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Fluvial seeding of cyanobacterial blooms in oligotrophic Lake Superior

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ABSTRACT

Lake Superior has recently begun experiencing cyanobacterial blooms comprised of *Dolichospermum lemmermannii* near the Apostle Islands and along the southern shore of the western arm. Little is known about the origin of these blooms. Experiments were conducted during the summers of 2017 and 2018 to identify sources of propagules and characteristics of sites that were potential sources. The 2017 experiments were conducted using a factorial design with three source zones ('River', 'Lake', and 'Harbor'), two nutrient conditions (high and low N:P), and three temperatures (15, 20, and 25°C). At the end of the experiment, cyanobacteria were most abundant from the 'River' and 'Harbor' zones at low N:P and 20 and 25°C, with *D. lemmermannii* most abundant at 20°C. Subsequently, in 2018 we evaluated 26 specific inland locations from three waterbody types ('River', 'Lake/Pond', and 'Coastal') and explored similarities among those sites that produced cyanobacteria in high abundance when samples were incubated under optimal conditions (low N:P and 25°C). Under these growing conditions, we found high cyanobacteria abundance developed in samples from river sites with low ambient temperatures and high conductivity. Field monitoring showed that Lake Superior nearshore temperatures were higher than rivers. These observations suggest that blooms of *D. lemmermannii* in Lake Superior are initiated by fluvial seeding of propagules and highlight the importance of warmer temperatures and favorable nutrient and light conditions for subsequent extensive cyanobacterial growth. We argue that the watershed is an important source of biological loading of *D. lemmermannii* to Lake Superior and that when those cells reach the nearshore where there are warmer water temperatures and increased light, they can grow in abundance to produce blooms.

1. Introduction

Cyanobacterial blooms are a major threat to the beneficial use of marine and freshwater ecosystems globally. Along with the rise in the spatial and temporal extent of nuisance cyanobacterial blooms (Huisman et al., 2018; Taranu et al., 2015), there has also been a rise in the number of blooms that are toxic (Anderson, 1989; Sukenik et al., 2015), posing a significant threat to public health. It is widely recognized that cyanobacterial blooms occur in four of the five Laurentian Great Lakes (LGL); however, less well known is that localized surface blooms of *Dolichospermum lemmermannii* (formerly known as *Anabaena lemmermannii* (Wacklin et al., 2009)) have been observed along the southern shore of the western arm in oligotrophic Lake Superior. In 2012 and 2016–2018, cyanobacterial blooms were detected in the nearshore with varying size and duration, most of which were very localized and dissipated quickly, but in 2018 the bloom persisted for approximately one week in early August and extended approximately 100 km alongshore from Duluth to the Apostle Islands region and 3 km offshore with the highest densities being observed at the shoreline (Sterner et al., 2020).

Blooms of cyanobacteria are often associated with increased loading of nutrients, particularly phosphorus (Lürling et al., 2018; Schindler, 1975; Steinberg and Hartman, 1988); however, Lake Superior is a cold oligotrophic system that is low in phosphorus (Sterner, 2010). The watershed in the region where algal blooms have been observed in Lake Superior is primarily forested, but even small differences in land cover and position influence nearshore water quality (Yurista et al., 2011). The western arm has become increasingly subject to extreme precipitation events delivering large amounts of sediment and nutrients to the nearshore (Cooney et al., 2018). Extreme storms provide large fluxes of nutrients and organic material to the lake (Cooney et al., 2018; Kling et al., 2000; Minor et al., 2014). Two 500–1000 year rainfall events that created unusually high runoff from the south shore of Lake Superior in 2012 and 2018 (Cooney et al., 2018; Sterner et al., 2020) were followed

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by cyanobacterial blooms varying in size and duration with a lag of 25 days in 2012 and 53 days in 2018. Small blooms have also occurred in 2016 and 2017 without such major rain events. Cyanobacterial blooms are also generally associated with higher water temperatures (Paerl and Huisman, 2008), and Lake Superior has been warming at the fastest rate of the LGL by maximum summer surface temperature (Austin and Colman, 2007), which may be contributing to the emergence of cyanobacterial blooms (Sterner et al., 2020). As summer epilimnetic temperatures increase, conditions can favor cyanobacteria and result in subsequent blooms (Konopka and Brock, 1978; Kosten et al., 2012; Paerl and Huisman, 2009; Robarts and Zohary, 1987). Despite being episodic and relatively small in spatial scale, algal blooms in Lake Superior have generated public concern due to their unprecedented nature and their potential impacts on public health, aesthetics, and the local tourism economy.

Coastal regions of both marine and freshwater systems can be breeding grounds for cyanobacterial blooms (Cloern, 1996; Cloern and Jassby, 2010, 2008). Coastal regions are transitional areas that are significantly influenced by watershed inputs as well as exchange with offshore waters (Cloern, 1996; Howell et al., 2012). Thus, consideration of the surrounding landscape is critical to understanding bloom development in coastal areas (Kratz et al., 1997).

Rivers are often recognized as sources of nutrients for algal growth in lakes, but seldom are inflowing waters considered as sources of living cells that initiate blooms. The riverine input of propagules to receiving lakes is referred to as "fluvial seeding" and the importance of such seeding is referred to as the algal loading hypothesis (ALH) (Conroy, 2007; Loftin et al., 2016). Discussion of the ALH so far is a relatively unexplored question (Conroy et al., 2017). Nearly all of the work done regarding this hypothesis has been in Lake Erie and its watershed, despite any evidence that this process is unique to that system (Bridgeman et al., 2012; Conroy et al., 2014; Davis et al., 2014; Kutovaya et al., 2012). Conroy et al. (2014) conducted a study evaluating major tributary inputs as sources of seed populations of cyanobacteria propagules to the Lake Erie blooms and found elevated concentrations of *Microcystis* in the rivers and observed visible riverine blooms, concluding that the rivers were playing a key role in the delivery of seed populations and bloom material to Lake Erie. In contrast, studies using DNA fingerprinting techniques have found that the cyanobacterial bloom communities found in the lake are not very similar to those sampled in the tributaries (Chaffin et al., 2014; Kutovaya et al., 2012). This disagreement in findings in Lake Erie demonstrates the lack of knowledge regarding fluvial seeding and illustrates the need for further research in this area. Answers to these questions are critical in effectively addressing current and future threats of cyanobacterial blooms in coastal waters.

In this work, we investigate the two-part question, "What are the sources of propagules leading to cyanobacterial blooms in Lake Superior, and are there identifiable characteristics among the locations that are the most likely potential sources? We hypothesize first that the watershed is an important source of cyanobacteria to Lake Superior. Second, we hypothesize that cyanobacterial growth will be highest in samples from locations with warm temperatures and low N:P ratios, as these conditions have been shown to be favorable to growth of *Dolichospermum* species, and thus are more likely to support a cyanobacterial population capable of initiating blooms (Downing et al., 2001; Paerl and Huisman, 2008). With these foci, we explore how land-lake interactions relate to the emergence of cyanobacterial blooms in Lake Superior and provide insights into mechanisms responsible for their appearance.

2. Material and methods

Laboratory experiments were conducted in the summer of 2017 and 2018 to identify potential sources of cyanobacterial blooms in western Lake Superior and to identify common characteristics of potential upstream sources. The 2017 experiment aimed to identify the major habitat types that are sources of cyanobacteria and determine whether Lake Superior blooms likely originate in the lake or were initiated in waterbodies further inland (Fig. 1). The 2018 experiment was informed by results from the 2017 experiments and examined 26 specific inland locations as potential sources of blooms. The 2018 experiments also identified characteristics of sites with higher potential for cyanobacterial growth (Fig. 1).

Experiments were designed to assess the propensity of a biological community from a given location to develop into a cyanobacterial bloom over ecologically realistic time scales. Inocula were brought into the lab and incubated in a wide range of nutrient and temperature conditions capable of supporting cyanobacteria. The abundance of major algal groups was recorded over time. This experimental approach was chosen because if a viable cyanobacterial population was present, we would likely observe growth during the experimental time frame. We concluded that inocula that did not develop blooms under bloom-promoting conditions within the experimental period were unlikely to be the source of bloom propagules *in situ*.

2.1. Sample collection and pre-processing

We defined three "zones" or habitat types during the 2017 experiments: 1) Lake Superior near the Apostle Islands ('Lake'), 2) inflowing rivers ('River'), and 3) the Duluth-Superior harbor ('Harbor'). Each of these three zones was comprised of two nearby locations: 'Lake' included two nearshore (max depth = 5–10 m) locations in Lake Superior, 'River' included the Bois Brule and Siskiwit Rivers, and 'Harbor' included Wisconsin Point and Superior Bay (Fig. 1). Water samples that were collected in each zone during the 2017 experiments were composited to increase the chances of collecting viable cyanobacterial propagules. Chemical analyses were conducted on the composited water samples. Habitat types were defined differently in 2018. The 26 sites studied in 2018 included 2 coastal waters (characterized by influence from both inland rivers/streams and Lake Superior) ('Coastal'), 11 upland lakes/ponds ('Lake/Pond'), and 13 rivers ('River'), and were selected for their accessibility and connectivity to Lake Superior.

Water, net tows, and sediment were collected from each location to be used as an inoculant in the laboratory experiments. Whole surface water was collected using a clean, acid washed carboy and filtered through a 150 μm mesh to remove large debris. Horizontal surface net tows were collected with an 80-μm mesh plankton net, and surface sediment was collected via grab sample using a 50 ml centrifuge tube or first collected using a PONAR at deeper sites and then transferred to the centrifuge tube. Net tows were used to provide an additional concentrated inoculant. Supernatant from sediment samples that had been allowed to settle for 1 min after agitation was added to capture vegetative cells or akinetes that were in the sediment. Filtered water samples, net tows, and supernatant pipetted from sediment samples from each zone were composited with their respective sample and used as an inoculant. The amount of each sample type in the inoculant was 500 ml water, 17 ml net tows, and 2 ml sediment supernatant in 2017, and 200 ml of water, 30 ml of net tow, and 10 ml of sediment supernatant in 2018. Temperature and conductivity were also measured from all sampling sites in July and August in 2018. Electrical conductivity (EC) was measured using an UBANTE™ total dissolved solids (TDS) meter in parts per million (ppm). The TDS data was converted to EC in mS/cm and then normalized to 25°C (EC_{25}) to account for temperature effects using Eq (1) (Atkins and de Paula, 2006), where T is the temperature in °C.

$$\begin{aligned} EC_{25} &= EC \big/ \left(0.889 \cdot 10^{A/B}\right), \text{ where} \\ A &= 1.37023\,(T-20) + 8.36 \cdot 10^{-4}\,(T-20)^2 \\ B &= 109 + T \end{aligned} \quad (1)$$

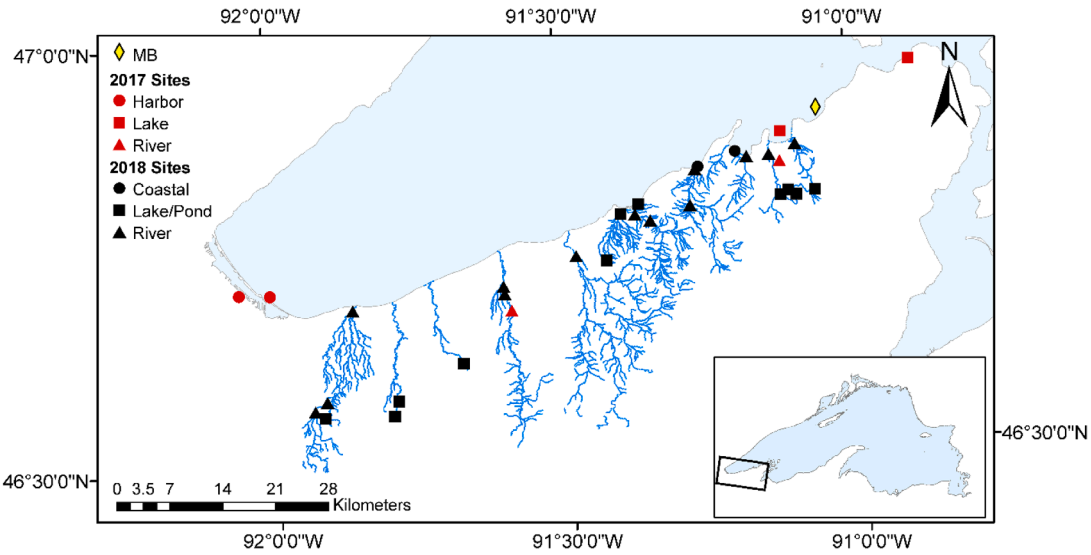

Fig. 1. Map of study sites. Sites from 2017 are shown in red with shapes denoting the zone, and 2018 sites are shown in black with shapes denoting the waterbody type. Stream systems associated with 2018 sites are also shown. A yellow diamond denotes the location of the Mawikwe Bay (MB) field sampling site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Chemical analyses

Water chemistry for 2017 was analyzed for composited water samples from each zone (3 zones) and 2018 samples were analyzed for individual sites (26 sites). Particulate fractions of carbon (POC) and nitrogen (PON) were collected on 25 mm pre-ashed GF/F filters and particulate phosphorus (PP) was collected on 25 mm pre-ashed, acid-rinsed GF/F filters. POC and PON filters were then dried at 60°C until analysis on a Costech Elemental Analyzer. Chlorophyll-a (chl-a) samples were filtered using 25 mm 0.2 μm cellulose nitrate filters and frozen in the dark until analyzed according to Welschmeyer (1994) on a Turner Design 10-AU fluorometer after extraction in 90% acetone for 20–22 hrs in the dark at 4°C. Total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), dissolved organic carbon (DOC), ammonia (NH_3), and soluble reactive silica (Si) samples were filtered through 0.22-μm filters, and frozen until analyzed, except for DOC which was acidified to pH of 2. Total phosphorus (TP) and nitrate (NO_3^-), were collected as whole-water samples and then frozen for preservation. PP, TP, and TDP samples were digested using potassium persulfate (modification of EPA method 365.1) and then analyzed on a SEAL AQ400. SRP samples were analyzed on a SEAL AQ400 on the undigested sample (EPA method 365.1, and Murphy and Riley (1962)). NO_3^- and Si were analyzed according to EPA method 353.2 and Standard Methods 4500-SiO_2, respectively, and also processed on a SEAL AQ400. DOC and TDN were analyzed using a Shimadzu TOC-Vcsh and a TNM-1 module. NH_3 was analyzed according to Taylor et al. (2007), modified from (Holmes et al., 1999), except for samples that exceeded the upper limit for those methods, in which case they were analyzed on the SEAL AQ400 using EPA method 350.1. Total nitrogen (TN) and was calculated as the sum of TDN and PON, and total organic carbon (TOC) was calculated as the sum of DOC and POC.

2.3. Laboratory conditions

In 2017, cultures were exposed to high and low nitrogen:phosphorus (TN:TP) nutrient conditions (high: 50:1, low:1.5:1) (all ratios are molar), and three temperatures (15, 20, and 25°C). This broad set of conditions was chosen to ensure we would observe cyanobacterial growth and to take a coarse look at ideal conditions because the species of cyanobacteria observed in Lake Superior blooms has been observed in both cold, oligotrophic and warm, eutrophic waters. Cultures were run in triplicate for a total of 54 samples (3 locations x 2 N:P x 3 temperatures x 3 replicates). Cultures were incubated in 1-L polycarbonate bottles (previously acid-washed), continuously bubbled slowly with room air, and exposed to approximately 250 μmol photons/m^2/s for 24 hrs per day for 21 days. However, the 15°C incubator failed 3 days before the scheduled end of the experiment, and the last growth measurement was taken 3 days prior to that; therefore, no data were used for the 15°C treatment in the final 6 days of the experiment, including samples for taxonomic identification. The experiment began with 100% environmental sample (500 ml water, 17 ml net tows, and 2 ml sediment supernatant) and then was run as a semi-continuous culture, with 150 ml of the sample volume being exchanged for new sterile media every other day, after thoroughly swirling the flasks. Culture flasks were exchanged to discourage wall growth when visible. Cultures were preserved using Lugol's fixing solution (Wetzel and Likens, 2000) and were examined for phytoplankton identification and counts (cells and colony units) by the Wisconsin State Lab of Health and Hygiene, Madison, WI. Filtered and acetone-extracted chl-a concentration was also measured in each culture at the end of the experiment.

In 2018, cultures were incubated under the same conditions as in 2017 with the following modifications. In 2018 only the low N:P and 25°C conditions were used, as they proved to be the best conditions for cyanobacterial growth in the previous year's experiment. Cultures were also run as batches, duplicates were used instead of triplicates due to space limitations, and the experiment was run for 10 days. Each bottle received 700 ml of low N:P medium and was inoculated with 200 ml of water, 30 ml of net tow, and 10 ml of sediment supernatant. In 2018, a subset of 14 samples from sites with the highest cyanobacteria biomass, as measured by the PHYTO-PAM (Walz, 2003), were examined for identification and counts due to cost limitations.

2.4. Growth media

The medium used in these experiments was originally designed for an experiment aimed at evaluating competition in natural algal communities in western Lake Superior, western Lake Erie, and Lake Huron, but here we only report its use in experimental work for Lake Superior. The Average Laurentian Great Lakes (A-LGL) Medium (Table S1) was developed for this work based on WC (Guillard, 1975), COMBO (Kilham et al., 1998), SuFr (Twiss et al., 2004), and HH—COMBO (Baer and Goulden, 1998) media, as well as major ion concentrations in the

Laurentian Great Lakes (LGL) (Chapra et al., 2012). Table S2 shows the total concentration of major ions in several media and LGLs. Each of the media were compared to LGL major ion concentrations and used to create a new media recipe, optimized for meeting LGL averages.

2.5. Growth measurements

Due to the large number of measurements necessary, a PHYTO-PAM (Walz, GmbH) was used to monitor growth along with Phyto-Win software (V 1.45) and an emission detector (ED) unit. The PHYTO-PAM employs multi-spectral fluorescence techniques to differentiate between "blue-green", "green", and "brown" algal groups which generally correspond to cyanobacteria, chlorophytes, and diatoms, respectively. High-frequency pulses of light are emitted and the excitation of chlorophylls at different wavelengths is measured to separate algal groups. This measurement has proven to provide reliable information on the abundance of major groups, provided certain precautions are taken and samples are validated (Lürling et al., 2018; Lürling et al., 2013; Lürling, 2006; Walz, 2003). To measure growth in our samples, each bottle was subsampled and left in the dark for approximately 30 min to be dark-adapted, and all PHYTO-PAM measurements were taken in the dark. Each sample was pipetted into a clean cuvette and placed in the ED unit. The gain was adjusted for each sample, and the amount of chlorophyll in each algal group was measured using chlorophyll measuring frequency (Chl (MF)) mode with a measuring frequency of 32.

The PHYTO-PAM, like virtually all measures of chlorophyll, is imperfect. Measurements rely on proper reference values, which can prove complicated when dealing with natural samples with unknown community composition. To combat this uncertainty, we took the following steps. First, we validated measurements on the PHYTO-PAM using pure cultures of *Microcystis, Ankistrodesmus*, and *Cyclotella*, to confirm that the PHYTO-PAM was correctly identifying "blue-green", "green", and "brown" algal groups, respectively (Figure S1). We also preserved samples at the end of each experiment for identification. Finally, we treated PHYTO-PAM measurements as a relative measure, meaning that we did not interpret absolute values, but instead evaluated relative changes over time (growth). Though the PHYTO-PAM should not be relied on to give precise biomass estimates, it can provide useful information on the abundances of major algal groups (Lürling et al., 2018; Lürling et al., 2013).

2.6. Data analysis

A 3-way MANOVA was used to test for differences in the response of cyanobacterial growth in the 2017 experiments. Each treatment was a different factor (temperature, zone, and N:P) and the response variables were growth rates of the blue-green, green, and brown algal groups. Because the last 15°C treatment measurements were taken 6 days before the end of the experiment, only the measurements up to day 13 were used to calculate growth rates for the 15°C treatment. Growth rates were calculated as the slope of the natural log of PHYTO-PAM measurements over time. Growth rates were calculated for the full duration of the experiment for all treatments as well as up to day 13 to determine if there were differences in results under the two windows of time and none were found. We also tested for differences among treatments using taxonomic observations of the relative abundance of *D. lemmermannii* by applying the non-parametric Kruskal-Wallis and Kruskal-Wallis multiple comparisons (adjusted to avoid type I errors (Siegel and Castellan, 1988)) tests using the *stats* and *pgirmess* packages in R (CoreTeam, 2017), respectively.

Due to the spatial nature of the 2018 data, we tested for spatial autocorrelation in site characteristics and experimentally derived growth rates by calculating Moran's I (Moran, 1950) using the inverse distance squared method in ArcMAP (V 10.4.1). Moran's I tests for the tendency of units to be similar to its neighbors by calculating a correlation coefficient ranging from −1 to 1, where values near 1, 0, and −1, are clustered, random, or dispersed, respectively. We found that only one response variable showed minor spatial autocorrelation, but many of the potential predictors were autocorrelated as clusters. We conducted a visual assessment of semivariograms with varied correlation structures to assess the severity of autocorrelation and concluded that corrective measures were not required. We applied paired two-sided t-tests to test for differences in site characteristics and growth rate between months, and one-way ANOVAs to test for differences among waterbody types. If differences were found from the ANOVA test, the Tukey HSD test was applied to identify which groups were different. These tests were performed using the *stats* package in R (CoreTeam, 2017). Four parameters (PP, NH_3, N:P, and chl-a) did not meet ANOVA criteria for equal variance, so the non-parametric Kruskal-Wallis and Kruskal-Wallis multiple comparison tests were applied to those parameters to test for differences among waterbody types.

To identify relationships between growth rates and site characteristics we applied a model selection approach using the Akaike Information Criterion (AIC). Simple linear regressions were used initially to determine which parameters should be included in the selection process, and multiple linear regressions were used for model selection. One outlier was removed from the blue-green growth rate data to evaluate regression models, and the mean growth rate from July and August experiments was used in the analyses. Non-normally distributed parameters were transformed when necessary. Unless otherwise noted, all analyses were conducted in R for Statistical Computing (V 3.5.0) using the *MASS* and *nlme* packages.

3. Results

3.1. Site characteristics

In 2017, water chemistry for composited samples was analyzed for each of the three zones ('River', 'Lake', and 'Harbor') (Table 3). The most notable difference in water chemistry among the zones was in NO_3^- and Si, where the 'River' zone had much lower and higher concentrations than the 'Lake' and 'Harbor' zones, respectively. Also notable is the lower chl-a observed in the 'River' zone compared to the others. We compared water quality data from 2018 for each waterbody type ('River', 'Lake/Pond', and 'Coastal') using boxplots to illustrate differences among the three groups (Fig. 2) in addition to testing for statistical differences (Table 1). Note that the 'Coastal' waterbody type in the 2018 experiment refers to sites that are influenced by Lake Superior and inland rivers/streams. TP, TDP, and PP were similar among groups (Fig. 2C, I, and F), as were particulate fractions of carbon and nitrogen (Fig. 2D, E). TOC, DOC, TN, and TDN were lower in the 'River' than in the 'Coastal' and 'Lake/Pond' waterbody types (Fig. 2A, G, B, and H), with statistical differences ($p<0.05$) between the 'Lake/Pond' and 'River' for all except TN. The N:P ratio was also lower in the 'River' group and had a much narrower range (Fig. 2M), and NO_3^- was highest in the 'River' group (Fig. 2K). The chl-a was highest in the 'Coastal' waterbody type, followed by 'Lake/Pond' and then 'River', with statistical differences between the 'Coastal' and 'River', and the 'River' and 'Lake/Pond' waterbody types. Temperature was lowest in the 'River' group and highest in the 'Lake/Pond' waterbodies (Fig. 2O), with a statistical difference between the 'Lake/Pond' and 'River' waterbodies. EC_{25} was highest in the 'Coastal' waterbodies (Fig. 2P), followed by the 'River' and then 'Lake/Pond'. Significant ($p<0.05$) differences between July and August were observed for all parameters except for N:P, POC, PON, PP, chl-a, and growth rate (Table 2).

3.2. Experimental results

The results of the 2017 experiments showed that cyanobacteria grew fastest and achieved the highest abundance in low N:P conditions at 20 and 25°C from the 'River' and 'Harbor' zones (Fig. 3N, O, Q, and R, Table 4). Significant differences in main effects and all two-way

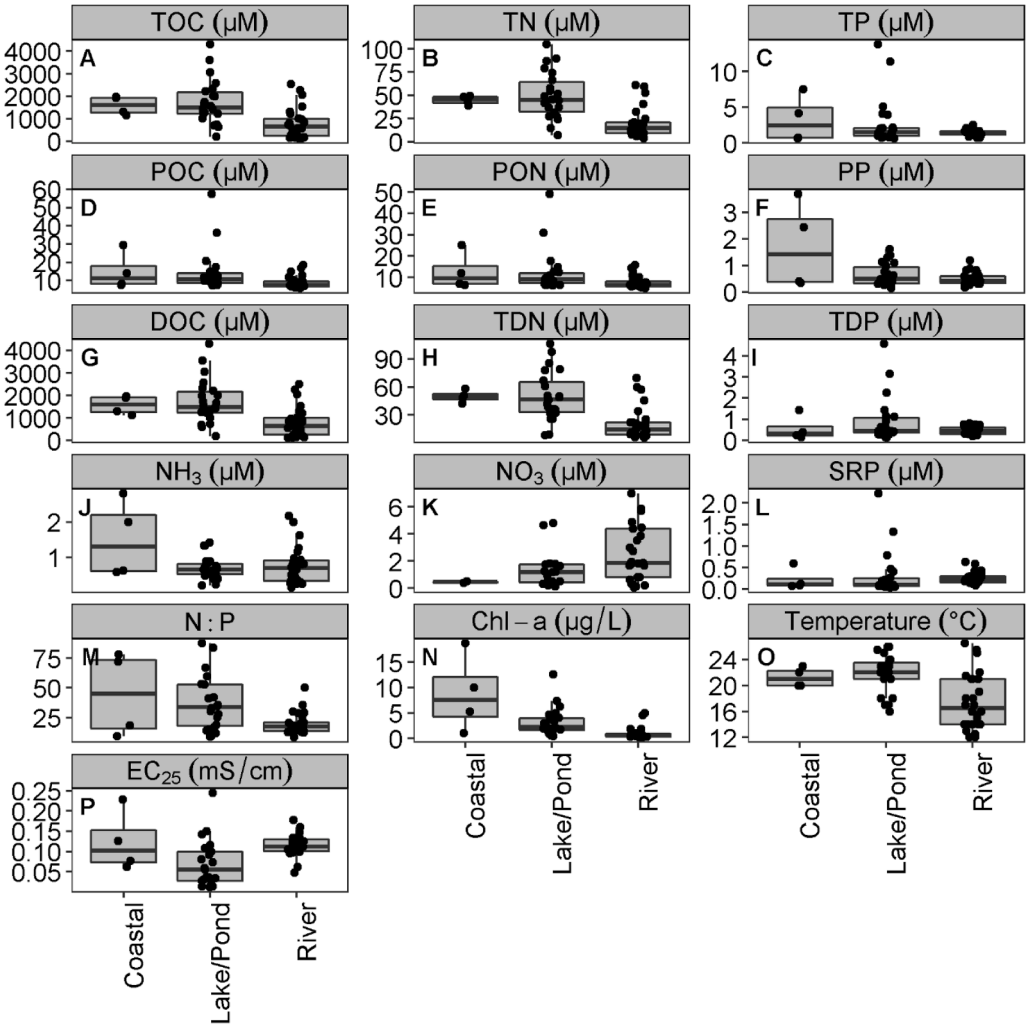

Fig. 2. Boxplots of water quality parameters for each waterbody type in the 2018 experiments. The central box line indicates the median, the upper and lower box hinges correspond to the 75% and 25% quartiles, respectively, and the whiskers correspond to 1.5 times the interquartile range. Black dots indicate sample points.

interactions were observed for cyanobacterial growth (Table 4). Temperature alone had a significant effect on the "green" algal group, and all treatments (temperature and nutrient conditions) and interactions of treatments had a significant effect on the "brown" algal group except for the interactions between N:P and temperature, and location and temperature.

Taxonomic identification showed that cyanobacteria were present in cultures with high "blue-green" growth rates, and that they were in the highest abundance in the Low N:P and the 20°C and 25°C treatments (Fig. 4). The results of the Kruskal-Wallis tests (Table S3) agree with those found in the MANOVA, that there were significant differences in the relative abundance of *D. lemmermannii* between the high and low N:P treatments and the 20°C and 25°C temperature treatments. However, there were not always significant differences among locations for a given nutrient and temperature treatment combination. The relative abundance between the 'River' and 'Harbor' locations in the 20°C and low N:P treatment conditions were not different, and locations were not different in both temperature levels in the high N:P treatment with exception to —20°C-High-River and —25°C-High-Lake, where the abundance was negligible. The taxonomic results support those found using the PHYTO-PAM and also provide additional information. The species of cyanobacteria found in the 'River' and 'Harbor' locations is the same species observed in Lake Superior blooms, *D. lemmermannii,* and while cyanobacterial growth was high in both 20°C and 25°C at low N:P, *D. lemmermannii* specifically grew in the greatest relative abundance in 20°C and low N:P.

The results from the 2017 experiments indicated that upland waters contribute to cyanobacterial blooms in the lake; therefore, in 2018 we evaluated 26 specific locations ('River', 'Lake/Pond', and 'Coastal' waterbodies) as potential sources of cyanobacterial propagules. We also identified characteristics shared among sites that could be potential sources. The results from 2018 showed that sites with the highest cyanobacterial growth rates were widely scattered across the study region (Fig. 5). Results of the spatial autocorrelation analyses showed that for the "blue-green" and "green" algal groups using Moran's I were not significant, and some slight clustering was present in the "brown" algal group (Green: Moran's I = −0.07, $p = 0.9$; Blue-green: Moran's I = 0.18, $p = 0.3$; Brown: Moran's I = 0.53, $p = 0.004$).

Out of 17 water quality parameters tested, four were significant predictors for cyanobacterial growth: temperature ($p = 0.006$, $R^2 = 0.26$, slope = −0.026), EC_{25} ($p = 0.0004$, $R^2 = 0.41$, slope = 2.70), ln (SRP) ($p = 0.002$, $R^2 = 0.34$, slope = 0.17), and ln(N:P) ($p = 0.02$, $R^2 = 0.20$, slope = −0.17). These results mean that cyanobacteria grew fastest under laboratory conditions from sites that had higher conductivity and SRP and lower temperature and N:P. Those parameters were then used with AIC and backward model selection, and the optimized model included only *in situ* EC_{25} and temperature (Table 5). Model selection could only be performed using additive models (*i.e.* without interactions), as the number of comparisons with multiplicative models would be too computationally expensive. We tested the best additive

Table 1
Results of one-way ANOVA with Tukey HSD and Kruskal Wallis tests for differences in site parameters among waterbody types in 2018. No F-value was provided for Kruskal-Wallis tests (PP, NH_3, N:P, and Chl-a), and no p-values were provided for Kruskal-Wallis multiple comparisons.

Parameter	F value	p-value	Comparison	p-value	Significant ($p<0.05$)
TOC	5.7	0.0094	Lake/Pond-Coastal	0.96	
			River-Coastal	0.31	
			River-Lake/Pond	0.0080	*
TN	0.48	0.50	–	–	
TP	1.2	0.31	–	–	
POC	2.6	0.093	–	–	
PON	2.6	0.093	–	–	
PP	–	0.75	–	–	
DOC	5.7	0.0095	Lake/Pond-Coastal	0.95	
			River-Coastal	0.31	
			River-Lake/Pond	0.0081	*
TDN	6.2	0.0067	Lake/Pond-Coastal	1.0	
			River-Coastal	0.19	
			River-Lake/Pond	0.0067	*
TDP	1.3	0.30	–	–	
NH_3	–	0.70	–	–	
NO_3^-	3.2	0.090	–	–	
SRP	0.15	0.86	–	–	
N:P	–	0.053			
Temp	5.6	0.011	Lake/Pond-Coastal	0.95	
			River-Coastal	0.32	
			River-Lake/Pond	0.0093	*
chl-a	–	0.00095	Lake/Pond-Coastal	–	
			River-Coastal	–	*
			River-Lake/Pond	–	*
EC25	3.4	0.052	–	–	
Cyanobacterial growth rate (d^{-1})	1.9	0.17	–	–	

Table 2
Results of the paired two-sided *t*-test for differences in site parameters and laboratory cyanobacterial growth rates between July and August.

Parameter	t-value	p-value	Significant ($p<0.05$)
DOC (μM)	8.6314	5.7×10^{-09}	*
TDN (μM)	3.27	3.1×10^{-3}	*
NH_3 (μM)	2.11	0.045	*
NO_3 (μM)	6.0218	1.1×10^{-5}	*
TDP (μM)	3.38	2.4×10^{-3}	*
SRP (μM)	2.53	0.018	*
TOC (μM)	7.96	2.8×10^{-8}	*
TN (μM)	2.3	0.030	*
TP (μM)	2.29	0.031	*
N:P (μM)	−0.9	0.36	
chl-a (μg/L)	1.195	0.24	
POC (μM)	−1.19	0.067	
PON (μM)	−1.19	0.067	
PP (μM)	0.7	0.49	
Temperature (°C)	5.594	9.3×10^{-6}	*
EC_{25} (mS/cm)	−3.474	1.9×10^{-3}	*
Cyanobacterial growth rate (d^{-1})	0.55	0.59	

Table 3
Water chemistry means and standard deviations (analytical variation across a given sample) for composited water samples from each zone in the 2017 experiments.

Parameter	Lake Mean	SD	River Mean	SD	Harbor Mean	SD
chl-a (μg/L)	1.92	0.02	0.79	0.01	3.47	0.34
POC (μM)	26.49	1.37	43.30	1.19	41.11	3.17
PON (μM)	3.02	0.14	4.66	0.68	4.95	0.06
NH_3 (μM)	0.85	0.10	0.21	0.01	1.44	0.11
NO_3^- (μM)	25.81	-	0.95	-	30.27	-
Si (μM)	25.42	0.04	121.45	0.71	50.53	0.02
PP (μM)	0.10	0.00	0.27	0.00	0.32	0.03
TDP (μM)	0.12	-	0.01	-	0.34	-
SRP (μM)	0.05	-	0.37	-	0.26	-
TP (μM)	0.16	-	0.52	-	0.76	-

model for interaction terms and found a significant interaction between temperature and EC_{25}, which resulted in a final model with $R^2 = 0.57$ (Table 6). We also tested for the impact of waterbody type ('River', 'Lake/Pond', and 'Coastal') in the model and found that there was no significant effect.

Preserved samples from 14 sites with the highest final "blue-green" biomass were surveyed to validate measurements using the PHYTO-PAM. This group included one 'Coastal' waterbody, four 'Lake/Pond', and nine 'River' sites. Seven of these samples were found to be comprised of over 75% *D. lemmermannii* and three sites had greater than 50% (Fig. 6). Ten out of the total 14 sites were also among sites with the highest experimental growth rates for "blue-green" algae (calculated using PHYTO-PAM data). Three out of the four sites that were not among the highest observed growth rates were 'Lake/Pond' waterbody types, and two of those four sites were comprised of less than 25% *D. lemmermannii* (1 'River' and 1 'Lake/Pond').

4. Discussion

The appearance of cyanobacterial blooms in Lake Superior is surprising, given that it is a cold, oligotrophic system with much less anthropogenic nutrient inputs relative to other lakes where blooms are often observed. The current paradigm for the role of rivers in lake cyanobacterial blooms is that rivers supply nutrients to algal populations in the receiving lakes (Bridgeman et al., 2012; Carpenter et al., 1998; Conroy et al., 2017). However, this study points to other potentially important roles for inflowing waters. This work is a critical step toward understanding why we have begun observing cyanobacterial blooms in Lake Superior and it indicates a likely important role of upland waterbodies, particularly rivers, in seeding blooms. There is a wealth of evidence in marine and freshwater systems that bloom-forming cyanobacteria can persist in rivers and estuaries (Conroy et al., 2017, 2014; Paerl and Otten, 2013; Reif, 1939; Schwartz, 2007; Steinberg and Hartman, 1988). What is not as well understood is how the presence of cyanobacteria in rivers may affect downstream lakes or ponds. The lack of research on this topic may be due to the greater likelihood of observing cyanobacterial blooms in lentic environments than in their lotic counterparts or because riverine conditions (including high turbulence, low light, short residence times, and cooler temperatures) are not typically associated with high algal growth rates (Paerl and Otten, 2013). Nevertheless, a recent study of 11 major rivers in the US showed that cyanobacteria were present in all rivers and comprised up to 52% of the community composition (Graham et al., 2020). The present study shows that rivers may play a critical role in the promotion of newly occurring cyanobacterial blooms in Lake Superior.

Our work assumes that samples producing high cyanobacterial growth under ideal laboratory conditions also have potential to seed blooms under favorable environmental conditions. This approach has the advantage of not relying on quantifying potentially rare populations

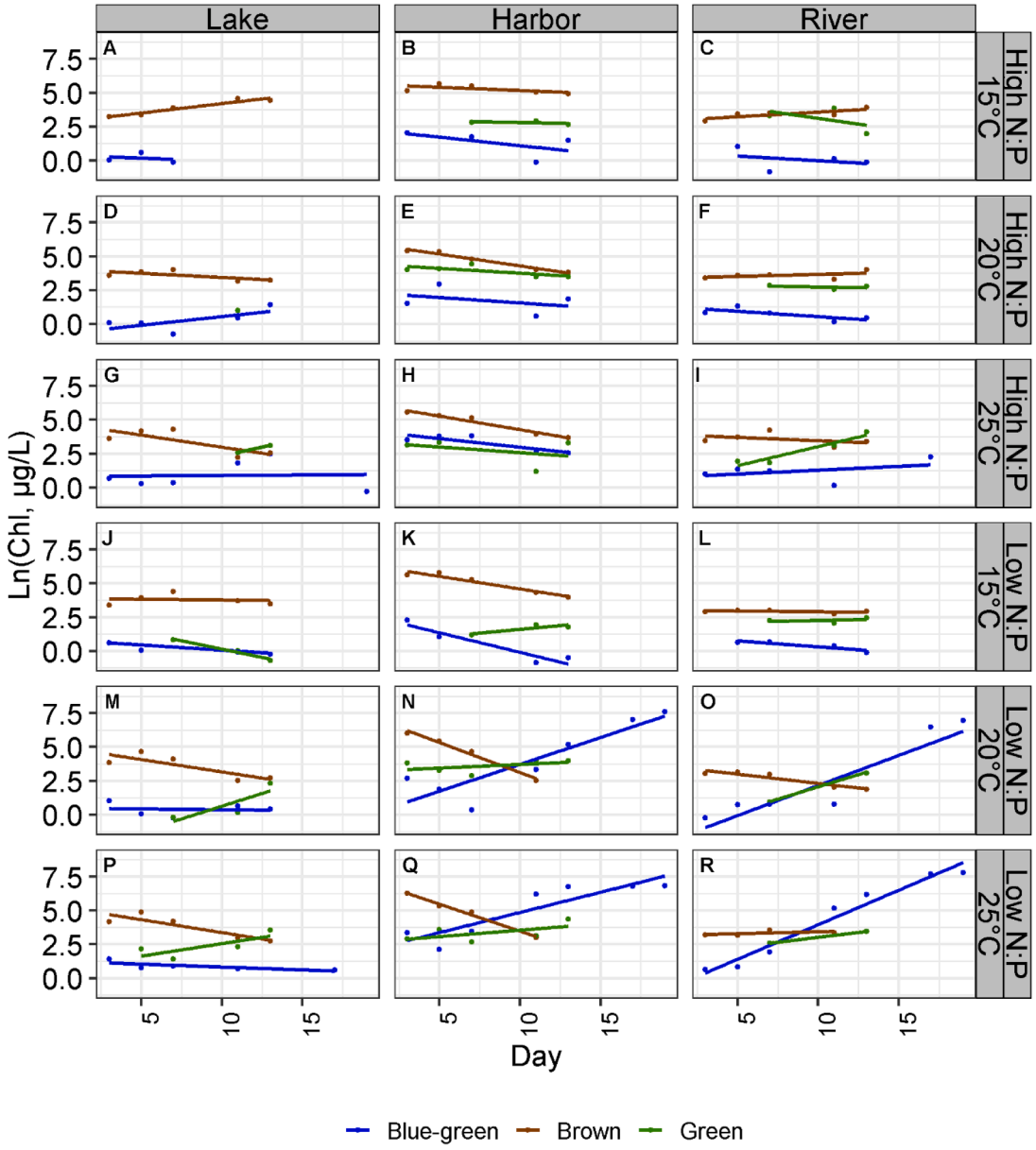

Fig. 3. The natural log of chlorophyll concentration of the blue-green, green, and brown algal groups throughout the experiment for each treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

that initiate blooms, and it avoids the potential that a given algal taxon may be present but not be viable, issues that could arise under a simple sampling regime no matter how sensitive it may be. The experiments we conducted in 2017 and 2018 provided insight into the origins and the conditions that promote cyanobacterial blooms along the south shore of Lake Superior. The conclusions we draw from the 2017 experiment are that bloom-forming cyanobacteria grew abundantly in low N:P in combination with moderate to high temperature, and more importantly, only from 'River' and 'Harbor' locations and not from the 'Lake' samples. This finding provides evidence that blooms are unlikely to have been sourced just from the lake, and that upstream sources are likely important contributors of living cells to generate blooms. The subsequent 2018 work then began to narrow down characteristics of upstream environments that are most likely to be involved in the generation and delivery of algal seed populations for Lake Superior blooms.

Cyanobacteria in the Nostocales order are diazotrophic; thus, it is not surprising that *D. lemmermannii* grew better than the other algal groups in these nitrogen-limiting conditions during laboratory experiments. However, growth under lab conditions does not necessarily indicate the conditions most responsible for high growth in a natural setting. PHYTO-PAM measurements in both 2017 and 2018 did not show a dominance of cyanobacteria in river samples at the beginning of the experiments. In fact, the initial total biomass of samples was low and typically dominated by the "brown" algal group while the "blue-green" only comprised approximately 1–5% of the total biomass (Fig. 3). This was also true for 'Lake' and 'Harbor' samples from 2017. This may mean that while similar concentrations of cyanobacteria were initially present from all zones, cells from 'River' and 'Harbor' samples were more likely *D. lemmermannii* and samples from the 'Lake' sites were not, given that this species did not grow in high abundance in any treatment combination from this location. An analysis of Lake Superior nearshore and offshore phytoplankton survey data from 2011 to 2016 (Kovalenko et al., 2019) found that diatoms accounted for over 75% of the total biovolume for all samples areas, with some codominance in 2016. Codominance of *D. lemmermannii* (by biovolume) was observed for part of 2016 at one site in central Lake Superior offshore (depth > 200 m),

Table 4
Results of the 3-way MANOVA test for each algal group using growth rate as the response variable. P-values marked with an asterisk (*) are significant at α=0.05).

Treatment	Blue-green algae		Green algae		Brown algae	
	F value	p-value	F value	p-value	F value	p-value
Temp	6.09	0.0079*	5.01	0.016*	21.6	6.5×10^{-6}*
Location	7.71	0.0029*	2.34	0.12	79.7	8.4×10^{-11}*
N:P	13.0	0.0015*	0.940	0.34	66.2	4.5×10^{-8}*
Location x Temp	2.92	0.044*	1.15	0.36	1.89	0.15
N:P x Temp	12.7	0.00022*	0.380	0.69	2.38	0.12
N:P x Location	8.03	0.0024	0.290	0.75	18.1	2.2×10^{-5}*
N:P x Location x Temp	1.21	0.31	0.0260	0.97	6.10	0.0078*

indicating that *D. lemmermannii* is not prominent in the offshore Lake Superior phytoplankton community. 'Harbor' samples did grow cyanobacteria in abundance, but a closer look at the taxonomic data showed that the species present from 'Harbor' samples was not predominately *D. lemmermannii* with exception to the low N:P and 20°C treatment. Analysis of 2018 data revealed that 'River' sites had the lowest total N:P ratio of the three waterbody types (mean of approximately 19:1); therefore, from an N:P standpoint, rivers feeding into nearshore Lake Superior where blooms have been observed may provide conditions to promote diazotrophic cyanobacteria like *D. lemmermannii*. Consequently, even though the relative abundance of cyanobacteria is not greatest in the overall community composition, the conditions in rivers support a community of cyanobacteria that is capable of proliferating under laboratory conditions.

In addition to a clear N:P preference, higher water temperatures stimulated growth during the 2017 experiments, and more specifically, taxonomic data showed that the 20°C treatment resulted in higher *D. lemmermannii* abundances than 25°C. We also observed high cyanobacterial growth rates in the 2018 experiments under laboratory conditions (25°C and low N:P), but the sites that produced those high growth rates under laboratory conditions, namely rivers, were characterized by low water temperatures *in situ* (12–26°C, mean of approx. 17°C). These findings indicate that fast-growing cyanobacteria originated in colder waters, but our 2017 experiments showed that 15°C was too cold for extensive growth of *D. lemmermannii*, even at optimal nutrient conditions. Evidence from subalpine lakes has shown the ability of *D. lemmermannii* to persist in cold water temperature systems and then grow extensively as temperatures warm to greater than 15°C (Salmaso et al., 2015), which is consistent with the temperature dependence we observed in our study.

Routine water quality monitoring during the 2018 field season (May-October) showed that the lake is typically warmer than inflowing rivers by several degrees (Fig. 7). Cooler river temperatures can be attributed to several factors including size, flowrate, canopy cover, hydrologic inputs, and drainage pattern, whereas water in shallow nearshore embayments is exposed to direct light and the surface layer warms throughout the summer, resulting in higher surface water temperatures in the nearshore than in rivers. Temperature has been identified as a primary control on *D. lemmermannii* growth in other cold, oligotrophic lakes (Callieri et al., 2014; Capelli et al., 2017; Salmaso et al., 2015, 2012), and has also been identified as an important driver in Lake Superior (Sterner et al., 2020). So, while rivers are more likely to yield viable *D. lemmermannii* cells that could initiate growth in the lake, the warmer temperature conditions in Lake Superior's nearshore are generally more favorable for growing high concentrations of biomass.

Light conditions in the nearshore and rivers were not measured during routine monitoring; however, river plumes in Lake Superior have been studied and it has been shown that light is lower in Lake Superior waters impacted by river plumes (Cooney et al., 2018; Minor et al., 2014), indicating that rivers have higher turbidity and thus, lower light compared to the nearshore. Detenbeck et al. (2004) also document high turbidity in south shore streams due to forest type and erosion of fine sediment with mean field turbidity measurements near approximately 100 NTU, which corresponds to an extinction coefficient of 5 m^{-1} (Brown, 1984). Typical Lake Superior values are less than approximately 0.4 m^{-1} (Cooney et al., 2018; Sterner, 2010). We hypothesize that when *D. lemmermannii* propagules from rivers with low temperature and light

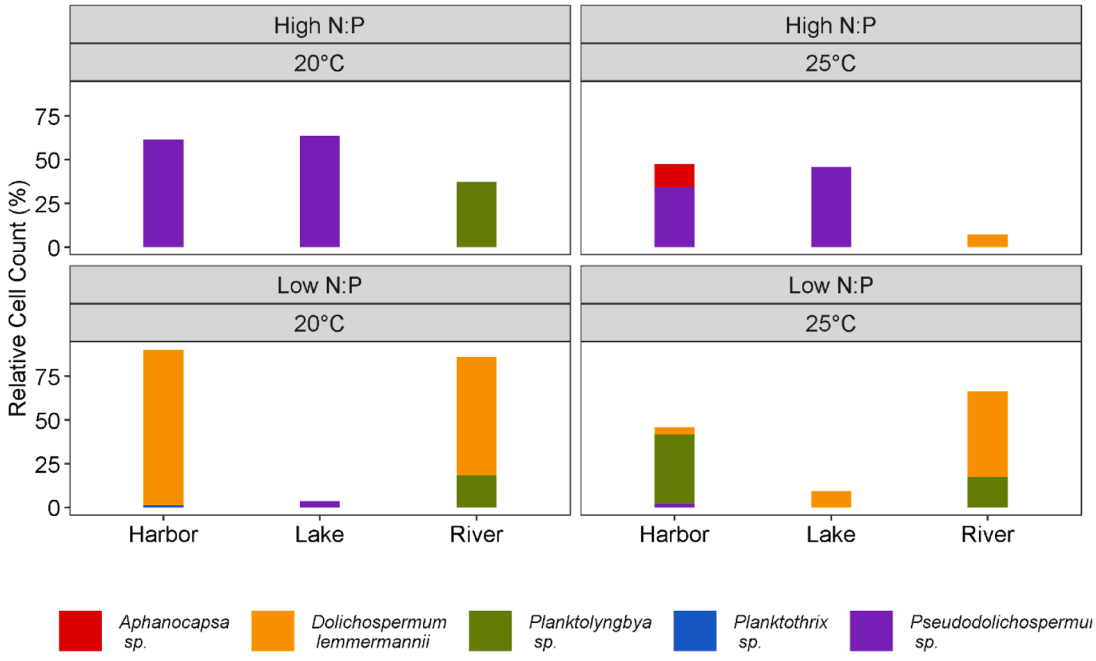

Fig. 4. Relative abundance of cyanobacterial species by treatment combinations. The 15°C treatment was not analyzed due to the mechanical failure of the incubator.

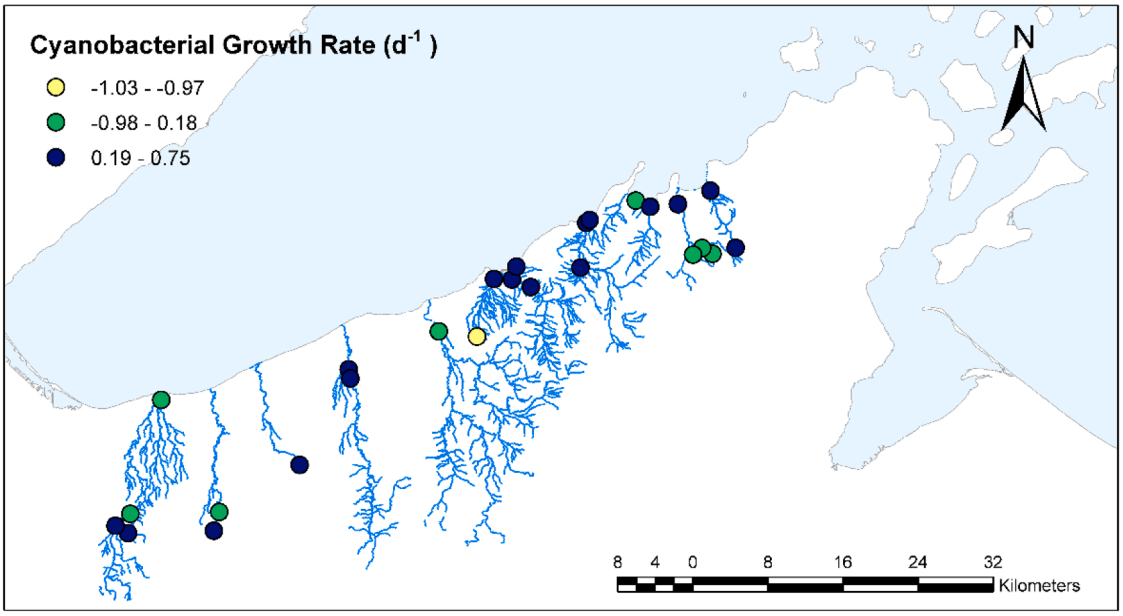

Fig. 5. Sampling locations of experimental cyanobacterial growth rate for July and August. Growth rates are divided by natural breaks.

Table 5
Results of cyanobacterial growth regression model selection using the Akaike Information Criterion (AIC). The lowest value indicates the best model. Soluble reactive phosphorus, N:P ratio, temperature, and conductivity are denoted by SRP, NP, Temp, and EC_{25}, respectively.

Model ($N = 24$)	AIC
Ln(SRP) + Ln(NP) + Temp + EC_{25}	−85.45
Ln(SRP) + Temp + EC_{25}	−87.41
Temp + EC_{25}	−88.58
EC_{25}	−88.84

Table 6
Summary of the interaction model using the optimal cyanobacterial growth model using Akaike Information Criterion AIC: temperature (Temp) and conductivity (EC_{25}).

Parameter	Slope Estimate	p-value	Overall R^2	Overall p-value
EC_{25}	15.7	0.00384	0.57	0.00015
Temp	0.0542	0.0384		
Temp x EC_{25}	0.227	0.00990		

conditions reach the nearshore of Lake Superior, where temperature is seasonally warmer and there is greater light exposure, they can grow in greater abundance and ultimately produce blooms. Further work is needed, however, to understand the relative contributions of temperature, light, and nutrients, as well as hydrodynamics, in promoting cyanobacterial blooms once the cells have entered the lake. Additional work is also needed to determine whether rivers are supporting a community of *D. lemmermannii* or acting as a transport mechanism from other upstream areas.

The other predictor that helped explain which sites are likely sources of cyanobacterial propagules to Lake Superior was higher conductivity. Conductivity is related to salinity and pH, both of which can affect cyanobacterial growth. There is some evidence that salinity can inhibit growth (Apte et al., 1987; Rai, 1990), but the concentrations used in these studies (55 mM – 500 mM NaCl) typically far exceed those we observed in our study sites (approximately 2.2 mM NaCl, assuming that all of the conductivity can be attributed to salinity). Similarly, pH conditions in five south shore Lake Superior streams were circumneutral during multiple sampling events in 2016, indicating that pH is not likely a factor influencing algal growth (J. Delvaux, unpublished data). Hence, it is unlikely that the levels of conductivity we observed are high enough to have an inhibitory effect on growth, and the more likely explanation is that conductivity is related to some other unmeasured parameter that is related to sites with high potential. Conductivity may be related to the geological substrates in the region. The predominant soil types in the study area are Alfisols and Spodosols, commonly referred to as 'red clay' due to their rich iron content, and are highly susceptible to erosion and the main contributor of dissolved ions to Lake Superior (Cooney et al., 2018; Stortz et al., 1976). We considered the high iron content of the soil as a possible linkage between high potential sites and increased conductivity, but the iron in these soils is present as iron oxides and are highly insoluble in water, and so is not likely stimulating algal growth (Tonello et al., 2019).

While we have provided a first look at potential sources of cyanobacterial blooms in Lake Superior, additional work is needed to better understand the appearance of these blooms and their drivers. Future work should include using molecular tools, such as the methods used in Kutovaya et al. (2012), to identify sources of *D. lemmermannii* to Lake Superior. Further, additional work is needed to identify the role of hydrodynamic processes (*e.g.*, development of prevailing longshore currents, surface slick dynamics, upwelling, and wind/wave action) in the formation of surface scums. We also recommend continued monitoring of the lake and surrounding watershed to provide a detailed record of seasonal processes that may contribute to blooms, as well as interannual variability.

To summarize, evidence from this study strongly suggests that rivers play a critical role in priming cyanobacterial blooms on the south shore of Lake Superior, with some additional evidence that shifting growth conditions between rivers and lakes are also necessary for bloom formation. Our data showed that *D. lemmermannii* grew under laboratory conditions from water samples that were collected from inland locations, particularly rivers, and grew best in 20°C and low N:P conditions. Somewhat unexpectedly, sites that had the greatest cyanobacterial growth under optimal laboratory conditions were characterized by relatively low temperatures and high conductivity. We ascertain that rivers produce cells of *D. lemmermannii* capable of initiating extensive growth, but cold water temperatures and low light conditions inhibit overall growth in these habitats. When these cells enter the Lake Superior nearshore, where temperatures are seasonally warmer and light levels are higher, conditions are more favorable for growth.

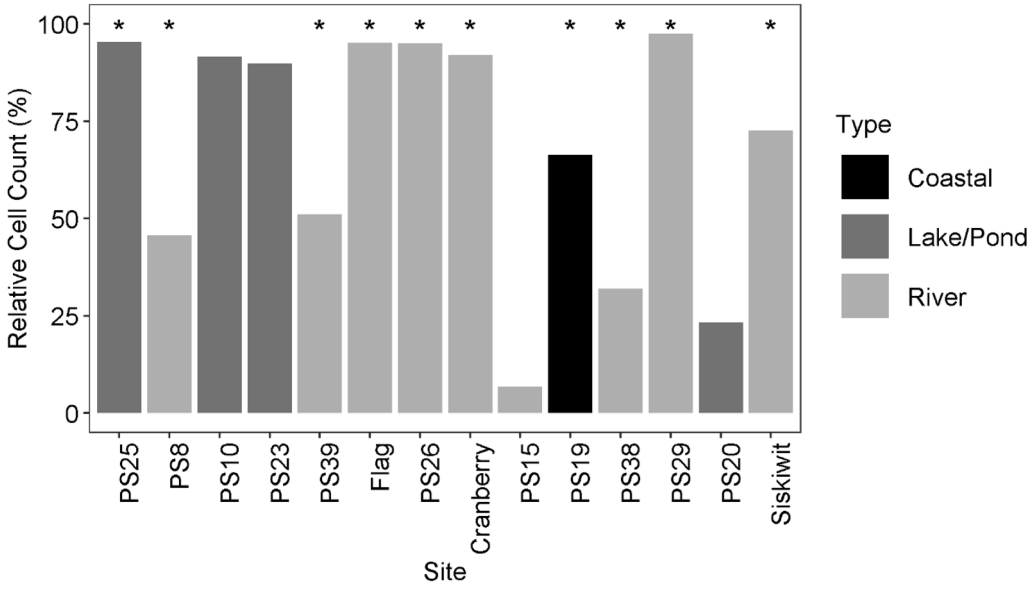

Fig. 6. Relative abundance of *D. lemmermannii* in 14 sites (ordered from west to east) with greatest cyanobacterial growth in culture. Colors indicate waterbody type and asterisks (*) denote the 10 sites that also had the highest cyanobacterial growth rates.

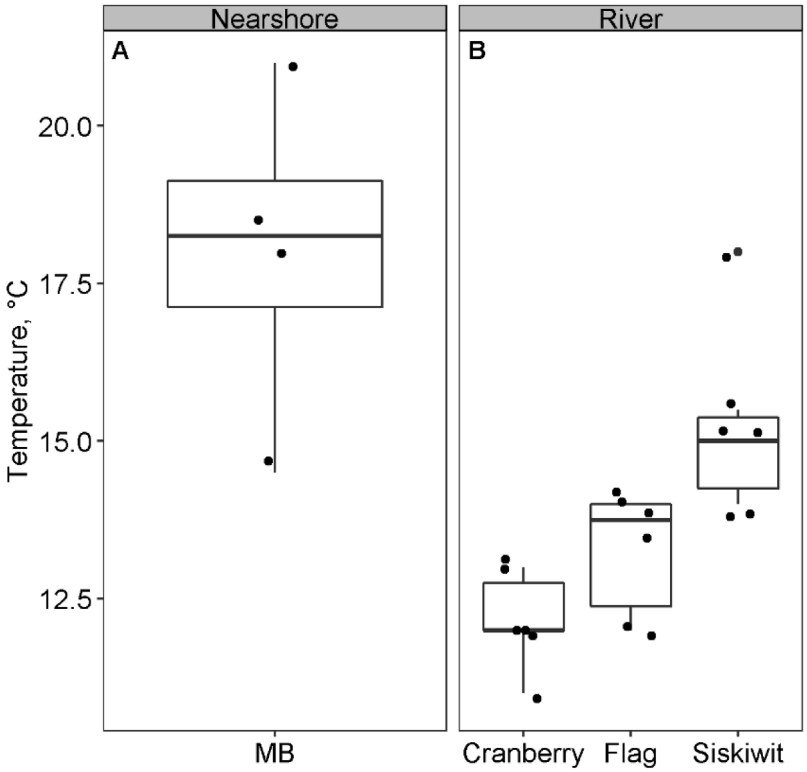

Fig. 7. Boxplots of July and August surface water temperature for nearshore Lake Superior (MB) and a subset of rivers included in the 2018 experiments. The central box line indicates the median, the upper and lower box hinges correspond to the 75% and 25% quartiles, respectively, and the whiskers correspond to 1.5 times the interquartile range. Black dots indicate sample points.

Based on this evidence, we argue that rivers provide a consistent source of viable propagules of *D. lemmermannii* to Lake Superior and that the appearance of blooms in the lake is dependent on in-lake conditions. Lake Superior has already begun experiencing significant warming (Austin and Colman, 2007) and extreme precipitation events (Cooney et al., 2018), and these trends are expected to continue. If water temperatures and nutrient inputs continue to increase, along with consistent seeding from rivers, cyanobacterial blooms may become a persistent feature of Lake Superior's nearshore.

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Author contribution statement

All authors have read and approved the final submission. KLR developed experimental design, carried out laboratory and field research, conducted data analysis, and prepared this manuscript. RWS advised on experimental design and data analysis and provided significant comments on the manuscript. BLM advised on experimental design, assisted with data collection, and commented on data analyses and manuscript. SLB assisted with field and laboratory research, sample analysis, and commented on the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2020.101941.

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