WRP Project: Identifying, predicting and managing the occurrence of harmful cyanobacterial blooms in Wyoming reservoirs

2020-2023

PI: Sarah Collins, UW Dept of Zoology and Physiology
Co-PIs: William Fetzer, UW Dept of Zoology and Physiology
            Lindsay Patterson, Wyoming DEQ
            Matt Ross, Colorado State U. Dept of Ecosystem Science & Sustainability
            Annika Walters, UW Co-op Unit and Dept Zoology and Physiology

Abstract: Harmful cyanobacterial blooms (HCBs) can lead to water quality problems and adverse health effects in humans, livestock, wildlife, and pets. The Wyoming Department of Environmental Quality (WY DEQ), Wyoming Department of Health, and the Wyoming Livestock Board have identified many reservoirs across Wyoming that have experienced HCBs in recent years; and have increased from only a few water bodies in 2017 to >40 water bodies in 2022 and 2023. Nutrients and climate have been identified as the primary drivers of blooms in other parts of the world, but research specifically targeted toward this area of the United States was needed to identify, predict, and manage HCBs in Wyoming. Specifically, we proposed to generate knowledge specific to Wyoming reservoirs to accurately: 1) verify the effectiveness of remote sensing data to identify HCBs, 2) predict what environmental conditions lead to HCBs, 3) identify nutrient management targets to prevent HCBs. Our field sampling campaign also allowed us to explore impact of blooms on reservoir food webs and water quality in Wyoming lakes and reservoirs.

      MS student Sam Sillen's thesis focused on the remote sensing aspects of this project, aiming to understand long-term trends in chlorophyll in lakes across the Intermountain West from the 1980s-present. He also conducted field sampling to verify the use of remote sensing tools for detecting blooms in Wyoming. Our results suggest that lake algal biomass is not increasing in most lakes since the mid 1980's, suggesting that the increasing number of algal blooms in Wyoming is more likely a result of increased awareness than widespread eutrophication. However, these estimates focus on measurements for lake centroids, not beaches, and further investigations should identify whether nearshore areas have different patterns. We also found that the EPA CYaN product used by WY DEQ is generally effective to identify blooms, and most errors are "false positive", where satellite imagery indicates a bloom but no bloom exists in field data collections.

      Other aspects of this research are in progress, including an analysis of the timing of nitrogen and phosphorus loading to Boysen Reservoir (led by collaborating PhD student Linnea Rock), and analyses of how blooms influence lake food webs (led by project PhD student Ashleigh Pilkerton). This project trained two graduate students (Samuel Sillen, MS 2022 and Ashleigh Pilkerton, PhD in progress), and six undergraduate students in field, laboratory, and computational approaches to research. The first manuscript from Sillen's thesis is in revision to be published at *Water Resources Research*, and a second manuscript is in preparation, and Pilkerton's in progress dissertation will include two manuscripts related to this work. We have presented this work at a diverse suite of local, state, national and international conferences, including the Wyoming Watershed Conference, the Society for Freshwater Science, and the American Geophysical Union.

Students trained under this project, including completed or in progress thesis/dissertations

Samuel Sillen   MS Zoology, December 2022

Sam is currently a Research Analyst for the University of Pittsburgh
Thesis title: Tracking harmful algal bloom (HAB) trends in Wyoming lakes using remote sensing

Ashleigh Pilkerton   PhD Ecology, Expected 2024

Ashleigh's dissertation generally focuses on water quality issues in Wyoming, and her work is split between a previous WRP project related to sediment and fisheries and this project. As part of this project, she has focused on impacts of HCBs on lake food webs. Thesis title: Water quality perturbations in aquatic ecosystems: Ecological response and management implications

Macy Jacobson   Undergraduate, Summer 2021

Macy assisted with Sam Sillen's field sampling in lakes across Wyoming to verify satellite-derived estimates of cyanobacterial density with field data. She graduated from UW in 2023.

Cortney Borer   Undergraduate, Summer 2021

Cortney was funded by the McNair Scholars program at UW and she assisted with lab experiments led by Ashleigh that tested how nutrient amendments influenced cyanobacterial community composition and toxin production.

Taylor Skiles   Postgraduate, 2022-2023

Taylor (UW 2022 graduate) assisted with plankton and nutrient sample processing

Darby McMartin   Undergraduate, 2022-2023

Darby (UW 2022 graduate) assisted with plankton and fish sample processing

Trevor Knowles   Undergraduate, 2022-2023

Trevor (current UW student) assisted with plankton sample processing

Foss Williams   Undergraduate, 2023

Foss (current UW student) assisted with plankton sample processing

Project Outputs:

Manuscripts:

Sillen, S.J., M.R.V. Ross, and S.M. Collins. *In Revision*. Long-term trends in productivity across Intermountain West Lakes provide no evidence of widespread eutrophication. *Water Resources Research*. Preprint DOI: 10.22541/essoar.168121606.64073311/v1

Conference Presentations:

Collins, S.M., W.W. Fetzer, K. Gauthier, A. Walters, M. Ross, S.J. Sillen. 2023. Lake productivity trends and harmful algal blooms in the high-elevation Western United States. American Geophysical Union, San Francisco, CA, USA (planned for December 2023, abstract accepted)

Collins, S.M., S.J. Sillen, A. Pilkerton, W.W. Fetzer, K. Hurshman, I. Oleksy, L Patterson, M. Ross, A. Walters. 2023. Harmful algal blooms in the high-elevation Western United States. Freshwater Sciences, Brisbane, Australia.

Pilkerton, A, T. Knowles, S. Sillen, W. Fetzer, A. Walters, S.M. Collins. 2023. Exploring zooplankton community response to harmful algal blooms in the Intermountain West, USA. Association for the Sciences of Limnology and Oceanography, Palma de Mallorca, Spain.

Sillen, S., J. Gardner, G. Zuccolotto, S.M. Collins. 2023. New capabilities in using remote sensing data to predict algal blooms in large rivers and reservoirs. Association for the Sciences of Limnology and Oceanography, Palma de Mallorca, Spain.

Skiles, T., A. Pilkerton, C. Brucker, A. Fuhman, S.M. Collins. 2023. Spatiotemporal variation of zooplankton community assemblages across water column TN and TP gradients. Conference on Biological Stoichiometry, Lincoln, NE.

Sillen, S.J. 2022. HCB project update from the University of Wyoming. Wyoming Watershed Conference. Riverton, WY, USA.

Sillen, S.J. 2022. Tracking chlorophyll trends in intermountain west lakes using remote sensing. Pecora Remote Sensing Symposium. Denver, CO, USA

Sillen, S.J., M. Ross, S.M. Collins. 2022. Tracking Chlorophyll Trends in Intermountain West Lakes Using Remote Sensing. Joint Aquatic Science Meeting, Grand Rapids, MI.

Web Apps:

https://sam-sillen.shinyapps.io/LandsatWaterQualityTrends

News Features:

"Toxic blooms again prompt health worries, warnings," Andromeda Erikson, WyoFile, August
   19, 2022

"Researchers look for clues as toxic blooms plague Wyo waters" Christine Peterson, WyoFile.
   September 14, 2021.

"The toxic mystery of Wyoming's backcountry cyanobacteria blooms" Mike Koshmrl, WyoFile.
   October 6, 2023.

Progress:

The primary goals of our project were to use satellite data to investigate long-term trends in lake
productivity, and to conduct field sampling in lakes and reservoirs across Wyoming to verify the
effectiveness of EPA CyAN estimates of cyanobacterial density with field samples. Both of
those projects have been completed, and the motivation, methods, results, and implications are
described in two attachments. First a publication resulting from the first chapter of Sam Sillen's
MS thesis (preprint in Appendix A) describes trophic state trends for lakes across the
intermountain west. The results suggest no evidence of widespread eutrophication across the
region, but rather that the majority of lakes are not changing and more are improving in water
quality (*i.e.*, becoming more oligotrophic since the mid-1980s) than are declining (*i.e.*, becoming
more eutrophic). Second, the second chapter of Sillen's thesis (Appendix B) is still in preparation
to be published, with an anticipated submission date in early 2024. This work details our field
sampling across Wyoming that allowed us to verify whether cyanobacterial densities from field
samples match cyanobacterial density estimates from the EPA CyAN remote sensing product
that is used by WY DEQ. Our result reflect a strong relationship between field and satellite data,
and that most mismatches are false positives, where the CyAN product suggests a bloom may be
occurring but the concurrently collected field samples have lower cyanobacterial densities.

Our project also includes ongoing research by current graduate students. First, PhD Student
Ashleigh Pilkerton took advantage of extensive field surveys in Sillen's thesis and repeated those
surveys in Summer 2021. She used those samples in two chapters of her dissertation, which
focus on the food web implications of HCBs in Wyoming lakes and reservoirs. The motivation,
methods, and anticipated results for these chapters are described in her dissertation proposal
(Appendix C).

All three graduate students involved in our project have contributed to the other objective of our
work, which was to understand how timing and intensity of nutrient loading to Boysen Reseroir
might influence cyanobacterial bloom development. First, Pilkerton conducted lab experiments
on water from Boysen in Summer 2021, where she added different amounts of nitrogen and
phosphorus to bottles and evaluated the algal community response. This experiment was difficult
to maintain in the laboratory and led us to conclude that a field data approach may be more
effective to understand this issue. Sillen began to run nutrient models to understand N and P
loading from the three major tributaries to Boysen Reservoir, but a lack of discharge data from
non-functional USGS gauges made it difficult to complete this before his defense. Because of

this, he passed the project to Collins Lab PhD student Linnea Rock, who is not funded by our project but has similar interests in aquatic biogeochemistry and water quality management. The second chapter of Rock's dissertation is in progress, and will use tributary nutrient samples, tributary discharge, and in-lake data to investigate priority questions defined in collaboration with WY DEQ staff. The proposal and preliminary results for this chapter are included in her PhD proposal for this chapter (Appendix D).

Summary and Significance

Our project applied novel methods for investigating the utility of remote sensing tools for understanding long-term trends and detection of harmful cyanobacterial blooms in Wyoming lakes and reservoirs. The results of this work suggest that the EPA CyAN estimates of bloom density are likely useful for surveillance in Wyoming, and that there is not strong evidence of widespread eutrophication or major shifts in lake trophic state since the 1980s. We continue to explore the connection between nutrient loading and in-lake processes, as well as the influence of HCBs on reservoir food webs in ongoing projects. This initial HCB-related project led our team to pursue a new WRP project (2023-2026) in which we will also continue to explore the connection between lake and bloom characteristics and cyanotoxin production.

Appendices:

Appendix A: Preprint version of Sam Sillen's first MS chapter. This manuscript describes multi-decadal trends in lake trophic state across Wyoming and surrounding states. A revision has been submitted to *Water Resources Research*.

Appendix B: Sam Sillen's second MS chapter, describing our field sampling in lakes across Wyoming to verify satellite derived HCB estimates.

Appendix C: Ashleigh Pilkerton's PhD proposal for the two chapters related to this project (3&4).

Appendix D: Linnea Rock's PhD proposal for the chapter related to Boysen Reservoir nutrient loading and data analysis.

Long-term trends in productivity across Intermountain West lakes provide no evidence of widespread eutrophication

Samuel James Sillen[1], Matthew Richard Voss Ross[2], and Sarah Collins[1]

[1]University of Wyoming
[2]Colorado State University

April 11, 2023

Abstract

Eutrophication represents a major threat to freshwater systems and climate change is expected to drive further increases in freshwater primary productivity. However, long-term in-situ data is available for very few lakes and makes identifying trends and drivers of eutrophication challenging. Using remote sensing data, we conducted a retrospective analysis of long-term trends in trophic status across the Intermountain West, a region with understudied water quality trends and limited long-term datasets. We found that most lakes (55%) were not exhibiting shifts in trophic status from 1984-2019. Our results also show that increases in eutrophication were rare (3% of lakes) during this period, and that lakes exhibiting negative trends in trophic status were more common (17% of lakes). Lakes that were not trending occupied a wide range of lake and landscape characteristics, whereas lakes that were becoming less eutrophic tended to be in more heavily developed catchments. Our results highlight that while there are well-established narratives that climate change can lead to more eutrophication of lakes, this is not broadly observed in our dataset, with more lakes becoming more oligotrophic than lakes becoming eutrophic.

Hosted file

959814_0_art_file_10846034_rsgr0g.docx available at https://authorea.com/users/603290/articles/633712-long-term-trends-in-productivity-across-intermountain-west-lakes-provide-no-evidence-of-widespread-eutrophication

Hosted file

959814_0_supp_10846026_rs3r0t.docx available at https://authorea.com/users/603290/articles/633712-long-term-trends-in-productivity-across-intermountain-west-lakes-provide-no-evidence-of-widespread-eutrophication

Hosted file

959814_0_supp_10846027_rspr0t.docx available at https://authorea.com/users/603290/articles/633712-long-term-trends-in-productivity-across-intermountain-west-lakes-provide-no-evidence-of-widespread-eutrophication

1

Long-term trends in productivity across Intermountain West lakes provide no evidence of widespread eutrophication

Samuel J. Sillen[1], Matthew R.V. Ross[2], and Sarah M. Collins[1]

[1]Department of Zoology and Physiology, University of Wyoming, Laramie, WY

[2]Department of Ecosystem Science and Sustainability, Colorado State University, Fort Collins, CO

Corresponding author: Samuel Sillen (ssillen@uwyo.edu)

Key Points:

- Remote sensing imagery captures long-term trends in lake productivity across the Intermountain West

- The majority of lakes observed in this dataset were not exhibiting shifts in trophic status from 1984-2019

- The incorporation of fine-scale lake climate data from new deep learning datasets results in substantial improvement to model accuracy

32 **Abstract**

33 Eutrophication represents a major threat to freshwater systems and climate change is expected to

34 drive further increases in freshwater primary productivity. However, long-term in-situ data is

35 available for very few lakes and makes identifying trends and drivers of eutrophication

36 challenging. Using remote sensing data, we conducted a retrospective analysis of long-term

37 trends in trophic status across the Intermountain West, a region with understudied water quality

38 trends and limited long-term datasets. We found that most lakes (55%) were not exhibiting shifts

39 in trophic status from 1984-2019. Our results also show that increases in eutrophication were

40 rare (3% of lakes) during this period, and that lakes exhibiting negative trends in trophic status

41 were more common (17% of lakes). Lakes that were not trending occupied a wide range of lake

42 and landscape characteristics, whereas lakes that were becoming less eutrophic tended to be in

43 more heavily developed catchments. Our results highlight that while there are well-established

44 narratives that climate change can lead to more eutrophication of lakes, this is not broadly

45 observed in our dataset, with more lakes becoming more oligotrophic than lakes becoming

46 eutrophic.

47 **Plain Language Summary**

48 Lakes are often classified by their productivity. Low productive lakes generally represent deep

49 lakes with low amounts of algae. Whereas lakes with high levels of productivity support more

50 plant growth and have higher amounts of algae. The accumulation of nutrients in freshwater

51 systems often results in increases in productivity and can lead to the development of algal

52 blooms. Algal blooms are a major concern due to their threat to ecosystem health, recreation, and

53 drinking water sources. Yet the lack of long-term field data across large scales has resulted in a

54 limited understanding of 1) what factors are driving productivity trends and the development of

55 algal blooms across regions, and 2) are increasing trends representative of widespread

56   intensification or an increase in awareness and reporting. Therefore, there is a pressing need to

57   effectively monitor and understand these trends in order to inform management actions that

58   address their frequency and intensity. Here, we use data obtained from satellite imagery from

59   1984 - 2019 to document lake productivity trends in 1,169 lakes across the Intermountain West.

60   We show that substantial increases in productivity were rare, and that the majority of lakes have

61   not undergone widespread change.

62   **1 Introduction**

63   Widespread eutrophication is a global phenomenon that threatens water quality,

64   recreational industries, and ecosystem function (Paerl et al., 2001; Gatz, 2020; Amorim and

65   Moura, 2021). A common outcome of eutrophication is an increase in the biomass of

66   phytoplankton, both algae and cyanobacteria, in freshwater, transitional, and ocean environments

67   (Anderson et al., 2008; Hudnell, 2010; Wurtsbaugh et al, 2019). In many cases, this rapid and

68   excessive growth can become severe and lead to the development of Harmful Algal Blooms

69   (HABs) (Smith, 2003; Heisler et al. 2008). HABs are of particular concern due to the threats they

70   pose to human health and drinking water sources (Fleming et al., 2002; Falconer and Humpage

71   et al., 2005; Christensen and Khan, 2020). Thus, the wide-ranging effects that eutrophication and

72   HABs have on aquatic systems and their threat to human health have highlighted the need to

73   understand the factors which drive them.

74   Generally, eutrophication and algal blooms are attributed to excessive loading of

75   nitrogen (N) and phosphorus (P) as well as high water temperatures (Rejmankova and

76   Komarkova, 2005; Paerl and Paul, 2012; Gobbler et al. 2016; Beaver et al. 2018). However, in

77   shallow lakes, warmer temperatures and higher light absorption have been found to be more

78   significant drivers of productivity (Kosten et al., 2012). In other words, the combination of

79   factors that drive rapid increases in lake productivity may differ between individual water bodies

80   or geographic regions, hence smaller and more focused state and regional scale studies may be

81   more useful in describing changes in lake productivity dynamics (Oleksy et al., 2022).

82   Large scale studies have highlighted that water quality trends are context dependent and

83   vary across regions (Beaver et al., 2018). However, some regions with unique landscape features

84   remain understudied regarding lake productivity trends. For example, the Intermountain West

85   region (including the US states Colorado, Idaho, Montana, Utah, and Wyoming) has very

86   different hydrological dynamics and landscape features compared with other regions, yet water

87   quality trends remain mostly undocumented. The region undergoes quick wet-dry seasonal

88   transitions, with most of the streamflow generated by snowmelt (Bales et al., 2006). Higher

89   gradients in temperature and precipitation with elevation make hydrologic processes significantly

90   different compared with low-elevation regions (Bales et al., 2006). Land use in this region also

91   differs, with substantial amounts of grassland pasture and range contributing to increased organic

92   nutrient loading to streams and rivers (Agouridis et al., 2005).

93   An increase in awareness and reporting of HABs in the Intermountain West suggests that

94   lakes in the region may be becoming more eutrophic, yet our understanding of lake productivity

95   trends is very limited. As nation-wide research and understanding of HABs has grown, so have

96   management and sampling plans, educational materials, and overall public awareness (Hudnell et

97   al. 2010). However, this increase in awareness and reporting has the potential to create a

98   perception that blooms are already increasing in intensity and frequency (Hallegraeff et al.,

99   2021). Recent work in the region highlights that lakes are experiencing roughly equal trends of

100   changing from blue to green or changing green to blue, indicating there is not overwhelming

101   evidence that they are getting more eutrophic, where eutrophic lakes are generally more green

102   (Oleksy et al., 2022). It remains unclear whether this is a result of representative increases in

103   intensity or a result of heightened monitoring. Therefore, retrospective data analyses and long-

104   term monitoring are needed to identify consistent productivity trends (Hudnell, 2008),

105   particularly in understudied regions like the Western US.

106   Remote sensing and long-term satellite imagery create opportunities to address key

107   research gaps surrounding what factors are driving freshwater productivity across regions. In-situ

108   sampling methods are often limited by resources such as time and funding. Therefore, in-situ

109   water quality data tends to be focused on relatively large lakes (> 20 ha) and long-term records

110   tend to be rare (Stanley et al. 2019). Importantly, leveraging remote sensing data can address

111   water quality dynamics over large spatial and temporal scales where in situ data is lacking (Topp

112   et al. 2020). Remote sensing data with high spatial and temporal coverage are also useful to

113   understand how global change is affecting productivity and bloom dynamics (Harvey et al. 2015;

114   Ho et al., 2017; Seegers et al. 2021). These tools can be used to determine water quality

115   parameters in freshwater systems such as chlorophyll-a (Boucher et al., 2018; Kuhn et al., 2019;

116   Papenfus et al., 2020), suspended sediments (Pavelsky and Smith, 2009), and organic matter

117   (Kutser et al., 2005; Slonecker et al., 2016).

118   In this study, we address two gaps in our understanding of lake productivity dynamics in

119   the Intermountain West. Specifically, we aimed to identify 1) the historical prevalence of

120   eutrophic lakes and whether this is an increasing trend of eutrophication, and 2) the drivers and

121   spatial distribution of changes in trophic state. We use remote sensing imagery and in-situ

122   chlorophyll-a data, covering 1984-2019, to predict chlorophyll-a and lake trophic state based

123   solely on satellite imagery. This approach allowed us to document productivity trends in 1,169

124   lakes over 35 years. By increasing the level of understanding of historical trends in lake

125   productivity and their drivers in this region, our analysis can also shed light on the intensification

126   of algal blooms in lakes and provide important information for water quality management.

127   **2 Materials and Methods**

128   2.1 Data Sources and Processing

129

130   Our analysis used various remote sensing, water quality, lake and landscape features, and

131   climate datasets. We opted for a machine-learning approach that uses paired satellite reflectance

132   from Landsat observations and in-situ water quality data. We acquired Landsat data and in-situ

133   chlorophyll-a samples for model training from the AquaSat dataset (Ross et al., 2019). AquaSat

134   joins Landsat Tier 1 surface reflectance to water quality samples from the Water Quality Portal

135   (Read et al. 2017) and LAGOS-NE (Soranno et al. 2017) that occurred ± 1 day of a Landsat

136   observation. We filtered AquaSat to only include observations over the Intermountain West

137   region and with Landsat scenes with less than 50% cloud cover. The resulting dataset included

138   1,340 observations across 249 lakes in the region. Reflectance values across the three different

139   Landsat satellites used (5, 7, and 8) were standardized using the methodology outlined in

140   Gardner et al. (2021). We then identified various open-source datasets that captured

141   environmental drivers we hypothesized might be important for predicting chlorophyll-a. We

142   merged Lake characteristics and catchment level metrics to our training dataset from the LakeCat

143   (Hill et al., 2018) and LAGOS-US (Cheruvelil et al., 2021), and HydroLAKES (Messager at al.,

144   2016) datasets. Initially we joined lakes in the training set to corresponding lake polygons

145   included in NHDPlusV2. LakeCat, LAGOS-US, and HydroLAKES datasets were then added

146   through common NHD identifiers. We selected metrics that were derived from these datasets

147   based on their potential to impact water quality (Table S1).

148   Daily surface water temperature and corresponding weather data (wind speed) were also

149   included in our model development. We extracted daily water temperature from Willard et al.

150   (2022), which includes estimated daily surface water temperature for 185,549 lakes across the

151   US. In addition to daily surface temperature, we calculated prior 14-day mean temperatures for

152   all 1,340 observations included in our training set. Then, we joined 14-day mean temperature and

153   meridional wind speed to our training set using common NHD identifiers and the date of

154   observation.

155   Using the same methods, we built our prediction dataset using LimnoSat-US (Topp et al.,

156   2021). LimnoSat-US includes Landsat Collection 1, Tier 1 surface reflectance for lakes greater

157   than 10 hectares in the U.S. spanning 1984 – 2020. Surface reflectance values represent the

158   median surface reflectance of a 120-meter buffer of the "deepest point" of a lake polygon. This

159   "deepest point" can be defined as the center of the largest circle that can fit within a lake

160   polygon. We joined the lake characteristics, catchment level metrics, and climate data described

161   above to our prediction dataset, resulting in 1,264,355 observations across 2,596 lakes in the

162   Intermountain West.

163   Lastly, we defined categories for three trophic states based on the following chlorophyll-a

164   thresholds: oligotrophic (0 - 2.6 ug /L), mesotrophic (2.7 – 7 ug / L), and eutrophic (> 7 ug / L).

165   These thresholds were inspired by the criteria outlined in the National Lakes Assessment (U.S.

166   Environmental Protection Agency, 2009). This categorical approach was taken because

167   predicting chlorophyll-a concentrations in freshwater systems with remote sensing has been

168   notably challenging, particularly with Landsat imagery (Salem et al., 2017; Smith et al. 2021).

169   Landsat bands are relatively broad with a low signal-to-noise ratio, often resulting in predictions

170   of chlorophyll-a with high levels of uncertainty (Matthews, 2011). Furthermore, the accurate

171 prediction of chlorophyll-a is affected by complex optical conditions in various waterbodies with

172 higher levels of turbidity (Ruddick et al. 2001; Alvain et al. 2005). These challenges were

173 addressed by focusing on broad, trophic level predictions of chlorophyll-a.

174   2.2 Model Development

175

176   We developed an Extreme Gradient Boosting (XgBoost) model to classify categories of

177 chlorophyll-a. These models build on machine learning concepts such as decision trees and

178 ensemble learning (Cheng and Guesterin, 2016). Decision trees represent a supervised learning

179 approach where training features are split into internal nodes and evaluated to form

180 homogeneous groups (terminal nodes) (Kotsiantis, 2013). Decision trees can comprise a single

181 univariate classifier or the combination of multiple classifiers, known as an ensemble classifier.

182 Gradient boosting is a method of ensemble learning where a series of models are built with

183 weights assigned to misclassified observations. Misclassified observations from the previous

184 model are used as training data for the next, and the result is an ensemble classifier that

185 represents an aggregation of individual classifiers and minimizes overall error (Pal, 2007).

186   We used a combination of optical and climatic variables to build a predictive model for

187 chlorophyll-a. Specifically, we calculated multiple band ratios that have been shown to explain

188 variation in phytoplankton blooms (Ho et al., 2017). We used a 14-day average of lake surface

189 temperature and daily meridional wind speed as additional predictor variables. We explored the

190 addition of static predictor variables (such as lake elevation or watershed land use) yet refrained

191 from including these in our final model because recent studies have shown that static predictor

192 variables can act as 'identifiers' and lead to overfitting and over-optimistic evaluation metrics

193 (Meyer et al., 2018). Thus, we selected only continuous predictor variables that we would not

194 expect to lead to substantial overfitting (Table 1).

195

196 **Table 1**. Predictor variables used for model training.

Predictor variable	Description
Blue	Surface reflectance of blue band
Dwl	Dominant wavelength
Nir	Surface reflectance of Nir band
Swir2	Surface reflectance of Swir2 band
Red / Blue	Red / Blue
Red / Nir	Red / Nir
Nir / Red.	Nir / Red
Green / Blue	Green / Blue
Nir Sac	(Nir – 1.03) * Swir1
Nir – Red	Nir - Red
Red - Green	Red - Green
EVI	2.5*((Nir – Red)/(Nir + ((6*Red) – (7.5*Blue)) + 1))
GCI	Nir / (Green – 1)
Mean 14-day Temp	14- day average surface water temperature (deg. C)
Wind	Meridional wind speed (m/s)

197

198   We partitioned our training set to reserve 20% for model testing and evaluation and 80%

199 for model training and parameter tuning. XgBoost models include a wide range of

200 hyperparameters and are one of the main tools used to reduce model variance. Hyperparameters

201 were tuned by first establishing a grid of conservative values (to prevent overfitting) and then

202 extracting the hyperparameters that resulted in the lowest validation loss. After training the final

203   model with these hyperparameters, model performance was evaluated through a confusion

204   matrix which shows the relative accuracy of predictions across different categories.

205   2.3 Data Analysis

206

207   To summarize lake trends and capture long-term changes in chlorophyll-a, we analyzed

208   the percent occurrence of trophic state observations. First, lakes included in our trend analysis

209   had to have at least two summer observations (June – September) for each year (1984-2019).

210   More conservative filtering criteria, such as at least 5 observations per year, was explored yet

211   had negligible effects on overall results and resulted in fewer lakes being included in our

212   analysis. We specifically focused our analysis on summer observations to limit the effect that

213   snow and ice may have on our results. As a result, 1,169 lakes were included in our analysis

214   based on these criteria. For each summer, the percent occurrence of each trophic state

215   observation was recorded. Then, the average percent occurrence for each trophic state was

216   recorded across two time periods: 1984 – 2004; and 2005 – 2019. Lastly, lakes were grouped

217   into the following categories based on the shift (if any) in trophic state during these two time

218   periods:

219   1)   **No trend:** Change in % oligotrophic, % mesotrophic, and % eutrophic was less

220   than 10% across all three categories (Figure 1A)

221   2)   **Increasing in % Eutrophic:** Number of eutrophic observations increased by

222   10% or more while the number of oligotrophic observations decreased by 10% or

223   more (Figure 1B)

224   3)   **Increasing in % Oligotrophic:** Number of oligotrophic observations increased

225   by 10% or more while the number of eutrophic observations decreased by 10% or

226   more (Figure 1C).
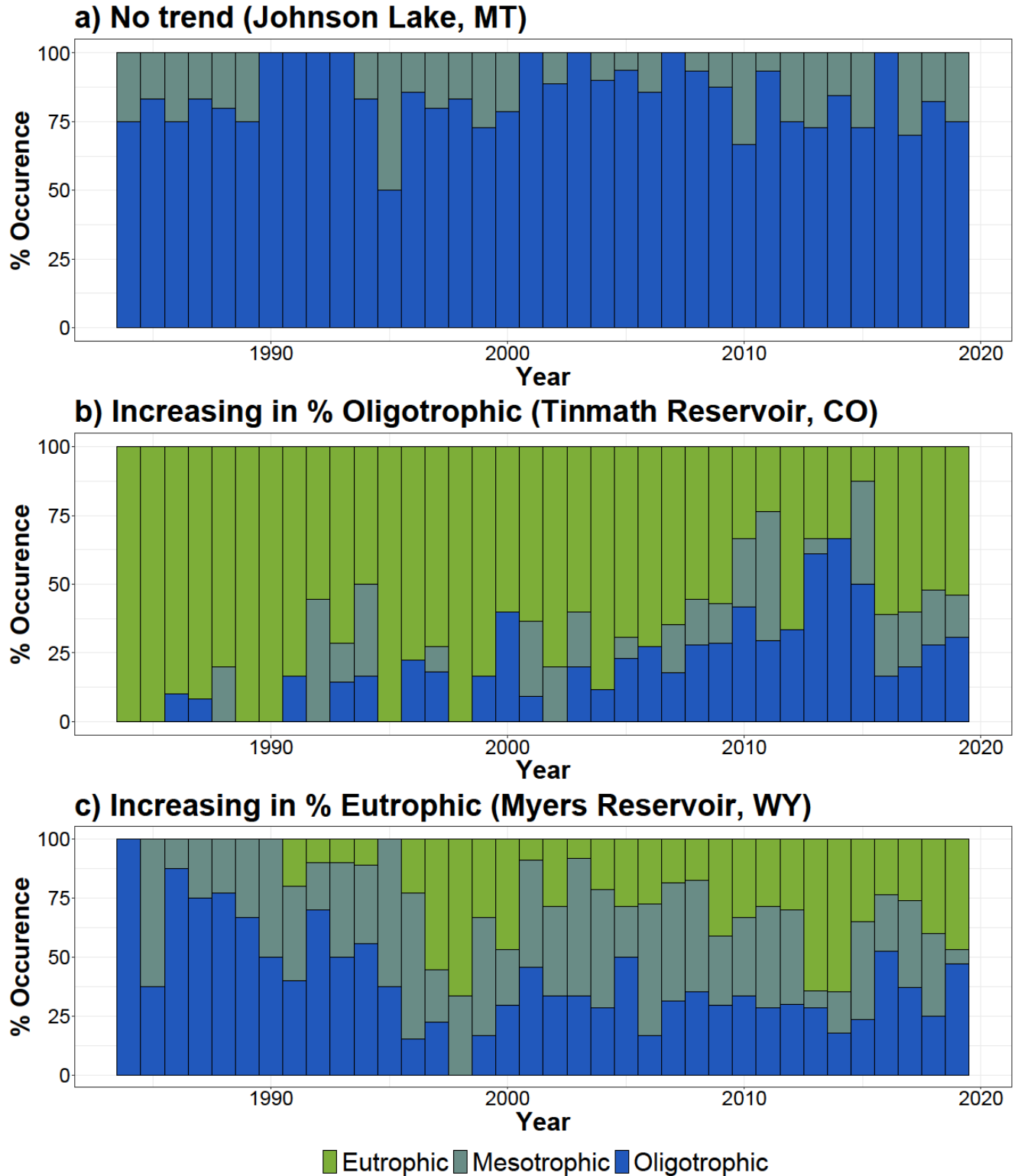
Figure 1. Examples of three possible trend categories based on the trends in % occurrence of oligotrophic, mesotrophic, and eutrophic observations. Each panel included in this plot represents the trends observed across three different lakes.

231   Lastly, trend-specific drivers were examined by determining how lake catchment,

232   hydrologic, and climate metrics explained differences across trends. We calculated variable

233   importance across trend categories by applying a random forest model using the randomForest

234   package in R (Liaw and Wiener, 2002). With this approach, we were able to classify the

235   reduction in accuracy that occurred across all three responses when certain variables were

236   excluded. All data processing, model development, statistical analysis, and visualizations were

237   done in Program R (R Core Team, 2022).

238   **3 Results**

239   3.1 Model Performance

240

241   Model performance was evaluated through a confusion matrix as well as various

242   accuracy and error metrics (Table 2, Figure 2). In the range of oligotrophic values (0 - 2.6 ug/L),

243   observations had a balanced accuracy of 78% and only 7% of these observations were

244   misclassified as eutrophic (Table 2). Mesotrophic observations (2.7 - 7 ug/L) represented the

245   range of values with the lowest prediction accuracy. Our model reported a balanced accuracy of

246   69% for mesotrophic classifications (Table 2). The most common misclassification among

247   mesotrophic predictions was with observed classes that were oligotrophic (30%) (Figure 2).

248   Lastly, eutrophic observations (> 7 ug/L) represented the class with the highest prediction

249   accuracy (85%) (Table 2). In addition, there was relatively low prediction error with oligotrophic

250   classes (6%). Overall, our model reported a global accuracy of 70% with a 95% confidence

251   interval of between 63% and 76% (Table S2).

252

253   **Table 2**. Model evaluation metrics for each predicted class.

Statistic	Oligotrophic	Mesotrophic	Eutrophic
Sensitivity	0.7500	0.5397	0.8426
Specificity	0.8167	0.8440	0.8912
Neg Pred Value	0.8235	0.8041	0.9291
Pos Pred Value	0.7412	0.6071	0.7460
Prevalence	0.4118	0.3088	0.2794
Balanced Accuracy	78.33%	69.18%	85.79%

254
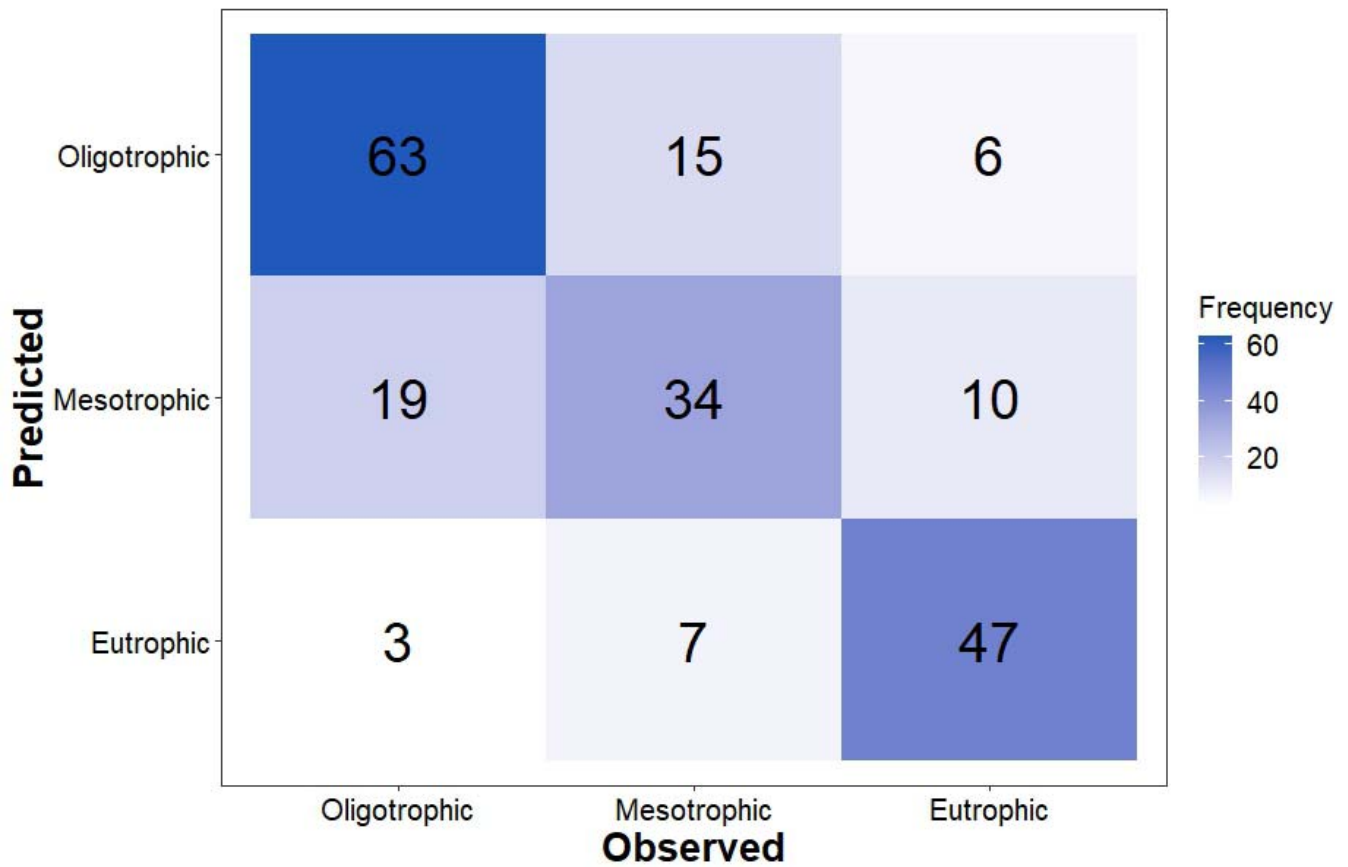
255
256   **Figure 2**. Confusion matrix illustrating the frequency and accuracy of predictions across all three
257   trophic states. The most common misclassification was among mesotrophic predictions that had
258   observed classes of oligotrophic (middle panel, far left). Overall, our model had a global
259   accuracy of 70% with a 95 % confidence interval of 63% - 76%.
260

261   The integration of fine-scale, daily temperature and climate features significantly

262   improved our ability to predict across these trophic states. In terms of feature importance

263   measured by model gain, mean 14 – day surface water temperature and meridional wind speed

264   were the second and fourth most important predictor variables, behind the band ratio of blue to

265   green and dominant wavelength (Figure 3). In addition, model scenarios without climate

266   variables reported global accuracies of around 63%, with a 95% confidence interval of between

267   57 – 69%.
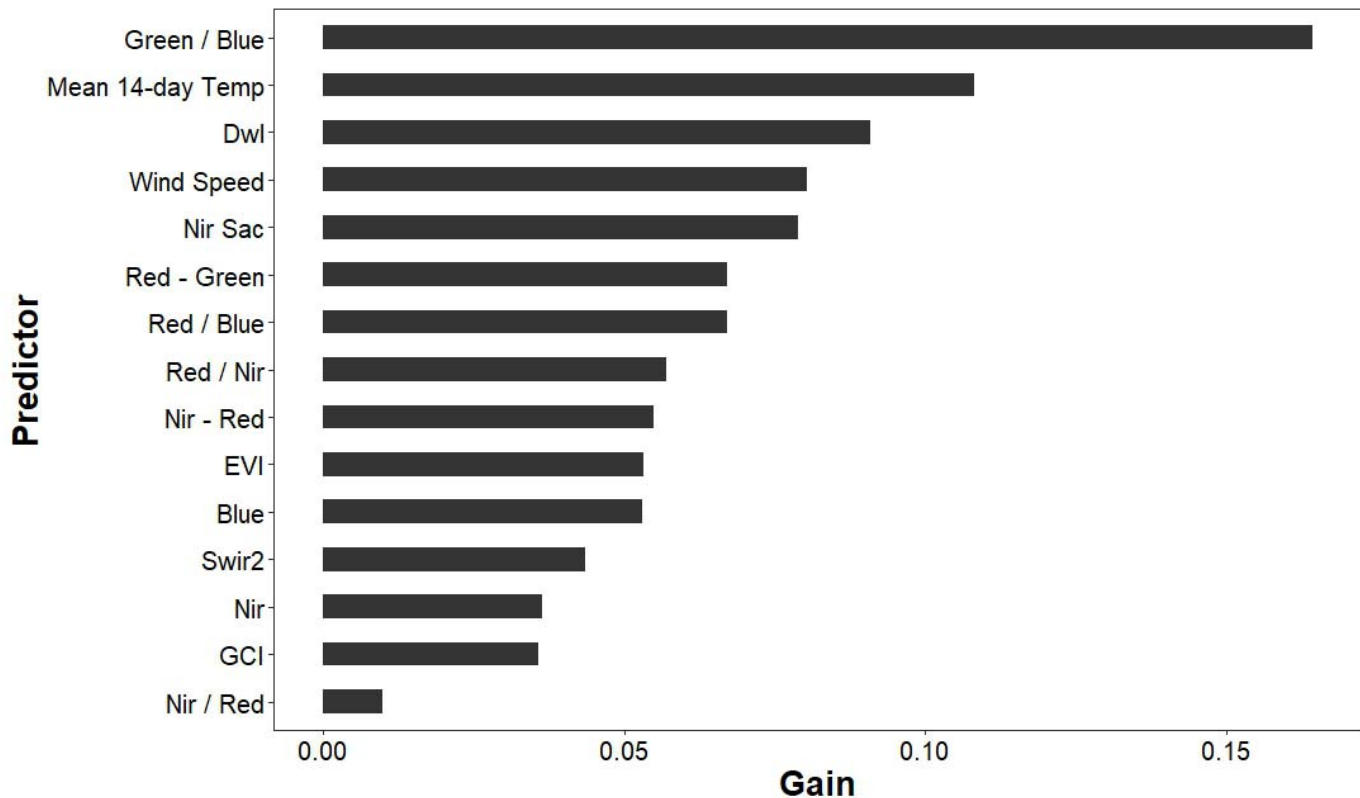
268   **Figure 3**. Feature importance, measured as model gain, for the predictor variables included in
269   model development.

270

271

272   3.2 Productivity Trends

273

274   Most lakes included in this study did not show trends in chlorophyll-a (Figure 4). Overall,

275   a total of 651 lakes (55%) did not meet our 10% thresholds for shifts across all three categories

276   More than half of the lakes that weren't changing from 1985-2019 were oligotrophic lakes with

277   most observations classified as oligotrophic. In contrast, 24% of lakes within this category were

278   eutrophic lakes. The remaining lakes (16%) in this trend category likely represent a more

279   complex, mesotrophic lake status.

280   The second most common trend we observed were lakes that had substantial shifts in

281   trophic status by becoming more oligotrophic. We found that 17% of lakes switched from

282   predominantly being classified as eutrophic to being classified primarily as oligotrophic. Most of

283   these lakes tended to be dominated by eutrophic observations, suggesting that they are eutrophic

284   lakes that are improving in water quality. Few lakes showed evidence of extreme (>30%) shifts

285   in oligotrophic observations. In other words, shifts in oligotrophic observations within this lake

286   trend was relatively moderate (10 - 30%, Figure S1).

287   Lastly, a minority (3%) of all lakes were shifting towards becoming more eutrophic.

288   Interestingly, these trends were equally distributed across lakes with high levels of eutrophic

289   observations and those with high levels of oligotrophic observations. In other words, lakes that

290   were predominately oligotrophic and were becoming more eutrophic were equally as common as

291   lakes that were eutrophic and were intensifying in this way. The magnitude of change was

292   similar to that of lakes that trended oligotrophic, with little evidence of extreme shifts in

293   eutrophic observations (Figure S1).

294
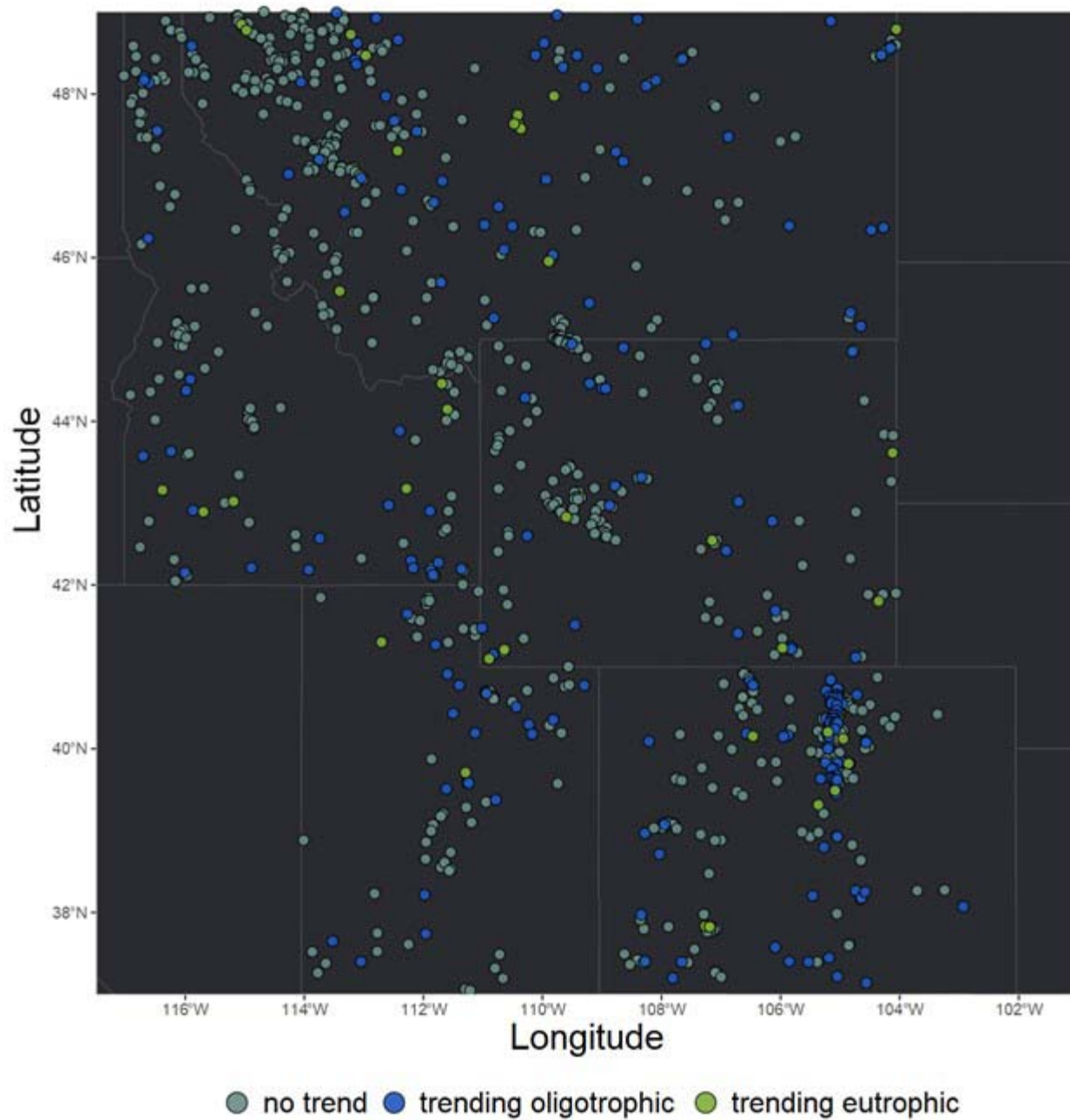
Figure 4. Spatial distribution of trophic state trends across the five states included in this analysis.

299   The remaining lakes that were included in this analysis and did not fit into these rigid

300   categories reflect various levels of trophic state change. For example, 7% of lakes could be

301   described as becoming more oligotrophic and less mesotrophic by the same thresholds outlined

302   in Figure 1. In contrast, few lakes (1%) were found to be becoming more mesotrophic during this

303   time. The 12% of lakes that did not fit into these categories displayed slight trends in certain

304   categories (such as becoming more oligotrophic), but did not satisfy thresholds for trends in

305   other categories such that we would be confident of defining clear trends in productivity.

306   3.3 Drivers of Trends

307

308   Our random forest model was able to identify partially important variables for explaining

309   trends in productivity. Lake catchment data such as 30 year normal mean temperature, base flow

310   index, and mean runoff were more important in explaining overall lake trends (Figure 5).

311   Specifically, lakes becoming more oligotrophic tended to have longer residence times and were

312   located in catchments that were generally less forested and more developed (Figure 6). Whereas,

313   lakes that were becoming more eutrophic also tended to be less forested but were located in

314   smaller catchments and were shallower on average (4.13 m) compared with lakes that were not

315   trending (9.12 m). Lastly, a number of climate and landscape metrics displayed a high level of

316   variation across trophic state trends, however some of these metrics had significant cross

317   correlation with other variables (Figure S2).
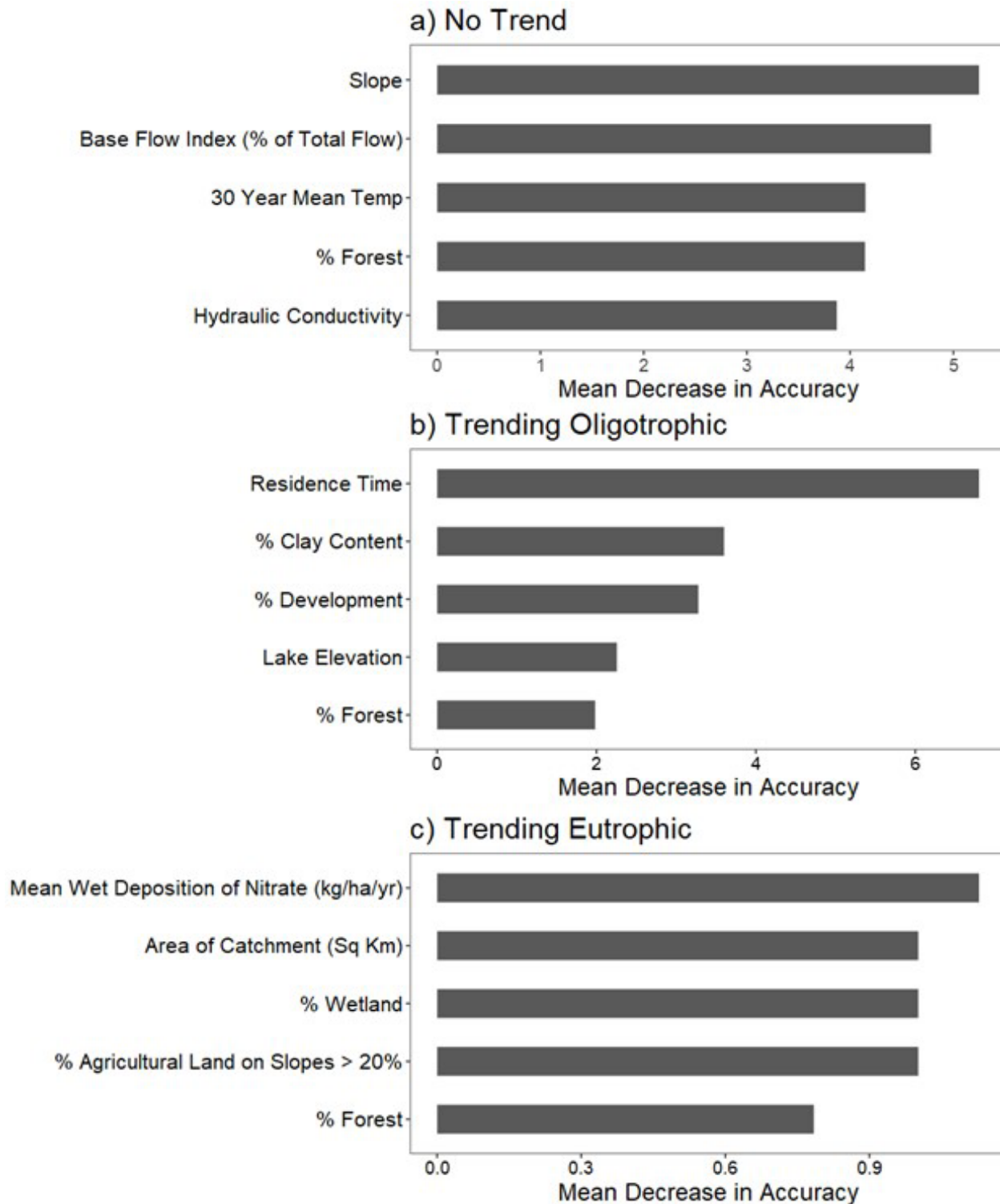
318

319

320

321

322
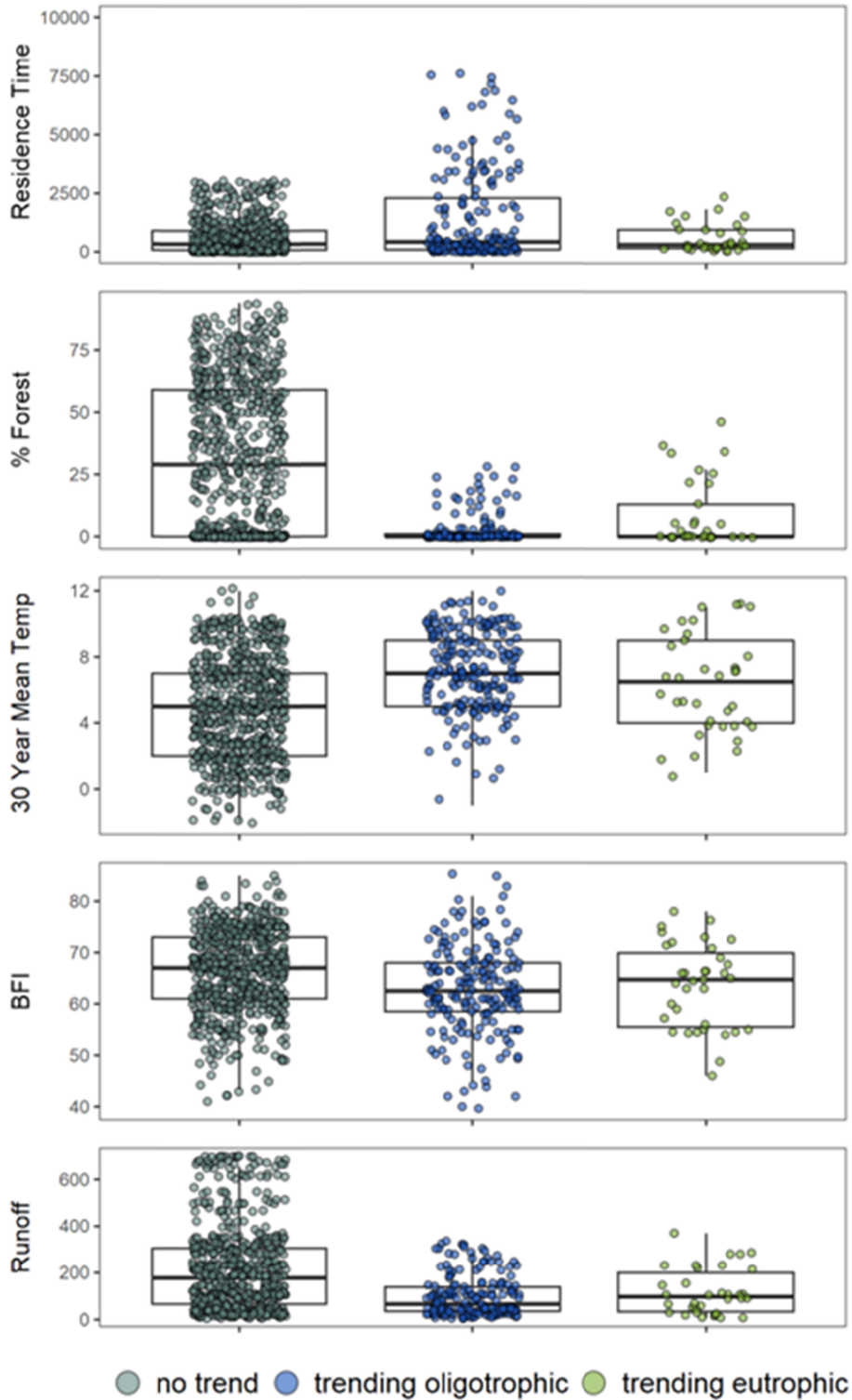
Figure 5. Boxplots across trend categories of the top five most important variables based on the decrease in accuracy from the overall (global) random forest model.

Figure 6. Mean decrease in accuracy of the top five variables used to explain each trend category in the random forest model. The mean decrease in accuracy describes variable importance by quantifying how much accuracy is lost by excluding that particular variable.

4 Discussion

Eutrophication and the development of algal blooms are global phenomena that threaten aquatic systems. Given the effects of global change and the expected increasing intensity of these disturbances, there has been a substantial level of interest in investigating recent productivity trends in lakes and reservoirs. Our analysis found that most lakes in the Intermountain West region have remained relatively static in terms of their productivity over the last 35 years. In addition, we found that a greater percentage of lakes were improving with regards to productivity, as opposed to becoming more eutrophic.

4.1 Productivity Trends

The majority of lakes included in this analysis showed no evidence of substantial changes in trophic state and supplement other regional-scale analyses of in-situ chlorophyll-a data. This is consistent with previous analyses demonstrating that magnitude, severity, and duration of algal blooms are not intensifying in US lakes (Wilkinson et al. 2022). Similarly, long-term trends of Florida lakes have indicated that a majority (73%) have not shown evidence of changes in chlorophyll-a and trophic state (Canfield et al., 2018). While there is a growing concern of eutrophication and HABs becoming pervasive in the Intermountain West, our results build on recent studies that suggest no indication of widespread intensification in algal blooms. Rather, the large percentage of lakes not trending combined with the presence of algal blooms across the region suggest a historical baseline of eutrophication and that blooms could have predated the 1980s.

Our analysis revealed that, in fact, the smallest percentage (3%) of lakes were trending eutrophic. Global analyses of long-term phytoplankton blooms have shown a substantial (68 %) number of lakes to be increasing in bloom intensity (Ho et al., 2019). However, only 5% of U.S.

353   lakes have been shown to be increasing in the same metric over the past 40 years (Wilkinson et

354   al., 2022). In addition, a minority of lakes (13%) in the Rocky Mountain region have shown to be

355   shifting from blue to greener wavelengths during this time (Oleksy et al., 2022). With our

356   analysis, we show that concerns regarding the widespread intensification of algal blooms are not

357   captured in our analysis of chlorophyll-a and trophic state.

358   Our analysis of lakes that were trending eutrophic revealed several important hydrologic

359   and climate factors associated with eutrophication. Specifically, 30-year normal mean

360   temperatures tended to be higher among lakes trending eutrophic and an important variable for

361   explaining overall trends. In addition, hydrologic variables such as lake depth and lake area

362   revealed that lakes trending eutrophic tended to be smaller and shallower than other lakes. Small,

363   shallow lakes are often more productive than deeper lakes because of the effects that lake

364   morphology can have on ecosystem structure (Richardson et al., 2022; Henderson et al., 2021).

365   Shallow lakes have also been shown to be more sensitive to climate conditions (Mooij et al.,

366   2007) and could explain the interaction between climate and depth driving these trends.

367   In contrast, 19 % of study lakes were found to be improving by simultaneously becoming

368   less eutrophic and more oligotrophic. Lake-specific characteristics reveal that lakes improving in

369   water quality were in more developed and less forested catchments, as well as at lower

370   elevations. These results are consistent with studies on water clarity (Topp et al., 2021), lake

371   color (Oleksy et al., 2022), and chlorophyll-a (Wilkinson et al., 2022), that highlight

372   improvements in water quality metrics over the same time period. These trends have been

373   hypothesized to be the result of management actions or restoration projects (Wilkinson et al.,

374   2022), although we lacked the information to make conclusions about the mechanisms of these

375   trends. However, concentrations of nutrients across urban watersheds have significantly

376   decreased over the past 20 years and have been directly attributed to the Clean Water Act (Stets

377   et al., 2020). Given the greater variable importance of developed land use across lakes becoming

378   more oligotrophic (3.9 compared to 1.6 among no trend lakes), it is possible that water quality

379   implementation projects have had a positive effect on mitigating eutrophication in the region.

380   Despite the 35-year study period and wide range of lakes involved, the remote sensing

381   data used in this study may not capture various spatial and temporal characteristics of

382   eutrophication or algal blooms. Algal blooms tend to have high temporal and spatial variance in

383   the short term, as wind dynamics drive the spatial distribution of phytoplankton blooms (Bosse et

384   al., 2019). Therefore, the 16-day return period for Landsat observations may not capture short-

385   term peaks in chlorophyll-a. Furthermore, some images can be unusable due to extensive cloud

386   cover and may extend the period between observations up to months at a time. However, given

387   that our analysis includes 35 years of data across 1,169 lakes, we would expect to capture

388   widespread eutrophication and the spatial clustering of eutrophication trends if it were present.

389   Additionally, Landsat's long-term record restricted us to coarse analyses of chlorophyll-a

390   and trophic state. Our analysis does not capture cyanobacteria dynamics or those of cyanotoxins

391   directly. Satellites with spectral resolution to capture cyanobacteria abundance, such as MERIS

392   and Sentinel-3, have lacked the data availability for long-term, retrospective analyses (Coffer et

393   al., 2020). However, future studies that are able to capture trends in cyanobacteria blooms

394   specifically will help provide further context regarding the concerns of bloom intensification.

395   4.2 Modeling Approach

396

397   Our research focused on leveraging long-term remote sensing and environmental datasets

398   that would supplement the ongoing debate regarding recent trends in phytoplankton blooms.

399   While the application of remote sensing for inland water quality monitoring has grown over the

400   past decade (Topp et al., 2020), the retrieval of certain optical properties such as chlorophyll-a

401   has remained a challenge (Matthews, 2011). However, by incorporating daily surface

402   temperature and meridional wind speed from datasets leveraging modern deep learning

403   techniques we were able to show substantial improvements in model accuracy. The incorporation

404   of fine-scale lake climate data over the 35-year time span of this study was instrumental to our

405   ability to document trophic state changes and add evidence to the ongoing debate regarding the

406   recent trends in increasing eutrophication and HABs.

407   Most notably, surface water temperature was the second most important predictor

408   variable of our trophic state model and could be important for a wide range of remote sensing

409   based water quality models. Water temperature has proven to be an important predictor of

410   chlorophyll-a across inland lakes (Liu et al. 2019; Karcher et al. 2020) as well as oceans

411   (Dunstan et al. 2018). However, applied remote sensing models that predict chlorophyll-a are

412   often limited to strictly optical predictors such as band-ratio (blue-green) models. These models

413   work well in waterbodies where other parameters such as colored dissolved organic matter co-

414   vary with chlorophyll-a (O'Reilly et al., 1998). However, in optically complex waterbodies with

415   higher levels of turbidity and dissolved organic matter band-ratio models struggle to accurately

416   retrieve chlorophyll-a concentrations (Tzortziou et al., 2007; Zheng and DiGiacomo, 2007;

417   Witter et al., 2009). Thus, relying on surface reflectance for predictive models has resulted in a

418   lack of generalizability across a wide range of waterbodies. However, the incorporation of

419   surface water temperature seems to have supplemented existing band-ratio features to better

420   predict across a wide range of lake types.

421   Wind speed was another climate predictor variable that was substantially important in

422   predicting trophic state. Correlations between wind speed and chlorophyll have been shown

423   using remote sensing at global scales (Kahru et al., 2010). In addition, wind speed has been

424   documented as an important driver of cyanobacterial bloom development with blooms favoring

425   warm, calm weather (Kanoshina et al. 2003). Overall, the integration of daily, fine-scale weather

426   data greatly improved our ability to predict trophic state and is likely to have a positive impact

427   on similar approaches that leverage remote sensing data.

428   **5 Conclusions**

429   With increases in global lake temperatures (Maberly et al., 2020), lakes globally are

430   expected to become more eutrophic as a response to climate change (Yang et al., 2020). Yet,

431   there have been conflicting results thus far regarding intensifying eutrophication and algal

432   blooms in U.S. and global lakes (Ho et al., 2019, Wilkinson et al., 2022, Topp et al., 2021).

433   While increasing eutrophication is a major threat to freshwaters, our analysis found that lakes in

434   the Intermountain West region have not undergone widespread change. Rather, we found that

435   most lakes were not changing, and a substantial number of lakes were becoming less eutrophic

436   and more oligotrophic over this time period. In addition, the number of eutrophic lakes that have

437   not undergone substantial change over this time period suggests algal blooms have been present

438   in the region since at least the early 1980s. These results highlight the complex nature of

439   observing changes in freshwater lakes across large scales. However, our results suggest that

440   despite the processes that drive eutrophication (warmer temperatures, nutrient accumulation,

441   etc.) which have increased over the past several decades, we haven't yet observed a concurrent

442   increase in eutrophication from a large, unbiased sample of 1,169 lakes in the Intermountain

443   West. This suggested suggesting controls on eutrophication in this region are complex and need

444   further additional study.

445

Acknowledgments

447

448   This research was supported by the Wyoming Water Research Program. We thank Kelsee

449   Hurshman, Fabian Nippgen, William Fetzer, Lindsay Patterson, and Annika Walters for valuable

450   feedback with this project.

451

Open Research

453

454   The data used for this paper (Hydrolakes, LakeCat, AquaSat, LimnoSat, and LAGOS) are all

455   freely available to download in online repositories (Messager et al., 2016; Hill et al., 2018; Ross

456   et al., 2019; Topp et al., 2021; Cheruvelil et al., 2021). Links to the where this data can be

457   downloaded can be found in the code for this analysis. The code used for this analysis can be

458   found at https://github.com/SamSillen/ProductivityTrends.

459

460

461

462

463

464

465

466

References

468

Agouridis, C. T., Workman, S. R., Warner, R. C., & Jennings, G. D. (2005). LIVESTOCK
GRAZING MANAGEMENT IMPACTS ON STREAM WATER QUALITY: A
REVIEW1. *JAWRA Journal of the American Water Resources Association*, *41*(3), 591–606.
https://doi.org/10.1111/J.1752-1688.2005.TB03757.X

Alvain, S., Moulin, C., Dandonneau, Y., & Bréon, F. M. (2005). Remote sensing of
phytoplankton groups in case 1 waters from global SeaWiFS imagery. *Deep Sea Research
Part I: Oceanographic Research Papers*, *52*(11), 1989–2004.
https://doi.org/10.1016/J.DSR.2005.06.015

Amorim, C. A., & Moura, A. do N. (2021). Ecological impacts of freshwater algal blooms on
water quality, plankton biodiversity, structure, and ecosystem functioning. *Science of the
Total Environment*, *758*. https://doi.org/10.1016/j.scitotenv.2020.143605

Anderson, D. M., Burkholder, J. M., Cochlan, W. P., Glibert, P. M., Gobler, C. J., Heil, C. A., …
Vargo, G. A. (2008). Harmful algal blooms and eutrophication: Examining linkages from
selected coastal regions of the United States. *Harmful Algae*, *8*(1), 39–53.
https://doi.org/10.1016/j.hal.2008.08.017

Bales, R. C., Molotch, N. P., Painter, T. H., Dettinger, M. D., Rice, R., & Dozier, J. (2006).
Mountain hydrology of the western United States. *Water Resources Research*, *42*(8), 8432.
https://doi.org/10.1029/2005WR004387

Beaver, J. R., Tausz, C. E., Scotese, K. C., Pollard, A. I., & Mitchell, R. M. (2018).
Environmental factors influencing the quantitative distribution of microcystin and common

489   potentially toxigenic cyanobacteria in U.S. lakes and reservoirs. *Harmful Algae*, *78*, 118–

490   128. https://doi.org/10.1016/j.hal.2018.08.004

491 Bosse, K. R., Sayers, M. J., Shuchman, R. A., Fahnenstiel, G. L., Ruberg, S. A., Fanslow, D. L.,

492   … Burtner, A. M. (2019). Spatial-temporal variability of in situ cyanobacteria vertical

493   structure in Western Lake Erie: Implications for remote sensing observations. *Journal of*

494   *Great Lakes Research*, *45*(3), 480–489. https://doi.org/10.1016/J.JGLR.2019.02.003

495 Boucher, J., Weathers, K. C., Norouzi, H., & Steele, B. (2018). Assessing the effectiveness of

496   Landsat 8 chlorophyll a retrieval algorithms for regional freshwater monitoring. *Ecological*

497   *Applications*, *28*(4), 1044–1054. https://doi.org/10.1002/EAP.1708

498 Canfield, D. E., Bachmann, R. W., & Hoyer, M. V. (n.d.). Long-term chlorophyll trends in

499   Florida lakes. *J. Aquat. Plant Manage*, *56*, 47–56. Retrieved from

500   http://water.epa.gov/lawsregs/rulesregs/upload/

501 Chen, T., & Guestrin, C. (2016). XGBoost: A scalable tree boosting system. *Proceedings of the*

502   *ACM SIGKDD International Conference on Knowledge Discovery and Data Mining*, *13-*

503   *17-August-2016*, 785–794. https://doi.org/10.1145/2939672.2939785

504 Christensen, V. G., & Khan, E. (2020). Freshwater neurotoxins and concerns for human, animal,

505   and ecosystem health: A review of anatoxin-a and saxitoxin. *Science of the Total*

506   *Environment*, *736*. https://doi.org/10.1016/j.scitotenv.2020.139515

507 Coffer, M. M., Schaeffer, B. A., Salls, W. B., Urquhart, E., Loftin, K. A., Stumpf, R. P., …

508   Darling, J. A. (2021). Satellite remote sensing to assess cyanobacterial bloom frequency

509   across the United States at multiple spatial scales. *Ecological Indicators*, *128*.

510   https://doi.org/10.1016/J.ECOLIND.2021.107822

511   Dunstan, P. K., Foster, S. D., King, E., Risbey, J., O'Kane, T. J., Monselesan, D., … Thompson,

512   P. A. (2018). Global patterns of change and variation in sea surface temperature and

513   chlorophyll a. *Scientific Reports 2018 8:1*, *8*(1), 1–9. https://doi.org/10.1038/s41598-018-

514   33057-y

515   Falconer, I. R., & Humpage, A. R. (2005). Health Risk Assessment of Cyanobacterial (Blue-

516   green Algal) Toxins in Drinking Water. *Int. J. Environ. Res. Public Health*, *2*(1), 43–50.

517   Retrieved from www.ijerph.org

518   Fleming, L. E., Rivero, C., Burns, J., Williams, C., Bean, J. A., Shea, K. A., & Stinn, J. (2002).

519   *Blue green algal (cyanobacterial) toxins, surface drinking water, and liver cancer in*

520   *Florida*. *Harmful Algae* (Vol. 1).

521   Gardner, J. R., Yang, X., Topp, S. N., Ross, M. R. V., Altenau, E. H., & Pavelsky, T. M. (2021).

522   The Color of Rivers. *Geophysical Research Letters*, *48*(1), e2020GL088946.

523   https://doi.org/10.1029/2020GL088946

524   Gatz, L. (2020). *Freshwater Harmful Algal Blooms: Causes, Challenges, and Policy*

525   *Considerations*. Retrieved from https://crsreports.congress.gov

526   Gobler, C. J., Burkholder, J. A. M., Davis, T. W., Harke, M. J., Johengen, T., Stow, C. A., &

527   Van de Waal, D. B. (2016). The dual role of nitrogen supply in controlling the growth and

528   toxicity of cyanobacterial blooms. *Harmful Algae*, *54*, 87–97.

529   https://doi.org/10.1016/J.HAL.2016.01.010

530   Graham, J. L., Jones, J. R., Jones, S. B., Downing, J. A., & Clevenger, T. E. (2004).

531   Environmental factors influencing microcystin distribution and concentration in the

532   Midwestern United States. *Water Research*, *38*(20), 4395–4404.

533   https://doi.org/10.1016/J.WATRES.2004.08.004

534   Hallegraeff, G. M., Anderson, D. M., Belin, C., Bottein, M. Y. D., Bresnan, E., Chinain, M., …

535   Zingone, A. (2021). Perceived global increase in algal blooms is attributable to intensified

536   monitoring and emerging bloom impacts. *Communications Earth & Environment 2021 2:1*,

537   *2*(1), 1–10. https://doi.org/10.1038/s43247-021-00178-8

538   Harvey, T., Kratzer, S., & Philipson, P. (2015). Satellite-based water quality monitoring for

539   improved spatial and temporal retrieval of chlorophyll-a in coastal waters. *Remote Sensing*

540   *of Environment*, *158*, 417–430. https://doi.org/10.1016/J.RSE.2014.11.017

541   Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C.,

542   … Suddleson, M. (2008). Eutrophication and harmful algal blooms: A scientific consensus.

543   *Harmful Algae*, *8*(1), 3–13. https://doi.org/10.1016/j.hal.2008.08.006

544   Henderson, K. A., Murdock, J. N., & Lizotte, R. E. (2021). Water depth influences algal

545   distribution and productivity in shallow agricultural lakes. *Ecohydrology*, *14*(6), e2319.

546   https://doi.org/10.1002/ECO.2319

547   Hill, R. A., Weber, M. H., Debbout, R. M., Leibowitz, S. G., & Olsen, A. R. (2018). The Lake-

548   Catchment (LakeCat) Dataset: characterizing landscape features for lake basins within the

549   conterminous USA. *Https://Doi.Org/10.1086/697966*, *37*(2), 208–221.

550   https://doi.org/10.1086/697966

551   Ho, J. C., Michalak, A. M., & Pahlevan, N. (2019). Widespread global increase in intense lake

552   phytoplankton blooms since the 1980s. *Nature 2019 574:7780*, *574*(7780), 667–670.

553   https://doi.org/10.1038/s41586-019-1648-7

554   Ho, J. C., Stumpf, R. P., Bridgeman, T. B., & Michalak, A. M. (2017). Using Landsat to extend

555   the historical record of lacustrine phytoplankton blooms: A Lake Erie case study. *Remote*

556   *Sensing of Environment*, *191*, 273–285. https://doi.org/10.1016/J.RSE.2016.12.013

557   Hudnell, H. K. (2008). Cyanobacterial harmful algal blooms: State of the science and research

558   needs. *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs*, 1–

559   957. https://doi.org/10.1007/978-0-387-75865-7/COVER

560   Hudnell, H. K. (2010, May). The state of U.S. freshwater harmful algal blooms assessments,

561   policy and legislation. *Toxicon*. https://doi.org/10.1016/j.toxicon.2009.07.021

562   Kahru, M., Gille, S. T., Murtugudde, R., Strutton, P. G., Manzano-Sarabia, M., Wang, H., &

563   Mitchell, B. G. (2010). Global correlations between winds and ocean chlorophyll. *J.

564   Geophys. Res*, *115*, 12040. https://doi.org/10.1029/2010JC006500

565   Kanoshina, I., Lips, U., & Leppänen, J. M. (2003). The influence of weather conditions

566   (temperature and wind) on cyanobacterial bloom development in the Gulf of Finland (Baltic

567   Sea). *Harmful Algae*, *2*(1), 29–41. https://doi.org/10.1016/S1568-9883(02)00085-9

568   Kärcher, O., Filstrup, C. T., Brauns, M., Tasevska, O., Patceva, S., Hellwig, N., … Markovic, D.

569   (2020). Chlorophyll a relationships with nutrients and temperature, and predictions for lakes

570   across perialpine and Balkan mountain regions. *Inland Waters*, *10*(1), 29–41.

571   https://doi.org/10.1080/20442041.2019.1689768/SUPPL_FILE/TINW_A_1689768_SM161

572   1.XLSX

573   Kosten, S., Huszar, V. L. M., Bécares, E., Costa, L. S., van Donk, E., Hansson, L. A., …

574   Scheffer, M. (2012). Warmer climates boost cyanobacterial dominance in shallow lakes.

575   *Global Change Biology*, *18*(1), 118–126. https://doi.org/10.1111/J.1365-

576   2486.2011.02488.X

577   Kotsiantis, S. B. (2011). Decision trees: a recent overview. *Artificial Intelligence Review 2011

578   39:4*, *39*(4), 261–283. https://doi.org/10.1007/S10462-011-9272-4

579   Kuhn, C., de Matos Valerio, A., Ward, N., Loken, L., Sawakuchi, H. O., Kampel, M., …

580   Butman, D. (2019). Performance of Landsat-8 and Sentinel-2 surface reflectance products

581   for river remote sensing retrievals of chlorophyll-a and turbidity. *Remote Sensing of*

582   *Environment*, *224*, 104–118. https://doi.org/10.1016/J.RSE.2019.01.023

583   Kutser, T., Pierson, D. C., Tranvik, L., Reinart, A., Sobek, S., & Kallio, K. (2005). Using

584   satellite remote sensing to estimate the colored dissolved organic matter absorption

585   coefficient in lakes. *Ecosystems*, *8*(6), 709–720. https://doi.org/10.1007/S10021-003-0148-6

586   Liaw A, Wiener M (2002). "Classification and Regression by randomForest." *R*

587   *News*, **2**(3), 18-22. https://CRAN.R-project.org/doc/Rnews/.

588   Liu, X., Feng, J., & Wang, Y. (2019). Chlorophyll a predictability and relative importance of

589   factors governing lake phytoplankton at different timescales. *Science of The Total*

590   *Environment*, *648*, 472–480. https://doi.org/10.1016/J.SCITOTENV.2018.08.146

591   Maberly, S. C., O'Donnell, R. A., Woolway, R. I., Cutler, M. E. J., Gong, M., Jones, I. D., …

592   Tyler, A. N. (2020). Global lake thermal regions shift under climate change. *Nature*

593   *Communications*, *11*(1). https://doi.org/10.1038/S41467-020-15108-Z

594   Matthews, M. W. (2011). A current review of empirical procedures of remote sensing in inland

595   and near-coastal transitional waters. *Http://Dx.Doi.Org/10.1080/01431161.2010.512947*,

596   *32*(21), 6855–6899. https://doi.org/10.1080/01431161.2010.512947

597   Messager, M. L., Lehner, B., Grill, G., Nedeva, I., & Schmitt, O. (2016). Estimating the volume

598   and age of water stored in global lakes using a geo-statistical approach. *Nature*

599   *Communications*, *7*. https://doi.org/10.1038/NCOMMS13603

600 Meyer, H., Reudenbach, C., Hengl, T., Katurji, M., & Nauss, T. (2018). Improving performance

601 of spatio-temporal machine learning models using forward feature selection and target-

602 oriented validation. *Environmental Modelling & Software*, *101*, 1–9.

603 https://doi.org/10.1016/J.ENVSOFT.2017.12.001

604 Mooij, W. M., Janse, J. H., De Senerpont Domis, L. N., Hülsmann, S., & Ibelings, B. W. (2007).

605 Predicting the effect of climate change on temperate shallow lakes with the ecosystem

606 model PCLake. *Hydrobiologia*, *584*(1), 443–454. https://doi.org/10.1007/S10750-007-

607 0600-2/FIGURES/3

608 O'Reilly, J. E., Maritorena, S., Mitchell, B. G., Siegel, D. A., Carder, K. L., Garver, S. A., …

609 McClain, C. (1998). Ocean color chlorophyll algorithms for SeaWiFS. *Journal of*

610 *Geophysical Research: Oceans*, *103*(C11), 24937–24953.

611 https://doi.org/10.1029/98JC02160

612 O'Reilly, J. E., Maritorena, S., Mitchell, B. G., Siegel, D. A., Carder, K. L., Garver, S. A., …

613 McClain, C. (1998). Ocean color chlorophyll algorithms for SeaWiFS. *Journal of*

614 *Geophysical Research: Oceans*, *103*(C11), 24937–24953.

615 https://doi.org/10.1029/98JC02160

616 Oleksy, I. A., Collins, S. M., Sillen, S. J., Topp, S. N., Austin, M., Hall, E. K., … Ross, M. R. V.

617 (2022). Heterogenous controls on lake color and trends across the high-elevation U.S.

618 Rocky Mountain region. *Environmental Research Letters*, *17*(10).

619 https://doi.org/10.1088/1748-9326/AC939C

620 Paerl, H. W., & Paul, V. J. (2012). Climate change: Links to global expansion of harmful

621 cyanobacteria. *Water Research*, *46*(5), 1349–1363.

622 https://doi.org/10.1016/j.watres.2011.08.002

623   Pal, M. (2007). Ensemble Learning with Decision Tree for Remote Sensing Classification.

624   https://doi.org/10.5281/ZENODO.1328942

625   Papenfus, M., Schaeffer, B., Pollard, A. I., & Loftin, K. (2020). Exploring the potential value of

626   satellite remote sensing to monitor chlorophyll-a for US lakes and reservoirs.

627   *Environmental Monitoring and Assessment*, *192*(12), 1–22. https://doi.org/10.1007/S10661-

628   020-08631-5/FIGURES/11

629   Pavelsky, T. M., & Smith, L. C. (2009). Remote sensing of suspended sediment concentration,

630   flow velocity, and lake recharge in the Peace-Athabasca Delta, Canada. *Water Resources*

631   *Research*, *45*(11), 11417. https://doi.org/10.1029/2008WR007424

632   R Core Team (2022). R: A language and environment for statistical computing. R Foundation

633   for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

634   Read, E. K., Carr, L., De Cicco, L., Dugan, H. A., Hanson, P. C., Hart, J. A., … Winslow, L. A.

635   (2017). Water quality data for national-scale aquatic research: The Water Quality Portal.

636   *Water Resources Research*, *53*(2), 1735–1745. https://doi.org/10.1002/2016WR019993

637   Rejmánková, E., & Komárková, J. (2005). Response of cyanobacterial mats to nutrient and

638   salinity changes. *Aquatic Botany*, *83*(2), 87–107.

639   https://doi.org/10.1016/j.aquabot.2005.05.011

640   Richardson, D. C., Holgerson, M. A., Farragher, M. J., Hoffman, K. K., King, K. B. S., Alfonso,

641   M. B., … Sweetman, J. N. (2022). A functional definition to distinguish ponds from lakes

642   and wetlands. *Scientific Reports 2022 12:1*, *12*(1), 1–13. https://doi.org/10.1038/s41598-

643   022-14569-0

644   Salem, S. I., Higa, H., Kim, H., Kobayashi, H., Oki, K., & Oki, T. (2017). Assessment of

645   Chlorophyll-a Algorithms Considering Different Trophic Statuses and Optimal Bands.

646   *Sensors (Basel, Switzerland)*, *17*(8). https://doi.org/10.3390/S17081746

647   Seegers, B. N., Werdell, P. J., Vandermeulen, R. A., Salls, W., Stumpf, R. P., Schaeffer, B. A.,

648   … Loftin, K. A. (2021). Satellites for long-term monitoring of inland U.S. lakes: The

649   MERIS time series and application for chlorophyll-a. *Remote Sensing of Environment*, *266*,

650   112685. https://doi.org/10.1016/J.RSE.2021.112685

651   Slonecker, E. T., Jones, D. K., & Pellerin, B. A. (2016). The new Landsat 8 potential for remote

652   sensing of colored dissolved organic matter (CDOM). *Marine Pollution Bulletin*, *107*(2),

653   518–527. https://doi.org/10.1016/J.MARPOLBUL.2016.02.076

654   Smith, B., Pahlevan, N., Schalles, J., Ruberg, S., Errera, R., Ma, R., … Kangro, K. (2021). A

655   Chlorophyll-a Algorithm for Landsat-8 Based on Mixture Density Networks. *Frontiers in*

656   *Remote Sensing*, *0*, 5. https://doi.org/10.3389/FRSEN.2020.623678

657   Smith, V. H. (2003). Eutrophication of Freshwater and Coastal Marine Ecosystems A Global

658   Problem. https://doi.org/10.1065/esor2002.12.142

659   Soranno, P. A., Bacon, L. C., Beauchene, M., Bednar, K. E., Bissell, E. G., Boudreau, C. K., …

660   Yuan, S. (2017). LAGOS-NE: a multi-scaled geospatial and temporal database of lake

661   ecological context and water quality for thousands of US lakes. *GigaScience*, *6*(12), 1–22.

662   https://doi.org/10.1093/GIGASCIENCE/GIX101

663   Stanley, E. H., Collins, S. M., Lottig, N. R., Oliver, S. K., Webster, K. E., Cheruvelil, K. S., &

664   Soranno, P. A. (2019). Biases in lake water quality sampling and implications for

665   macroscale research. *Limnology and Oceanography*, *64*(4), 1572–1585.

666   https://doi.org/10.1002/LNO.11136

667   Stets, E. G., Sprague, L. A., Oelsner, G. P., Johnson, H. M., Murphy, J. C., Ryberg, K., …

668   Riskin, M. L. (2020). Landscape Drivers of Dynamic Change in Water Quality of U.S.

669   Rivers. *Environmental Science and Technology*, *54*(7), 4336–4343.

670   https://doi.org/10.1021/ACS.EST.9B05344/ASSET/IMAGES/LARGE/ES9B05344_0005.J

671   PEG

672   Tilstone, G., Gons, H. J., Ruddick, K. G., & Rijkeboer, M. (2001). Optical remote sensing of

673   chlorophyll a in case 2 waters by use of an adaptive two-band algorithm with optimal error

674   properties. *Applied Optics, Vol. 40, Issue 21, Pp. 3575-3585*, *40*(21), 3575–3585.

675   https://doi.org/10.1364/AO.40.003575

676   Topp, S. N., Pavelsky, T. M., Dugan, H. A., Yang, X., Gardner, J., & Ross, M. R. V. (2021).

677   Shifting Patterns of Summer Lake Color Phenology in Over 26,000 US Lakes. *Water*

678   *Resources Research*, *57*(5), e2020WR029123. https://doi.org/10.1029/2020WR029123

679   Topp, S. N., Pavelsky, T. M., Jensen, D., Simard, M., & Ross, M. R. V. (2020). Research Trends

680   in the Use of Remote Sensing for Inland Water Quality Science: Moving Towards

681   Multidisciplinary Applications. *Water 2020, Vol. 12, Page 169*, *12*(1), 169.

682   https://doi.org/10.3390/W12010169

683   Topp, S. N., Pavelsky, T. M., Stanley, E. H., Yang, X., Griffin, C. G., & Ross, M. R. V. (2021).

684   Multi-decadal improvement in US Lake water clarity. *Environmental Research Letters*,

685   *16*(5). https://doi.org/10.1088/1748-9326/ABF002

686   Tzortziou, M., Subramaniam, A., Herman, J. R., Gallegos, C. L., Neale, P. J., & Harding, L. W.

687   (2007). Remote sensing reflectance and inherent optical properties in the mid Chesapeake

688   Bay. *Estuarine, Coastal and Shelf Science*, *72*(1–2), 16–32.

689   https://doi.org/10.1016/J.ECSS.2006.09.018

690   U.S. Environmental Protection Agency (USEPA). 2009. National Lakes Assessment: A

691   Collaborative Survey of the Nation's Lakes. EPA 841-R-09-001. U.S. Environmental

692   Protection Agency, Office of Water and Office of Research and Development,

693   Washington, D.C.

694   Wilkinson, G. M., Walter, J. A., Buelo, C. D., & Pace, M. L. (2022). No evidence of widespread

695   algal bloom intensification in hundreds of lakes. *Frontiers in Ecology and the Environment*,

696   *20*(1), 16–21. https://doi.org/10.1002/FEE.2421

697   Willard, J. D., Read, J. S., Topp, S., Hansen, G. J. A., & Kumar, V. (2022). Daily surface

698   temperatures for 185,549 lakes in the conterminous United States estimated using deep

699   learning (1980–2020). *Limnology and Oceanography Letters*, *7*(4), 287–301.

700   https://doi.org/10.1002/LOL2.10249

701   Witter, D. L., Ortiz, J. D., Palm, S., Heath, R. T., & Budd, J. W. (2009). Assessing the

702   application of SeaWiFS ocean color algorithms to Lake Erie. *Journal of Great Lakes*

703   *Research*, *35*(3), 361–370. https://doi.org/10.1016/J.JGLR.2009.03.002

704   Wurtsbaugh, W. A., Paerl, H. W., & Dodds, W. K. (2019). Nutrients, eutrophication and harmful

705   algal blooms along the freshwater to marine continuum. *WIREs Water*, *6*(5).

706   https://doi.org/10.1002/wat2.1373

707   Yang, X., O'Reilly, C. M., Gardner, J. R., Ross, M. R. V., Topp, S. N., Wang, J., & Pavelsky, T.

708   M. (2022). The Color of Earth's Lakes. *Geophysical Research Letters*, *49*(18),

709   e2022GL098925. https://doi.org/10.1029/2022GL098925

710   Zheng, G., & DiGiacomo, P. M. (2017). Remote sensing of chlorophyll-a in coastal waters based

711   on the light absorption coefficient of phytoplankton. *Remote Sensing of Environment*, *201*,

712   331–341. https://doi.org/10.1016/J.RSE.2017.09.008

CHAPTER TWO

Validation of the Cyanobacteria Assessment Network (CyAN) in Wyoming Lakes

Abstract

Harmful cyanobacterial blooms (HCBs) pose a major threat to both ecosystem and human health and have led to a need for frequent water quality monitoring. However, traditional in-situ monitoring programs are limited in their capacity to address the spatial and temporal coverage of HCBs. Remote sensing tools, such as the Cyanobacteria Assessment Network (CyAN), can provide daily estimates of cyanobacteria abundance in large lakes, yet the accuracy of these tools is still not fully understood. In particular, the relatively few existing validation studies have utilized existing datasets which have varying degrees of consistency and lack information on taxonomy. Our study aimed to address some of the limitations in the initial validation of CyAN and provide useful information that supports the most effective use of this tool for lake water quality monitoring in Wyoming. Using a targeted validation approach, we found that there was a positive relationship (R^2 0.62) between remote sensing estimates and in-situ cell counts. However, our analysis revealed the tendency for CyAN to overestimate cell counts (prediction bias of -11,070) and predict false positives. Given that this tool lacks the capability to provide information in near-shore environments or on toxicity, it is unlikely that CyAN will replace in-situ monitoring efforts. However, the spatial and temporal coverage of remote sensing tools such as CyAN can still provide useful information on the spatial and temporal patterns cyanobacteria blooms and help inform targeted monitoring approaches.

Introduction

Algal blooms are expected to increase in frequency and intensity due to climate change, but detecting blooms poses challenges to the land and water management agencies that have a critical role in monitoring aquatic ecosystems. Accurate detection of Harmful Cyanobacterial Blooms (HCBs) is of particular importance due to the potentially toxic effects they can have on human health (Chorus and Welker, 2021; Duy et al., 2000; Buratti et al., 2017). Water quality impairment in lakes and reservoirs, including detecting HCBs, has historically been monitored and evaluated through in situ and laboratory sampling procedures (Averett and Schroder, 1994). Although in-situ methods are effective for the detection of HCBs, they struggle to account for the spatial and temporal distribution of cyanobacteria due to the complex biotic and abiotic interactions that drive their concentration (Graham et al. 2004).

Cyanobacteria abundance can be highly variable and respond rapidly to short-term environmental fluctuations in nutrients, precipitation, and temperature (Heisler et al., 2008; Egerton et al., 2014; Reinl et al., 2021). As such, cyanobacterial blooms can develop and persist on a wide range of timescales (days, weeks, or months) (Heisler et al., 2008). This poses a challenge for in-situ monitoring programs that are often designed for broad-scale monitoring on a monthly or seasonal basis. In-situ monitoring programs are also limited by resources because identifying trends usually takes years of continued sampling across multiple sites. Hence, most studies focus on a small number of lakes, and are typically biased toward larger lakes in populated areas, as large-scale and prolonged sampling can be difficult and expensive (Stanley et al. 2019).

In recent years, the application of remote sensing and satellite imagery to water quality monitoring has overcome these limitations of in situ sampling programs and helped address cyanobacterial dynamics over larger spatial and temporal scales (Shi et al., 2019; Coffer et al. 2021; Ignatius et al. 2022; Schaeffer et al. 2022). The application of remote sensing methods for water quality studies began in the 1980s (Gordon and Morel 1983). Spectrometers onboard satellites measure the radiance (surface reflectance) of visible light observed over the Earth's surface. However, when measuring reflectance over a water body there are several constituents within the water column that affect reflectance values. The relationship regarding how different water column constituents reflect or absorb light, known as the spectral response curves of different spectra (constituents), can be used to obtain information regarding the concentrations of certain water quality parameters.

The unique absorption and fluorescence characteristics of chlorophyll-a has been the focus of numerous algorithms that retrieve chlorophyll-a concentrations from satellite imagery. For example, waterbodies with high phytoplankton biomass have a spectral response that results in peaks in reflectance spectra at 709 nm (Gower et al. 1999). The height of this peak, termed the fluorescence line height, has been used to define the Maximum Chlorophyll Index (MCL) to characterize harmful algal blooms (Gower et al. 1999). The MCI was designed for the detection of algal blooms with a minimum chlorophyll-a concentration of 30 ug / L and has been used to monitor phytoplankton blooms across the globe (Gower et al. 2008; Schaeffer et al. 2022). However, chlorophyll-based algorithms such as the MCI flag high chlorophyll pixels regardless of what functional group is present (e.g., diatoms as opposed to cyanobacteria).

The continued development of these retrieval algorithms has resulted in the Cyanobacteria Index (CI) and CIcyano algorithms that focus on cyanobacterial presence. The CI

was developed by Wynne et al. (2008) and utilizes the peaks in reflectance spectra at 681 nm, as opposed to 709 nm included in the MCI. This approach covers peaks in chlorophyll absorption and fluorescence. Importantly, reflectance signatures or spectra in remote sensing and in-situ data have been shown to differ among various phytoplankton species. Specifically, cyanobacteria have been shown to display significantly lower fluorescence values than other phytoplankton genera (Seppala et al., 2007, Wynne et al., 2008). Therefore, where chlorophyll absorption peaks and fluorescence drops the CI algorithm is able to provide estimates of cyanobacterial presence. This method has proven to be effective where blooms are primarily dominated by *Microcystis sp.* (Wynne et al., 2008). However, this method was also shown to overpredict concentrations of cyanobacteria abundance due to the instances where green algae (not cyanobacteria) do not fluoresce (Wynne et al. 2013). To address this, the CIcyano algorithm was developed by Lunetta et al. (2015) which includes the 620 nm band that is sensitive to phycocyanin, a pigment unique to cyanobacteria.

The development of such algorithms has resulted in a capacity to specifically estimate cyanobacterial density and provide helpful information regarding the presence of potentially toxic species. This has simultaneously resulted in the development of numerous programs by NASA, the National Oceanic and Atmospheric Administration (NOAA), and the Environmental Protection Agency (EPA) to deliver useful products to resource managers. The Cyanobacteria Assessment Network (CyAN), uses images from the Sentinel-3 satellite and the CIcyano algorithm to provide estimations of cyanobacteria abundance (Wynne et al., 2018). The spatial resolution of Sentinel 3 pixels is relatively large (300 meters x 300 meters), compared to the spatial resolution of Landsat imagery (30 meters by 30 meters). Thus, CyAN estimates are limited to relatively large waterbodies to avoid adjacency effects and pixel mixing (Clark et al.,

2017). This remote sensing product provides daily and 7-day maximum composite observations of cyanobacteria estimates, and currently serves as a major synoptic tool for resource managers. However, matchups of in situ data and remote sensing estimations are still needed to determine the accuracy of these methods.

Previous validation efforts of CyAN have been limited to one species, *Microcystis sp.*, or provide no information on taxonomic differences (Mishra et al., 2021; Lunetta et al., 2015). Furthermore, published validation locations for CyAN studies have been restricted to 14 US states (Mishra et al., 2021). In the western US, only California, Oregon, Utah, and Idaho are represented. In this study, our goal was to validate the effectiveness of the CyAN product and CIcyano algorithm (used interchangeably here) for estimating cyanobacteria abundance in Wyoming lakes and reservoirs. Specifically, we address gaps in understanding regarding the model performance across different cyanobacteria species and across a wide range of lakes with different trophic status. Overall, we aim to provide information that supports the most strategic use of this product as a tool to monitor cyanobacterial blooms in Wyoming waterbodies.

Materials and Methods

Study Design and Data Collection

We conducted a remote sensing verification study during the summer of 2021 across 12 lakes and reservoirs in Wyoming. We selected 12 out of 40 resolvable waterbodies to capture a wide range of lake and landscape features (Table 1, Figure 1). For each waterbody, three sampling locations were identified that reduced the potential for adjacency effects and pixel mixing from shoreline pixels. A one pixel buffer (300 m x 300 m) was used to select open water

sites across pelagic, riverine, and transitional environments (Figure 2). We collected water quality samples monthly, when feasible, at each waterbody from June 2021 to September 2021.

We collected phytoplankton, chlorophyll-a, nutrients, zooplankton, and physicochemical parameters (such as pH and dissolved oxygen) across all sites. Phytoplankton samples were collected for remote sensing verification at each sampling location at depths just below the surface using 250 milliliter (mL) polypropylene bottles. Phytoplankton samples were preserved using a Lugol's iodine solution and stored in a dark container until processing. In addition, we collected chlorophyll-a samples by immediately filtering 500 mLs of lake water taken at 0.5 meters depth using a van Dorn sampler. Filters were placed inside sterile film canisters to prevent light penetration. We also collected water samples for total nitrogen (TN) and total phosphorus (TP) analysis and preserved them using sulfuric acid (H_2SO_4). Chlorophyll-a, TN, and TP samples were all kept on ice (~4° C) while in the field and frozen upon return to the laboratory. Zooplankton samples were collected using a zooplankton net with vertical tows were taken within the water column at each site. We preserved zooplankton samples with a 90 % ethanol solution and stored samples in a dark container until processing. Lastly, we recorded vertical profiles of various water quality parameters such as temperature, dissolved oxygen, conductivity, and pH at 0.5 - 1.0 meter intervals using a Yellow Springs Instrument (YSI) ProDSS handheld multimeter. The was calibrated at each sampling location prior to each sampling event.

Laboratory Methods

We enumerated cyanobacteria in phytoplankton samples using a Sedgewick-Rafter counting chamber. Counting chambers are the most common method for phytoplankton

enumeration and are well adapted for samples of high population density, such as algal blooms. We examined the presence of cyanobacterial species in each sample and recorded filaments or colonies of these species as objects or units. The average number of cells per filament or colony was estimated by counting the number of cells in the first 10 filaments or colonies. If this was not feasible, we used average cell number / unit from literature (Olenina et al., 2016). A minimum of four rows containing 50 squares was counted per sample. Then, Equation 1 was used to calculate the number of cells per unit of volume, where N represents the total number of cells counted, V is the volume of the chamber in mm^3, A is area of a field in mm^2, N_f is number of fields counted, d is depth of chamber in mm, and C is the concentration factor (if used).

$$Equation\ 1 \qquad Cells/mL = \frac{N * V}{A_f * N_f * C}$$

We processed chlorophyll-a samples using a Turner Designs AquaFluor Handheld Fluorometer/Turbidimeter. We extracted samples in 10 milliliters of 90 % buffered ethanol (EtOH) for 12-24 hours. 2.7 mL of sample was added to a glass cuvette and fluorescence units were recorded. Then, we added 90 microliters (uL) of 0.1 N hydrochloric acid and recorded the fluorescence units 90 seconds later. We determined the concentration of chlorophyll-a in the sample by 1) determining the amount of chlorophyll-a in the extraction using an instrument-specific standard curve that relates chlorophyll-a concentration to the difference between initial fluorescence and fluorescence after acidification and 2) correcting for the volume of lake water filtered for the extraction (usually 500 mL). All chlorophyll-a samples were processed within a month of sample collection.

Data Processing and Analysis

Estimates of cyanobacteria abundance derived from CyAN were obtained from the National Aeronautics and Space Administration (NASA) Earth Science Data Systems (ESDS) web portal. The CyAN product represents a level 3 product, defined as any geophysical parameter (such as chlorophyll-a, or cyanobacteria abundance) mapped in uniform geo-spatial scales with completeness and consistency. CyAN data is made available through GeoTiff files of tiles that cover large areas of the continental US. For this study, we downloaded tiles covering the spatial extent of Wyoming (3_2, 3_3, 4_2, and 4_3) for dates that coincided with sampling events.

We extracted digital number (DN) values provided by CyAN over pixels that matched sampling locations and dates. We also extracted DN data from existing cell count data provided by the Wyoming Department on Environmental Quality (DEQ), although these represented few (5%) of all observations. These DN values were converted into CIcyano and then cyanobacteria cells / mL with the following equations (Equation 2; Equation 3).

$$Equation\ 2 \qquad CIcyano = 10^{\left(\frac{3.0}{250}\right)*DN-4.2}$$

$$Equation\ 3 \qquad Cells/mL = CIcyano * 1000000$$

To test the accuracy of the CyAN product at different spatial resolutions, we not only extracted 1 x 1 pixel windows that corresponded to sampling locations but also 3 x 3 and 5 x 5 pixel means surrounding sampling locations. All processing of remote sensing data and spatial analysis was done in ArcMap Version 10.7.1 and converted to text files for use in Program R. We visualized the relationship of in-situ cyanobacteria cells / mL and remote sensing cells / mL

in Program R (R Core Team, 2022). We calculated the mean absolute error (MAE) and root mean squared error (RMSE) as a measure of error between these two different methods. Additionally, we categorized both sets of data into two categories: 1) below bloom threshold (< 20,000 cells / mL) and 2) above bloom threshold (> 20,000 cells / mL) based on existing criteria (Chorus and Welker, 2021). We then examined the effectiveness of CyAN to correctly categorize bloom based on these thresholds through a confusion matrix using the *caret* package.

Results

In-situ Data

The sites included in our study covered a wide range of trophic status and productivity throughout the summer. Half (6 out of 12) of sites had chlorophyll-a values that reflected eutrophic conditions at some point during the summer (Figure 3). Whereas, four sites had chlorophyll-a reflected a more mesotrophic lake status during the summer (Figure 3). Chlorophyll-a was not collected at New Fork Lakes or Yellowstone Lake, however these lakes are assumed to be mostly oligotrophic. Phytoplankton samples exhibited a wide range of taxonomic diversity among both green algae and cyanobacterial species. The most common algae species observed were *Asterionella formosa* and *Fragilaria sp.*, both belonging to the class Bacillariophyceae (diatoms). *Aphanizomenon flos-aquae* and *Anabeana sp.* were the most common cyanobacterial species present and reflected the greatest proportion of cell counts. While the presence of non-cyanobacterial species were recorded (i.e. *Fragilaria sp.*), only units of cyanobacteria species were counted. Other cyanobacteria species observed included *Microcystis aeruginosa*, *Woronichinia*, and *Gleotrichia echinulata (*Figure S1*)*.

In-situ cell counts of cyanobacteria cell counts ranged from 0 cells / mL to 94,124 cells / mL. Cyanobacteria are naturally found in low densities across almost all lakes and reservoirs,

and cyanobacteria species were identified in 8 out of 12 sites. We found the presence of cyanobacteria to be rare in June and July months and peaked later in the summer during August and September. CIcyano values were highest among sites in transitional environments, followed by riverine and lacustrine sites, respectively.

Lastly, to determine the error associated with our counting methods we collected duplicate samples to enumerate and compare with original samples. We observed a significant positive correlation (R^2 of 0.9) between cyanobacteria cell counts and duplicate samples taken at sites (Figure S2). Sample duplicates covered the observed range of cell abundance across all samples and included multiple cyanobacteria species.

Remote sensing verification

CIcyano values derived from CyAN ranged from 0 - 120 covering cell counts from ~ 6,500 cells / mL - 173,780 cells / mL. However, cell counts corresponded primarily to relatively low ranges (10,000 - 109,999). The highest cell counts derived from CyAN were observed in August months across the majority of sites. The majority of CIcyano values were extracted from the corresponding sampling date of in-situ data. In instances where cloud cover resulted in invalid pixels, the CIcyano value from the previous or following day was extracted. There was a positive correlation between the cell counts derived from CIcyano and from in-situ data (R^2 of 0.62). Mean absolute error (MAE), the difference between observed and predicted values, was 12,434 (Table 2). Whereas, root mean squared error (RMSE) was 24,833 cells / mL (Table 2). However, prediction bias, the amount by which in-situ observations were greater than CIcyano was -11,070, indicating the over estimation of cell densities by CIcyano (Figure 4). In addition, we observed that prediction error tended to be higher when samples displayed mixed cyanobacteria assemblages (Figure 5). Lastly, we found that CIcyano values extracted across

pixel means (3 x 3 and 5 x 5) had substantially higher prediction errors. For example, matchups that included 3 x 3 pixel means had an RMSE of 109,753 cells / mL, compared to that of 1 x 1 pixels (24,833 cells / mL) (Table 2).

Regardless of the accuracy of CIcyano cell counts compared to in-situ cell counts, we found that CIcyano effectively classified algal bloom thresholds. Bloom classification was evaluated through a confusion matrix as well as various accuracy and error metrics (Table 3; Figure 6). For observations below the threshold for bloom presence (<20,000 cells / mL), CIcyano had an overall accuracy of 88 %. For observations above the threshold for bloom presence (>20,000 cells / mL), CIcyano had a balanced accuracy of 71 %. Overall, CIcyano had a global accuracy of 86 % with a 95% confidence interval between 79 % and 94 % in its ability to classify cyanobacterial bloom thresholds.

Lastly, we also evaluated the relationship between cyanobacteria density and chlorophyll-a concentrations. Cyanobacteria density varied across a wide range of chlorophyll-a concentrations and reflected a poor relationship between the two metrics. We observed that high cyanobacteria cell densities (> 20,000 cells / mL) tended to have chlorophyll-a concentrations in the range of 0 – 20 ug/L (Figure 7). However, these high cyanobacteria densities could be observed with chlorophyll-a values as low as 5 ug / L. Overall, high chlorophyll-a concentrations (> 30 ug/L) did not necessarily indicate the presence of cyanobacteria (Figure 7).

Discussion

Algorithm Validation

Our primary motivation for this study was to supplement the initial validation of the CIcyano algorithm by addressing its effectiveness at capturing HCB dynamics in Wyoming lakes

and reservoirs. Given the interest in remote sensing for water quality monitoring, a thorough understanding of the uncertainty of these methods at regional scales is critical to guide the appropriate use of these tools for state management agencies. Our study was designed to fill in the gaps from the few existing validation studies by using a targeted approach. Our analysis revealed that CIcyano tended to overestimate cell counts when compared to in-situ data, yet still was robust enough to provide accurate estimates of bloom status when compared to in-situ thresholds.

The initial validation of CIcyano derived cell counts was conducted by Lunetta et al. (2015) and utilized the National Lakes Assessment Program (NLAP) inventory as well as various data sets from state agencies for remote sensing validation. Lunetta et al. (2015) reported a RMSE of 225,369 cells / mL and an R^2 of 0.87 when comparing in-situ cell counts to CIcyano cell counts. However, our analysis showed an RMSE of 24,833 cells / mL with an R^2 of 0.62. While considerably lower, the Lunetta et al. (2015) analysis used in-situ cell count data that covered much larger cell densities (300,000 - 1,000,000). They reported a more even distribution of prediction error (under prediction and over prediction). However, their study used temporal windows of up to 7 days. Given previous studies that have shown the potential for CIcyano to overestimate and report false-positives and the results of this study, it is possible that the greater time window used in Lunetta et al. (2015) resulted in a more random distribution of cell count estimates.

The temporal stability of cyanobacteria abundance varies depending on factors that interact to drive bloom formation. In particular, wind speed has been shown to drive the spatial distribution of cyanobacteria blooms in the short-term (Bresciani et al., 2013; Xue et al., 2022). Given the sensitivity of cyanobacteria bloom dynamics to daily climate factors such as wind

speed and light availability, a 7-day temporal window for satellite matchups likely introduces

substantial variation. The ability of wind speed to drive the spatial distribution of cyanobacteria

densities within lakes could have also influenced the results presented in this study.

Investigations into available wind energy have shown Wyoming to be one of the regions in the

U.S. with the greatest wind speed (Martner and Marwitz, 1982). However, we attempted to

minimize the effect of wind speed on our results by sampling during clear, calm conditions.

The evaluation of CIcyano across different taxa provides new context regarding the

uncertainty of this remote sensing method. The CI was originally developed for the use of

detecting *Microcystis sp.* in the Great Lakes region (Wynne et al. 2008). CIcyano has been

developed to apply to a wide range of cyanobacteria taxa, yet no study to our knowledge has

reported differences in prediction accuracy across different cyanobacteria taxa. The majority of

our samples present with cyanobacteria were dominated by *Aphanizomenon flos-aquae.* This

species has fundamentally different morphological characteristics compared to *Microcystis sp.*

and represents a filamentous type of cyanobacteria. Differences in taxonomic biovolumes and

the effects on reflectance as determined by the quantity of pigment and scattering of individual

species has been shown to be substantial (Wood et al., 2009). For example, the biovolume of

Microcystis sp. can be 19 um^3 and *Anabaena circinalis* can be 208 um^3 (Wood et al. 2009).

Furthermore, published bio volumes can differ based on the size class of specific cyanobacteria

taxa (small *Microcystis sp.* = 19 um^3 vs. large *Microcystis sp.* = 93 um^3). The dominant presence

of *Aphanizomenon flos-aquae* in our samples, combined with our results that CIcyano tends to

overpredict cell counts may shed light on potential differences in accuracy across different

cyanobacteria assemblages. In other words, CIcyano may be more prone to overfitting when it

comes to cyanobacteria assemblages consisting of filamentous species (such as *Aphanizomenon flos-aquae* or *Anabaena circinalis*), as opposed to species with spherical morphologies.

While cell counts are particularly useful in determining risk based on published thresholds, the evaluation of CIcyano across broad categories may be of particular use. For example, if in-situ cell counts were enumerated at 70,000 cells / mL but CIcyano reported a cell density of 200,000 cells/mL, there is limited utility in the large difference when both methods indicate a bloom is present (> 20,000 cells / mL). Therefore, we evaluated the ability of CIcyano to classify bloom presence based on the available bloom thresholds. CIcyano was capable of classifying bloom presence with a global accuracy of 88% and a kappa of 0.52. This is consistent with the only other known analysis where CIcyano has been validated across categories (bloom presence vs. bloom absence). Similarly, Mishra et al. (2021) found that CIcyano was capable of classifying bloom presence with a global accuracy of 84 %. Importantly, the most common misclassification (31%) was the tendency for CIcyano to predict bloom presence when in-situ data reflected absence (Mishra et al., 2021). Our study also indicated that the most common misclassification were instances of false positives, where CIcyano predicted bloom presence where in-situ data did not.

Management Implications

The deployment of in-situ water quality monitoring plans to monitor HCBs is limited by the available resources (time and funding) required to cover high spatial and temporal variation of HCBs, especially in a large, sparsely populated state such as Wyoming. Remote sensing products, such as CyAN, have been developed as an early warning tool for land and water management agencies to provide estimates of cyanobacteria densities. The data collected in this

study provides insight into the effectiveness of CyAN compared to in-situ cyanobacteria collection and processing.

CyAN was found to be particularly effective in classifying the presence or absence of HCBs as defined by existing thresholds (20,000 cells / mL). There were few instances where in-situ data indicated a bloom was present, but CyAN failed to flag pixels as such (false-negatives). However, when cyanobacteria are present above the bloom threshold of 20,000 cells / mL, our results indicated that CyAN often overpredicts the abundance of cyanobacteria compared to in-situ data. Furthermore, we found that CyAN tends to overpredict when there is a high level of taxonomic diversity among phytoplankton species present with in-situ samples. These results are consistent with previous results of the Cyanobacteria Index and CIcyano which highlight overprediction in instances where green algae do not fluoresce (Wynne et al., 2013).

These findings provide important context for resource managers to determine the best way to utilize this remote sensing tool. The major benefits of CyAN are the ability to explore cyanobacterial estimates across a large spatial area without having to spend time and funding on in-situ sample collection and enumeration. This is particularly useful for understanding cyanobacterial variation within lakes and across waterbodies in high elevation or remote environments that may require additional resources. However, CyAN provides no information regarding the toxicity of cyanobacteria and does not capture near-shore environments where recreational exposure is assumed to be highest. Therefore, CyAN will likely not replace in-situ sampling methods for cyanobacteria that are focused on addressing the harmful exposure to cyanotoxins.

Given the inability for CyAN to capture near-shore pixels and toxicity, CyAN has the greatest potential to capture the broad lake-wide distribution of cyanobacteria and long-term

trends in bloom occurrence. Current cyanobacteria in-situ monitoring is often constrained to shorelines due to the ease of sampling and available resources, yet CyAN can provide important context regarding the size of blooms. Furthermore, the existing and continued record of daily CyAN observations will allow for trend analyses that shine light on the spatial and temporal trends of HCBs. Overall, CyAN has the greatest potential to classify blooms and understand elusive spatial and temporal trends of HCBs, yet in-situ sampling methods will likely remain the most effective way to monitor and evaluate HCB exposure.

Conclusion

This study provides data designed to evaluate the effectiveness of the CyAN program and CIcyano algorithms in Wyoming lakes and reservoirs. We found that cell counts derived from the CIcyano algorithm were positively correlated with in-situ cell counts, however CIcyano tended to overestimate at higher cell concentrations. The inclusion of mixed phytoplankton assemblages and different taxa of cyanobacteria supplement previous studies that show in instances where multiple species are present, CIcyano can overestimate cell counts. Regardless, we found CIcyano to be effective in classifying bloom presence as defined by existing bloom thresholds. While in-situ sampling monitoring is still needed to address toxicity and presence of cyanobacteria in high-use shoreline areas, CyAN provides the opportunity to classify blooms in open-water areas and understand elusive spatial and temporal trends of HCBs.

Bibliography:

Averett, R. C., & Schroder, L. J. (1994). *A Guide to the Design of Surface-Water-Quality Studies*. *U.S. Geological Survey*. https://doi.org/10.3133/ofr93105

Bresciani, M., Rossini, M., Morabito, G., & Matta, E. (n.d.). Analysis of within-and between-day chlorophyll-a dynamics in Mantua Superior Lake, with a continuous spectroradiometric measurement Evaluation of different approaches for the retrieval of sun-induced fluorescence View project Trophic chain transfer of contaminants in aquatic environments View project. https://doi.org/10.1071/MF12229

Buratti, F. M., Manganelli, M., Vichi, S., Stefanelli, M., Scardala, S., Testai, E., & Funari, E. (2017). Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. *Archives of Toxicology 2017 91:3*, *91*(3), 1049–1130. https://doi.org/10.1007/S00204-016-1913-6

Chorus, I., & Welker, M. (2021). Toxic Cyanobacteria in Water. *Toxic Cyanobacteria in Water*. https://doi.org/10.1201/9781003081449

Clark, J. M., Schaeffer, B. A., Darling, J. A., Urquhart, E. A., Johnston, J. M., Ignatius, A. R., … Stumpf, R. P. (2017). Satellite monitoring of cyanobacterial harmful algal bloom frequency in recreational waters and drinking water sources. *Ecological Indicators*, *80*, 84–95. https://doi.org/10.1016/J.ECOLIND.2017.04.046

Coffer, M. M., Schaeffer, B. A., Salls, W. B., Urquhart, E., Loftin, K. A., Stumpf, R. P., … Darling, J. A. (2021). Satellite remote sensing to assess cyanobacterial bloom

frequency across the United States at multiple spatial scales. *Ecological Indicators*, *128*. https://doi.org/10.1016/j.ecolind.2021.107822

Duy, T. N., Lam, P. K., Shaw, G. R., & Connell, D. W. (2000). Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water. *Reviews of Environmental Contamination and Toxicology*, *163*, 113–185. https://doi.org/10.1007/978-1-4757-6429-1_3/COVER

Egerton, T. A., Morse, R. E., Marshall, H. G., & Mulholland, M. R. (2014). Emergence of Algal Blooms: The Effects of Short-Term Variability in Water Quality on Phytoplankton Abundance, Diversity, and Community Composition in a Tidal Estuary. *Microorganisms*, *2*(1), 33. https://doi.org/10.3390/MICROORGANISMS2010033

Gordon, H. R., & Morel, A. Y. (1983). Remote Assessment of Ocean Color for Interpretation of Satellite Visible Imagery: A Review, *4*. https://doi.org/10.1029/LN004

Gower, J. F. R., Doerffer, R., & Borstad, G. A. (1999). Interpretation of the 685nm peak in water-leaving radiance spectra in terms of fluorescence, absorption and scattering, and its observation by MERIS. *International Journal of Remote Sensing*, *20*(9), 1771–1786. https://doi.org/10.1080/014311699212470

Gower, J., King, S., & Goncalves, P. (2008). Global monitoring of plankton blooms using MERIS MCI. In *International Journal of Remote Sensing* (Vol. 29, pp. 6209–6216). Taylor and Francis Ltd. https://doi.org/10.1080/01431160802178110

Graham, J. L., Jones, J. R., Jones, S. B., Downing, J. A., & Clevenger, T. E. (2004).
Environmental factors influencing microcystin distribution and concentration in the
Midwestern United States. *Water Research*, *38*(20), 4395–4404.
https://doi.org/10.1016/J.WATRES.2004.08.004

Heisler, J., Glibert, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W.
C., … Suddleson, M. (2008). Eutrophication and Harmful Algal Blooms: A Scientific
Consensus. *Harmful Algae*, *8*(1), 3. https://doi.org/10.1016/J.HAL.2008.08.006

Hunter, P. D., Tyler, A. N., Willby, N. J., & Gilvear, D. J. (n.d.). *The spatial dynamics of
vertical migration by Microcystis aeruginosa in a eutrophic shallow lake: A case study
using high spatial resolution time-series airborne remote sensing*.

Ignatius, A. R., Purucker, S. T., Schaeffer, B. A., Wolfe, K., Urquhart, E., & Smith, D.
(2022). Satellite-derived cyanobacteria frequency and magnitude in headwaters &
near-dam reservoir surface waters of the Southern U.S. *Science of the Total
Environment*, *822*. https://doi.org/10.1016/j.scitotenv.2022.153568

Lunetta, R. S., Schaeffer, B. A., Stumpf, R. P., Keith, D., Jacobs, S. A., & Murphy, M. S.
(2015). Evaluation of cyanobacteria cell count detection derived from MERIS imagery
across the eastern USA. *Remote Sensing of Environment*, *157*, 24–34.
https://doi.org/10.1016/j.rse.2014.06.008

Martner, B. E., & Marwitz, J. D. (1982). Wind characteristics in southern Wyoming.
Journal of Applied Meteorology, *21*(12). https://doi.org/10.1175/1520
0450(1982)021<1815:WCISW>2.0.CO;2

Mishra, S., Stumpf, R. P., Schaeffer, B., Werdell, P. J., Loftin, K. A., & Meredith, A. (2021). Evaluation of a satellite-based cyanobacteria bloom detection algorithm using field-measured microcystin data. *Science of The Total Environment*, *774*, 145462. https://doi.org/10.1016/J.SCITOTENV.2021.145462

Reinl, K. L., Brookes, J. D., Carey, C. C., Harris, T. D., Ibelings, B. W., Morales-Williams, A. M., … Zhan, Q. (2021). Cyanobacterial blooms in oligotrophic lakes: Shifting the high-nutrient paradigm. *Freshwater Biology*, *66*(9), 1846–1859. https://doi.org/10.1111/FWB.13791

Schaeffer, B. A., Urquhart, E., Coffer, M., Salls, W., Stumpf, R. P., Loftin, K. A., & Jeremy Werdell, P. (2022). Satellites quantify the spatial extent of cyanobacterial blooms across the United States at multiple scales. *Ecological Indicators*, *140*. https://doi.org/10.1016/j.ecolind.2022.108990

Schaeffer, B., Salls, W., Coffer, M., Lebreton, C., Werther, M., Stelzer, K., … Gurlin, D. (2022). Merging of the Case 2 Regional Coast Colour and Maximum-Peak Height chlorophyll-a algorithms: validation and demonstration of satellite-derived retrievals across US lakes. *Environmental Monitoring and Assessment*, *194*(3). https://doi.org/10.1007/s10661-021-09684-w

Seppälä, J., Ylöstalo, P., Kaitala, S., Hällfors, S., Raateoja, M., & Maunula, P. (2007). Ship-of-opportunity based phycocyanin fluorescence monitoring of the filamentous cyanobacteria bloom dynamics in the Baltic Sea. *Estuarine, Coastal and Shelf Science*, *73*(3–4), 489–500. https://doi.org/10.1016/J.ECSS.2007.02.015

Shi, K., Zhang, Y., Qin, B., & Zhou, B. (2019, October 30). Remote sensing of

    cyanobacterial blooms in inland waters: present knowledge and future challenges.

    Science Bulletin. Elsevier B.V. https://doi.org/10.1016/j.scib.2019.07.002

Stanley, E. H., Collins, S. M., Lottig, N. R., Oliver, S. K., Webster, K. E., Cheruvelil, K. S.,

    & Soranno, P. A. (2019). Biases in lake water quality sampling and implications for

    macroscale research. *Limnology and Oceanography*, *64*(4), 1572–1585.

    https://doi.org/10.1002/lno.11136

Visser, P. M., Passarge, J., & Mur, L. R. (1997). *Modelling vertical migration of the*

    cyanobacterium Microcystis. *Hydrobiologia* (Vol. 349). Kluwer Academic Publishers.

Wood, S.A., D.P. Hamilton, W.J. Paul, K.A. Safi, and W.M. Williamson, 2009. New

    Zealand Guidelines for Managing Cyanobacteria in Recreational Freshwaters—

    interim Guidelines. Wellington, Ministry for the Environment and Ministry of Health

    Wellington.

Wynne, T. T., Stumpf, R. P., & Briggs, T. O. (2013). Comparing MODIS and MERIS

    spectral shapes for cyanobacterial bloom detection.

    Http://Dx.Doi.Org/10.1080/01431161.2013.804228, *34*(19), 6668–6678.

    https://doi.org/10.1080/01431161.2013.804228

Wynne, T. T., Stumpf, R. P., Tomlinson, M. C., Warner, R. A., Tester, P. A., Dyble, J., &

    Fahnenstiel, G. L. (2008). Relating spectral shape to cyanobacterial blooms in the

    Laurentian Great Lakes. *International Journal of Remote Sensing*, *29*(12), 3665–3672.

    https://doi.org/10.1080/01431160802007640

Xue, Z., Zhu, W., Zhu, Y., Fan, X., Chen, H., & Feng, G. (2022). Influence of wind and light on the floating and sinking process of Microcystis. *Scientific Reports 2022 12:1*, *12*(1), 1–12. https://doi.org/10.1038/s41598-022-08977-5

Table 1. Lake characteristics of the sites sampled during summer 2021.

Lake name	Elevation (m)	Lake Area (ha)	Watershed Area (ha)
Alcova Reservoir	1677	926	15824
Boysen Reservoir	1441	4794	952763
Fontenelle Reservoir	2028	3169	833301
Glendo Reservoir	1414	4880	845959
Grayrocks Reservoir	1343	1457	327284
Guernsey Reservoir	1351	784	173361
Lake Viva Naughton	2208	562	61533
New Fork Lakes	2385	511	4599
Ocean Lake	1596	2469	26226
Pilot Butte Reservoir	1667	338	440914
Wheatland Reservoir no. 3 / Post Lake	2117	1545	4573
Yellowstone Lake	2360	34172	20774

Table 2. Error metrics across the various pixel windows used for extraction

Pixel window	MAE	RMSE
1 x 1	12,434	24,833
3 x 3 mean	31,827	109,753
5 x 5 mean	33,220	119,475

Table 3. Confusion matrix statistics extracted from comparison of predictions across bloom thresholds (Figure 6).

Overall Accuracy	95 % CI	Kappa
88.37 %	(79.65 %, 94.28 %)	0.5243
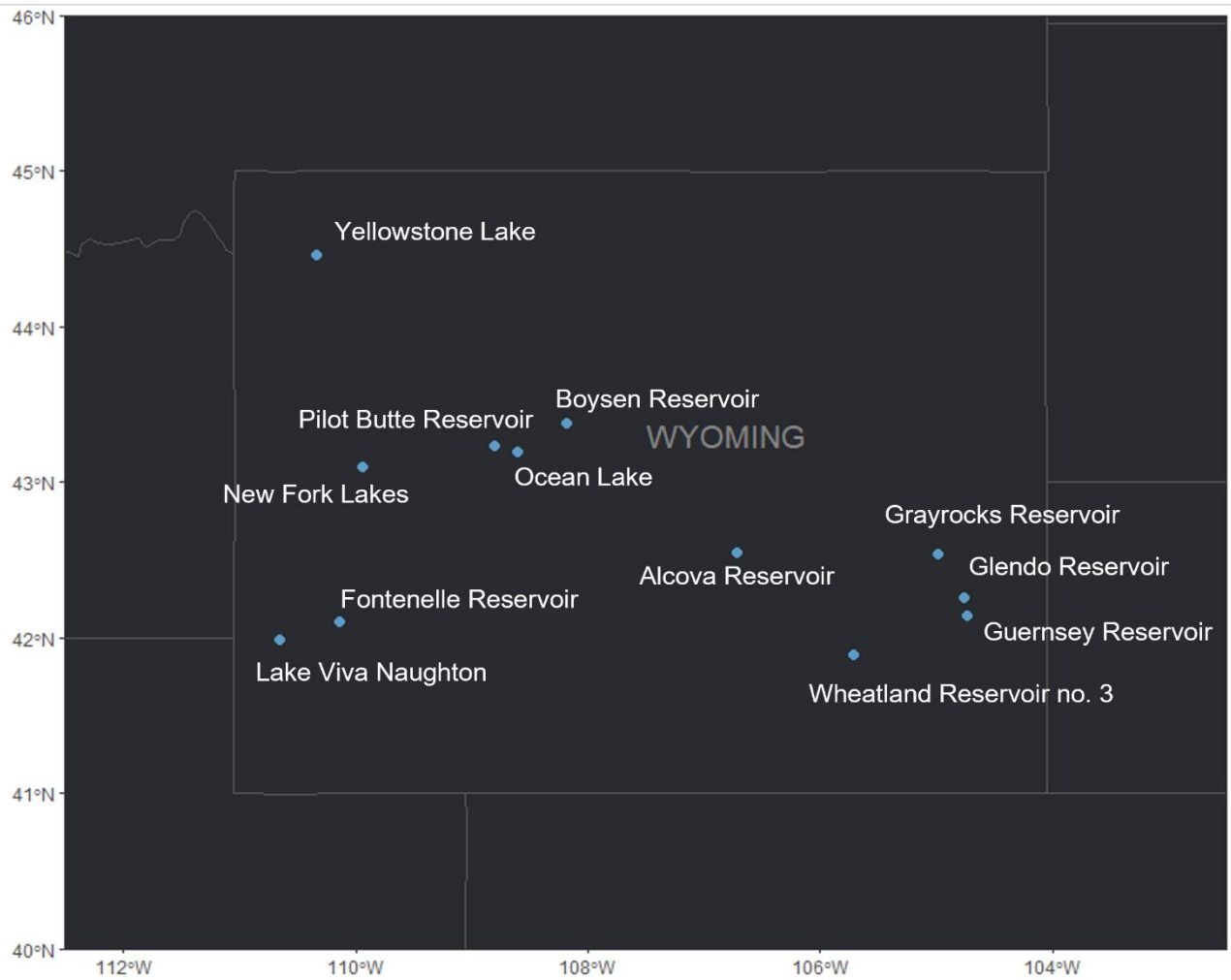
Figure 1. Map of lakes and reservoirs sampled during the summer of 2021.
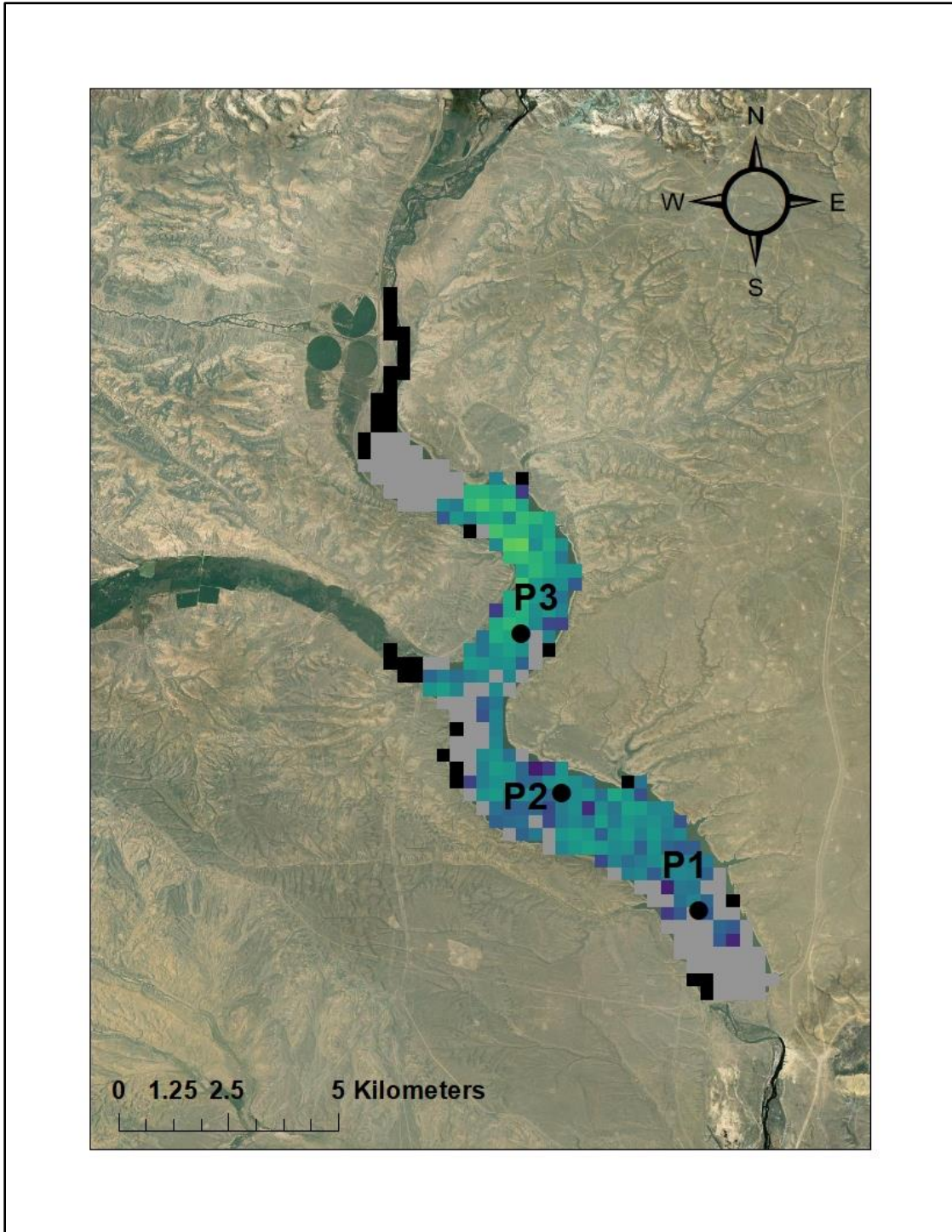
Figure 2. Example of CIcyano values extracted at Fontenelle Reservoir. P1, P2, and P3
represent the three sampling locations at Fontenelle Reservoir, with P3 representing a
more riverine environment and P1 representing a more lacustrine environment.
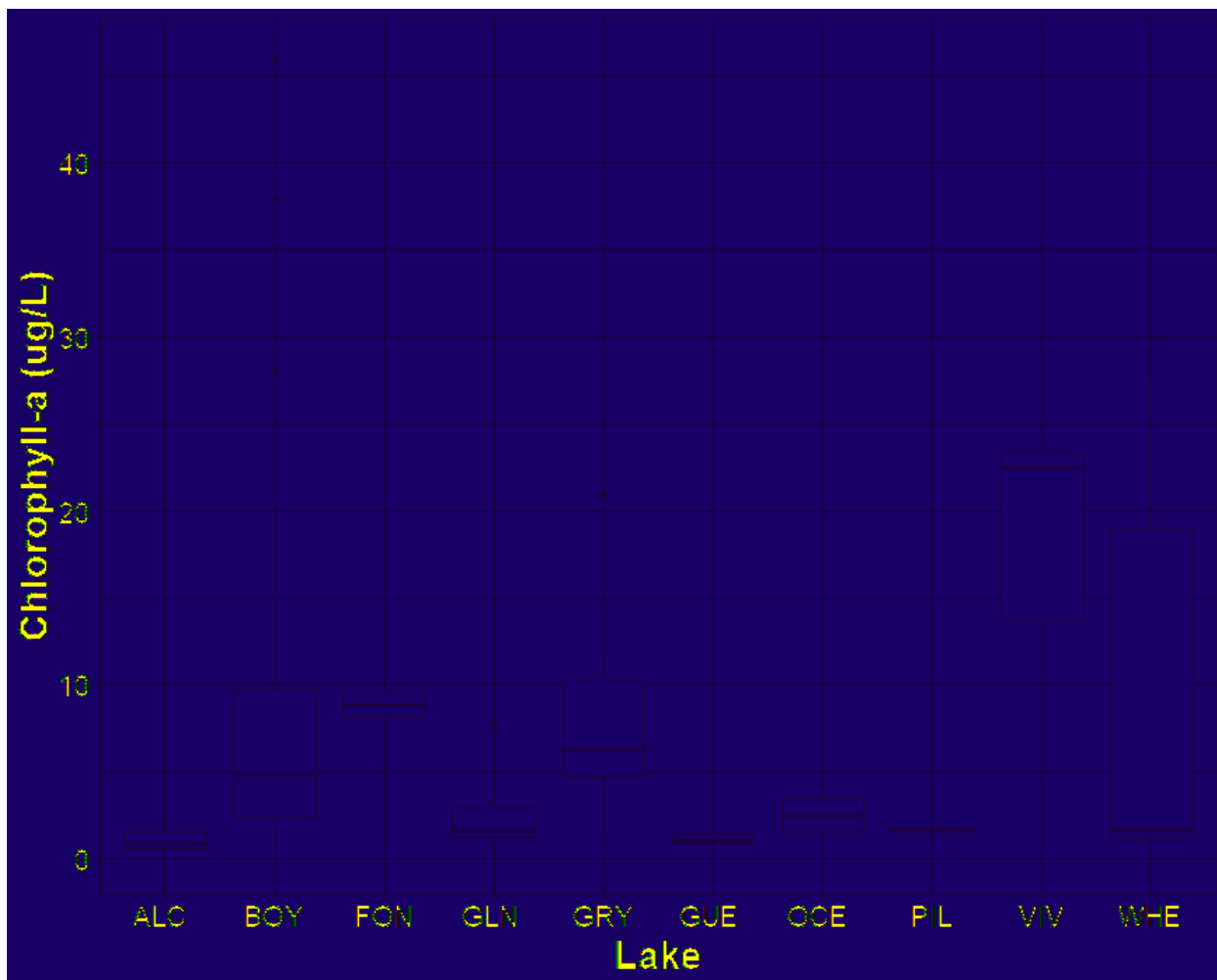
Figure 3. Boxplots of chlorophyll-a samples across sites. Chlorophyll-a was found to vary across sites with Boysen Reservoir, Fontenelle Reservoir, Grayrocks Reservoir, Lake Viva Naughton, and Wheatland Reservoir no. 3 being the most eutrophic of the study sites. Two sites, New Fork Lakes and Yellowstone Lake did not have chlorophyll-a data available.
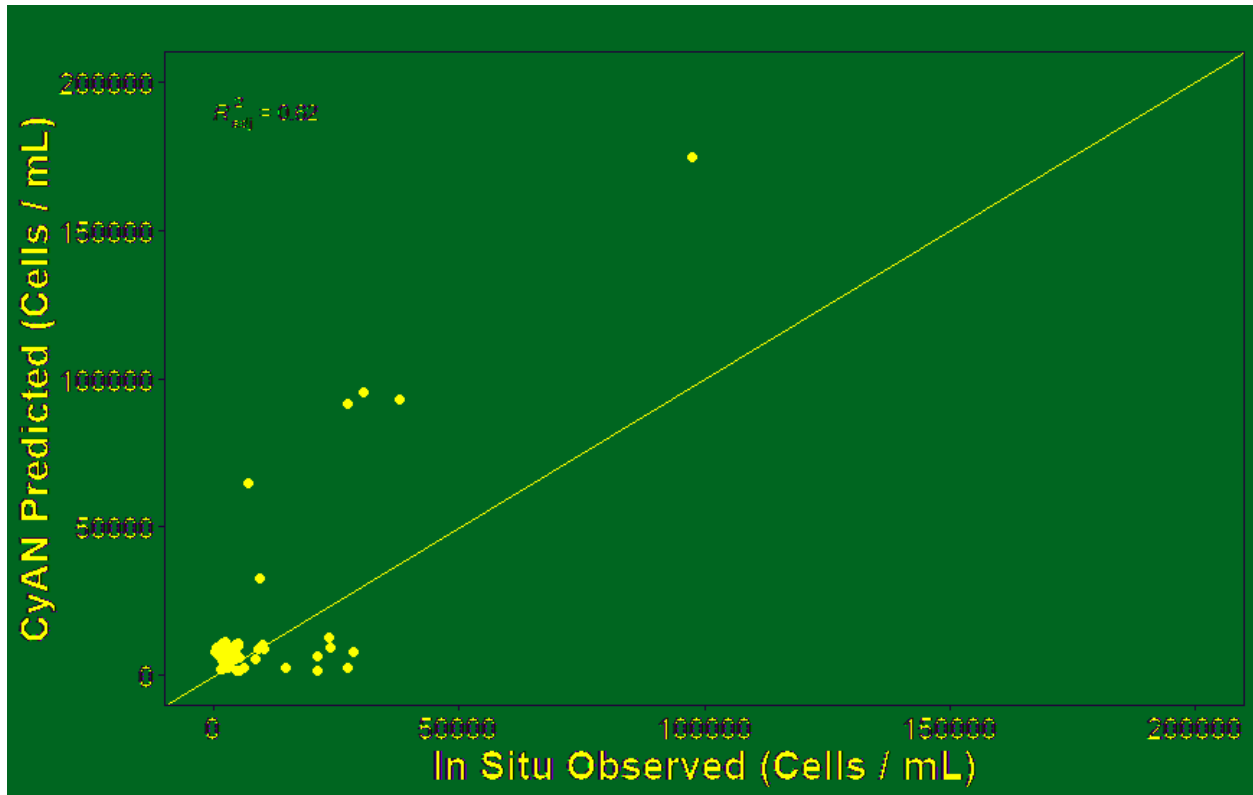
Figure 4. Regression of CIcyano predicted cells / mL vs in-situ cells / mL. The solid line represents a 1:1 line and shows a strong positive relationship between remote sensing estimates and in-situ cell counts.
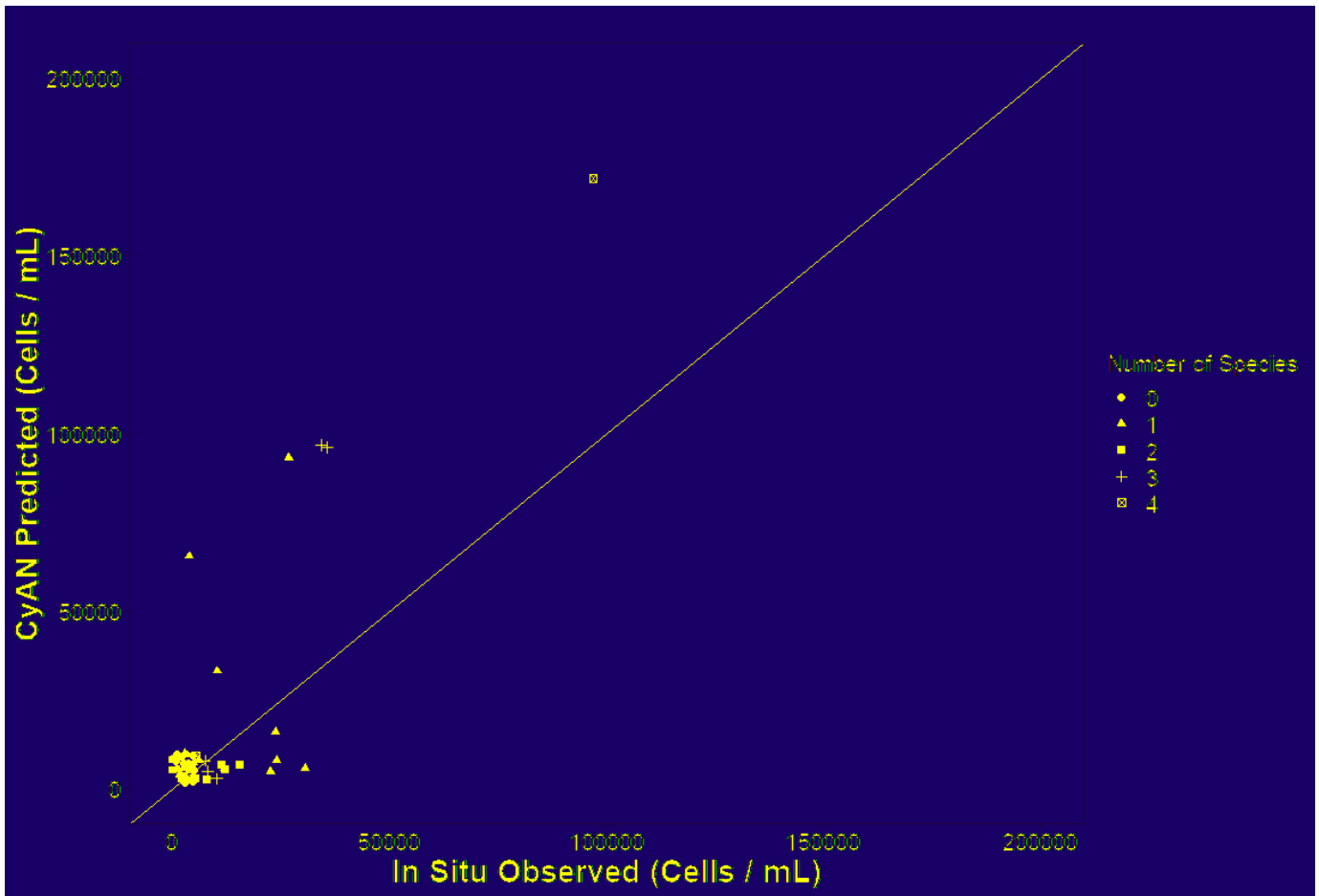
Figure 5. CyAN accuracy across samples with varying amounts of cyanobacteria taxa

present.
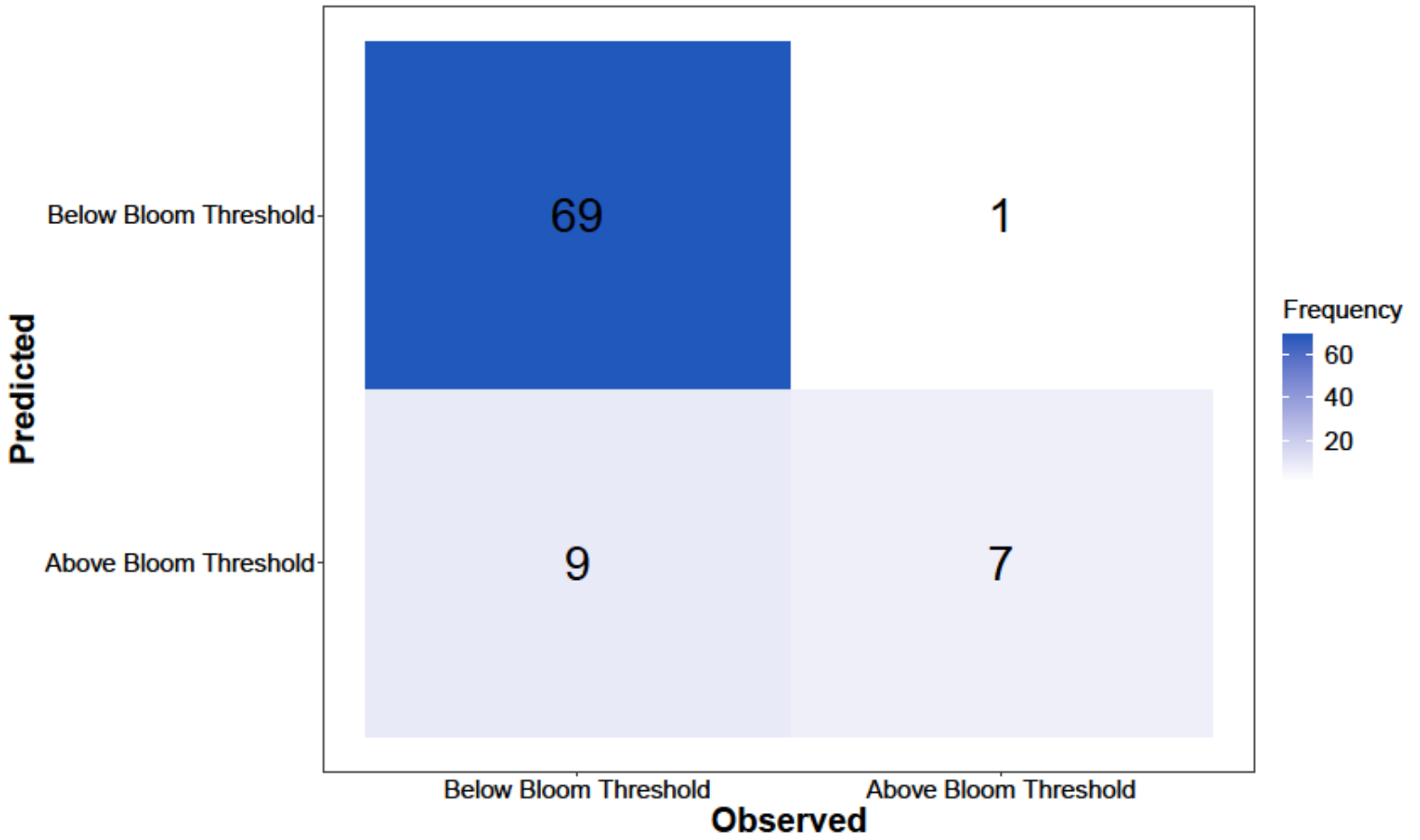
Figure 6. Confusion matrix illustrating the prediction accuracy of CIcyano to distinguish bloom presence based on the threshold of 20,000 cells / mL.
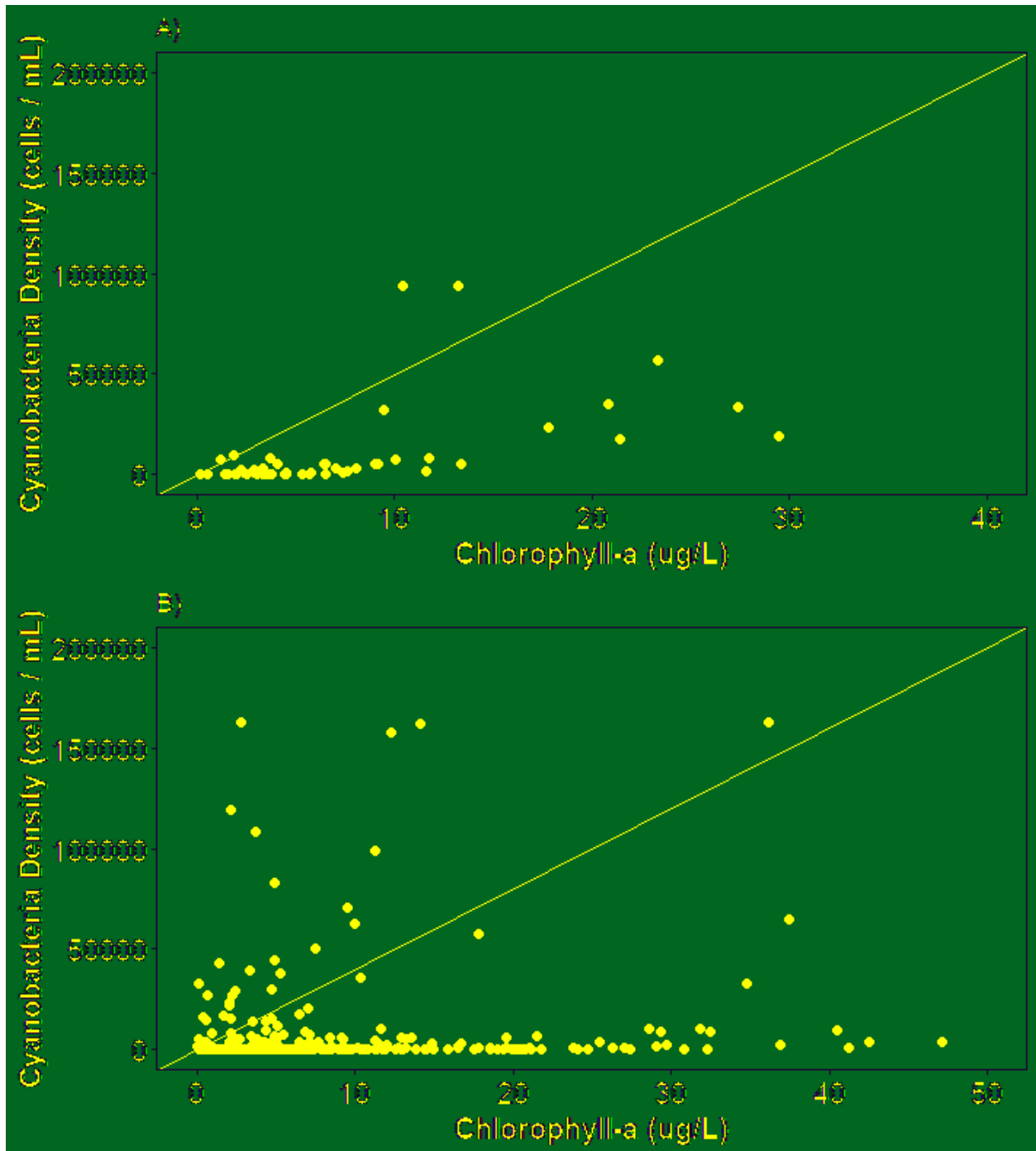
Figure 7. Relationship of chlorophyll-a and in-situ cyanobacteria density. The top panel (A) represents the data collected within this study. The bottom panel (B) represents data collected by the Department of Environmental Quality (DEQ).
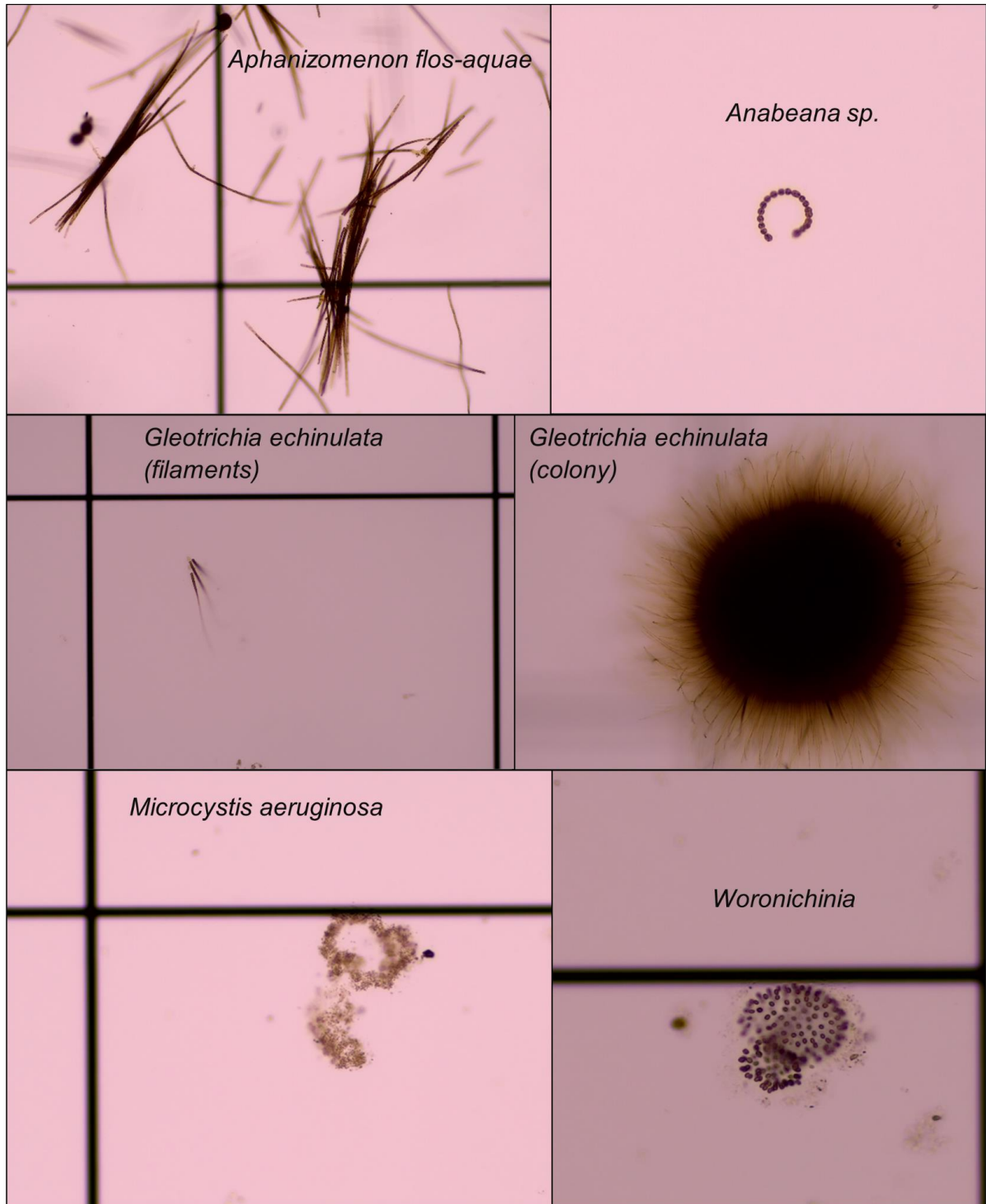
Supplementary figures:

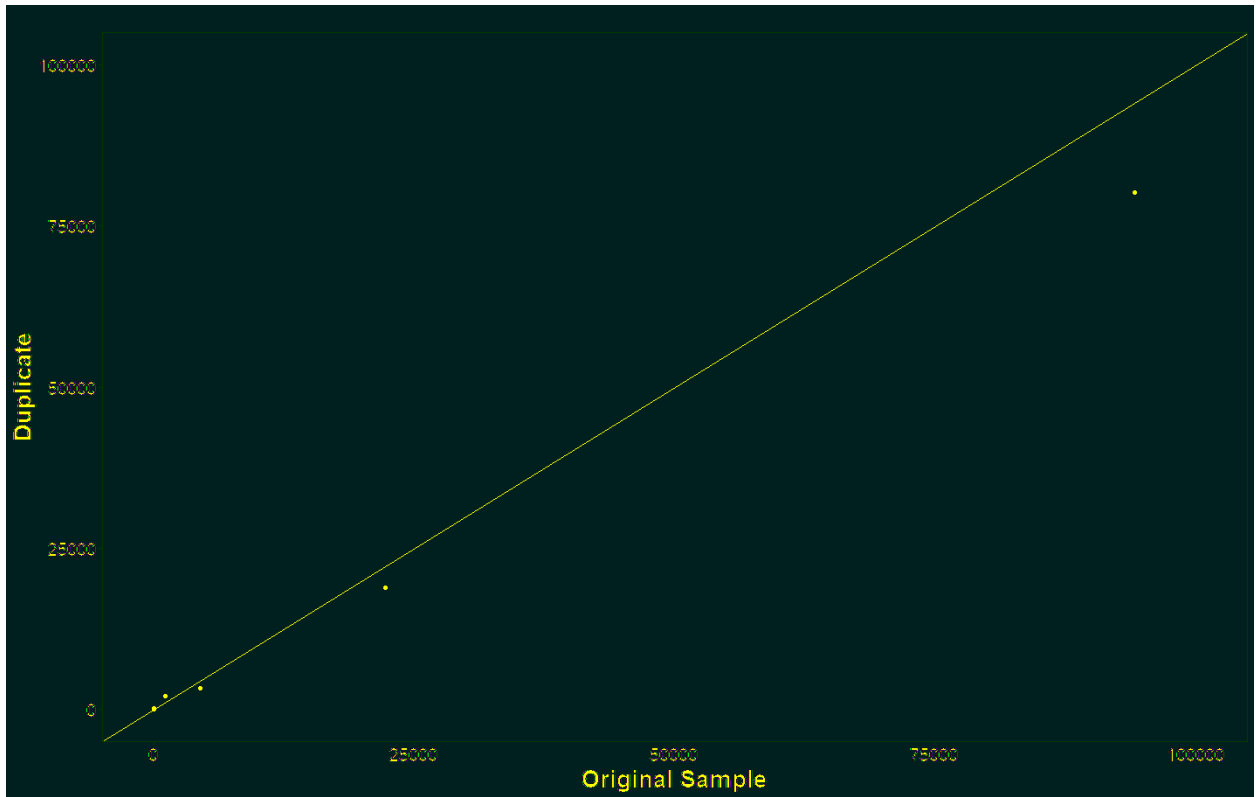Figure S1. Common cyanobacteria taxa observed across in-situ samples.

Figure S2. Regression of in situ cyanobacteria duplicate samples indicated a strong

positive relationship and reflect consistency in the laboratory and enumeration methods
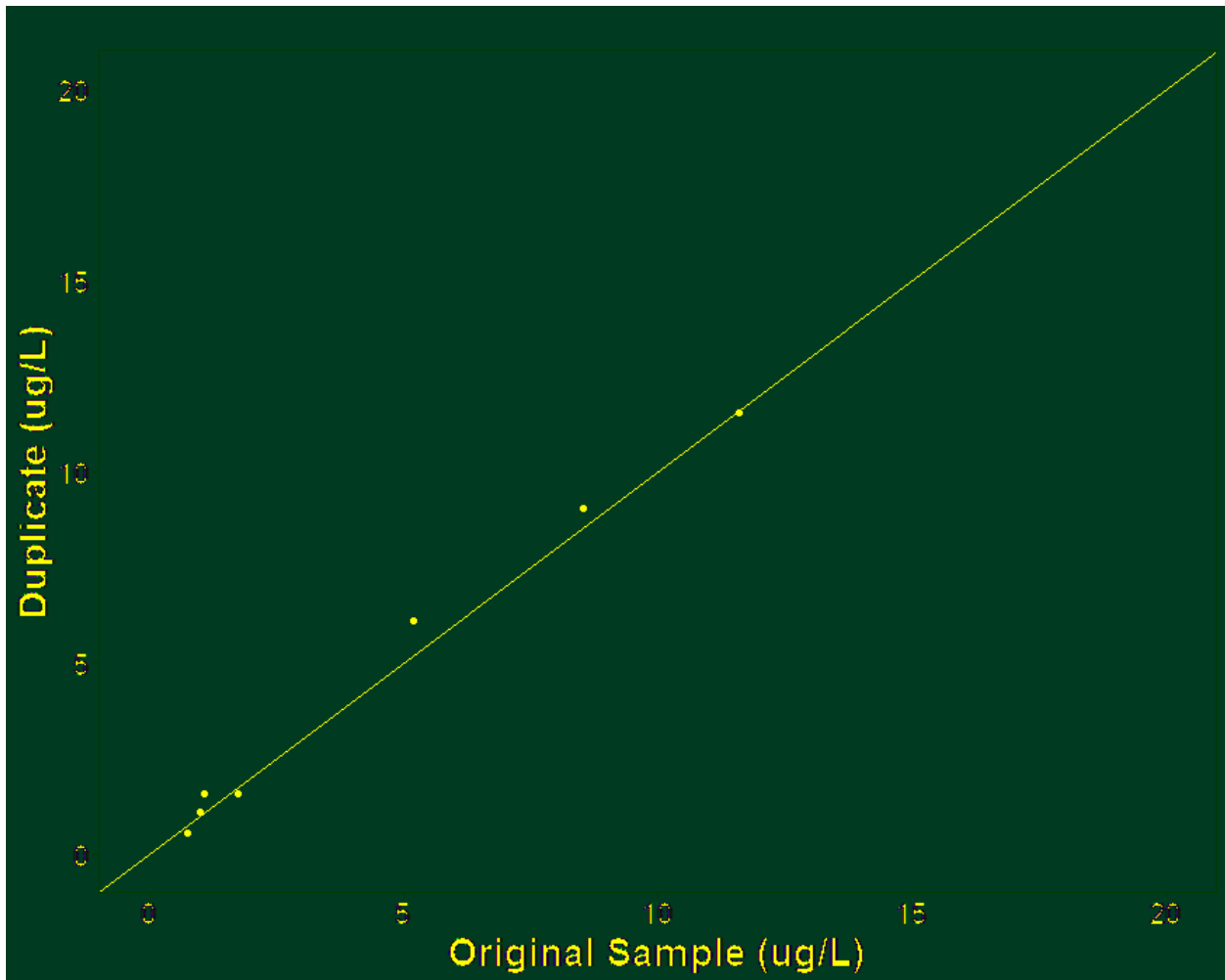
used for analysis.

Figure S3. Regression of Chlorophyll-a duplicate samples indicated a strong positive

relationship and reflect consistency in the field and laboratory methods used for analysis.

Water Quality Perturbations in Aquatic Ecosystems: Ecological Response and Management Implications

Ashleigh Pilkerton

PhD Dissertation Proposal

Program in Ecology, Department of Zoology and Physiology
University of Wyoming
December 2022

Dr. Annika Walters, Committee Co-Chair: _____

Dr. Sarah Collins, Committee Co-Chair: _____

Dr. Brandon McElroy, Outside Dept. Member: _____

Dr. Frank Rahel, UW Faculty: _____

Dr. William Fetzer, UW Faculty: _____

Dr. Melanie Murphy, Director, Program in Ecology: _____

Introduction

Ecosystems have regularly faced periodic and persistent changes (Steffen et al. 2015) with disturbance serving an important role in structuring communities and natural systems (Hobbs and Huenneke 1992). Disturbance, here defined as an event that disrupts ecosystem, community, or population structure and changes resources, substrate availability, or the physical environment (White and Pickett 1985), is essential for many organismal life histories and can contribute to increased species richness (Hobbs and Huenneke 1992; Reuling et al. 2019). However, the frequency, intensity, and size of a disturbance can have important implications for community response (Noble and Slayter 1976; Peereman et al. 2022). Understanding the mechanisms of disturbance on ecological patterns is often complicated due to the direct and indirect pathways in which alterations may be mediated. For example, a perturbation may be reflected immediately by direct mortality of organisms. However, a perturbation may also cause a cascade of biological or chemical changes and result in indirect implications, such as changes in resource availability. Increasingly, ecologists are faced with the challenge of predicting how novel and complex human perturbations will impact community structure and function (Bae and Park 2014). A better understanding of trophic interactions and biogeochemical impacts can aid in the prediction of perturbation effects on ecosystems, especially in circumstances when systems receive multiple disturbances simultaneously (Hobbs and Huenneke 1992; Caraco 1993). Through these more mechanistic approaches, ecologists can help identify systems and organisms that may be most sensitive to changes in the environment.

Human induced environmental perturbations constitute a novel disturbance to which species and ecosystems have not previously encountered or adapted. Anthropogenic activity (i.e. land use changes, biogeochemical cycle disruptions, nonnative species introductions, carbon emissions) is increasing both the rate and the intensity of environmental change (Dudgeon et al. 2006; Oliver et al. 2015; Steffen et al. 2015; Reid et al. 2019). Widespread changes to abiotic and biotic variables such as landscape cover and niche-based processes (i.e. environmental filtering and biotic interactions), have caused shifts in the global distribution of organisms and fluctuations in levels of biodiversity (Chapin III et al. 2000). The direct effects of anthropogenic disturbances are most pronounced in trophic groups highly sensitive to change, which varies considerably across taxa and ecosystem type (Voigt et al. 2003; Thackeray et al. 2016; Hu et al. 2022). However, the indirect effects on other species and trophic levels conferred through linkages within food webs has important implications for ecosystem functioning (Seibold et al. 2018). While previous work has demonstrated direct effects on sensitive species, there is a critical gap in our understanding of how indirect effects on more resilient species will restructure ecosystems, inhibiting our ability to fully predict the consequences of anthropogenic activities.

There has been increasing recognition of the importance of disturbance, both natural and anthropogenic, in the function of aquatic ecosystems (Paerl and Paul 2012; Hosen et al. 2019; Lehman et al. 2022; Williams et al. 2022). Aquatic ecosystems are well known for their role in buffering the effects of anthropogenic disturbance, such as the role of oceans in cycling atmospheric carbon dioxide and wetlands and riverine floodplains in mitigating flood risk (Hasse 2017; Jiang et al. 2019; Randall et al. 2020). However, the buffering capacity of aquatic ecosystems to environmental perturbations is increasingly limited, and in some cases, already declining (Palmer et al. 2008; Jiang et al. 2019; Hessen and Vandvik 2022) as a result of increasingly pervasive disturbances (Hasse 2017; Walters et al. 2018; Hessen and Vandvik 2022; Lehman et al. 2022). Environmental perturbations are often manifested as changes to water quality (Zampella 1994), which is an important driver of regional species distributions and diversity (Flynn et al. 2009; Kim et al. 2021; Dong et al. 2022). Emerging stressors to water quality include climate change, eutrophication and sedimentation (Hooper et al. 2005, 2012; Carpenter et al. 2011; Reid et al. 2019). The

ramifications of these interacting environmental perturbations can lead to changes in aquatic ecosystems, such as favoring the proliferation of harmful cyanobacterial bloom (HCB) species, and causing habitat degradation, which is a leading and persistent cause of population loss in freshwaters (Dudgeon et al. 2006).

Through my PhD dissertation research, I seek to quantify the indirect impacts of disturbances influencing species presence and distribution in aquatic ecosystems, understand mechanistic links between water quality impairments and community response, and evaluate the role of disturbance on ecological patterns. Abiotic freshwater ecosystem conditions such as physical habitat, sedimentation rates, hydrological patterns and water temperature are modified by human actions through their direct linkages to terrestrial environments, particularly the local and regional catchment scale (Vörösmarty et al. 2010). Similarly, aquatic ecosystem drivers, such as the input of nutrients, autochthonous production, food availability and quality, and species diversity, are also influenced by catchment scale anthropogenic disturbances (Parreira De Castro et al. 2016). In the four chapters of my PhD, I will investigate the ecological impacts associated with two types of aquatic disturbances: sediment, including habitat response to sediment flushing events and fishes variable sensitivity to suspended sediment, and harmful cyanobacterial blooms and the resulting food web response.

In my first two chapters, I will investigate both direct and indirect effects of sediment on habitat and fishes. Infrastructure such as dams and diversions, has altered natural flow regimes in rivers, changing water temperature (Clarkson and Childs 2000), discharge, and sediment-transport patterns (Holden 1979). Changes in sediment-transport patterns have important implications for aquatic ecosystems. For example, sedimentation on, or within, the riverbed can alter the substrate characteristics including the surface conditions and the hyporheic zone (Wood and Armitage 1997). At high concentrations, sedimentation can indirectly have deleterious effects on macrophyte communities (Clarke and Wharton 2000) and macroinvertebrate populations by disrupting filter feeding, impeding foraging and mobility, and increasing macroinvertebrate drift (Ward et al. 1998; Collins et al. 2011). Fishes are also affected by suspended sediment and aggradation including reduced fitness of free-swimming fish and decreased success of egg/larval survival (Jensen et al. 2009) with indirect effects prevalent at the habitat level (Greig et al. 2005). Understanding how dam management alters sediment transport and abiotic riverine conditions has important implications for predicting the effects of novel anthropogenic disturbance on biological processes.

In Chapter 1, "Understanding the Effects of Dam Sediment Management on Salmonid Spawning Habitat", I will explore the relationship between dam operations and downstream sediment dynamics in the context of riverine fisheries management. My goal is to provide managers with the tools and information to address these challenges. I will assess how experimental releases of sediment contrast with normal drawdown operations and quantify the seasonal impact of dam operations on downstream spawning habitat. I will evaluate the capacity of real-time, near real-time, and laboratory water column sediment metrics to predict the effects of sediment releases from dams and other seasonal dam operations on spawning habitat with implications for the youngest life stages of fish, including eggs and embryos. This chapter will present the results of three years of spatiotemporal monitoring and provides more precise quantitative clarification of the impact of sediment releases from dams on spawning habitat and downstream fisheries.

In Chapter 2, "An Updated Quantitative Assessment of Risk and Impact between Suspended Sediment and Fish", I will expand upon Newcombe and Jensen's (1996) seminal work exploring the severity of ill effects of suspended sediment exposure to various life stages and taxonomic groups of fishes. I will synthesize existing literature, including studies in Newcombe and Jensen (1996), and new studies to the present to update the severity index. I

will provide updated information on which fish species, life history stages, and time periods are most sensitive to sediment releases, and track fish species and life history stage. This work will allow us to assess the sensitivity of Wyoming fishes to suspended sediment and develop an understanding of key temporal windows with respect to fish life histories. The updated severity of ill effects models documented here will allow managers to make informed decisions and set regulatory thresholds to help ensure the amount and timing of sediment releases are reflective of ecological requirements.

In my last two chapters, I will explore how harmful cyanobacterial blooms affect ecosystem functioning by investigating changes in community composition and food web response. Anthropogenic induced changes in the hydrological cycle, and increases in nutrient input and water temperatures have led to a significant increase in the frequency, intensity, duration, and geographic range of HCBs (Robarts and Zohary 1987; O'Neil et al. 2012; Paerl and Paul 2012; Jia et al. 2017; Wells et al. 2020). Many bloom-forming cyanobacterial species produce toxins that can result in widespread socioeconomic implications such as contaminating drinking water supplies and reducing recreational opportunities (O'Neil et al. 2012; Glibert 2017; Angradi et al. 2018; Kouakou and Poder 2019). Further, HCBs compromise the integrity of aquatic ecosystems by dominating the phytoplankton community, disrupting food-web processes and inhibiting the transfer of primary production to primary consumers (Muller-Navarra et al. 2000; Zheng et al. 2013). Consequently, HCBs are known drivers of changes in diet selectivity with potential consequences for food web-scale interactions (Creed et al. 2018) and understanding the indirect impacts of HCBs is pressing.

In Chapter 3, "Effects of Harmful Cyanobacterial Blooms on Zooplankton Communities in Wyoming Reservoirs", I seek to evaluate the response of zooplankton communities to harmful algal blooms across 17 reservoirs spanning a gradient of oligotrophic to eutrophic lakes in Wyoming. I first set out to understand the spatial distribution of zooplankton species in large reservoirs in Wyoming, and evaluate temporal variation in zooplankton species dynamics across these systems to address a regional knowledge gap in zooplankton community composition. Second, I will evaluate how zooplankton species dynamics change in the presence of HCBs and third, quantify the underlying physiochemical factors related to HCBs that lead to changes in zooplankton communities and species abundance. Understanding the implications of blooms on zooplankton communities that play a critical role in the transfer of energy from primary producers to primary consumers will help mitigate the impacts of climate change on aquatic ecosystems.

In Chapter 4, "Isotopic Niche Space: Are Harmful Cyanobacterial Blooms Driving Resource Use Shifts in Small-Bodied Fishes?", I will evaluate the effects of HCBs on small-bodied fish trophic niche dimensions using stable isotopes to elucidate patterns of resource use. Understanding the complexities of biotic interactions, biodiversity, and the role of abiotic factors that shape species distribution across a landscape is pertinent for conservation of aquatic systems. I will use carbon and nitrogen stable isotope analyses to examine trophic niche width of three sympatric small-bodied fish, Brook Stickleback *Culaea inconstans* (an invasive species), Iowa Darter *Etheostoma exile* (a species of greatest conservation need), and Fathead Minnow *Pimephales promelas*, in two lakes in SE Wyoming where HCBs occur and do not currently occur. I will characterize fish and prey isotope compositions through time, space, and ontogeny. Then, I will examine isotope values in relation to prey availability and harmful cyanobacterial bloom occurrence. Quantifying trophic niche width and patterns of niche overlap will help us better understand how HCBs affect species interactions, trophic and food web dynamics, and overall impacts of HCBs on ecosystems.

Across all chapters of my PhD, I will investigate the effects of water quality disturbances on ecological patterns and evaluate variable sensitivity among taxa groups by quantifying abiotic and biotic drivers. Combined, these results will build on existing knowledge of how environmental perturbations influence biotic communities. Direct effects of anthropogenic impacts are poorly understood in Wyoming aquatic systems, and indirect effects have been understudied in the broader scientific community. It is important to quantify the multitrophic level effects to help identify what indirect effects anthropogenic stressors have on aquatic ecosystems. Knowing the effects of perturbations on ecosystems allows ecologists to better assess the most effective way to facilitate management and conservation and thus develop guidance for managing and mitigating the effects of environmental change (Oliver et al. 2015).

Chapter 3

The Effects of Harmful Cyanobacterial Blooms on Zooplankton Communities in Wyoming Reservoirs

1. Introduction

Harmful cyanobacterial blooms (HCBs) are intensifying globally and there is growing interest in their ecological effects (Ortiz et al. 2020). Aquatic ecosystems can be negatively impacted by HCBs through a suite of direct and indirect pathways and the associated water quality impairments can have wide ranging impacts (Paerl and Otten 2016; Reid et al. 2019). For example, HCB cyanotoxin production can and competitively exclude other species and diminish water quality (Jewel et al. 2003; Glibert 2017). Similarly, HCB species constitute a poor food resource for primary consumers, which can negatively impact higher trophic levels and lead to declines to biodiversity (Sukenik et al. 2015; Glibert 2017; Wells et al. 2020). However, our understanding of how HCBs affect the structure, function, and dynamics of aquatic food webs is incomplete, and the implications for primary and secondary consumers, notably zooplankton, and higher trophic levels are unclear (Ger et al. 2014; Briland et al. 2020).

Zooplankton play a crucial role in the redistribution of nutrients and regulation of energy flow through aquatic food webs and therefore can directly influence community structure (Zhang et al. 2022). The composition of zooplankton species is highly sensitive to environmental change (McNaught 1975) with disturbances causing detectable shifts in relative species abundance and body size distribution (US EPA 2021). Many zooplankton species are unable to consume cyanobacteria species due to their colonial or filamentous nature during blooms (Ger et al. 2014). In turn, shifts in the composition of primary producers towards the dominance of HCBs can result in changes in zooplankton community dynamics and declines in zooplankton biomass, though the patterns of zooplankton community composition are not consistent (Fulton and Paerl 1987).

In some systems, HCBs have been shown to cause a shift in zooplankton communities towards copepods, rotifers, and sometimes smaller cladocerans (Richman and Dodson 1983; Orcutt and Pace 1984; Fulton and Paerl 1987). One study found calanoid copepods dominated eutrophic lakes and it was hypothesized they were better adapted to utilize large cyanobacteria prey. The author notes the filamentous nature of some HCB species was found to inhibit foraging of cladocera, thereby reducing growth and production (Haney 1987). This work supports the hypothesis that shifts in zooplankton community could be attributed to zooplankton morphology and resource availability, or lack thereof due to the inedible nature of HCBs to some zooplankton. However, zooplankton community shifts were equivocal across systems. For example, another study found zooplankton community and size structure across lake trophic gradients indicated calanoid copepods declined and cyclopoid copepods increased in relative abundance, unrelated to size structure. The author suggests these results refute the notion that changes in resources during eutrophication favors smaller zooplankton (Pace 1986). Further complicating our understanding of the role of HCBs on zooplankton, strong positive relationships were found between cyanobacteria concentration and the biomass of several herbivorous zooplankton taxa (Briland et al. 2020). This suggests that herbivorous zooplankton may benefit from HCBs more than they are hampered by them (Briland et al. 2020) and supports the hypothesis that overall high food abundance during blooms may be beneficial to some zooplankton abundances. Other work found zooplankton grazers such as daphnia species may promote cyanobacterial bloom growth by selectively consuming competitive phytoplankton (Haney 1987).

Given HCBs can strongly affect the distribution, composition, and interactions of zooplankton, a better understanding of the indirect impacts of HCBs on aquatic food webs is important for quantifying critical linkages

between primary producers and higher trophic levels (Briland et al. 2020). I seek to evaluate the response of zooplankton communities to HCBs across 17 reservoirs spanning a gradient of oligotrophic to eutrophic lakes in Wyoming. I first seek to understand the spatial distribution of zooplankton species in large reservoirs in Wyoming, and evaluate temporal variation in zooplankton species dynamics across these systems to address a regional knowledge gap in zooplankton community composition. Second, I will evaluate changes in zooplankton species dynamics in the presence of HCBs by exploring changes in species diversity, size structure and biomass. Third, I will quantify the underlying physiochemical factors related to HCBs and how these relate to changes in zooplankton communities and species abundance.

I hypothesize zooplankton species diversity, size structure and biomass will change after the onset of an HCB due to changes in resource availability. I predict zooplankton community diversity, size structure, and biomass will decrease with the onset and persistence of an HCB and predict a more pronounced shift in these zooplankton metrics in eutrophic lakes that experience an HCB. Understanding the implications of HCBs on zooplankton communities and determining how environmental factors interact to shape biological assemblages has important implications for natural resource management and species conservation (Zhang et al. 2022) and will help mitigate the impacts of climate change on aquatic ecosystems.

2. Methods

I will conduct this study across 17 reservoirs in Wyoming spanning the gradient of trophic state, size, and elevation. Due to the recent implementation of HCB monitoring in 2017 by the Wyoming Department of Environmental Quality, it is unclear whether the frequency of blooms in Wyoming has increased, or if there is more reporting of the blooms because of the additional monitoring efforts. Across Wyoming, most HCB recreational use advisories are issued during late July or August and persist through fall and into winter (Wyoming Department of Environmental Quality, https://www.wyohcbs.org/). To understand the impacts of HCBs on zooplankton communities, I will sample the reservoirs before and after HCB onset. Reservoirs will be sampled monthly from May through October in 2021 and 2022 at three sites longitudinally spaced from reservoir inlet to outlet.

2.1 Zooplankton

At each site, I will use vertical zooplankton tows (64 um mesh, 0.5 m diameter net) conducted to the depth of the reservoir for lakes less than 10 meters deep, or to a maximum of 15 meters to sample zooplankton; at times, due to high phytoplankton biomass, zooplankton tows will be collected in the top 3 meters of the water column. All samples will be collected during daylight hours with best efforts made to sample the entire water column to remove the potential influence of diel vertical migration behavior of some species. Zooplankton samples will be stored in 250 mL containers and preserved in ethanol. In the laboratory, a minimum of 3 mL of subsample will be transferred to a 1 mL Sedgewick rafter cell and individuals will be identified and counted on a dissecting microscope (Leica SE6). Lengths of the first 30 individuals for each species or taxonomic group will be measured and taxon-specific length-dry mass regression equations will be used to estimate biomass (Culver et al.,1985).

2.2 Physiochemical Attributes

I will measure a suite of physicochemical attributes at each site using a combination of in situ and grab samplers. Water temperature, specific conductivity, dissolved oxygen and pH will be measured at 0.5 meter intervals to the same depth as the zooplankton tows using a multisensory probe (YSI ProDSS, Yellow Springs, OH). Water transparency will be measured using a Secchi disk at each site. Integrated water samples will be collected 0.5

meters below the water surface using a Van Dorn sampler. These samples will be used to quantify nutrients (total nitrogen and total phosphorus), cyanotoxins (microcystin and cylindrospermopsin), phytoplankton and chlorophyll-a. The samples intended for identifying and counting phytoplankton will be immediately preserved with Lugol's iodine and stored in the dark. All other water samples will be kept on ice after collection until laboratory processing. The whole-water nutrient and cyanotoxin samples will be stored frozen at -20 °C within 24 h of collection. To estimate chlorophyll-a, whole-water samples will be filtered from each site through 0.7 µm pore-size filter papers (GF/C Whatman). Filters will be stored in the dark at -20°C until analysis. Chlorophyll-a will be extracted in 10 milliliters of 90 % buffered ethanol for 12-24 hours. Subsequently, 2.7 mL of sample will be added to a glass cuvette and fluorescence units will be recorded using a Turner Designs AquaFluor Handheld Flourometer/Turbidimeter. Then, I will add 90 microliters of 0.1 N hydrochloric acid and record the fluorescence units 90 seconds later. I will determine the concentration of chlorophyll-a in the sample by 1) determining the amount of chlorophyll-a in the extraction using an instrument-specific standard curve that relates chlorophyll-a concentration to the difference between initial fluorescence and fluorescence after acidification and 2) correcting for the volume of lake water filtered for the extraction.

2.3 Phytoplankton

Whole-water samples preserved with Lugol's will be condensed by pouring a mixed sample into a 250 mL graduated cylinder. After these samples are allowed to settle, the top suspension portion will be removed. An aliquot of the concentrated sample will be transferred to a Sedgewick rafter cell. I will estimate the average number of individual units (e.g., cells, colonies, or filaments) by counting the number of cells in the first 10 filaments or colonies. Literature derived averages for cells per unit will be used when this is not feasible. A minimum of four transects will be examined and identified to species. I will then calculate the number of cells per unit volume.

2.4 Cyanotoxins

Total (intracellular plus extracellular) cyanotoxin (microcystin and cylindrospermopsin) water samples will be measured using enzyme-linked immunosorbent assay (ELISA) kits (96-well format, Abraxis). Samples will be exposed to two freeze/thaw cycles to rupture cells and release the toxins, and subsequently analyzed with a plate photometer.

2.5 Analysis

I will use three classical biodiversity indexes (species richness, Simpson's Diversity Index, and Shannon Diversity Index), three functional diversity indexes (functional richness, functional evenness, and functional divergence), and zooplankton community size structure to understand zooplankton communities dynamics. To understand the spatial distribution of zooplankton species, I will compare these community composition indices within and across reservoirs. Additionally, the before-after-control-impact study design will allow me to understand in what ways the zooplankton community composition changes in response to HCBs. I will use ordinary least-squares regression to relate zooplankton abundance and biomass to measures of lake trophy (Pace 1986). Zooplankton biomass will be calculated using mass-length equations from EPA Standard Operating Procedure for Zooplankton Analysis 2016 (U.S. Environmental Protection Agency 2016). Last, I will use non-metric multidimensional analysis or canonical correlation analyses to visualize abiotic and biotic drivers of community composition and species diversity.

3. Statement of Importance

Zooplankton species diversity and abundance can serve as a measure of biological condition and used as an indicator of water quality changes (McNaught 1975; US EPA 2021). Because HCBs can alter the structure of zooplankton communities and disrupt food web processes, understanding shifts in species and community composition is pertinent for predicting future impacts and developing adaptive management plans. Understanding the relationship between HCBs and zooplankton communities will help ecologists characterize the effects across multiple trophic levels and understand how environmental perturbations influence ecosystem services.

4. Research Progress

We collected all field samples for this research during 2021 and 2022. 2021 zooplankton samples are fully processed and we are currently working on processing 2022 zooplankton samples. I plan to process 2022 phytoplankton, water nutrient, chlorophyll-a and cyanotoxin samples in the next six months. I have not started analyses for this research.

Chapter 4

Isotopic Niche Space: Are Harmful Cyanobacterial Blooms Driving Resource Use Shifts In Small-Bodied Fishes?

1. Introduction

Niche-based processes, such as environmental filtering and biotic interactions, are important divers of community composition and diversity. Understanding an organism's niche allows us to better comprehend the structure of ecological assemblages including species distribution and range limits, co-occurrence and interspecific competition between species, and biodiversity dynamics across multiple gradients (Chase and Myers 2011). Interspecific interactions may be altered as a result of climate change (Ogloff et al. 2019), and in aquatic ecosystems, HCBs are known drivers of changes in diet selectivity with potential consequences on food web interactions (Creed et al. 2018).

HCBs affect plankton diversity, energy transfer across trophic levels, and can alter community composition in lakes (Paerl and Paul 2012; Visser et al. 2016; Krztoń et al. 2019; Amorim and Moura 2021). For example, secondary metabolites have been found to inhibit growth and photosynthesis in non-HCB species of phytoplankton and macrophytes (Zheng et al. 2013; Xu et al. 2016; Wang et al. 2017; Zi et al. 2018) and greatly reduce zooplankton species richness (Richman and Dodson 1983; Orcutt and Pace 1984; Briland et al. 2020). Impacts at higher trophic levels include sub- or paralethal impacts, such as causing oxidative stress in bighead carp (Sun et al. 2013) and reduction in growth rate due to energetic costs of metabolizing cyanotoxins (Pääkkönen et al. 2008). Lethal impacts of HCBs include embryonic heart failure in fish embryos (Zi et al. 2018) and system wide fish kills (Jewel et al. 2003). Further, HCBs can impact diet selectivity with potential consequences for food web-scale interactions (Creed et al. 2018). However, key questions remain unanswered with respect to how HCBs affect the structure of aquatic food webs and the foraging abilities of higher consumers (Briland et al. 2020).

Stable isotope analysis is a useful tool for characterizing patterns of resource use and quantifying an organism's trophic niche (Boecklen et al. 2011; Carter et al. 2019; Borrell et al. 2021). Given organismal tissue stable isotope signatures reflect diet during the corresponding tissue synthesis time period, consumer tissue is composed of the relative proportions of each prey species assimilated, and in some instances, foraging location (Bearhop et al. 1999; Yeakel et al. 2016). For example, in aquatic ecosystems, pelagic consumers are often depleted in δ13C relative to littoral consumers; the difference in these consumers has been attributed to reliance on different food sources (France 1995). Similarly, stable isotope δ15N signatures can reveal an organism's trophic position, which is critical for understanding food web interactions and human-mediated ecosystem disturbance (Hussey et al. 2014). Niche parameters, such as niche width, can also respond rapidly to changes in intra- and interspecific competition and prey availability (Boecklen et al. 2011; Jackson et al. 2011) and help elucidate patterns in food webs that are attributable to abiotic and/or biotic variables.

Disentangling the complexities of how biotic interactions, biodiversity, and abiotic environmental factors shape species distributions across a landscape is pertinent for conservation of aquatic systems. Research completed by Lyles and colleagues (2022) suggests Brook Stickleback *Culaea inconstans* (an invasive species) and Fathead Minnow *Pimephales promelas* switched from pelagic to littoral resources coinciding with the onset of the HCB at Saratoga Lake during 2021. Further, the niche space of Brook Stickleback contracted and subsequently overlapped with that of Iowa Darter *Etheostoma exile*, a species of greatest conservation need, post HCB implying the threat of invasive species may be compounded by bloom onset. If the trophic niche space of small-bodied fishes is affected by HCBs, the ecosystem wide implications of HCBs could be much more pervasive than previously

thought. The findings of Lyle and colleagues (2022) inspired me to further evaluate the effects of HCBs on small-bodied fish using carbon and nitrogen stable isotope analyses in two lakes in SE Wyoming, one where HCBs occur (Saratoga Lake) and one where they do not currently occur (Alsop Lake).

The objectives of this work are to:
1. Explore the spatiotemporal variation of fish trophic niche dimensions and position across two lakes in Wyoming to understand patterns of resource use with respect to species and ontogeny
2. Evaluate fish trophic niche dimensions with respect to the onset and persistence of HCBs
3. Quantify trophic niche width and patterns of niche overlap to infer how HCBs affect the potential for competitive interactions between species

I hypothesize the trophic niche of Brook Stickleback, Fathead Minnows and Iowa Darter will shift during the spawning season as these three species breed in shallow water and may move from deeper waters into the shoreline during spawning season; I predict the trophic niche will reflect a shift from pelagic to littoral resource use at both Alsop and Saratoga Lake. I also hypothesize fish resource use will shift in Saratoga Lake with the onset of the HCB as pelagic zooplankton prey availability will decrease and fishes may migrate to nearshore water to optimize foraging opportunity. I predict we will see an additional signature of resource use shift from pelagic to littoral with the onset of the HCB in Saratoga Lake; I do not predict a secondary shift in resource use from pelagic to littoral at Alsop Lake, which is serving as my HCB control as we currently do not observe HCBs there. Additionally, I predict trophic niche overlap will be greater between species in Saratoga Lake than Alsop Lake due to the presence of HCBs and the indirect food web implications. Quantifying trophic niche width and patterns of niche overlap will help us better understand how harmful algal blooms affect interactions between fish species.

2. Methods

2.1 Study Sites
Samples for stable isotope analysis will be collected biweekly from May to October 2022 in two small reservoirs in SE Wyoming: Saratoga Lake and Alsop Lake. Brook Stickleback, Iowa Darter, and Fathead Minnow are present in Saratoga Lake; Iowa Darter and Fathead Minnows are present in Alsop Lake. Saratoga Lake experiences severe harmful cyanobacterial blooms annually, with water cyanotoxin levels routinely exceeding thresholds set by the Wyoming Department of Health (Wyoming Department of Environmental Quality 2022). Alsop Lake does not have a history of HCBs and serves as the control in this study.

2.2 Fish Sampling

Fish will be collected using a dip-net and miniature fyke net. 20 fish from each species of interest will be collected for stable isotope analysis; fish will be euthanized with an overdose of Aqui-S20E and placed in a jar with native lake water. Samples will immediately be placed on ice and frozen at -20 C until future processing.

2.3 Prey Sampling
To assess stable isotope composition of prey, I will collect whole-body invertebrate samples during each fish sampling event. Nearshore horizontal zooplankton tows and off-shore vertical zooplankton tows (64 um mesh, 0.5 m diameter net) will be collected and immediately placed on ice. Macroinvertebrates will be collected from

nearshore environments using dip nets and placed on ice. All samples will be frozen at -20 C until future processing.

2.4 Stable Isotope Processing

Fish muscle fillets and livers from each individual will be extracted and oven dried at 60 C for 48 hours in centrifuge tubes. Fish stomachs will be collected and stored in ethanol. I will dry muscle tissue and homogenize it using a pestle in centrifuge tubes. Zooplankton samples will be sieved at 153 microns, dried at 60C for 48 hours, then sonicated for two minutes and manually sorted to remove phytoplankton. Macroinvertebrate samples will be separated by taxonomic order and placed in centrifuge tubes. Prey samples will be dried at 60 C for 48 hours. All stable isotope samples will be weighed to the nearest 0.001 mg before being packed into 3.5-mm x 5-mm tin capsules. Samples will be analyzed for stable isotopes of carbon and nitrogen at the University of Wyoming Stable Isotope Facility using an elemental analyzer (Carlo Erba 1110) connected to a continuous flow isotope ratio mass spectrometer (Finnigan Delta V). Carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic composition will be reported with respect to standards of Vienna PeeDee Belemnite and atmospheric nitrogen, respectively, and expressed in parts per thousand (per mille, ‰).

2.5 Statistical Analysis

I will use values of δ^{13}C and δ^{15}N to describe trophic niche metrics including niche area, overlap between species, and to understand both resource source (i.e., pelagic vs littoral; 13C range) and trophic range (δ^{15}N range). All measures of trophic niche will be grouped by pre- and post-HCB onset to understand how resource use varies in the presence of HCBs. Trophic niche widths will be compared using a simple variance ratio test (F-test, Bearhop et al. 1999). Wilcoxon Rank-Sum tests will be used to examine differences between stable carbon and nitrogen isotope values (Ogloff et al. 2019). Niche overlap will be quantified using standard ellipse areas corrected for small sample sizes, which represent the standard deviation around the bivariate mean and encompass ~40% of the data points (Ogloff et al. 2019). Niche overlap will also be quantified using 95% ellipse areas (encompassing 95% of the data points), which represent the isotopic niche of each population as a whole rather than the core isotopic niche. Additionally, muscle δ^{13}C and δ^{15}N will be analyzed using the SIBER package (Jackson et al. 2011) to quantify isotopic niche breadth and overlap. To test for potential ontogenetic shifts in isotopic composition, I will use linear regressions of isotope values on fish body length and use analyses of variance to compare isotope values among groups (i.e. temporal timing pre HCB vs post HCB, and with respect to spawning).

3. Expected Outcomes

Using stable isotope analysis to estimate trophic niche width provides a robust and powerful metric for understanding resource acquisition and allocation, prior diet, trophic relationships and constructing food webs (Boecklen et al. 2011; Jackson et al. 2011; Ben-David and Flaherty 2012). Quantifying the trophic level impacts of HCBs is pertinent for understanding ecosystem wide impacts of this increasingly prevalent threat to aquatic ecosystems. This research will provide insight to how the niche dimensions and position across space, time, and ontogeny, reveal potential overlap between species, and elucidate patterns of resource use allocation in small-bodied fishes in light of harmful cyanobacterial blooms.

4. Research Progress

I sampled Saratoga and Alsop Lakes bimonthly from May to September 2022 and collected fish and prey samples. All fish samples have been processed and we submitted fish muscle samples to the University of Wyoming Stable Isotope Facility (UW SIF) for δ^{13}C and δ^{15}N analysis in late October 2022. Macroinvertebrate prey samples are

dried, tinned, and ready for submission to the UW SIF. We are currently processing zooplankton prey samples and refining our methods for separating zooplankton from phytoplankton for samples collected during severe HCBs. I plan to submit our prey samples to the UW SIF for analysis after we finish processing the zooplankton samples. I have not received stable isotope results and have not started the analysis phase.

Introduction References

Angradi, T. R., P. L. Ringold, and K. Hall. 2018. Water clarity measures as indicators of recreational benefits provided by U.S. lakes: Swimming and aesthetics. Ecological Indicators 93:1005–1019. Elsevier B.V.

Bae, M. J., and Y. S. Park. 2014. Biological early warning system based on the responses of aquatic organisms to disturbances: A review. Science of the Total Environment 466–467:635–649.

Caraco, N. F. 1993. Disturbance of the phosphorus cycle: A case of indirect effects of human activity. Trends in Ecology and Evolution 8(2):51–54.

Carpenter, S. R., J. J. Cole, M. L. Pace, R. Batt, W. A. Brock, T. Cline, J. Coloso, J. R. Hodgson, J. F. Kitchell, D. A. Seekell, L. Smith, and B. Weidel. 2011. Early warnings of regime shifts: A whole-ecosystem experiment. Science 332(6033):1079–1082.

Chapin III, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds||, D. U. Hooper, S. Lavorel#, O. E. Sala$, S. E. Hobbie, M. C. Mack, and S. Díaz. 2000. Consequences of changing biodiversity. Nature 405(May):234–242.

Clarke, S. J., and G. Wharton. 2000. Sediment nutrient characteristics and aquatic macrophytes in lowland English rivers. Page The Science of the Total Environment.

Clarkson, R. W., and M. R. Childs. 2000. Temperature effects of hypolimnial-release dams on early life stages of Colorado River Basin big-river fishes. Copeia 2000(2):402–412.

Collins, A. L., P. S. Naden, D. A. Sear, J. I. Jones, I. D. L. Foster, and K. Morrow. 2011. Sediment targets for informing river catchment management: International experience and prospects. Hydrological Processes 25(13):2112–2129.

Creed, I. F., A. K. Bergström, C. G. Trick, N. B. Grimm, D. O. Hessen, J. Karlsson, K. A. Kidd, E. Kritzberg, D. M. McKnight, E. C. Freeman, O. E. Senar, A. Andersson, J. Ask, M. Berggren, M. Cherif, R. Giesler, E. R. Hotchkiss, P. Kortelainen, M. M. Palta, T. Vrede, and G. A. Weyhenmeyer. 2018, August 1. Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. Blackwell Publishing Ltd.

Dong, M., S. Yang, X. Yang, M. Xu, W. Hu, B. Wang, Y. Huang, J. Xu, H. Lu, Y. Yang, X. Chen, H. Huang, and G. Sun. 2022. Water quality drives the distribution of freshwater cable bacteria. Science of the Total Environment 841(June):156468. Elsevier B.V.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan. 2006. Freshwater biodiversity: Importance, threats, status and conservation challenges. Biological Reviews of the Cambridge Philosophical Society 81(2):163–182.

Flynn, D. F. B., M. Gogol-Prokurat, T. Nogeire, N. Molinari, B. T. Richers, B. B. Lin, N. Simpson, M. M. Mayfield, and F. DeClerck. 2009. Loss of functional diversity under land use intensification across multiple taxa. Ecology Letters 12(1):22–33.

Glibert, P. M. 2017. Eutrophication, harmful algae and biodiversity — Challenging paradigms in a world of complex nutrient changes. Marine Pollution Bulletin 124(2):591–606. Elsevier Ltd.

Greig, S. M., D. A. Sear, and P. A. Carling. 2005. The impact of fine sediment accumulation on the survival of incubating salmon progeny: Implications for sediment management. Science of the Total Environment 344(1-3 SPEC. ISS.):241–258.

Hasse, D. 2017. Urban Wetlands and Riparian Forests as a Nature-Based Solution for Climate Change Adaptation in Cities and Their Surroundings. Pages 111–122 in N. Kabisch, H. Korn, J. Stadler, and A. Bonn, editors. Nature-based Solutions to Climate Change Adaptation in Urban Areas, Theory and Practice of Urban Sustainability Transitions,.

Hessen, D. O., and V. Vandvik. 2022. Buffering Climate Change with Nature. Weather, Climate, and Society 14(2):439–450.

Hobbs, R. J., and L. F. Huenneke. 1992. Disturbance, Diversity, and Invasion: Implications for Conservation. Conservation Biology 6(3):324–337.

Holden, P. B. 1979. Ecology of Riverine Fishes in Regulated Stream Systems with Emphasis on the Colorado River.

Pages 57–74 The Ecology of Regulated Streams.

Hooper, D. U., E. C. Adair, B. J. Cardinale, J. E. K. Byrnes, B. A. Hungate, K. L. Matulich, A. Gonzalez, J. E. Duffy, L. Gamfeldt, and M. I. Connor. 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486(7401):105–108. Nature Publishing Group.

Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs 75(1):3–35.

Hosen, J. D., K. S. Aho, A. P. Appling, E. C. Creech, J. H. Fair, R. O. Hall, E. D. Kyzivat, R. S. Lowenthal, S. Matt, J. Morrison, J. E. Saiers, J. B. Shanley, L. C. Weber, B. Yoon, and P. A. Raymond. 2019. Enhancement of primary production during drought in a temperate watershed is greater in larger rivers than headwater streams. Limnology and Oceanography 64(4):1458–1472.

Hu, N., P. E. Bourdeau, C. Harlos, Y. Liu, and J. Hollander. 2022. Meta-analysis reveals variance in tolerance to climate change across marine trophic levels. Science of the Total Environment 827:154244. Elsevier B.V.

Jensen, D. W., E. A. Steel, A. H. Fullerton, and G. R. Pess. 2009. Impact of Fine Sediment on Egg-To-Fry Survival of Pacific Salmon: A Meta-Analysis of Published Studies. Reviews in Fisheries Science 17(3):348–359.

Jia, J., W. Shi, Q. Chen, and T. L. Lauridsen. 2017. Spatial and temporal variations reveal the response of zooplankton to cyanobacteria. Harmful Algae 64:63–73. Elsevier B.V.

Jiang, L. Q., B. R. Carter, R. A. Feely, S. K. Lauvset, and A. Olsen. 2019. Surface ocean pH and buffer capacity: past, present and future. Scientific Reports 9(1).

Kim, M. S., S. H. Ahn, I. J. Jeong, and T. K. Lee. 2021. Water quality drives the regional patterns of an algal metacommunity in interconnected lakes. Scientific reports 11(1):13601.

Kouakou, C. R. C., and T. G. Poder. 2019. Economic impact of harmful algal blooms on human health: A systematic review. Journal of Water and Health 17(4):499–516. IWA Publishing.

Lehman, P. W., T. Kurobe, and S. J. Teh. 2022. Impact of extreme wet and dry years on the persistence of Microcystis harmful algal blooms in San Francisco Estuary. Quaternary International 621:16–25.

Muller-Navarra, D. C., M. T. Brett, A. M. Liston, and C. R. Goldman. 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers 403:74–77.

Newcombe, C. P., and J. O. T. Jensen. 1996. Channel Suspended Sediment and Fisheries: A Synthesis for Quantitative Assessment of Risk and Impact. North American Journal of Fisheries Management 16(4):693–727. Wiley.

Noble, I. R., and R. O. Slayter. 1976. The Use of Vital Attributes to Predict Successional Changes in Plant Communities Subject to Recurrent Disturbances. Vegetatio 43:5–21.

O'Neil, J. M., T. W. Davis, M. A. Burford, and C. J. Gobler. 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful Algae 14:313–334. Elsevier B.V.

Oliver, T. H., M. S. Heard, N. J. B. Isaac, D. B. Roy, D. Procter, F. Eigenbrod, R. Freckleton, A. Hector, C. D. L. Orme, O. L. Petchey, V. Proença, D. Raffaelli, K. B. Suttle, G. M. Mace, B. Martín-López, B. A. Woodcock, and J. M. Bullock. 2015. Biodiversity and Resilience of Ecosystem Functions. Trends in Ecology and Evolution 30(11):673–684. Elsevier Ltd.

Paerl, H. W., and V. J. Paul. 2012. Climate change: Links to global expansion of harmful cyanobacteria. Water Research 46(5):1349–1363. Elsevier Ltd.

Palmer, M. A., C. A. Reidy Liermann, C. Nilsson, M. Flörke, J. Alcamo, P. S. Lake, and N. Bond. 2008. Climate change and the world's river basins: Anticipating management options. Frontiers in Ecology and the Environment 6(2):81–89.

Parreira De Castro, D. M., D. Reis De Carvalho, P. Dos, S. Pompeu, M. Z. Moreira, G. B. Nardoto, and M. Callisto. 2016. Land Use Influences Niche Size and the Assimilation of Resources by Benthic Macroinvertebrates in Tropical Headwater Streams.

Peereman, J., | J Aaron Hogan, and T.-C. Lin. 2022. Disturbance frequency, intensity and forest structure modulate cyclone-induced changes in mangrove forest canopy cover. Global Ecol Biogeography 31:37–50.

Randall, C. J., L. T. Toth, J. J. Leichter, J. L. Maté, and R. B. Aronson. 2020. Upwelling buffers climate change impacts on coral reefs of the eastern tropical Pacific. Ecology 101(2).

Reid, A. J., A. K. Carlson, I. F. Creed, E. J. Eliason, P. A. Gell, P. T. J. Johnson, K. A. Kidd, T. J. MacCormack, J. D. Olden, S. J. Ormerod, J. P. Smol, W. W. Taylor, K. Tockner, J. C. Vermaire, D. Dudgeon, and S. J. Cooke. 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biological Reviews 94(3):849–873.

Reuling, L. F., A. W. D'amato, B. J. Palik, K. J. Martin, and D. S. A. Fassnacht. 2019. Initial tree regeneration response to natural-disturbance-based silviculture in second-growth northern hardwood forests. Canadian Journal of Forest Research 49(6):628–639.

Robarts, R. D., and T. Zohary. 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. New Zealand Journal of Marine and Freshwater Research 21(3):391–399.

Seibold, S., M. W. Cadotte, J. S. MacIvor, S. Thorn, and J. Müller. 2018. The Necessity of Multitrophic Approaches in Community Ecology. Trends in Ecology and Evolution 33(10):754–764. Elsevier Ltd.

Steffen, W., W. Broadgate, L. Deutsch, O. Gaffney, and C. Ludwig. 2015. The trajectory of the Anthropocene: The Great Acceleration. The Anthropocene Review:1–18.

Thackeray, S. J., P. A. Henrys, D. Hemming, J. R. Bell, M. S. Botham, S. Burthe, P. Helaouet, D. G. Johns, I. D. Jones, D. I. Leech, E. B. MacKay, D. Massimino, S. Atkinson, P. J. Bacon, T. M. Brereton, L. Carvalho, T. H. Clutton-Brock, C. Duck, M. Edwards, J. M. Elliott, S. J. G. Hall, R. Harrington, J. W. Pearce-Higgins, T. T. Høye, L. E. B. Kruuk, J. M. Pemberton, T. H. Sparks, P. M. Thompson, I. White, I. J. Winfield, and S. Wanless. 2016. Phenological sensitivity to climate across taxa and trophic levels. Nature 535(7611):241–245. Nature Publishing Group.

Voigt, W., J. Perner, A. J. Davis, T. Eggers, J. Schumacher, R. Bährmann, B. Fabian, W. Heinrich, G. Köhler, D. Lichter, R. Marstaller, and F. W. Sander. 2003. Trophic levels are differentially sensitive to climate. Ecology 84(9):2444–2453.

Vörösmarty, C. J., P. B. Mcintyre, M. O. Gessner, D. Dudgeon, A. Prusevich, P. Green, S. Glidden, S. E. Bunn, C. A. Sullivan, C. R. Liermann, and P. M. Davies. 2010. Global threats to human water security and river biodiversity. Nature 467:555–561.

Walters, A. W., C. P. Mandeville, and F. J. Rahel. 2018. The interaction of exposure and warming tolerance determines fish species vulnerability to warming stream temperatures. Biology Letters 14(9).

Ward, J. V., G. Bretschko, M. Brunke, D. Danielopol, J. Gibert, T. Gonser, and A. G. Hildrew. 1998. The boundaries of river systems: The metazoan perspective. Freshwater Biology 40(3):531–569.

Wells, M. L., B. Karlson, A. Wulff, R. Kudela, C. Trick, V. Asnaghi, E. Berdalet, W. Cochlan, K. Davidson, M. De Rijcke, S. Dutkiewicz, G. Hallegraeff, K. J. Flynn, C. Legrand, H. Paerl, J. Silke, S. Suikkanen, P. Thompson, and V. L. Trainer. 2020. Future HAB science: Directions and challenges in a changing climate. Harmful Algae 91. Elsevier B.V.

White, P. S., and S. T. A. Pickett. 1985. Natural disturbance and patch dynamics: an introduction. Pages 3–13 The Ecology of natural disturbance and patch dynamics.

Williams, A. P., B. I. Cook, and J. E. Smerdon. 2022. Rapid intensification of the emerging southwestern North American megadrought in 2020–2021. Nature Climate Change 12(3):232–234. Springer US.

Wood, P. J., and P. D. Armitage. 1997. Biological Effects of Fine Sediment in the Lotic Environment:203–217.

Zampella, R. A. 1994. Characterization of Surface Water Quality Along a Watershed Disturbance Gradient. Water Resources Bulliten 30(4):605–611.

Zheng, G., R. Xu, X. Chang, S. Hilt, and C. Wu. 2013. Cyanobacteria can allelopathically inhibit submerged macrophytes: Effects of Microcystis aeruginosa extracts and exudates on Potamogeton malaianus. Aquatic Botany 109:1–7. Elsevier.

Chapter 3 References

Briland, R. D., J. P. Stone, M. Manubolu, J. Lee, and S. A. Ludsin. 2020. Cyanobacterial blooms modify food web structure and interactions in western Lake Erie. Harmful Algae 92:101586. Elsevier.

Fulton, R. S., and H. W. Paerl. 1987. Toxic and inhibitory effects of the blue-green alga Microcystis aeruginosa on

herbivorous zooplankton. Page Journal of Plankton Research.

Ger, K. A., L.-A. Hansson, and M. Lurling. 2014. Understanding cyanobacteria-zooplankton interactions in a more eutrophic world. Freshwater Biology 59:1783–1798.

Glibert, P. M. 2017. Eutrophication, harmful algae and biodiversity — Challenging paradigms in a world of complex nutrient changes. Marine Pollution Bulletin 124(2):591–606. Elsevier Ltd.

Haney, J. F. 1987. Field studies on zooplankton-cyanobacteria interactions. New Zealand Journal of Marine and Freshwater Research 21(3):467–475.

Jewel, M. A. S., M. A. Affan, and S. Khan. 2003. Fish Mortality Due to Cyanobacterial Bloom in an Aquaculture Pond in Bangladesh. Pakistan Journal of Biological Sciences 6(12):1046–1050.

McNaught, D. C. 1975. A hypothesis to explain the succession from calanoids to cladocerans during eutrophication. Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen 19(1):724–731.

Orcutt, J. D., and M. L. Pace. 1984. Seasonal dynamics of rotifer and crustacean zooplankton populations in a eutrophic, monomictic lake with a note on rotifer sampling techniques. Hydrobiologia 119:73–80.

Ortiz, D., J. Palmer, and G. Wilkinson. 2020. Detecting changes in statistical indicators of resilience prior to algal blooms in shallow eutrophic lakes. Ecosphere 11(10).

Pace, M. L. 1986. An empirical analysis of zooplankton community size structure across lake trophic gradients'. Limnol. Oceanogr 31:45–55.

Paerl, H. W., and T. G. Otten. 2016. Duelling "CyanoHABs": Unravelling the environmental drivers controlling dominance and succession among diazotrophic and non-N2-fixing harmful cyanobacteria. Environmental Microbiology 18(2):316–324.

Reid, A. J., A. K. Carlson, I. F. Creed, E. J. Eliason, P. A. Gell, P. T. J. Johnson, K. A. Kidd, T. J. MacCormack, J. D. Olden, S. J. Ormerod, J. P. Smol, W. W. Taylor, K. Tockner, J. C. Vermaire, D. Dudgeon, and S. J. Cooke. 2019. Emerging threats and persistent conservation challenges for freshwater biodiversity. Biological Reviews 94(3):849–873.

Richman, S., and S. I. Dodson. 1983. The effect of food quality on feeding and respiration by Daphnia and Diaptomus. Limnol. Oceanogr 28(5):948–956.

Sukenik, A., A. Quesada, and N. Salmaso. 2015. Global expansion of toxic and non-toxic cyanobacteria: effect on ecosystem functioning. Biodivers Conserv 24:889–908.

US EPA. 2021. Ambient Water Quality Criteria to Address Nutrient Pollution in Lakes and Reservoirs. Us Epa (August).

Wells, M. L., B. Karlson, A. Wulff, R. Kudela, C. Trick, V. Asnaghi, E. Berdalet, W. Cochlan, K. Davidson, M. De Rijcke, S. Dutkiewicz, G. Hallegraeff, K. J. Flynn, C. Legrand, H. Paerl, J. Silke, S. Suikkanen, P. Thompson, and V. L. Trainer. 2020. Future HAB science: Directions and challenges in a changing climate. Harmful Algae 91. Elsevier B.V.

Zhang, Z., Z. Shi, Z. Yu, K. Zhou, J. Lin, J. Wu, and J. Mu. 2022. Spatio-Temporal Variations of Zooplankton and Correlations with Environmental Parameters around Tiaowei Island, Fujian, China. International Journal of Environmental Research and Public Health 19(19).

Chapter 4 References

Amorim, C. A., and A. do N. Moura. 2021. Ecological impacts of freshwater algal blooms on water quality, plankton biodiversity, structure, and ecosystem functioning. Science of the Total Environment 758. Elsevier B.V.

Bearhop, S., D. R. Thompson, S. Waldron, I. C. Russell, G. Alexander, and R. W. Furness. 1999. Stable isotopes indicate the extent of freshwater feeding by cormorants Phalacrocorax carbo shot at inland fisheries in England. Journal of Applied Ecology 36(1):75–84.

Ben-David, M., and E. A. Flaherty. 2012. Stable isotopes in mammalian research: A beginner's guide. Journal of Mammalogy 93(2):312–328.

Boecklen, W. J., C. T. Yarnes, B. A. Cook, and A. C. James. 2011. Use of stable isotopes in foraging ecology and

food web analysis. Annual Review of Ecology, Evolution, and Systematics 42:411–440.

Borrell, A., M. Gazo, A. Aguilar, J. A. Raga, E. Degollada, P. Gozalbes, and R. García-Vernet. 2021. Niche partitioning amongst northwestern Mediterranean cetaceans using stable isotopes. Progress in Oceanography 193(March).

Briland, R. D., J. P. Stone, M. Manubolu, J. Lee, and S. A. Ludsin. 2020. Cyanobacterial blooms modify food web structure and interactions in western Lake Erie. Harmful Algae 92:101586. Elsevier.

Carter, W. A., U. Bauchinger, and S. R. McWilliams. 2019. The importance of isotopic turnover for understanding key aspects of animal ecology and nutrition. Diversity 11(5).

Chase, J. M., and J. A. Myers. 2011. Disentangling the importance of ecological niches from stochastic processes across scales. Philosophical transactions of The Royal Society 366:2351–2363.

Creed, I. F., A. K. Bergström, C. G. Trick, N. B. Grimm, D. O. Hessen, J. Karlsson, K. A. Kidd, E. Kritzberg, D. M. McKnight, E. C. Freeman, O. E. Senar, A. Andersson, J. Ask, M. Berggren, M. Cherif, R. Giesler, E. R. Hotchkiss, P. Kortelainen, M. M. Palta, T. Vrede, and G. A. Weyhenmeyer. 2018, August 1. Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. Blackwell Publishing Ltd.

France, R. L. 1995. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnology and Oceanography 40(7):1310–1313.

Hussey, N. E., M. A. Macneil, B. C. Mcmeans, J. A. Olin, S. F. J. Dudley, G. Cliff, S. P. Wintner, S. T. Fennessy, and A. T. Fisk. 2014. Rescaling the trophic structure of marine food webs. Ecology Letters 17(2):239–250.

Jackson, A. L., R. Inger, A. C. Parnell, and S. Bearhop. 2011. Comparing isotopic niche widths among and within communities: SIBER — Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology 80(3):595–602.

Jewel, M. A. S., M. A. Affan, and S. Khan. 2003. Fish Mortality Due to Cyanobacterial Bloom in an Aquaculture Pond in Bangladesh. Pakistan Journal of Biological Sciences 6(12):1046–1050.

Krztoń, W., J. Kosiba, A. Pociecha, and E. Wilk-Woźniak. 2019. The effect of cyanobacterial blooms on bio- and functional diversity of zooplankton communities. Biodiversity and Conservation 28:1815–1835.

Lyles, C. 2022. Going Green ? Seasonal changes in resource use and catch rates of small- bodied fishes coincide with timing of a cyanobacterial bloom. University of Wyoming.

Ogloff, W. R., D. J. Yurkowski, G. K. Davoren, and S. H. Ferguson. 2019. Diet and isotopic niche overlap elucidate competition potential between seasonally sympatric phocids in the Canadian Arctic. Marine Biology 166(8):1–12. Springer Berlin Heidelberg.

Orcutt, J. D., and M. L. Pace. 1984. Seasonal dynamics of rotifer and crustacean zooplankton populations in a eutrophic, monomictic lake with a note on rotifer sampling techniques. Hydrobiologia 119:73–80.

Pääkkönen, J. P., S. Rönkkönen, M. Karjalainen, and M. Viitasalo. 2008. Physiological effects in juvenile three-spined sticklebacks feeding on toxic cyanobacterium Nodularia spumigena-exposed zooplankton. Journal of Fish Biology 72(3):485–499. John Wiley & Sons, Ltd.

Paerl, H. W., and V. J. Paul. 2012. Climate change: Links to global expansion of harmful cyanobacteria. Water Research 46(5):1349–1363. Elsevier Ltd.

Richman, S., and S. I. Dodson. 1983. The effect of food quality on feeding and respiration by Daphnia and Diaptomus. Limnol. Oceanogr 28(5):948–956.

Sun, H., W. Wang, L. Geng, Y. Chen, and Z. Yang. 2013. In situ studies on growth, oxidative stress responses, and gene expression of juvenile bighead carp (Hypophthalmichthys nobilis) to eutrophic lake water dominated by cyanobacterial blooms. Chemosphere 93(2):421–427. Pergamon.

Visser, P. M., J. M. H. Verspagen, G. Sandrini, L. J. Stal, H. C. P. Matthijs, T. W. Davis, H. W. Paerl, and J. Huisman. 2016. How rising CO_2 and global warming may stimulate harmful cyanobacterial blooms. Elsevier B.V.

Wang, L., J. Zi, R. Xu, S. Hilt, X. Hou, and X. Chang. 2017. Allelopathic effects of Microcystis aeruginosa on green algae and a diatom: Evidence from exudates addition and co-culturing. Harmful Algae 61:56–62. Elsevier.

Wyoming Department of Environmental Quality. 2022. Harmful Cyanobacterial Bloom (HCB) Action Plan for Publicly Accessible Waterbodies in Wyoming.

Xu, R., S. Hilt, Y. Pei, L. Yin, X. Want, and X. Chang. 2016. Growth phase-dependent allelopathic effects of cyanobacterial exudates on Potamogeton crispus L. seedlings. Hydrobiologia 767:137–149.

Yeakel, J. D., U. Bhat, E. A. Elliott Smith, and S. D. Newsome. 2016. Exploring the isotopic niche: Isotopic variance, physiological incorporation, and the temporal dynamics of foraging. Frontiers in Ecology and Evolution 4(JAN):1–16.

Zheng, G., R. Xu, X. Chang, S. Hilt, and C. Wu. 2013. Cyanobacteria can allelopathically inhibit submerged macrophytes: Effects of Microcystis aeruginosa extracts and exudates on Potamogeton malaianus. Aquatic Botany 109:1–7. Elsevier.

Zi, J., X. Pan, H. J. MacIsaac, J. Yang, R. Xu, S. Chen, and X. Chang. 2018. Cyanobacteria blooms induce embryonic heart failure in an endangered fish species. Aquatic Toxicology 194:78–85. Elsevier.

Freshwater nutrient dynamics across spatial scales

Dissertation Proposal

Student: Linnea Ann Rock[1]
Advisor: Dr. Sarah M. Collins[1]
Committee Members: Dr. David G. Williams[2],
Dr. Amy C. Krist[1], Dr. Shannon E. Albeke[3], Dr. Jessica R. Corman[4]

[1]Program in Ecology, Department of Zoology and Physiology, University of Wyoming
[2]Program in Ecology, Botany Department, University of Wyoming
[3]Program in Ecology, Geography Department, University of Wyoming
[4]School of Natural Resources, University of Nebraska

"Water is the driving force of all nature." - Leonardo da Vinci

INTRODUCTION

Fresh surface waters, or lakes and streams, are among the world's most important and most threatened resources. Only 0.26 % of all available freshwater on the globe is surface water (Shiklomanov, 2000), which must be shared among all living things. Freshwaters experience the greatest and fastest changes of all ecosystems, after lands converted for agriculture (Carpenter et al., 2011). Among the serious forces that threaten freshwater health is the dramatically altered global nutrient flux (Falkowski et al., 2000). Nitrogen (N) and phosphorus (P) in particular are heavily associated with eutrophication of freshwaters. Eutrophication is a natural process of aging surface waters and results in 'greening,' through which productivity is enhanced by nutrient enrichment. Cultural eutrophication is the acceleration of the process via anthropogenic nutrient inputs (Dodds & Whiles, 2020). Despite the potential to cause eutrophication, nutrients are essential for freshwater quality and biodiversity.

N and P are inherently linked through organisms, food webs, and ecosystems because these nutrients are required for protein synthesis, cellular metabolism, growth, bone synthesis, and other biological structures and functions. N and P also regulate each other's dynamics at every point along the continuum of cells to the biosphere. Because N and P are used by all organisms on Earth, they are constantly recycled through food webs and ecosystems. Nutrient dynamics and stoichiometry provide tools to connect various components of ecosystems. Ecological stoichiometry aims to assess if/how much variation in ecology can be understood by simplifying the intricate details and complexities of ecological interactions into fundamental chemical basis of life (e.g., N and P) (Sterner & Elser, 2002).

In this context, it is imperative to emphasize the need for a holistic approach when studying nutrient dynamics in freshwater ecosystems. While in-depth studies focusing on specific mechanisms and processes within niche systems hold significant value, there is equal importance in adopting a broader perspective that encompasses entire watersheds, networks, and regional to continental scales. While N:P stoichiometry is notoriously difficult to predict (Collins et al., 2017), stoichiometric variation drives research questions. For example, while there are many biogeochemical processes that can influence N:P stoichiometry, nutrient sourcing composition (which can be infinitely varied within a watershed) can be a large determinant (Downing & McCauley, 1992).

I have developed a research plan that addresses the roles of co-nutrient dynamics at a range of spatial scales. My research engages the biogeochemical coupling of N and P (Sterner & Elser, 2002), the intimate connection of freshwaters to each other and the landscape (Wurtsbaugh et al., 2019), and societal and cultural relevance of freshwaters (Kondolf & Pinto, 2017). My work asks fundamental questions on reservoir management (chapter 2) and lake-river network connectivity (chapter 3) to broad-scale nutrient limitation (chapter 1) and productivity (chapter 4).

My work is conducted at multiple scales, including broadly across stolen land (https://native-land.ca/). My acknowledgement seeks to honor the rich culture of the indigenous people and remind us that the land is sacred. I recognize my work is centered around the natural world, which has been negatively impacted by colonialism.
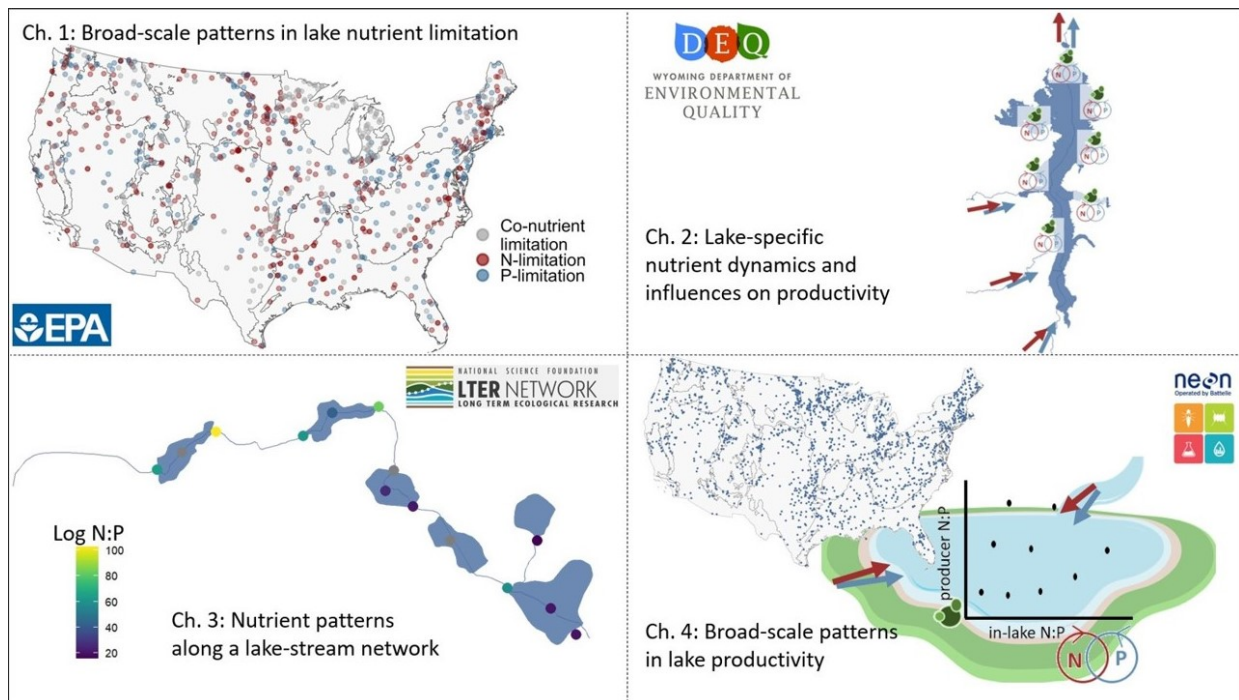
Figure 1: Freshwater nutrient dynamics vary across spatial and temporal scales. Patterns in nutrient limitation and trophic state vary across space and time and may influence management decisions (chapter 1). But sometimes in-lake dynamics and local context matter most for mitigating water quality problems (chapter 2). Nutrient dynamics exhibit variability along a continuum of linked freshwater lakes and rivers, which is further diversified by seasonal influences (chapter 3). Finally, in-lake nutrient and productivity trends may integrate and form larger scale patterns, revealing underlying factors contributing to stoichiometric imbalances and water quality (chapter 4). Within each chapter, understanding the interconnectedness of nutrients is paramount, as these elements are tightly linked across various biological levels, from cellular processes to the functioning of entire ecosystems. Moreover, N and P play pivotal roles in regulating each other's dynamics, highlighting the intricate balance that exists within nutrient cycles. By recognizing these fundamental connections, we can gain deeper insights into the complexities of ecological interactions and better appreciate the chemical underpinnings of life itself.

CHAPTER 2: Nutrient budgets and influences on productivity in a critical Wyoming water source

Collaborators: Sarah M. Collins, Kelsee Hurshman, Lindsay Patterson, Ron Steg, Samuel J. Sillen
Status: Planning with DEQ, data analyses ongoing

We respectfully acknowledge and recognize this work is conducted on the stolen lands and water of the Tséstho'e (Cheyenne), Newe Sogobia (Eastern Shoshone), Apsáalooke (Crow), Očhéthi Šakówiŋ, and Cayuse, Umatilla and Walla Walla People. Boysen reservoir currently resides on the Wind River Reservation.

Research Questions

The goal of this chapter is to understand nutrient dynamics in Boysen Reservoir and to assist the Wyoming Department of Environmental Quality (DEQ) in reaching their nutrient reduction and harmful algal bloom mitigation goals. We aim to answer the following questions:

- What spatial frequency is sufficient to characterize nutrient loading and water quality?
    - What information do we get from each of the 7 monitored sites?
- What (reservoir heterogeneity, timing of loading, water level, in-reservoir dynamics) is driving changes or lack of changes in water quality?
- How are nutrients influence phytoplankton dynamics?
    - Does nutrient loading and/or nutrient stoichiometry correlate with phytoplankton density dynamics?
    - Are there nutrient thresholds associated with changepoints in phytoplankton community structure shifts or densities?
    - What are nutrient management targets to prevent harmful densities of cyanobacteria?

Introduction

Reservoirs serve as critical human resources for drinking water, irrigation, navigation, flood control, hydropower, recreation, and fisheries (Marzolf & Robertson, 2006). These man-made waterbodies (via dams) fundamentally alter biology and nutrient dynamics by changing the movement of water, biota, and solutes (Hayes et al., 2017). Additionally, reservoirs typically serve as settling basins for sediments, nutrients, and other solutes (Syvitski et al., 2009). It is crucial to understand drivers of water quality within reservoirs because of the importance of reservoirs and high demand for water. And while some parts of reservoirs behave similarly to lakes, there are physical differences that can lead to variation in drivers.

Reservoirs are even more intimately connected to the landscape than lakes because they drain substantially greater catchment areas and have greater perimeters, thus more land-water connection, than lakes (Hayes et al., 2017). Reservoirs are particularly critical in the arid mountain western U.S. and have a played a large role in full development of the western U.S. as they allowed for boat travel, agricultural irrigation, and water storage (Marzolf & Robertson, 2006). Reservoirs can be divided into three zones illustrating key areas within a single reservoir that vary in their physical structure and biogeochemical function: river, transitional, and lacustrine (Kennedy et al., 1985). The riverine zone is narrow and channelized and often has greater nutrient input, turbidity, and lower

light availability, and is typically more eutrophic than other portions of the reservoir. The lacustrine zone is the broadest and deepest zone that functions most similarly to a natural lake. There is little flow and it tends to be more oligotrophic with less direct nutrient input and more internal nutrient recycling. The transitional zone is characterized as a gradient between the riverine and lacustrine zone with intermediate physical and biogeochemical characteristics (Marzolf & Robertson, 2006).

Cultural eutrophication and harmful algal blooms are among the top threats to water quality and biodiversity worldwide with excess N and P as arguably the most important contributor to eutrophication (Conley et al., 2009; Reid et al., 2019; Smith & Schindler, 2009). In the Western U.S., increased awareness and monitoring of eutrophication and harmful algal blooms in freshwaters has pointed toward eutrophication in this region. However, long-term satellite imagery has suggested there is no evidence of changing water quality and rather most lakes in this region have been static for the past several decades (Oleksy et al., 2022; Sillen et al., 2023). Since reservoirs typically drain greater catchments than natural lakes, eutrophication can occur more rapidly with variable nutrient dynamics in the reservoir zones (Marzolf & Robertson, 2006) and photic zones of reservoirs have been found to be shallower (Hayes et al., 2017).

Over the past 6 years, Wyoming Department of Environmental Quality (DEQ) has routinely listed a Harmful Cyanobacterial Bloom Advisories for Boysen Reservoir (see https://wdeq.maps.arcgis.com/apps/Shortlist/index.html?appid=342d22d86d0048819b8dfa61dd3ff061). It is a culturally significant waterbody that is used for recreation and drinking water supply, but harmful cyanobacterial blooms threaten the water quality and ecosystem services provided by the reservoir (of Environmental Quality, 2023). Thus, Boysen is considered a high priority for implementation of proactive nutrient reduction as part of the Wyoming Nutrient Strategy.

Methods

Data Description

Boysen Reservoir located on the Wind River, about 23 km south of The Wedding of the Waters, where the Bighorn River changes names to Wind River. Since 2020, the DEQ collects monthly data between May and October from seven locations along the reservoir (Figure 5). Collections include alkalinity, orthophosphate, total ammonia, secchi depth, chlorophyll-a, total P, total N, and nitrate, and water column profile data for specific conductance, dissolved oxygen, pH, and water temperature. Additionally, monthly counts of phytoplankton are identified to the genus/species/variety level. The U.S. Geological Survey (USGS) monitors the three major tributaries for continuous discharge and nutrients on a monthly basis (total N, orthophosphate, and total P). The USGS also monitors the outlet for monthly nutrient concentrations. Wind River outlet discharge water level data are available from the U.S. Department of the Interior Bureau of Reclamation. The University of Wyoming operates a buoy within the pelagic section of the reservoir with water column profile temperature data, and surface water productivity data (see https://limno.io/station/boysen/).

Data Analysis

As part of my collaboration with the Wyoming DEQ, this chapter will address how monitoring sites differ and if seven sites is necessary to fully characterize water quality in Boysen Reservoir. We will examine lake heterogeneity, biogeochemical loading, and in-reservoir dynamics and their influence on water quality. We will also analyze nutrient concentration and stoichiometry thresholds associated with changes in phytoplankton community structure changes.
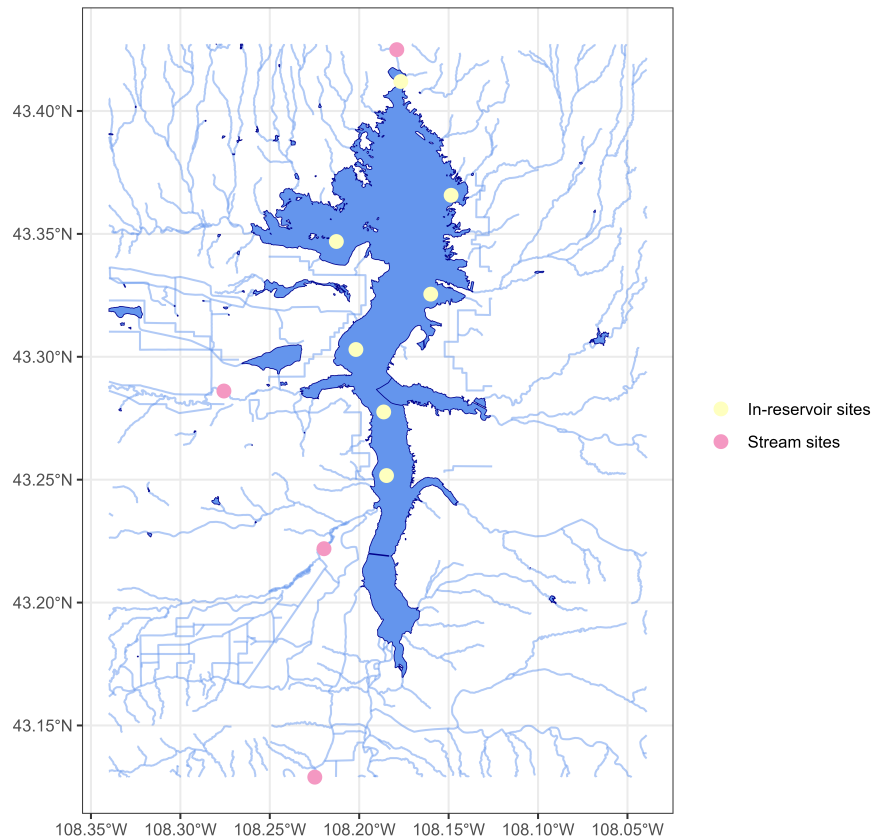
Figure 5: Map with Boysen Reservoir and tributaries. The yellow points are the sampling locations in Boysen Reservoir that represent various parts of the reservoir (i.e., river, transitional, and lacustrine). The pink points represent the tributaries and outlet. The direction of flow is toward the north.

We plan to develop monthly tributary and outlet nutrient budgets to quantify spatial and temporal input and output dynamics. Additionally, we will quantify in-reservoir nutrient dynamics using bathymetry data and monthly nutrient concentrations. We will then examine corresponding phytoplankton communities and assess correlations between changes in community dynamics and nutrient inflows using changepoint analyses. We will also assess at which nutrient concentrations and ratios cyanobacteria are present/abundant.

We will examine differences at each of the seven monitoring locations, including in nutrient concentrations and stoichiometry, phytoplankton communities, and water column profiles of the water quality parameters to understand the differences in the various parts of the reservoir and if any monitoring locations are redundant. To examine in-reservoir dynamics that might influence water quality, we plan to test for statistical differences in profiles and Schmidt's Stability (a measure of resistance to mixing (Idso, 1973; Schmidt, 1928)). Correlations between parameters and spatial patterns will be assessed using Empirical Orthogonal Function (EOF) and Principle Component (PC) analyses. EOF analyses are useful for examining spatiotemporal and multivariate patterns, and may help us determine which parts of the reservoir are most influenced by nutrients or other in-lake dynamics. PC analyses summarize multivariate trends into digestible formats and are especially useful when exploring many parameters.

Expectations

We expect to see distinct differences in nutrient concentrations and stoichiometry, productivity, and water quality parameters between the sampling locations within the reservoir based on whether they are riverine (narrow basin, channelized like a river), transitional (intermediate portion between riverine and lacustrine), or lacustrine zones (most similar to a natural lake, broadest and deepest portion) (Kennedy et al., 1985), but less differences between sites of the same type. Additionally, we expect in-reservoir stratification and mixing dynamics to have high correlation with productivity, rather than nutrient loading, which likely occurs in extreme values early in summer.
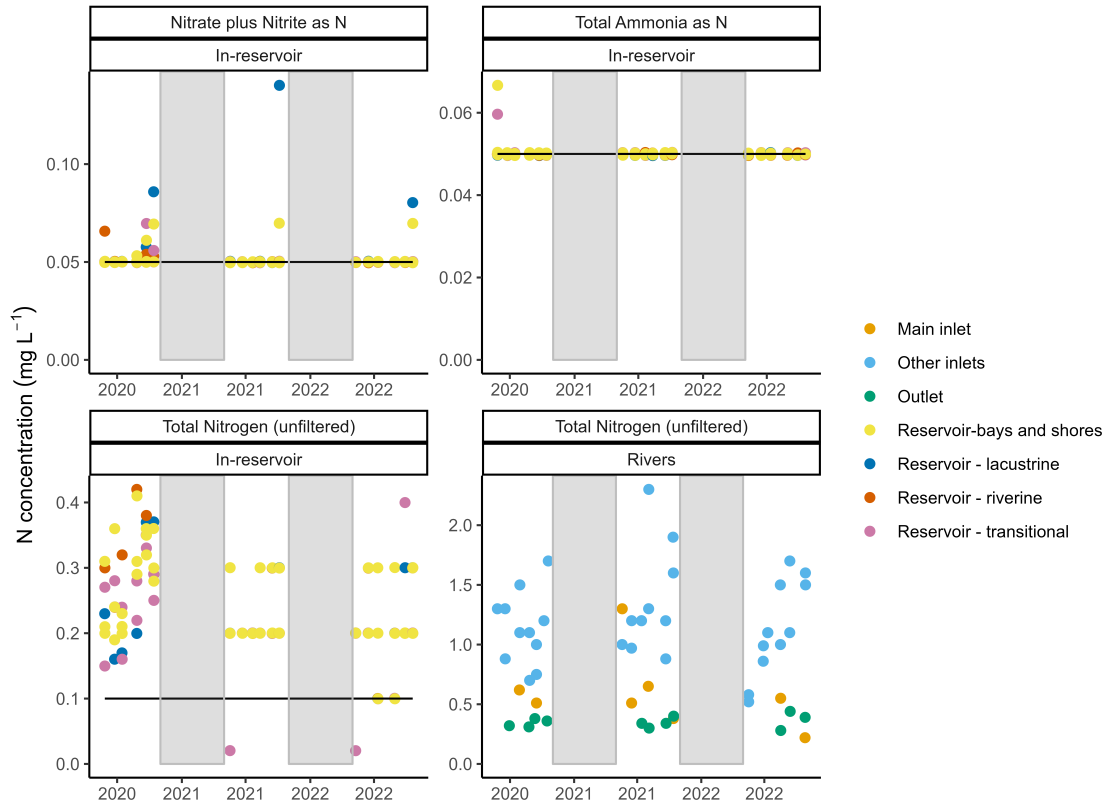
Selected data exploration
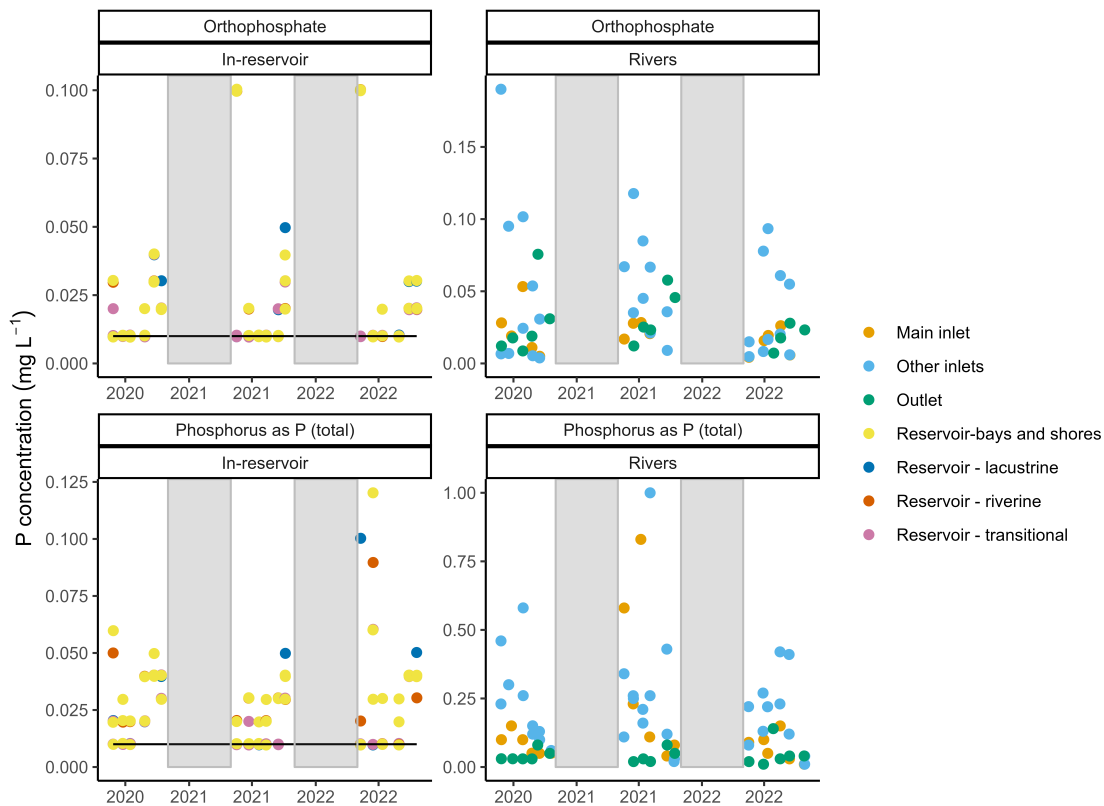
Figure 6: Raw P data from all sampling locations and events, May-October. November-April are greyed out on figures. Orthophosphate concentrations in the top row and total P concentrations in the bottom row. Various sampling location types are represented by colors and in-reservoir concentrations are in the first column with river concentrations in the second column. Detection limits for reservoirs are represented by horizontal lines.

There were consistently higher nutrient concentrations in the inlets than in-reservoir or in the outlet, which more closely matched the in-reservoir concentrations (Figures 6, 7). Outlet concentrations were especially similar to the lacustrine concentrations, which is close in proximity to the outlet. Despite not being the main sources of water, the minor inlets tended to have higher nutrient concentrations than the Wind River major inlet.

There were also substantial annual and inter-annual variation in nutrient concentrations. Ammonia concentrations were typically less than nitrate, with the lacustrine portion of the reservoir

Figure 7: Raw N data from all sampling locations and events, May-October. November-April are greyed out on figures. Nitrate plus nitrite in-reservoir concentrations are presented in the top left panel, total ammonia in-reservoir concentrations are in the top right panel, and total N concentrations in the bottom panel, with in-reservoir and river concentrations separated for better viewing. Various sampling location types are represented by colors. Detection limits for reservoirs are horizontal lines. The three forms of N are collected from the reservoir but only total N is collected in the rivers.

having the highest nitrate concentrations in October of each year (Figure 7). For total N and P, outlet concentrations show little seasonal variation, suggesting the reservoir serves as a nutrient sink, buffering the outflow concentrations. However, orthophosphate shows seasonality with the highest inlet and in-reservoir concentrations during the spring months and highest outlet concentrations during the fall months, suggesting an abundance of P in this system (Figure 6).

Significance Statement

Tailoring one of my studies to directly address the questions and work toward the goals of the Wyoming DEQ is a great way to expand my skills into the management realm. This study will translate the needs of DEQ into quantifiable results that will work toward the development of reservoir-specific nutrient thresholds to protect the water quality of a culturally important water resource. Furthermore, eutrophication can have serious consequences for aquatic habitats and ecosystem services including decreased water clarity, decreased water column oxygen, increase in toxic compounds (Camargo & Alonso, 2006; Conley et al., 2009). Eutrophication can also result in high economic costs (Dodds et al., 2008; Keiser et al., 2019). Increased knowledge of the context-dependent water quality drivers in Boysen Reservoir may inform management decisions.