2-0.3 mg/L at both sampling locations.

Iron is generally found in very low concentrations under oxygenated conditions because ferric iron is not soluble in water and is quickly lost through sedimentation (Wetzel 2001). The iron measured from the near-surface in Kentuck Lake starting in mid-summer was likely iron that had been incorporated into or was attached to phytoplankton which kept it within the epilimnion.

Phytoplankton & Water Clarity

In 2015, water samples were collected from sites 1 and 5 to measure chlorophyll-*a* concentration and to conduct phytoplankton taxa analysis. As was discussed earlier, chlorophyll-*a* concentrations in Kentuck Lake were found to be positively correlated with near-surface total phosphorus concentrations. In 2015, chlorophyll-*a* concentrations increased as near-surface total phosphorus concentrations increased (Figure 12). Chlorophyll-*a* concentrations were low in late-April through early-June, ranging from 2.2 to 4.8 µg/L. By mid-June with the onset of stratification and anoxia, chlorophyll-*a* concentrations began to increase at a rate of 1.1 µg/L/day reaching a concentration of 32.9 µg/L in late-June.

In mid-July, chlorophyll-*a* concentrations declined to 17.2 µg/L despite increasing near-surface total phosphorus concentrations. It is not known why chlorophyll-*a* concentrations declined in mid-July, but phytoplankton abundance can vary across different areas in a lake in response to changes in wind and temperature. During the mid-July sampling event, southerly winds may have carried phytoplankton to the extreme northern end of the lake and/or mixed them down further into the water column decreasing phytoplankton density at the surface. However, following calmer and warmer weather, chlorophyll-*a* concentrations quickly increased in late-July to the maximum concentration measured of 74.4 µg/L. With cooler temperatures and declining day length from August through October, chlorophyll-*a* concentrations declined and ranged from 48.7 to 33.0 µg/L, averaging 38.8 µg/L.

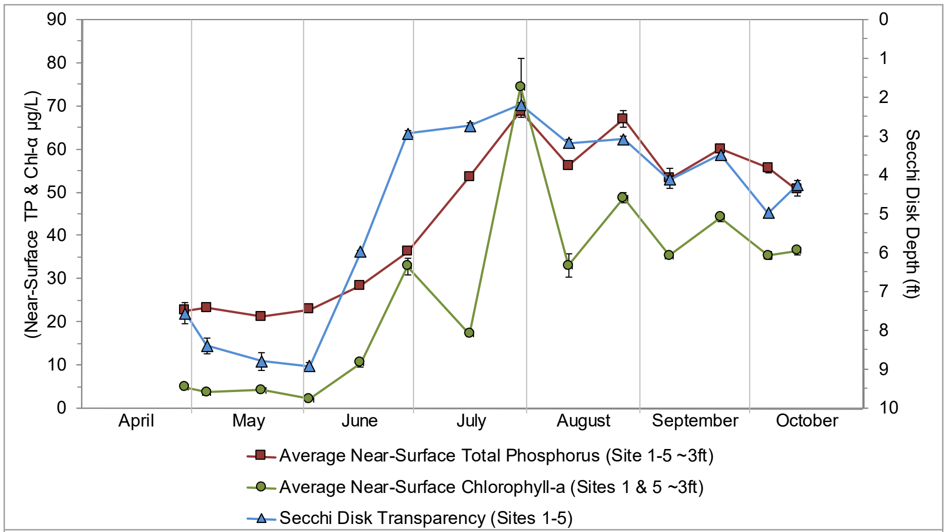

Figure 12. Kentuck Lake 2015 average chlorophyll-α concentrations from sites 1 and 5, average near-surface total phosphorus concentrations from sites 1-5, and average Secchi disk transparency from sites 1-5. Error bars indicate one standard error.

Phytoplankton taxa analysis by Jim Kreitlow (WDNR) indicated that eukaryotic algae (not cyanobacteria) dominated the phytoplankton community across all sample depths from late-April through early-June (Table 2). Cyanobacteria began to dominate in near-surface samples in mid-June soon after the onset of stratification and anoxia, and were dominant or codominant with eukaryotic algae for the remainder of the sampling period in October. Cyanobacteria became codominant in in near-oxycline and near-bottom samples beginning in late-July and continued to be codominant into October. The complete list of taxa within the samples collected in 2015 can be found in Appendix A. The cyanobacteria *Anabaena* sp. and *Gloeotrichia echinulata* were dominant in mid-June through mid-July, and *Anabaena* sp., *Aphanizomenon flos-aquae*, and *Gomphosphaeria* sp. were the dominant cyanobacteria from mid-July through October.

While living cyanobacteria were observed in near-bottom samples, it is not believed this was due to active migration from the epilimnion. As is discussed in the next section, it is believed that migration would not have been necessary as sediment-released nutrients were delivered to phytoplankton from bottom waters to the epilimnion through physical entrainment (see definition in next section). The phytoplankton collected in in anoxic, near-bottom samples were most likely dying colonies that were settling to the bottom.

Table 2. Dominant groups (eukaryotes and cyanobacteria) in Kentuck Lake's phytoplankton community over the 2015 sampling period (April-October). Samples collected from site 1 (deep hole). Analysis conducted by Jim Kreitlow (WDNR).

Sample Depth	April				May				June				July				August				September				October			
Near-Surface (~3ft)				E	E	E	E	E	E	E	CE	C	C	C	C	CE	C	C	CE	CE	CE	CE	CE	CE	CE	CE		
Near-Oxycline (Depth Varies)				E	E	E	E	E	E	E	E	E	E	E	E	CE	CE	CE	CE	CE	CE	CE	CE	CE	CE	CE		
Near-Bottom (~37 ft)				E	E	E	E	E	E	E	E	E	E	E	E	CE	CE	CE	CE	CE	E	CE	CE	CE	CE	CE		

C = Cyanobacteria Dominant; E = Eukaryotic Algae Dominant; CE = Cyanobacteria & Eukrayotic Algae Codominant

As discussed earlier, water clarity in Kentuck Lake is mainly determined by phytoplankton biomass. With a low chlorophyll-*a* concentration in late-April through early-June, water clarity was relatively high with an average of 8.4 feet (Figure 12). As chlorophyll-*a* concentrations increased in June, Secchi disk depth declined at a rate of 0.2 feet/day to 2.9 feet by the end of June. Secchi disk depth remained low through July at around 2.5 feet and began to slowly increase as chlorophyll-*a* concentrations declined beginning in early-August. Secchi disk depth increased from 3.2 feet in early-August to around 4.6 feet by early- to mid-October.

2015 Evidence for Internal Nutrient Loading

The 2015 water chemistry data previously presented provides empirical evidence that internal nutrient loading occurs in Kentuck Lake. The increasing concentrations of total phosphorus, soluble reactive phosphorus, ammonia nitrogen, and total iron measured in near-bottom waters with the onset of anoxia confirm that these nutrients are being released from the sediments into the overlying water. Unlike 2014 where a complete mixing of the water column occurred in early summer, a complete mixing event did not occur in Kentuck Lake until late-August 2015. However, nutrient concentrations measured at the surface in 2015 increased concurrently with those measured in near-bottom anoxic waters while the lake was still thermally stratified. This simultaneous increase in near-bottom and near-surface nutrient concentrations with the onset of stratification indicates that sediment-released nutrients within anoxic bottom waters are being delivered to the epilimnion via some mechanism.

While complete mixing events certainly deliver sediment-released nutrients to Kentuck Lake's surface, the 2015 data indicate that wind-induced *entrainment* was likely the mechanism of transport of these nutrients from anoxic bottom waters to the surface. Entrainment is the continual erosion of the metalimnion and deepening of the epilimnion (Wetzel 2001). For example, southwesterly winds blowing across Kentuck Lake will cause epilimnetic water to pile up on the northeast end, physically raising the lake's surface elevation on this end of the lake. Gravity pulls this built-up water downward where it encounters the cooler, denser metalimnion and it flows back across the metalimnion towards the other end of the lake. The water moving in the metalimnion will generate turbulence across the barrier between the epilimnion or the metalimnion can be eroded downward, mixing sediment-released nutrients into the epilimnion above.

Entrainment can be a significant source of nutrients in polymictic lakes, and the amount of nutrients delivered from anoxic bottom waters to the epilimnion can vary from year to year depending on climatic conditions which govern the duration and strength of stratification (Kamarainen et al. 2009 and James et al. 2015). Conditions in 2015 allowed Kentuck Lake to remain thermally stratified and anoxia to persist from mid-June through late-August, and winds caused a gradual erosion of the metalimnion over this period entraining nutrients from anoxic bottom waters into the epilimnion. Rather than receiving a 'pulse' of nutrients from a complete mixing event as was observed in the early summer of 2014, in 2015 nutrients were continually being loaded to the epilimnion from June until the late-August mixing event.

Figure 13 displays the average daily air temperature, average daily wind speed and direction, and Kentuck Lake water temperature at various depths over the course of the 2015 sampling period. The gradual increase in mid-depth water temperatures (green and yellow lines) and eventual similarity with surface temperatures illustrates the gradual deepening of the epilimnion and erosion of the metalimnion. The complete mixing event in late-August is indicated where temperatures across all depths are relatively equal.

To determine if the phosphorus released from the area of sediment exposed to anoxia could account for the increase in phosphorus concentration observed within the epilimnion in 2015, the estimated phosphorus release rate (mg/m^2/day) was calculated and compared to release rates documented from other lakes. This release rate was calculated by determining the mass of phosphorus within the lake just prior to stratification (June 2, 2015) and the mass of phosphorus within the lake following the late-August mixing event (August 27, 2015). Using the concentration of phosphorus measured on these two dates and the estimated volume of the lake, it was estimated that phosphorus within the lake had increased by approximately 1,596 pounds over this 86-day period. The estimated area of anoxia during stratification based on dissolved oxygen measurements was approximately 101 acres. Using this information, the estimated phosphorus release rate from the area of anoxia was approximately 20.6 mg/m^2/day.

This estimated sediment-release rate of phosphorus for Kentuck Lake is higher than the release rate of 12 mg/m^2/day measured by James et al. (2015) in Cedar Lake, a polymictic lake in Polk and St. Croix Counties that has similar morphology to Kentuck Lake. The estimated phosphorus release rate from 2015 is also higher than the median release rate (~10.0 mg/m^2/day) for eutrophic lakes reported by Nürnberg (1988) in a review of phosphorus release rates from anoxic sediments in lakes worldwide. The calculated sediment phosphorus release rate from anoxic sediments may be overestimated as it is believed once pH is elevated above 9.0 from excessive phytoplankton growth, phosphorus is likely being released from shallower, oxic sediments as well.

Transport of nutrients from anoxic sediments to the epilimnion initially fuels the growth of phytoplankton. As the biomass of phytoplankton increases, dissolved carbon dioxide is consumed for photosynthesis faster than it can be replenished by diffusion from the atmosphere and pH rises. Once pH rises above 9.0, phosphorus is then also released from oxic sediments increasing epilimnetic concentrations further. Epilimnetic pH was measured at 9.6 and 9.4 in July and August of 2015, respectively. The exponential increase (R^2 = 0.99) of epilimnetic total phosphorus concentrations in 2015 is believed to be an indicator of initial loading from anoxic bottom sediments followed by additive loading from oxic sediments once pH is above 9.0.

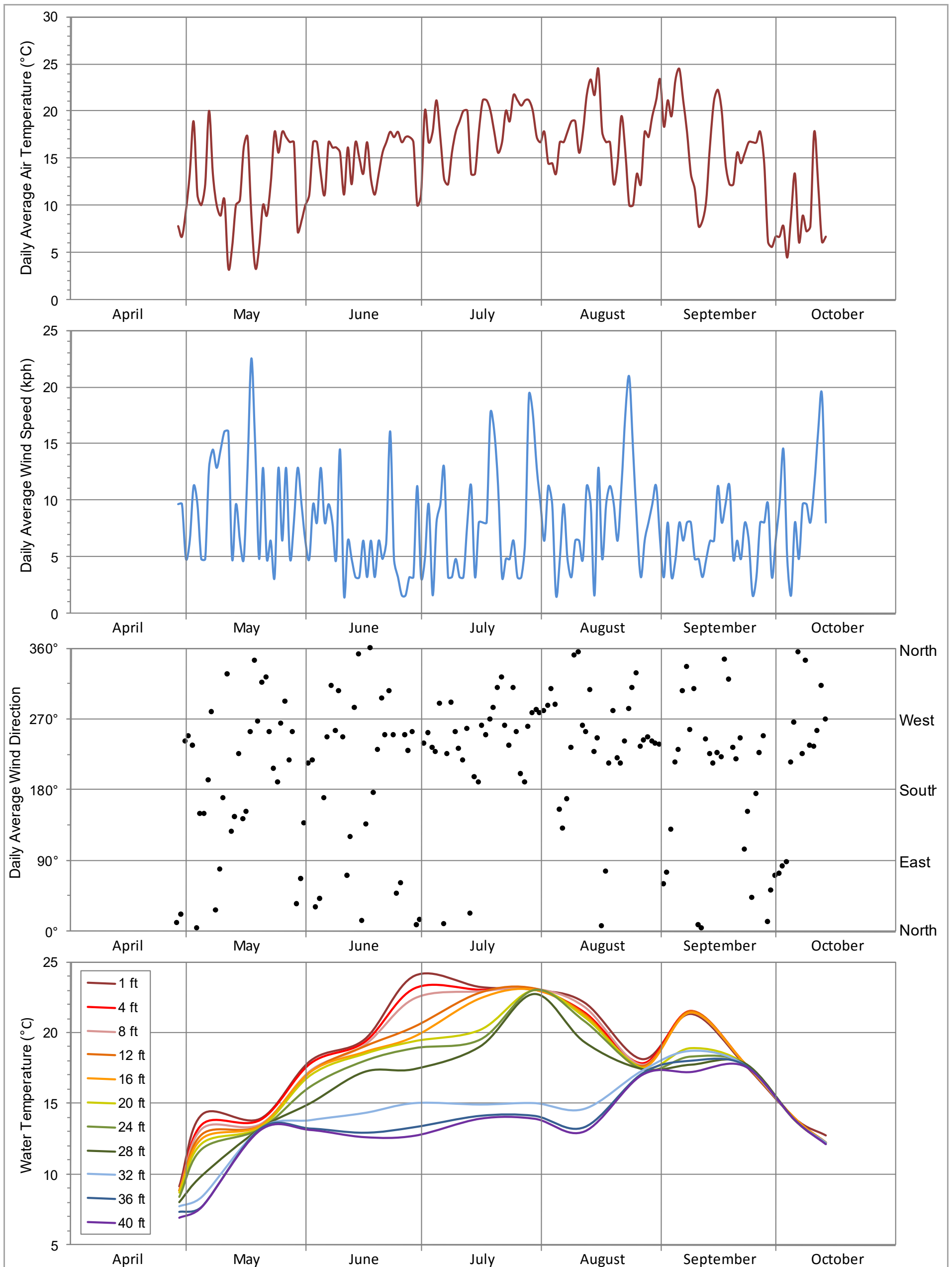

Figure 13. Daily average air temperature, wind speed and direction, and Kentuck Lake water temperature over the 2015 sampling period. Climatic data obtained from the Eagle River Union Airport. Water temperature data collected at site 1 (deep hole) by KLPRD volunteers.

As discussed in the Introduction Section, internal loading of phosphorus can also occur through the translocation of phosphorus from the sediment to the water by benthic migration of cyanobacteria. *Gloeotrichia echinulata*, one of the dominant cyanobacteria in Kentuck Lake in June and July, is known to be able to cause a significant increase in epilimnetic phosphorus through uptake of sediment phosphorus and migration into the water column (Barbiero and Welch 1992; Cottingham et al. 2015). This species has also been documented to stimulate the growth of other phytoplankton by releasing sediment-obtained nutrients into the water (Carey and Rengefors 2010). However, biomass of

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Gloeotrichia was not quantified from Kentuck Lake in 2015, and its contribution to the increase in epilimnetic phosphorus and growth of other phytoplankton is not known.

Gloeotrichia and other species of cyanobacteria are present in Kentuck Lake every year, but their abundance is going to depend on the availability of sediment-released nutrients, specifically phosphorus and ferrous iron. It is known that in phosphorus-limited lakes like Kentuck Lake phosphorus regulates phytoplankton production and it is believed that the availability of ferrous iron will favor a phytoplankton community that is dominated by cyanobacteria (Molot et al. 2014). Phosphorus and phytoplankton abundance would be relatively low in Kentuck Lake if internal nutrient loading did not occur. If phosphorus were to increase in Kentuck Lake apart from iron, phytoplankton biomass would increase but cyanobacteria would likely comprise a smaller portion of the phytoplankton community. However, because anoxia causes the release of both phosphorus and ferrous iron, and entrainment and/or mixing makes these nutrients available to phytoplankton at the surface, a high-biomass of phytoplankton dominated by cyanobacteria develops.

Drivers of Annual Variation in Water Quality Since 2011

Kentuck Lake experienced an abrupt change in water quality from 2010-2011, and the presumed causes of this change will be discussed in the next section. While concentrations of phosphorus and chlorophyll-*a* have been higher from 2011-2015 compared with 2001-2010, concentrations of phosphorus and chlorophyll-*a* have varied between years from 2011-2015. Of these five years, 2011, 2013, and 2015 have seen average phosphorus and chlorophyll-*a* concentrations that are above historical averages for Kentuck Lake, while concentrations in 2012 and 2014 fall near historical averages. One of the objectives of this study is to determine why phosphorus, and consequently chlorophyll-*a* concentrations have varied between years since 2011.

As discussed previously, in Kentuck Lake phosphorus and other nutrients are released from bottom sediments when they are exposed to anoxic conditions. Through entrainment and/or complete mixing events these nutrients become available to phytoplankton at the surface. Excessive phytoplankton growth raises water pH releasing additional phosphorus from oxic sediments around the lake. The amount of phosphorus released from bottom sediments each year is going to depend initially on the duration of anoxia. The longer the duration of anoxia, the more phosphorus and other nutrients will be released into the overlying water.

The annual onset, strength, and duration of thermal stratification and thus anoxia in Kentuck Lake is going to be dependent on local weather conditions, primarily a combination temperature, wind speed, and wind direction. To determine what combination of daily air temperature, wind speed, and wind direction leads to periods of stratification and periods of mixing in Kentuck Lake, an index termed the Daily Climate Index (DCI) was developed which incorporates all three of these climatic variables to produce a single daily value (equation found in Appendix A). Higher DCI values indicate days with higher average air temperatures and lower average wind speeds which increase thermal stability, while lower values indicate days with lower average temperatures and/or higher wind speeds which reduce thermal stability.

The DCI was then compared against Kentuck Lake's measured Schmidt Stability (equation found in Appendix A), a measure of how much work (g/cm) is needed to completely mix the water column calculated from the temperature profiles collected from 2014 and 2015. A stability value of 0 indicates the entire water column is of uniform temperature and no work is needed to mix it. The higher the stability value, the stronger the temperature/density gradient is between surface and bottom waters and more work energy (wind) is needed to mix them together. The goal of this analysis was to determine the conditions that lead to the onset of stratification, the conditions that maintain stratification, and conditions that result in mid-summer mixing events in Kentuck Lake. With this analysis, the annual duration of anoxia could be estimated based upon climatic data. Analysis methods comparing the DCI and Schmidt Stability along with the equations for each index can be found in Appendix A.

Comparing the 2014 and 2015 DCI against Kentuck Lake's 2014 and 2015 Schmidt Stability indicated that seven consecutive days of climatic data prior to the collection of a temperature profile (7-day mean DCI value) was the best predictor of Kentuck Lake's thermal stability (stratified versus mixed) (Pearson pairwise correlation $r = 0.927$). This analysis indicated that anoxia begins to develop in Kentuck Lake when Schmidt Stability is approximately 64 g/cm, and Schmidt Stability of 64 g/cm develops when the 7-day mean DCI value reaches a value of approximately 5.3 in the spring. If the 7-day mean DCI value is maintained at or above 5.3, Schmidt Stability will increase and anoxic conditions will be maintained where nutrients will be released from bottom sediments. However, if the 7-day mean DCI value falls below 5.3 (i.e. cooler air temperatures with strong winds) after the lake has already thermally stratified, a complete mixing event will likely occur ending the period of anoxia.

For example, in 2014 Schmidt Stability reached 64 g/cm in late-May and reached a maximum of 120 g/cm in late-June. In early-July, the 7-day mean DCI declined below 5.3 and a complete mixing of the water column was observed. In 2015, Schmidt Stability reached 64 g/cm in mid-June; however, in contrast to 2014, the 7-day mean DCI did not fall below 5.3 until late-August, causing a complete mixing event. In 2014, climatic conditions facilitated a mixing event earlier in the summer, shortening the duration of anoxia and lessening the amount of nutrients released from bottom sediments. In 2015, climatic conditions maintained strong thermal stability until late-summer, creating a longer period of anoxia and allowing more nutrients to be released from bottom sediments and consequently, larger phytoplankton blooms occurred in 2015.

Using climatic data from 2011-2015, the 7-day mean DCI was calculated for each year and used to estimate the annual duration of anoxia. The estimated duration of anoxia was then compared against the measured average growing season near-surface total phosphorus concentration and revealed a strong, positive relationship (Figure 14). This relationship illustrates that historical climatic data can be used to estimate the annual duration of anoxia and average growing season near-surface total phosphorus concentrations in Kentuck Lake. The longer the duration of anoxia, the more phosphorus is released from bottom sediments. Historical climatic data can be used to estimate the duration of anoxia in past years when little or no dissolved oxygen data are available. In the following section, climatic data are used to estimate the annual duration of anoxia and to compare predicted versus measured phosphorus values in years prior to 2011.

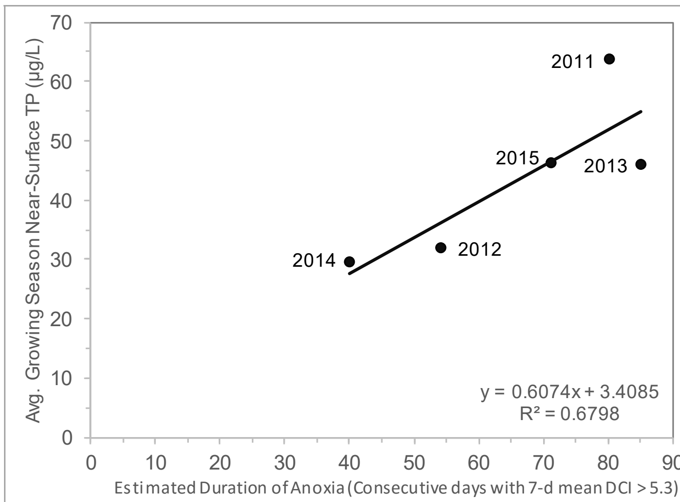

Figure 14. Kentuck Lake linear regression of average growing season near-surface total phosphorus and measured 2011-2015.

Drivers of Long-term Variations in Water Quality

One of the primary objectives of this study is to determine the drivers of longer-term phosphorus dynamics in Kentuck Lake and the cause of transitions between these periods. Average near-surface growing season total phosphorus concentrations from 1988-2000 were 36 µg/L, declined to 22 µg/L from 2001-2010, and increased again to 44 µg/L from 2011-2015. In the previous section, it was shown that climatic conditions, primarily temperature and wind, determine the duration of anoxia each year. Based upon this information, it was hypothesized that the differences in phosphorus concentrations between these periods was driven by differences in the annual duration of anoxia; the periods with higher phosphorus concentrations had longer annual durations of anoxia while the period with lower phosphorus concentrations had shorter annual durations of anoxia.

To test this idea, historical climatic data were used to calculate the 7-day mean DCI, and using the 7-day mean DCI, the annual duration of anoxia was estimated during the higher phosphorus period of 1988-2001 (1999 was excluded because only two phosphorus samples were collected that year) and the lower phosphorus period from 2001-2010. Using the linear model developed between the estimated annual duration of anoxia and the annual average growing season near-surface total phosphorus concentration from 2011-2015, annual average near-surface total phosphorus concentrations were predicted for each year from 1988-2010 using the estimated annual duration of anoxia. The predicted annual phosphorus concentrations were then plotted against measured annual phosphorus concentrations.

In the higher phosphorus period from 1988-2000, the average estimated annual duration of anoxia was 34 days compared to an average of 57 days during the lower phosphorus period from 2001-2010. This goes against the hypothesis that the lower phosphorus period from 2001-2010 had lower annual durations of anoxia. Plotting the predicted phosphorus based upon the estimated annual duration of anoxia versus measured phosphorus indicated that the estimated duration of anoxia was a good predictor of phosphorus concentrations during the higher phosphorus period from 1988-2000 (R^2 = 0.842) (Figure 15). Within this period, the years with the highest phosphorus concentrations, 1988 and 1991, also had the longest estimated durations of anoxia.

In contrast, the estimated duration of anoxia was a poor predictor of annual phosphorus concentrations in the lower phosphorus period from 2001-2010 (R^2 = 0.331) (Figure 15). Six of the ten years within this period had significantly lower phosphorus concentrations than predicted based upon the estimated duration of anoxia. These results suggest that an additional factor(s) was regulating phosphorus concentrations within Kentuck Lake during the period from 2001-2010.

Figure 16 illustrates the relationship between the estimated duration of anoxia and measured average growing season near-surface total phosphorus concentrations from 2001-2010 and the combined data from 1988-2000 and 2011-2015. The exponential increase in phosphorus concentration with the duration of anoxia in the 1988-2000 and 2011-2015 dataset is likely the result of additional phosphorus being released from oxic sediments when phytoplankton reaches sufficient levels to elevate water pH above 9.0.

Because predicted phosphorus concentration was significantly higher than measured phosphorus concentration in six of the ten years within the period from 2001-2010 that had higher estimated durations of anoxia suggests that an additional variable(s) was tempering phosphorus and phytoplankton increase within the epilimnion during these years. It is hypothesized that unusually high walleye abundance and resulting lower planktivore (panfish) abundance are the likely driver of lower-than-predicted phosphorus concentrations during this period.

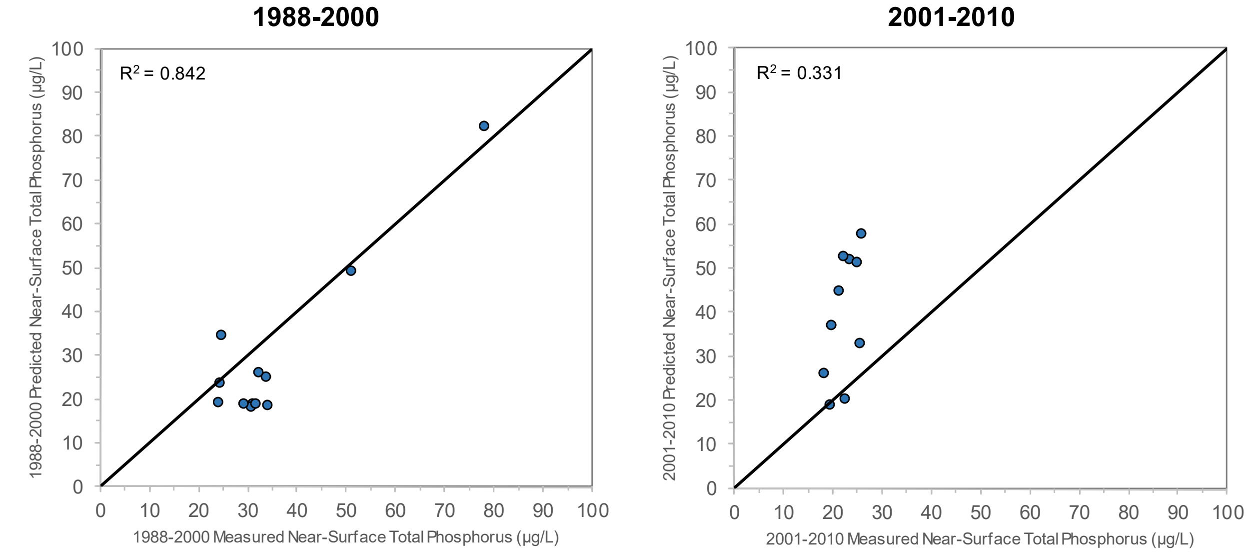

Figure 15. Kentuck Lake 1988-2000 (left) and 2001-2010 (right) measured versus predicted average growing season near-surface total phosphorus concentrations based upon estimated annual duration of anoxia.

It is well documented that food web interactions, specifically fish-zooplankton-phytoplankton interactions, can significantly affect a lake's water quality, particularly in shallow, mesotrophic lakes (Benndorf et al. 2002). At its basic level, high *piscivorous* fish abundance (e.g. walleye) decreases *planktivorous* fish (e.g. bluegill) abundance, increases herbivorous zooplankton abundance (e.g. *Daphnia* spp.), and decreases phytoplankton abundance (Carpenter et al. 1987). And in contrast, low piscivorous fish abundance increases planktivorous fish abundance, decreases herbivorous zooplankton abundance, and increases phytoplankton abundance (Figure 17).

Piscivorous is a term used to describe any organism which primarily feeds on fish (piscivore). **Planktivorous** is a term used to describe any organism that primarily feeds on plankton (planktivore), including zooplankton and phytoplankton.

Under high piscivore/low planktivore abundance, phytoplankton are reduced through two primary mechanisms: 1) increased predation by zooplankton and 2) reduced phosphorus availability through increased sedimentation (zooplankton fecal pellets) and accumulation within zooplankton (Benndorf et al. 2002). The combined reduction in phytoplankton, reallocation of phosphorus to the zooplankton community, and increased sedimentation of phosphorus under high piscivore/low planktivore abundance results in less phosphorus (and chlorophyll-*a*) being measured at the surface. While high piscivore/low planktivore abundance does not prevent the internal release of phosphorus due to anoxia, it increases phosphorus sedimentation and causes more phosphorus to be allocated to the zooplankton rather than the phytoplankton community.

Walleye were not historically found in Kentuck Lake and were introduced sometime in the 1970s (Steve Gilbert, personal comm. 2015). With some natural reproduction occurring, the population increased rapidly in the 1980s reaching adult densities of approximately 7.0 fish/acre (Figure 18). However, walleye abundance declined through the 1990s to a minimum density of approximately 1.0

fish/acre due to recruitment failures, the causes of which are unknown. With coordination from the WDNR and the Great Lakes Indian Fish and Wildlife Commission (GLIFWC), the tribal community began walleye stocking in 1999 and 2000.

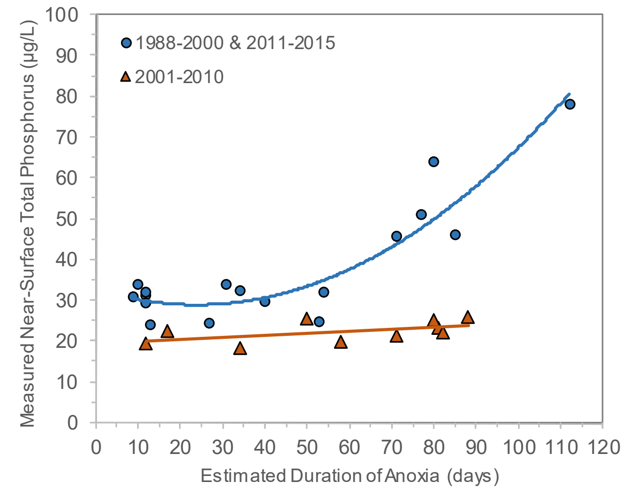

Figure 16. Relationship of annual estimated duration of anoxia and average growing season near-surface total phosphorus in Kentuck Lake from 2001-2010 (orange triangles) and combined data from 1988-2000 and 2011-2015 (blue circles).

Following stocking in 1999 and 2000 and natural reproduction, adult walleye abundance rapidly increased beginning in 2001, and from 2003-2009 walleye abundance ranged between 7.2 and 13.9 fish/acre. These high walleye abundances are considered to be atypical and unsustainable. To coordinate walleye management in Kentuck Lake, a walleye management plan was developed by the WDNR and GLIFWC in 2005 and set criteria for when stocking should be conducted and/or harvest restrictions should be put into place to rehabilitate a declining population. The goal for Kentuck Lake's walleye population is to maintain a population of at least 3.0 adult fish per acre. In 2010, adult walleye abundance declined to 3.4 fish/acre from 12.6 fish/acre in 2009, and continued to decline to approximately 1.6 fish/acre in 2011 and 2012 followed by a slight increase to 3.2 fish/acre in 2013. The WDNR stocked approximately 34,000 small fingerling walleye in 2014 as part of the Wisconsin Walleye Initiative, and stocking is scheduled to occur again in the spring of 2016 (Steve Gilbert, personal comm. 2015).

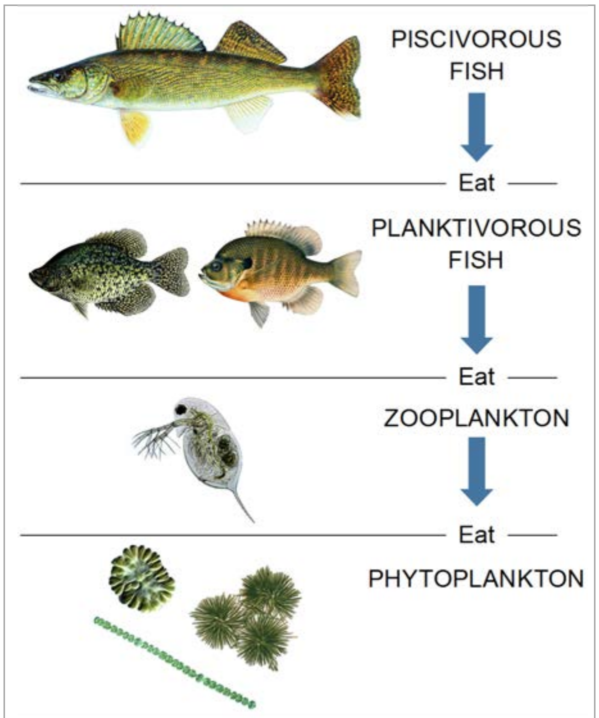

Figure 17. Simplified lake food web structure.

In the higher-phosphorus periods, 1988-2000 and 2011-2015, when annual near-surface total phosphorus concentrations were highly correlated with the estimated annual duration of anoxia, average adult walleye abundance was 2.9 fish/acre. It is important to note that in 2014 and 2015 the walleye population was almost entirely comprised of female fish. In the absence of males, the females will tend to congregate more in shallower areas in search of males where they are more prone to be sampled during the survey and inflating the estimated population abundance (Steve Gilbert, personal comm. 2016). During the lower-phosphorus period from 2001-2010, adult walleye abundance averaged 9.3 fish/acre.

Little data on planktivorous fish abundance is available until 1999 in Kentuck Lake. Figure 18 displays the combined annual fall survey catch per unit effort (CPUE) of the four-most abundant planktivorous fish in Kentuck Lake: black crappie, bluegill, pumpkinseed, and yellow perch. Within the lower phosphorus period from 2001-2010, the combined average CPUE for these four species was 43.6. Between 2010 and 2012, the combined average CPUE for these planktivores increased over five-fold to an average of approximately 219 from 2012-2015.

While empirical evidence of the zooplankton community during these periods of varying walleye and planktivore abundance are not available, it is believed that the fisheries data from Kentuck Lake support the hypothesis that phosphorus concentrations during the period from 2001-2010 were lower than predicted due to high walleye/low planktivore abundance tempering the effects of internal nutrient loading. With the decline in walleye abundance beginning in 2010 and concurrent increase in planktivore abundance, zooplankton abundance and predation upon phytoplankton was likely reduced and Kentuck Lake's nutrient concentrations were then largely dictated by the annual duration of anoxia.

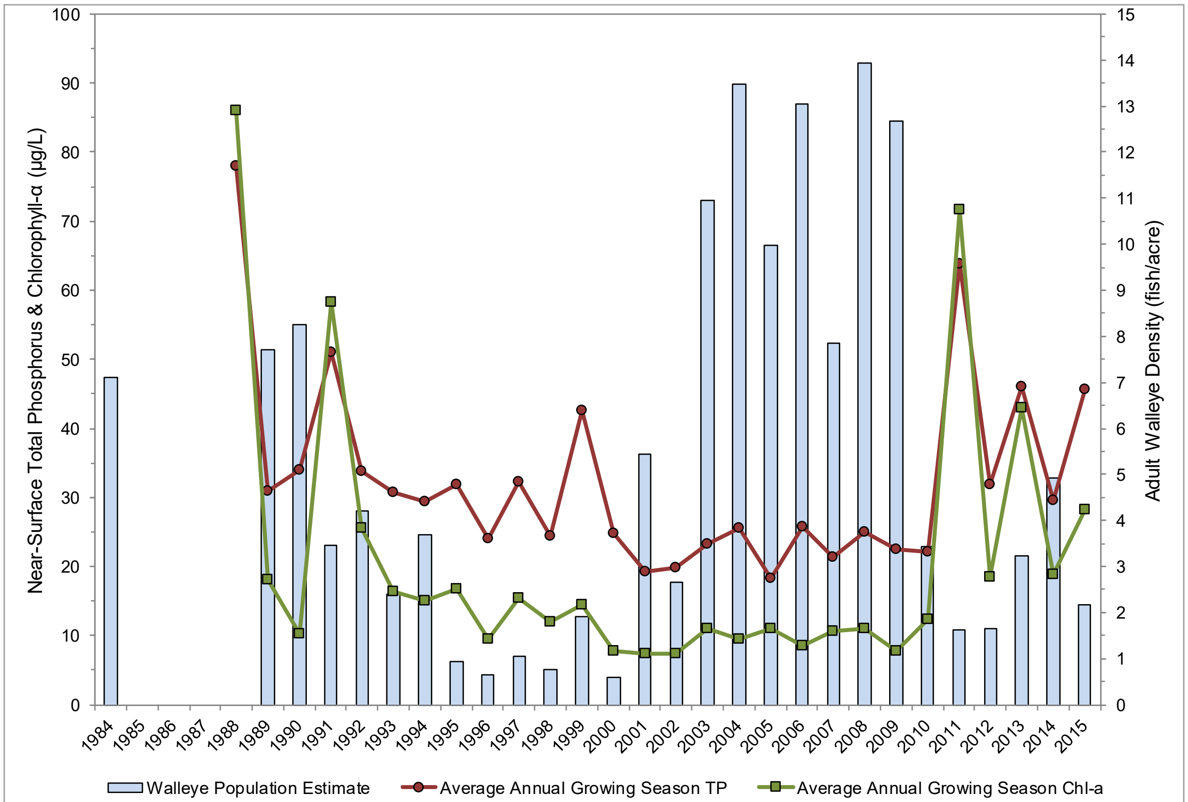

Figure 18. Kentuck Lake annual spring walleye population estimates and average growing season near-surface total phosphorus and chlorophyll-α. Fisheries data provided by Steve Gilbert, WDNR fisheries biologist.

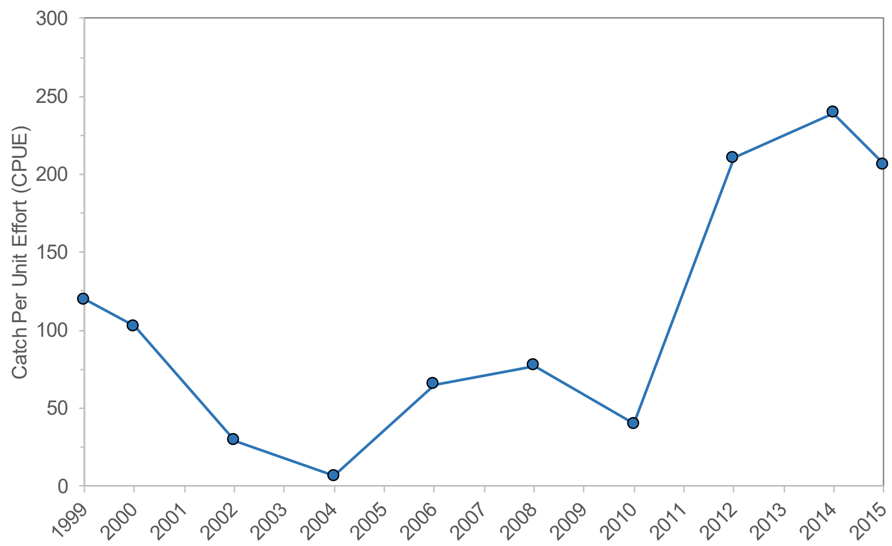

Figure 19. Combined annual fall catch per unit effort of four-most abundant planktivorous fish in Kentuck Lake (black crappie, bluegill, pumpkinseed, and yellow perch). Fisheries data provided by Steve Gilbert, WDNR fisheries biologist.

Management Strategies for Water Quality

The previous sections have shown that internal nutrient loading from anoxic bottom sediments occurs in Kentuck Lake, and the lake's large and shallow nature allow these nutrients to be delivered from bottom waters to the surface where they fuel nuisance phytoplankton blooms. The amount of nutrients released on an annual basis is largely dependent upon the duration of anoxia, and the duration of anoxia is going to depend on annual climatic conditions. However, it is believed there is also evidence to suggest that nutrient concentrations and phytoplankton abundance were lower than expected from 2001-2010 due to unusually high walleye abundance and the resulting trophic interactions. Fisheries survey data also show that planktivorous fish abundance was lower during this period, suggesting that zooplankton abundance and predation upon phytoplankton was likely higher.

The dramatic change in water quality observed from 2010-2011 is believed to be due in part to the rapid decline in walleye abundance and simultaneous increase in planktivore abundance. Without the moderating effect of high walleye/low planktivore abundance, phosphorus concentrations and phytoplankton abundance increased significantly in 2011 in response to a long duration of anoxia. With higher planktivore abundance, zooplankton abundance and predation upon phytoplankton is reduced, phosphorus sedimentation through zooplankton fecal pellets is reduced, and more phosphorus is available to phytoplankton which grow largely unregulated. In other words, under higher planktivore abundance, phytoplankton growth is largely regulated by the amount of phosphorus available, and will be more abundant in years when the duration of anoxia is longer. However, even under high planktivore abundance, phytoplankton abundance will still be relatively low in years when the duration of anoxia is smaller and less phosphorus is released from bottom sediments.

Because it is hypothesized that high walleye/low planktivore abundance has the capacity to mitigate against effects of internal nutrient loading and create better water quality conditions in Kentuck Lake, it would be logical to assume that a potential strategy to improve water quality in Kentuck Lake would be to increase the number of walleye to decrease the number planktivores. However, WDNR fisheries biologists do not believe that this is a realistic management option as the densities that were observed during the low-phosphorus period from 2001-2010 were considered to be unusually high and unsustainable. Fisheries data show that the walleye population in Kentuck Lake has the capacity to fluctuate widely, and WDNR fisheries biologists do not have a full understanding of what drives these rapid increases and decreases in the population. Following walleye stocking in 2014 and 2016, it is possible that the walleye population could increase again to levels observed from 2001-2010, but WDNR fisheries biologists are not certain.

Two additional management strategies that have been implemented on other lakes in Wisconsin and around the world that experience internal phosphorus loading are also being investigated. These strategies are aluminum sulfate (alum; $Al_2(SO_4)_3$) application and artificial de-stratification through aeration. As discussed previously, phosphorus is initially released from deeper areas of Kentuck Lake which are the first to experience anoxia with the onset of stratification. The phosphorus released from anoxic sediments fuels phytoplankton growth at the surface. Excessive growth of phytoplankton then raises water pH causing additional phosphorus release from shallower, oxic sediments. The goal of an alum treatment or artificial destratification would be to prevent the initial release of phosphorus from deeper, anoxic sediments.

The use of alum to inactivate sediment phosphorus involves the application of alum over areas of lake bottom determined to be releasing significant amounts of phosphorus. In Kentuck Lake, this would be conducted over deeper areas that frequently experience anoxia to prevent the release of phosphorus. The alum is applied as a liquid and once it enters the water hydrolysis begins and through numerous steps aluminum hydroxide ($Al(OH)_3$) is produced. As more $Al(OH)_3$ is created, a visible coagulant, or floc appears within the water column. As the floc increases in size and density, so does its mass and eventually it sinks to the bottom. Relatively quickly (within hours), the $Al(OH)_3$ binds with sediment phosphorus and forms a "blanket" that is integrated with the upper layer of the sediment, preventing further release phosphorus to the overlaying water. Unlike iron, aluminum continues to bind with phosphorus even during periods of anoxia.

Over time the $Al(OH)_3$ floc becomes buried in a new layer of sediment or all of the aluminum receptors are depleted. In either case, the treatment loses its ability to retard sediment phosphorus release and the treatment effectiveness decreases. However, if dosed correctly and if external sources of phosphorus are minimized, the benefits of an alum treatment may last for 15 or more years (Welch and Cooke 1999). While there are obviously great benefits to completing a successful alum treatment, there are risks that need to be considered. Dissolved aluminum is toxic to animals, including insects, fish, and humans. However, by controlling lake water pH, the risk is controlled. At pH levels between 5.5-9.0, insoluble (not dissolved) $Al(OH)_3$ is by far the most dominate form of aluminum. In fact, dissolved aluminum (Al^{3+}) does not form unless the pH falls below 5. Still, other forms of soluble (dissolved) aluminum can form at pH levels between 4 and 6 and above 8; therefore, by maintaining pH levels between approximately 6 and 8, toxicity issues are avoided.

As mentioned above, once the alum is added to the lake, hydrolysis begins and $Al(OH)_3$ is formed. Hydrolysis is essentially the release of hydrogen ions (H^+) into the water. As hydrogen ions increase, the pH within the lake falls. In soft water lakes this can be a problem because as the alum is added the pH drops and as described above, once it decreases to 6 or less, toxicity can become an issue. In those lakes, sodium aluminate may also be added to the lake as it buffers against hydrolysis and prevents the

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pH from falling. Lakes with high alkalinities have natural buffering capacity against the addition of acids (hydrogen ions). The treatments of lakes with alkalinities above 75 mg/L as $CaCO_3$ are not expected to have chronic or acute effects to biota because the lake's natural buffering capacity would maintain the pH well above 6. Historical data from Kentuck Lake indicates its alkalinity is around 34 mg/L as $CaCO_3$, lower than the 75 mg/L threshold.

Because of Kentuck Lake's lower alkalinity, sodium aluminate would likely also have to be applied to prevent the alum from lowering the lake's pH. Given Kentuck Lake's size, even if a treatment was conducted over the deeper area of the lake, the application of both alum and sodium aluminate would likely be expensive. An approximate 40-acre alum treatment was conducted on East Alaska Lake (Manitowoc County) without the simultaneous application of sodium aluminate in 2011 at a cost of approximately $165,000. In addition, Kentuck Lake has highly flocculent bottom sediments, and the alum may actually sink into these sediments rather than creating a layer on top of the sediments. Onterra ecologists collected sediment cores during the winter of 2016 that are currently undergoing nutrient release analysis. The results of this analysis will shed more light into the feasibility of completing an alum treatment in Kentuck Lake, but currently this does not appear to be a realistic option for reducing anoxic nutrient release.

Another strategy being investigated is the employment of an aeration system in the deeper areas of Kentuck Lake that would continually mix the water column in an effort to prevent thermal stratification and anoxia from developing. While some studies have shown that this strategy can actually exacerbate phosphorus loading (James et al. 2015), this has typically occurred in lakes with low iron to phosphorus ratios, meaning once phosphorus was transported to oxygenated waters there was not sufficient iron to bind with it and make it unavailable to phytoplankton. Kentuck Lake has a high iron to phosphorus ratio, and destratification through aeration is going to be investigated further as a management strategy as the project moves forward. The lake's flocculent sediments are also a concern for this management strategy has aeration could resuspend these sediments resulting in oxygen depletion.

As additional data are collected in the second year of the project in 2016, Onterra ecologists will continue to investigate these management strategies and their applicability to Kentuck Lake. As was discussed with the KLPRD at the start the project, one of the likely outcomes of this study was that there would be no feasible management strategies that could be implemented to improve water quality, and what would be gained would be a detailed understanding of Kentuck Lake's water quality dynamics. The KLPRD needs to communicate and be open to this possibility.

As was discussed during the lake management planning project, the KLPRD can ensure that they minimize development along the lake's shoreline and within its watershed to minimize external inputs of phosphorus. However, the KLPRD has to understand that internal nutrient loading in Kentuck Lake is a natural occurrence that results from the lake's shallow morphology, and its impact on water quality is largely going to be dependent on annual climatic conditions. Some years will see shorter durations of anoxia, lower phosphorus concentrations, and lower phytoplankton abundance, while some years will experience higher durations of anoxia, higher phosphorus concentrations, and higher phytoplankton abundance. With the data being collected as part of this project, it may be possible to predict when severe cyanobacteria blooms will occur in Kentuck Lake.

Curly-leaf Pondweed & Eurasian Water Milfoil

Curly-leaf pondweed (CLP) was first documented in Kentuck Lake in 1999, but plants were not observed in the lake again by KLPRD members until the summer of 2012. While subsequent surveys since 2012 have found that for unknown reasons the CLP population in Kentuck Lake remains very small, the KLPRD understands that this plant has the capacity to expand rapidly and has elected to continue to monitor the lake's population on an annual basis.

Eurasian water milfoil (EWM) was discovered more recently in Kentuck Lake in 2011. Onterra was contracted by the KLPRD in 2012 to conduct a Late-Summer EWM Peak-Biomass Survey to map areas of EWM and to develop a management strategy. During the 2012 survey, approximately 13.4 acres of colonized EWM (polygons) were located. Following discussions between the KLPRD, Onterra, and WDNR over the winter of 2012/2013, an 18.6-acre herbicide spot treatment was conducted in the spring of 2013. Post-treatment surveys conducted in the summer of 2013 and 2014 found that the spring 2013 herbicide treatment was mostly successful, with EWM density being reduced within the two of the three application areas one year following the treatment. In 2014, 3.7 acres of colonized EWM mainly delineated as *highly scattered* was located, and given this low level of EWM no herbicide control strategy was proposed for 2015.

It is important to understand the different herbicide control strategies that are currently used in Wisconsin's lakes and under what circumstances they are appropriate for employment. Before the 2015 AIS monitoring results are discussed, the following section provides background information on herbicide application strategies in Wisconsin.

Background on Herbicide Application Strategy

Herbicides that target submersed plant species are directly applied to the water, either as a liquid or an encapsulated granular formulation. Factors such as water depth, water flow, treatment area size, and plant density work to dilute herbicide concentration within aquatic systems. Understanding concentration-exposure times are important considerations for aquatic herbicides. Successful control of the target plant is achieved when it is exposed to a lethal concentration of the herbicide for a specific duration of time. Much information has been gathered in recent years, largely as a result of a joint research project between the WDNR, US Army Corps of Engineers (USACE), and private consultants. Based on their preliminary findings, lake managers have adopted two main treatment strategies; 1) whole-lake and 2) spot treatment strategies.

Whole-lake treatments are those where the herbicide is applied to specific sites but when the herbicide reaches equilibrium within the entire volume of water (entire lake, lake basin, or within the epilimnion of the lake or lake basin) it is at a concentration that is sufficient to cause mortality to the target plant within that entire lake or basin. The application rate of a whole-lake treatment is dictated by the volume of water in which the herbicide will reach equilibrium. Because exposure time is so much longer, target herbicide levels for whole-lake treatments are significantly less than for spot treatments. This strategy is utilized when the target plant is widespread throughout a lake or basin. Because the majority of the EWM in Kentuck Lake was isolated to three areas in 2012, the whole-lake treatment strategy was not warranted.

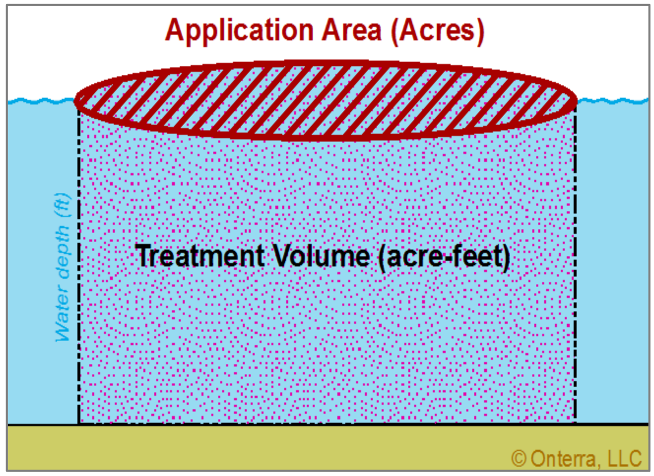

Figure 20. Herbicide Spot Treatment diagram.

Spot treatments are a type of control strategy where the herbicide is applied to a specific area (treatment site) such that when it dilutes from that area, its concentrations are insufficient to cause significant effects outside of that area. Herbicide application rates for spot treatment are formulated volumetrically, typically targeting EWM with 2,4-D at 3.0-4.0 ppm acid equivalent (ae). This means that sufficient 2,4-D is applied within the application area such that if it mixed evenly with the treatment volume, it would equal 3-4.0 ppm ae (Figure 20). This standard method for determining spot treatment use rates is not without flaw, as no physical barrier keeps the herbicide within the treatment volume and herbicide dissipates horizontally out of the area before reaching equilibrium. While lake managers may propose that a particular volumetric dose be used, such as 3.0-4.0 ppm ae, it is understood that actually achieving 3.0-4.0 ppm area within the water column is not likely due to dissipation and other factors. This was the EWM control strategy that was utilized in Kentuck Lake in 2013.

2015 AIS Survey Results

As is discussed in the Methods Section, the primary goal of the June 2015 Early-Season AIS (ESAIS) Survey was to locate and map occurrences of CLP as this time of year is when these plants are at or near their peak growth and the probability of locating them is highest. During the 2015 ESAIS Survey, Onterra ecologists observed a few floating fragments of CLP, but could not locate any rooted plants that were visible from the surface. This indicates that while CLP is present in Kentuck Lake, it remains at a level which is not causing adverse ecological or recreational impacts. The CLP population in Kentuck Lake will be reassessed again in June of 2016.

Please note that the following figure (Figure 21) represents the acreage of mapped EWM polygons, not EWM mapped within point-based methodologies (*single or few plants, clumps of plants*, or *small plant colonies*). Taken out of context, this figure can be misleading as it relates to the EWM population changes. For instance, large increases in colonized acreage between 2014 and 2015 may seem like a drastic change, but actually represent when a collection of point-based EWM occurrences increase in density to the point they require delineation with polygons (Figure 22).

The September 2015 Late-Summer EWM Peak-Biomass Survey found that the EWM population in Kentuck Lake has increased in area and density from previous surveys. Acreage of colonized EWM increased from approximately 3.7 acres in 2014 to 52.2 acres in 2015 (Figure 21 and 22). However, 88% of the colonized EWM located in 2015 (46.4 acres) was comprised of low-density EWM, classified as either *scattered* or *highly scattered*. The increase in EWM acreage in one year's time Kentuck Lake is not unprecedented, and has been observed in a number of northern lakes that contain EWM. Long-term aquatic plant data collected by WDNR and Onterra ecologists indicate that EWM not only has the capacity to rapidly increase in occurrence, but that populations can also rapidly decline despite no management action occurring.

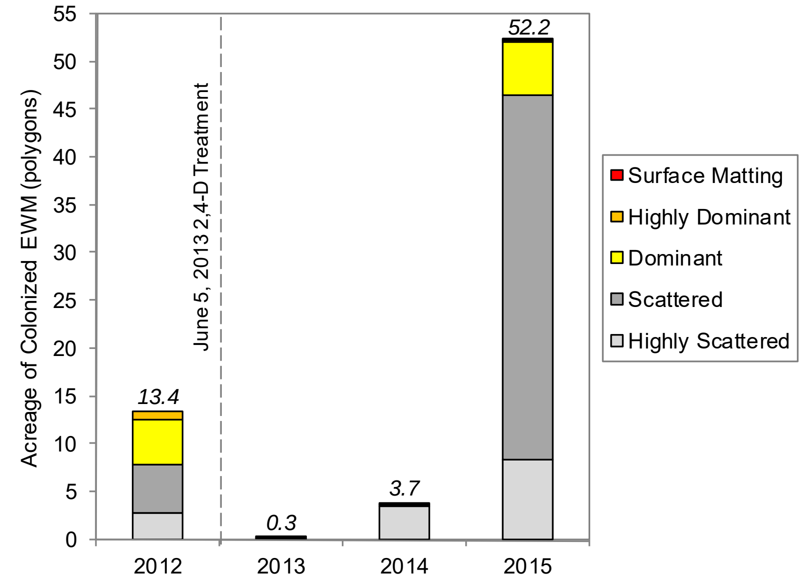

Figure 21. Kentuck Lake acreage of colonized EWM from 2012-2015.

Figure 23 displays the annual littoral occurrence of EWM from South Twin Lake (Vilas County) from 2008-2015 and HWM (hybrid water milfoil) from Little Bearskin Lake (Oneida County). Typically, a littoral occurrence of 10% or higher indicates that EWM is widespread throughout the lake, and this occurrence is usually used as a threshold when assessing whether or not to implement the whole-lake treatment strategy. In South Twin Lake, EWM has been actively managed with two whole-lake 2,4-D treatments occurring in 2009 and 2010. These treatments were effective at reducing the EWM population but also imparted damage to certain susceptible native aquatic plants as well. And while the whole-lake treatments in South Twin Lake succeeded at maintaining a small population of EWM for four years, the population began to rapidly increase again and exceeded pre-treatment levels in 2015. The North and South Twin Lakes Riparian Association is currently poised to conduct another whole-lake treatment in South Twin Lake in 2016 in an effort to reduce the EWM population.

In Little Bearskin Lake, HWM was first discovered in the lake in 2009 at an already relatively high occurrence of greater than 10% (Figure 23). The Little Bearskin Lake Association completed a lake management plan with one of the goals being to develop a HWM management strategy. However, due to a UW-Extension study of milfoil weevils within the lake, the association agreed to postpone the implementation of any management strategy until the study was completed. At the end of the weevil study, the HWM population was assessed again to determine if a control strategy was warranted. As Figure 22 illustrates, the HWM population in Little Bearskin Lake has been in decline since 2011 despite no control strategy being implemented. The reason for this population's decline is unknown, and a control strategy is not warranted at this time. However, it is believed the population will continue to fluctuate over time, and the association will be monitoring it into the future.

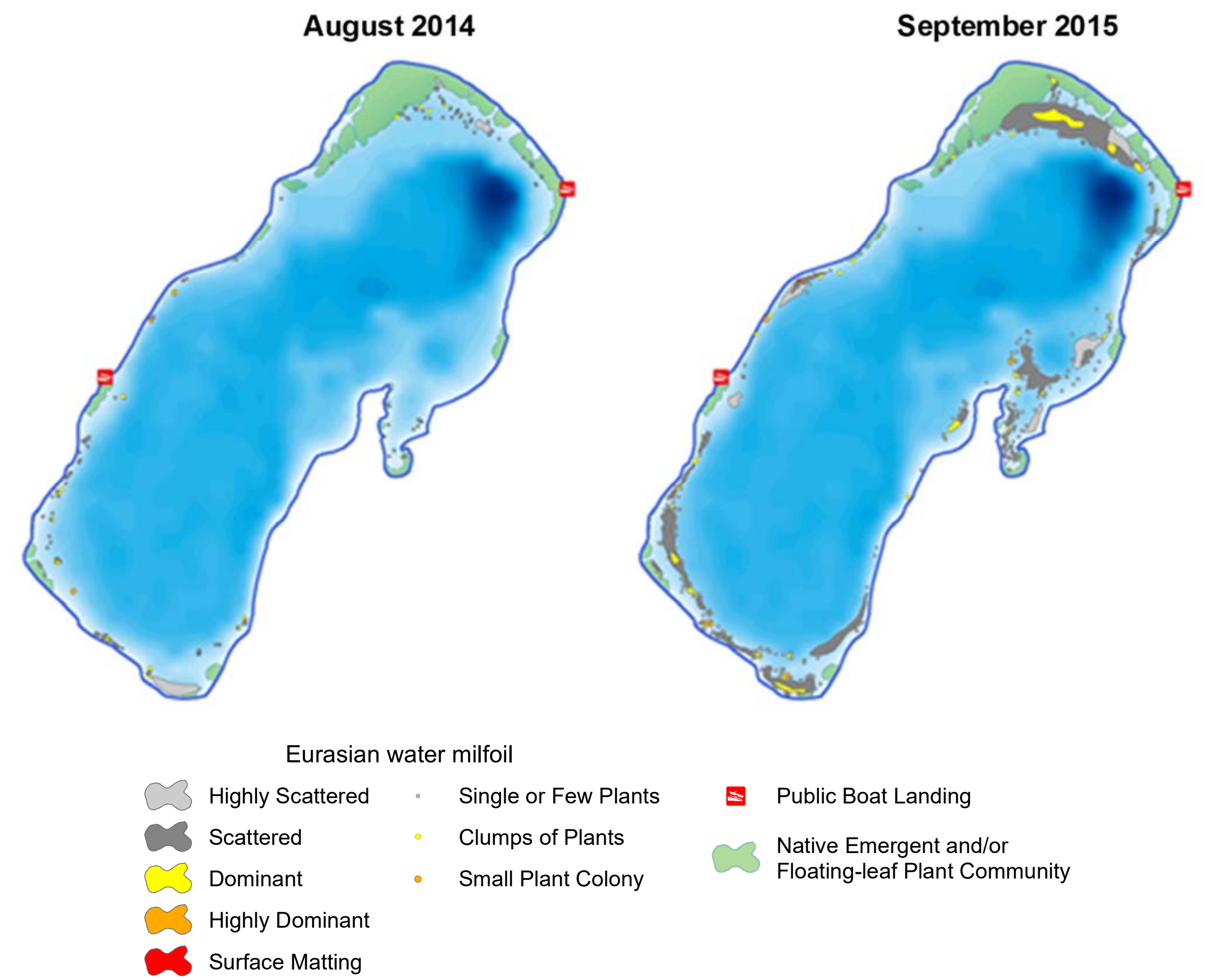

Figure 22. Kentuck Lake August 2014 and September 2015 EWM locations and densities.

The long-term data collected on lakes with EWM in Wisconsin that have not implemented any management actions are indicating that EWM occurrence tends to fluctuate over time around some equilibrium level. While EWM has spread in Kentuck Lake and is at its highest level since it was discovered in 2011, there is not a good understanding as to whether it will continue to increase, decrease, or remain the same in 2016 and beyond. The reason data from South Twin Lake and Little Bearskin Lake are presented here are to illustrate the different paths lake groups have chosen to take in terms of EWM management.

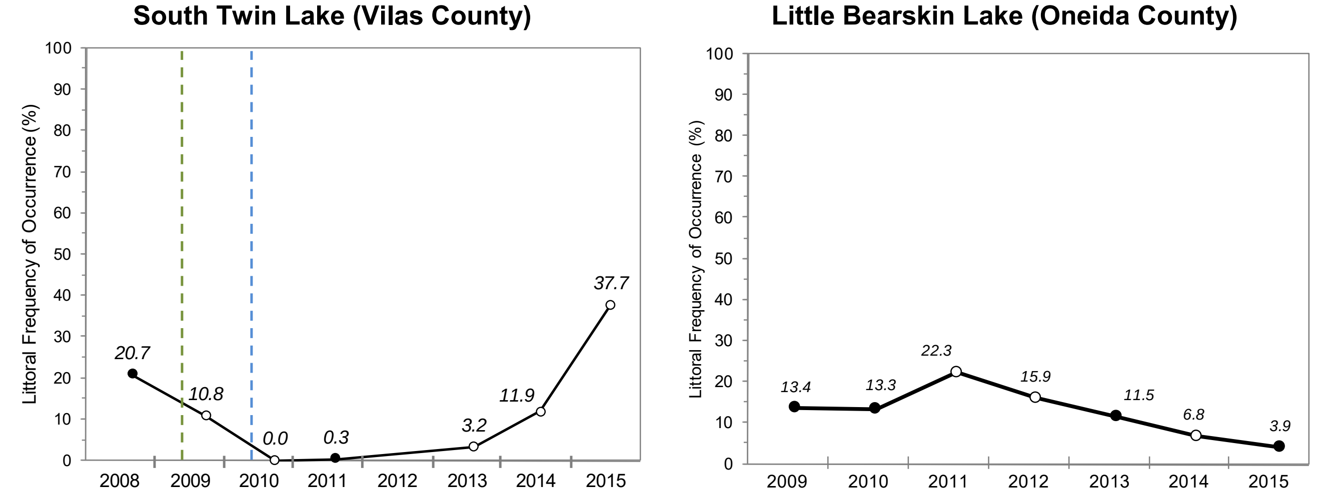

Figure 23. Littoral occurrence of EWM in South Twin Lake (left) and HWM in Little Bearskin Lake (right). Green and blue dashed lines represent the implementation of whole-lake 2,4-D treatments. Open circles indicate a statistically valid change in occurrence from the previous year (Chi-square α = 0.05).

Within Kentuck Lake's Comprehensive Management Plan (Onterra 2015) a trigger or threshold level of EWM was established that if met or exceeded would initiate herbicide application strategy development. The trigger level was established as areas of EWM of approximately three acres or greater with the majority of that area containing *dominant* or greater density of EWM. While over 50 acres of colonized EWM were mapped in Kentuck Lake in 2015, a combined total of only 5.7 acres contained EWM with a density of *dominant* or greater. And all of the colonies containing *dominant* or greater EWM densities were less than three acres, falling below the established treatment trigger.

The treatment trigger developed during the management planning process was designed so that isolated colonies of EWM could be targeted as needed. However, the EWM in Kentuck Lake has not remained contained to isolated areas but rather has expanded simultaneously throughout shallower areas around the lake. While the 2015 EWM did not meet the treatment threshold as discussed, its current wide-spread nature also does not make it conducive for a spot-treatment strategy. The WDNR's position on AIS management is to target the population at the lake-wide level, and if targeting one or two areas using the spot-treatment strategy does not achieve this, then the control effort will likely not be eligible for grant funding. For example, if the colony of EWM located in the northern portion of Kentuck Lake in 2015 was proposed to be targeted with a spot treatment in 2016, it would control EWM within this particular area but would not achieve the goal of reducing EWM lake-wide.

If all of the areas of EWM located in Kentuck Lake in 2015 were targeted for herbicide control, the resulting estimated lake-wide concentration following dissipation would likely have impacts lake-wide and not solely within the application areas. For this reason, a whole-lake treatment strategy would likely be the only applicable strategy to control EWM in Kentuck Lake given its current wide-spread nature. However, even though EWM is wide-spread in littoral areas of Kentuck Lake, it is still found in relatively low densities.

In a meeting with the KLPRD Board in December of 2015, Onterra ecologist Brenton Butterfield presented that Onterra does not believe that the EWM population within Kentuck Lake is currently at a level that warrants a whole-lake treatment strategy. As mentioned, it is not known whether the EWM population in Kentuck Lake will increase to a level at which a whole-lake herbicide treatment would be warranted. Also at the meeting in December, the cost of doing such a treatment on Kentuck Lake was presented. It is estimated that a whole-lake treatment on Kentuck Lake would cost around $92,000.

In addition, a whole-lake treatment on Kentuck Lake would likely have secondary impacts to the lake (native plant impacts, potential alterations to water quality parameters, and other less-understood impacts to other aquatic life). In Kentuck Lake, four of the five-most abundant native aquatic plant species (common waterweed, slender naiad, small pondweed, and northern water milfoil) have all been shown to be prone to decline following these treatments. Therefore, whole-lake treatments are typically postponed until the EWM population exceeds a certain threshold in order to balance these factors.

Many area lakes set a threshold at EWM occurring in 10-15% of the littoral zone as measured through the point-intercept survey. While quantitative whole-lake point-intercept surveys have not been conducted since 2011 on Kentuck Lake, approximately 6% of the littoral sampling locations from the point-intercept sampling grid were located within areas of EWM mapped in 2015. This indicates that the current littoral occurrence of EWM in Kentuck Lake is likely below 10%. On some area lakes, EWM may cause a small amount of localized recreational impact, but overall causes minimal ecological impacts to the lake. In these instances, the respective lake group may choose to tolerate the EWM within the lake and focus their attention away from EWM management and towards other meaningful lake management activities such as shoreline condition enhancement.

The current WDNR Lake Protection Grant includes funding for monitoring of EWM in Kentuck Lake in 2016, and Onterra proposes that the KLPRD continue to monitor the EWM population in the absence of an herbicide treatment to determine if further population increases may suggest whole-lake treatment strategies are warranted, or if the population plateaus (or declines) and the KLPRD can postpone management for another year or more. It is important to note that whole-lake treatments are all-encompassing, so the level of the pretreatment EWM population has minimal impact on the outcome of the treatment. This means that if the EWM population in Kentuck Lake increases exponentially in the next few years, a whole-lake treatment would still be effective and would not have any additional costs associated.

The following outlines a proposed strategy for moving forward with EWM in Kentuck Lake:

Summer 2016

1. Onterra ecologists conduct Early-Season AIS Survey in June of 2016 and map areas of EWM (and CLP). While the EWM will not likely be at its peak growth, it will provide Onterra ecologists with an idea of how the population has changed since 2015.
2. Following the ESAIS Survey, Onterra ecologists will present their findings to the KLPRD. If the EWM population is found to have significantly expanded and increased in density and the KLPRD wishes to pursue a whole-lake treatment in the spring of 2017, Onterra ecologists will conduct a quantitative, whole-lake point-intercept survey in July or August to determine the

littoral frequency of occurrence of EWM. This survey will also serve as a pre-treatment survey for both EWM and native aquatic plants. The point-intercept survey will be funded out-of-pocket as the 2013 WDNR AIS-Early Detection and Response Grant expired on June 30, 2015. However, if the EWM population has not significantly expanded, no herbicide treatment strategies will be proposed for 2017.

3. Onterra ecologists conduct the Late-Summer EWM Peak-Biomass Survey in late-August or early-September 2016.
4. If the littoral frequency of occurrence of EWM as determined from the point-intercept survey is ≥10%, a whole-lake treatment strategy may be warranted.

Fall-Winter 2016/2017

5. If a whole-lake treatment is justified, Onterra work with the KLPRD and WDNR to develop a treatment and monitoring strategy. Onterra would also assist the KLPRD in applying for a WDNR-Established Population Control Grant (grant deadline February 1st) to aid in funding the treatment and associated monitoring costs.

Spring 2017

6. Onterra ecologists complete the Spring Pre-treatment Confirmation and Refinement Survey. This survey would potentially (but not likely) result in refinements of herbicide application areas, assessments of growth stage of aquatic plants, and documentation of thermal stratification parameters that will ultimately influence the final dosing strategy. Volunteer-based monitoring of temperature profiles would be coordinated surrounding the treatment, as well as collection of post treatment herbicide concentration samples at multiple locations and sampling intervals.
7. Whole-lake treatment application occurs followed by herbicide concentration monitoring at various locations throughout the lake.

Summer 2017

8. While not a requirement of the application permit, Onterra ecologists recommend completing a year-of-treatment whole-lake point-intercept survey and Late-Summer EWM Peak-Biomass Mapping Survey in August of 2017.

Fall-Winter 2017/2018

9. Analysis of whole-lake pre- and post-treatment data and annual report created.

Summer 2018

10. Onterra ecologists complete ESAIS Survey in June 2017 to map CLP and EWM.
11. Onterra ecologists complete year after treatment whole-lake point-intercept survey and Late-Summer EWM Peak-Biomass Mapping Survey in August of 2018.
12. Analysis of 2018 data and final report with continued EWM management strategy created.

SUMMARY & CONCLUSIONS

The first of two years of water quality monitoring in Kentuck Lake has revealed greater insight into the drivers of its highly dynamic water quality. Kentuck Lake straddles the line between deep and shallow lakes, and consequently the lake is deep enough to thermally stratify and develop anoxia yet shallow enough to allow sediment-released nutrients to be transported to surface waters where they fuel phytoplankton growth. The data collected and analyzed thus far have shown that annual phosphorus concentrations in Kentuck Lake are highly correlated with the annual duration of anoxia, and the annual duration of anoxia is going to be determined by a combination of local temperature and wind conditions. The longer Kentuck Lake remains thermally stratified, the longer anoxia persists and the more phosphorus and other nutrients are released from bottom sediments. The relationship between the duration of anoxia and phosphorus concentration was found to be exponential, indicating that phytoplankton growth is initially fueled by nutrients released from deeper, anoxic sediments, but excessive phytoplankton growth then elevates water pH causing a subsequent release of phosphorus from shallower, oxic sediments.

However, in the period from 2001-2010 when walleye abundance was unusually high and planktivore abundance was low, phosphorus concentrations were lower than predicted given six of the ten years within this period had longer estimated annual durations of anoxia. Given the high walleye/low planktivore abundance, it is hypothesized that zooplankton abundance was greater during this period and mitigated against the effects of internal nutrient loading through two primary mechanisms: 1) increased predation upon phytoplankton, and 2) decreased phosphorus availability to phytoplankton through increased phosphorus sedimentation (zooplankton fecal pellets) and phosphorus accumulation within the zooplankton community. For unknown reasons, walleye abundance declined and planktivore abundance increased in 2010, and phosphorus concentrations increased markedly in 2011 in response to an estimated duration of anoxia of approximately 11 weeks. With the moderating effect of high walleye/low planktivore abundance removed, phosphorus concentrations have largely been dictated by the annual duration of anoxia since 2011.

As discussed within the Results and Discussion Section, one of the primary goals of this project was to determine if there are any management strategies that can be employed to prevent nuisance phytoplankton blooms like those observed in 2011, 2013, and 2015. Given the working hypothesis that high walleye abundance and the resulting trophic structure was the primary driver of the low and stable phosphorus concentrations from 2001-2010, it follows that increasing the walleye population in Kentuck Lake should be a logical strategy for improving water quality. However, walleye abundance during the period from 2001-2010 was considered atypical and unsustainable, and WDNR fisheries biologists to not believe the population can be sustained at these densities. In addition, it is not fully understood what drives wide fluctuations in walleye abundance (and other fish populations) in Kentuck Lake. However, walleye fingerlings were stocked in 2014 and will be stocked again in 2016, and it is possible that the population may increase again to densities observed from 2001-2010.

Onterra ecologists are going to continue to investigate the feasibility of other management strategies including the application of alum and artificial destratification through aeration. Many obstacles to these management strategies are already present, including cost and Kentuck Lake's lower alkalinity and highly flocculent sediments. Additional data collected in 2016 along with data collected from sediment cores in 2015/16 will provide further insight into Kentuck Lake's water quality dynamics and aid in determining the feasibility of management strategies for internal nutrient loading.

The 2015 AIS surveys revealed that Kentuck Lake's CLP population still remains very small, and is likely not imparting any adverse ecological impact to the lake. However, the lake's EWM population was found to have expanded in 2015, but is still mainly comprised colonies containing *scattered* or *highly scattered* EWM. As discussed within the AIS Results Section, EWM has been shown to fluctuate in abundance from year to year in the absence of any active management. At this time, Onterra is not recommending any active management strategy for EWM in Kentuck Lake. The current WDNR Lake Protection Grant includes funding for another assessment of Kentuck Lake's EWM population in 2016. Following this assessment, Onterra ecologists will work with the KLPRD to determine an appropriate management strategy for EWM moving forward.

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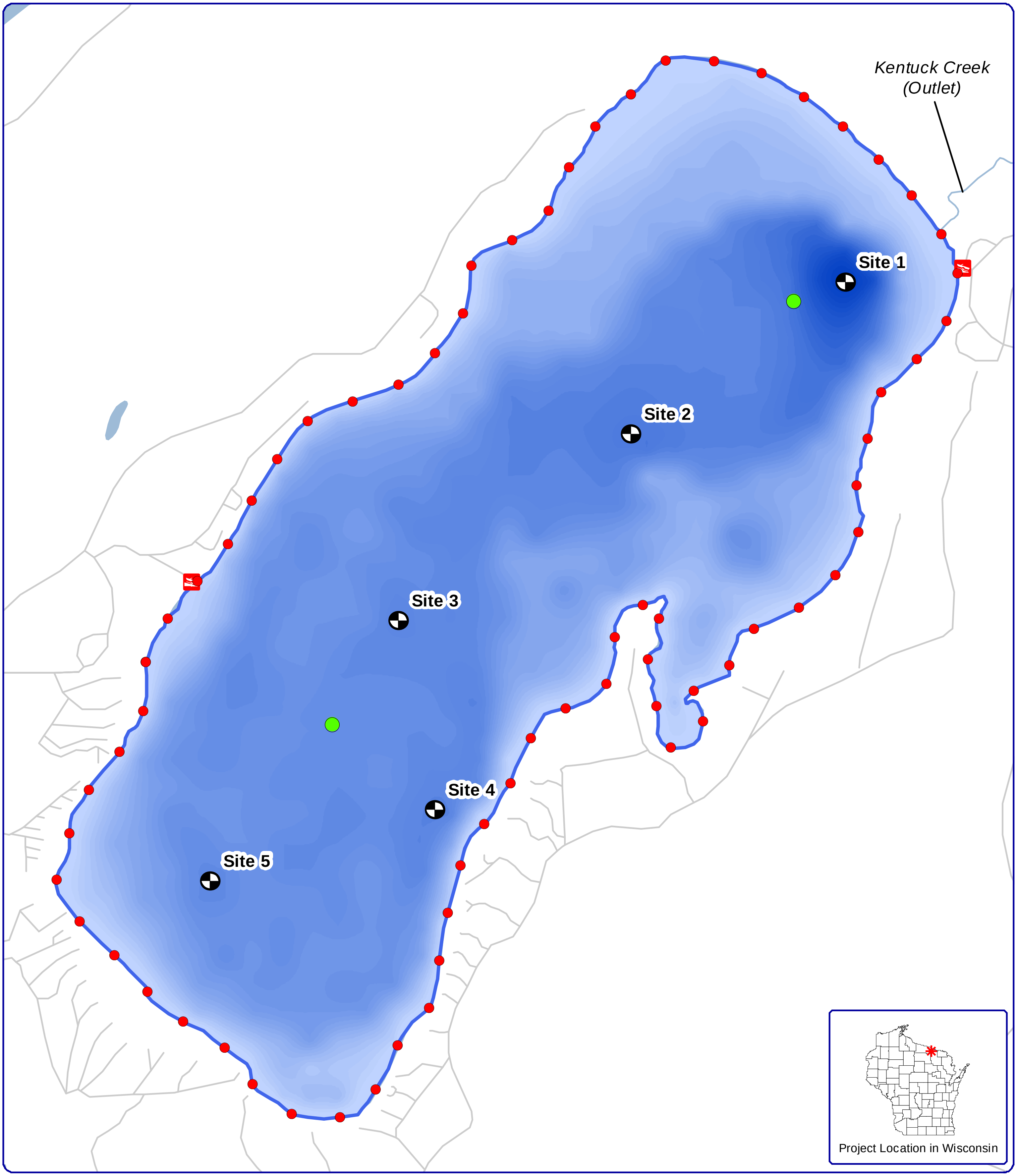

Legend

- Surface Water Sampling Location
- Groundwater Sampling Location *500-foot spacing, 67 total locations*
- Nutrient Analysis Sediment Core Collection Site
- Public Access

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Sources:
Roads and Hydro: WDNR
Bathymetry: WDNR 2011; digitized by Onterra
Map Date: January 11, 2016
Filename: Map1_Kentuck_2015_SamplingLocations.mxd

Map 1

Kentuck Lake

Forest & Vilas Counties, Wisconsin

2015 Surface & Groundwater Sampling Locations