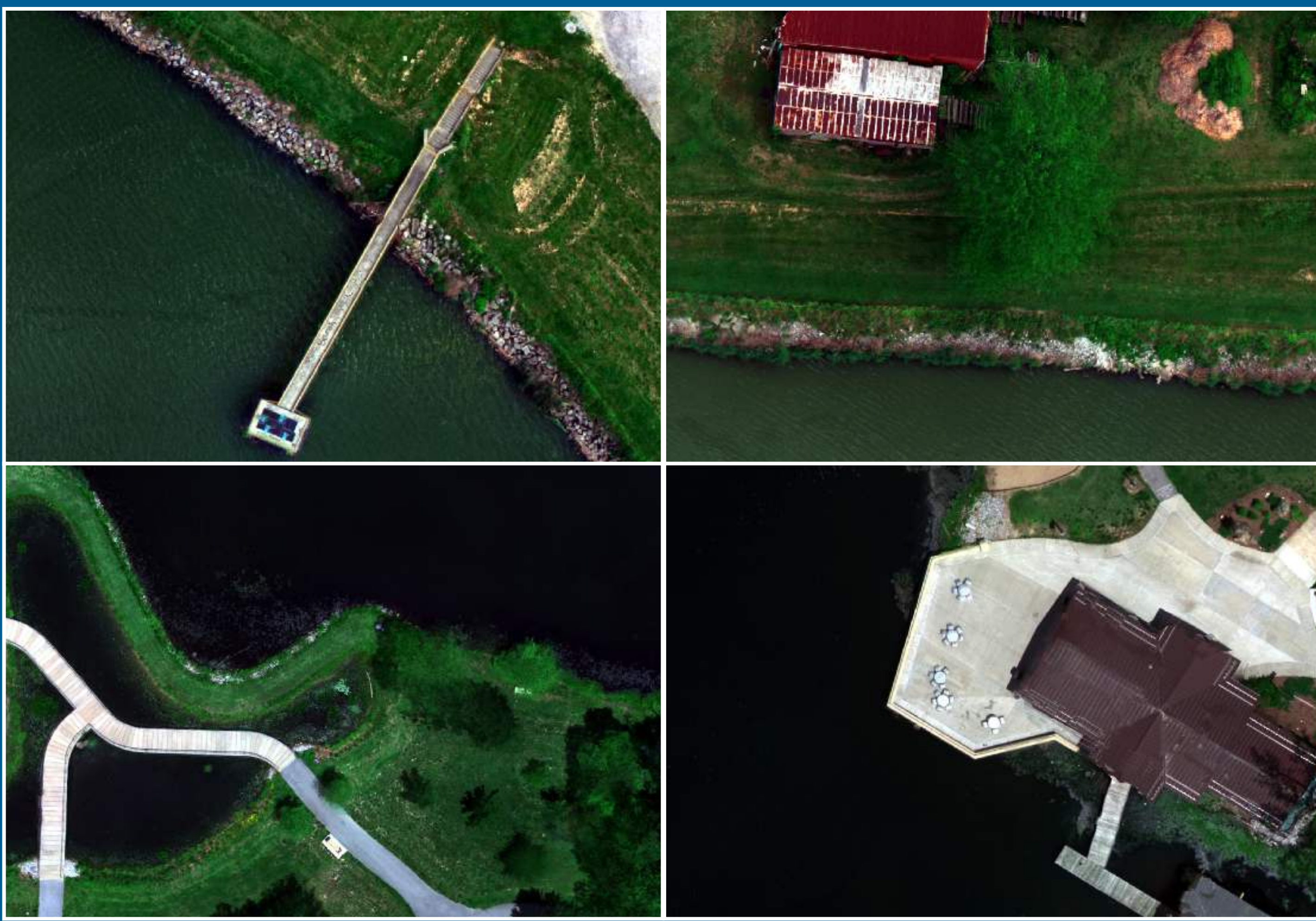
Journal of Contemporary

Water Research & Education

Issue 177
April 2023



Sponsored Research of the National Institutes for Water Resources

UCOWR
UNIVERSITIES COUNCIL ON WATER RESOURCES

A publication of the Universities Council on Water Resources with support from Southern Illinois University Carbondale

JOURNAL OF CONTEMPORARY WATER RESEARCH & EDUCATION

Universities Council on Water Resources
1231 Lincoln Drive, Mail Code 4526
Southern Illinois University
Carbondale, IL 62901
Telephone: (618) 536-7571
www.ucowr.org

ISSN 1936-7031

Cover photos: *Images taken using an unmanned aerial vehicle to monitor algal blooms in southern Illinois lakes*. Credit: Ruopu Li.
Back & inside back cover photos: Courtesy of Colorado State University

The *Journal of Contemporary Water Research & Education* is published by the Universities Council on Water Resources (UCOWR). UCOWR is not responsible for the statements and opinions expressed by authors of articles in the *Journal of Contemporary Water Research & Education.*

Journal of Contemporary Water Research & Education

Issue No. 177 **April 2023**

Universities Council on Water Resources
Journal of Contemporary Water Research & Education
Issue 177, Page 1, April 2023

Letter from the Special Issue Editor

I am pleased to report on the release of the National Institutes for Water Resources (NIWR) Special Issue in the *Journal of Contemporary Water Research & Education* (JCWRE) that features important water research by researchers and students studying at our collective institutions of higher learning. This JCWRE Special Issue is a partnership between NIWR, which consists of the 54 land-grant university water institutes in the United States, and UCOWR, which represents 63 of the best water research universities in the United States and Canada. This timely water research is supported by Sec. 104b and 104g grants from the Department of Interior and U.S. Geological Survey appropriated by Congress through the 1964 Water Resources Research Act as amended in 1988. This peer-reviewed research includes articles on water quantity and quality from universities that stretch from east to west and from coast to coast that focus on most of the large river basins and watersheds in America. I wish to especially thank Jackie Gillespie and Karl Williard, Co-editors of JCWRE, for pushing this collaboration forward. Upon rereading the articles published in this Special Issue of JCWRE, I am reminded that the future of our field is in good hands to tackle the critical water resources issues of the day as they appear more and more in the headlines and front pages of the news.

Warmly,

Gerald Kauffman
Director and Associate Professor
University of Delaware
Water Resources Center
Joseph R. Biden, Jr. School of Public Policy & Administration
DGS Annex 261 Academy St.
Newark, Delaware 19711

Universities Council on Water Resources
Journal of Contemporary Water Research & Education
Issue 177, Pages 2-16, April 2023

Statewide Assessment Reveals Spatiotemporal Variability of Iron in Iowa Lakes

Tania Leung[1] and *Elizabeth D. Swanner[1]

[1]*Department of Geological & Atmospheric Sciences, Iowa State University, Ames, Iowa*
**Corresponding Author*

Abstract: The micronutrient iron has been noted to play a crucial role in regulating phytoplankton growth; however, most studies have focused on large lakes with persistent phytoplankton blooms that are known to undergo iron limitation, such as Lake Erie. Iron abundance in boreal lakes is also known to correlate with dissolved organic carbon and increased iron concentrations causing "browning." To assess the spatial distribution of dissolved Fe (DFe) in lakes throughout Iowa, a landscape once dominated by prairies, DFe was measured in surface waters of 124 lakes distributed across the state over the 2018 summer season. Thirty lakes were selected for 15 weeks of weekly DFe monitoring to assess temporal trends over the summer season. Dissolved Fe concentrations in surface waters ranged from 5 to 1000 μg L^{-1}. Iowan lakes exhibited temporal trends in DFe, with decreasing concentrations from May to mid-July and an increase into August. Unsupervised learning method (k-means) identified three main groups of lakes based on temporal DFe trends. In this study, surface water temperature was associated with DFe trends in some lakes. This study serves as a baseline for DFe in Iowa's lakes and can provide insights into iron biogeochemical cycling and its role in phytoplankton blooms, which are important to ecosystem and public health.

Keywords: *dissolved iron, lakes, water quality, trace metal*

The rapid proliferation of phytoplankton biomass in lakes is a nuisance and can be harmful to ecosystem health. Iron (Fe) limitation has been observed to control phytoplankton growth in the Laurentian Great Lakes (McKay et al. 2004; North et al. 2007). The Great Lakes tend to have dissolved Fe (DFe) that exceeds what is found in the oceans (Klein 1975), likely reflecting greater proximity to terrestrial sources of Fe supplied through tributaries or groundwater. Therefore, the quantity of DFe in smaller lakes might be expected to be even greater, given the larger influence of the catchment. However, lacking widespread data on Fe in smaller lakes makes it difficult to evaluate the relationship between Fe and catchment area. It is also difficult to determine how Fe impacts phytoplankton proliferation for other lakes because Fe data are only occasionally collected.

Iron plays an active biogeochemical role in aquatic systems as a vital micronutrient for phytoplankton. It is a cofactor in proteins such as ferrodoxin and cytochrome, which act to transfer electrons during photosynthesis (Campbell et al. 1998). Iron is critical to macronutrient acquisition, as it is required for the synthesis of nitrogenase in diazotrophic organisms (Larson et al. 2018) and in phosphatases that acquire phosphorus (Browning et al. 2017). The amount and form of Fe available can induce changes in phytoplankton physiology. For instance, low Fe availability

Research Implications

- A mid-summer minimum in dissolved iron (DFe) was common and may link to internal iron cycling.
- Iowa lakes have similar amounts of DFe as lakes in other Midwest, U.S.A. states.
- DFe was poorly correlated with dissolved organic carbon (DOC), likely reflecting the dominance of autochthonous DOC.

lowered the efficiency of photosystem II in the chlorophyte, *Dunaliella tertiolecta* (Vassiliev et al. 1995) and cyanobacteria *Microcystis aeruginosa* and *Microcystis wesenbergii* (Xing et al. 2007). If bioavailable forms of Fe are abundant, they may enhance phytoplankton growth (Twiss et al. 2000), while bioavailable Fe scarcity can restrict phytoplankton proliferation (Xu et al. 2013).

Iron is the fourth most abundant element in the Earth's crust; however, only a small fraction of total Fe may be bioavailable to phytoplankton in aquatic systems (Wells et al. 1995). Iron availability in lakes is controlled by many factors, such as regional geology, soil composition, watershed characteristics, mixing regime, and periodic anoxia (Gorham et al. 1983; Davison 1993; Nürnberg 1995; Dillon and Molot 2005). Aside from physicochemical processes in waters (e.g., redox reactions, mineral precipitation, adsorption-desorption, complexation-dissociation), Fe availability is also regulated by the presence of phosphates, sulfides, and organic matter in lakes (Azzoni et al. 2005; Hoffman et al. 2013). Iron exists in two oxidation states: reduced (ferrous) Fe^{2+} and oxidized (ferric) Fe^{3+}. Ferrous Fe predominates in acidic and/or anoxic waters while Fe^{3+} predominates in oxygenated waters. However, the dissolved form of Fe accessible to phytoplankton [Fe^{3+} (aq)] is often in low abundance in aquatic systems. Oxidized Fe^{3+} is poorly soluble in oxygenated waters due to the formation of highly insoluble oxides and hydroxides. Furthermore, it can also be complexed with organic ligands and not directly available for phytoplankton uptake (Nagai et al. 2007; Hassler et al. 2009). This leaves inorganic, unchelated Fe^{3+} as the preferred form of Fe for phytoplankton uptake (Kranzler et al. 2011; Du et al. 2019); however, this form comprises only a small fraction of DFe in well-oxygenated environments with a pH > 7 (Wells et al. 1995; Liu and Millero 2002; Morel et al. 2008).

Lakes provide environmental and economic benefits, serving as a source for food, recreation, and habitat for aquatic animals. Lakes in the Midwest, U.S.A. are impaired from eutrophication, primarily due to their location in watersheds dominated by row crop agriculture. For instance, Iowa's lakes are susceptible to phytoplankton proliferation, which are often linked with toxin production (Iowa Department of Natural Resources 2018). It is unclear whether Fe is associated with phytoplankton growth in Iowa's lakes because there is a lack of data on the presence and abundance of Fe. In this study, we establish a spatial and temporal dataset of DFe in surface waters of both natural and artificial (borrow pits or impoundments) lakes throughout Iowa. A subset of these lakes were monitored for DFe trends in surface waters over the summer season. Data collected may be used to understand whether this micronutrient may influence phytoplankton biomass in these lakes. Herein, the term DFe refers to concentrations measured in lakes within this study, whereas Fe refers to concentrations reported in other studies. Lake surface water was analyzed for DFe and evaluated against Fe in regional lakes that were also susceptible to phytoplankton bloom events. Results were compared with other variables: chlorophyll *a* (chl-*a*), dissolved organic carbon (DOC), pH, and temperature. Data collected from this survey will aid researchers in understanding the biogeochemistry of Fe in freshwater systems, its relationship to phytoplankton bloom events, and can be used in lake modeling studies.

Methods

Study Sites and Sample Collection

Lakes in this study were participants of Iowa Department of Natural Resources' (IDNR) Ambient Lakes Monitoring (ALM) program. To assess spatial distribution of DFe in Iowa, 124 lakes were surveyed for one summer in 2018 (Figure 1). Of the ALM lakes, 24 lakes are natural and 100 lakes are artificial, designated as borrow pits or impoundments. Most lakes in this study are considered eutrophic, and the remainder are mesotrophic lakes. From May through August, composite water samples were collected from the epilimnion (up to 2 m depth, depending on thermocline) at the deepest part of the lake and transported (maintained at 4°C) back to Iowa State University for analysis.

To better observe temporal trends in DFe, an additional subset of lakes (n = 30; Figure 2) were monitored weekly from May through August 2018 (15 weeks). Of the 30 lakes, four are mesotrophic and the remaining lakes are eutrophic. These lakes

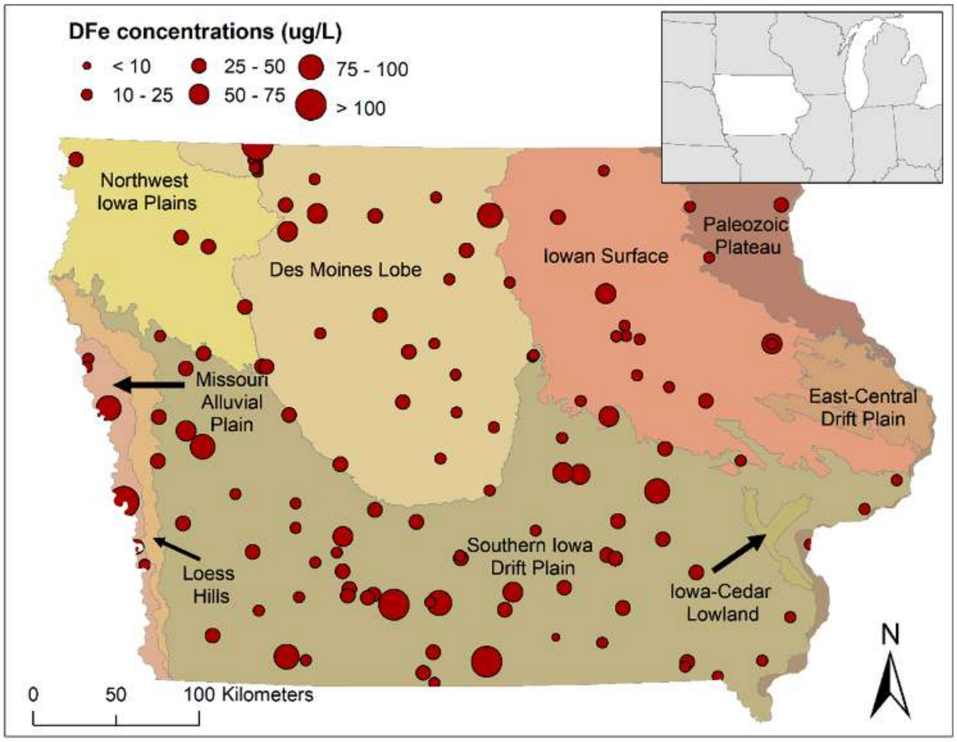


Figure 1. Mean DFe varied among ALM lakes, with no strong association with geological landforms. Measurements comprised of three sampling events in summer season 2018 (beginning, middle, and end of summer).

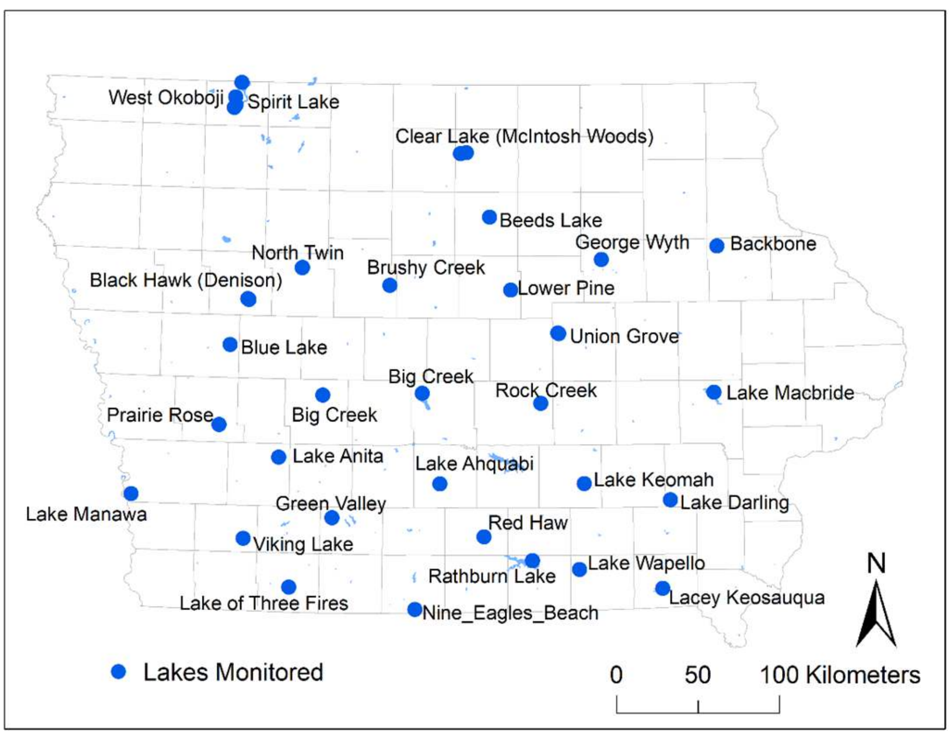


Figure 2. A subset of lakes (BM) which were monitored for temporal trends in DFe.

are part of IDNRs' statewide swimming beach monitoring program (BM), which monitors water quality conditions in shallow swimming areas (surface waters of littoral zone). Therefore, the DFe measurements for BM lakes are representative of near-shore surface water less than 1 m deep, and not representative of the whole lake. Homogenized surface water grab samples were collected between 7 am and 3 pm from the surface of the littoral zone. Three transects (maximum 6 m distance from lake shoreline) were established at the littoral zone. Next, three points were sampled per transect (250 mL volume at each sample point) and then mixed into a 2.25 L composite sample. This sampling protocol was adapted to monitor water quality conditions within littoral zones and inform lake swimmers in a timely and cost-effective manner. It needs to be noted that DFe measurements in this study are not representative of an entire lake or open waters. Physical parameters (surface water temperature, dissolved oxygen pH, and turbidity) were also measured during weekly sampling at the littoral zone. Surface water temperature and dissolved oxygen were measured by a YSI 55 (temperature resolution 0.1°C, accuracy ± 0.2°C; air saturation resolution 0.01 mg L^{-1}, accuracy ± 0.3 mg L^{-1}), pH was measured using an Oakton pH meter (mV resolution 0.01 to 1, with an accuracy of ± 2%), and a Hach P2100 measured turbidity (resolution at 0.01 FNU, with accuracy of ± 2%). All meters were calibrated on a weekly basis, one day prior to sample collection. Physical characteristics are available from the IDNR at https://programs.iowadnr.gov/aquia.

Dissolved Fe Analysis

Prior to sample collection, 15 mL polypropylene tubes were acid washed in 10% HCl (trace metal grade) overnight and rinsed three times with deionized water (> 18.0 MΩ·cm^{-1}). However, single-use nylon syringe filters (0.22 µm) did not undergo this cleaning procedure. To address the possibility of contamination from syringe filters, field blanks were also passed through nylon syringe filters. Furthermore, field duplicates (10%) were used to assess for variability and contamination.

Water samples were filtered (0.22 µm, nylon membrane) into 15 mL conical tubes and acidified with 2% trace metal grade nitric acid to pH < 2. Total DFe was analyzed by ICP-OES at the Iowa State University Environmental Engineering Research Laboratory (Shimadzu ICP-ES9800, detection limit of 0.2 µg L^{-1}, and quantification limit of 0.5 µg L^{-1}). For quality control and assurance, lake water samples were analyzed in duplicate to ensure readings were below 3% relative standard deviation. Blanks, calibration standard checks, and lab duplicates were measured every tenth sample. Dissolved Fe data collected in this study are accessible from Environmental Data Initiative (https://doi.org/10.6073/pasta/642c014e2af89958e42860c6712912fd).

Phytoplankton Data

A multi-wavelength Pulse Amplitude Modulated (PAM) II fluorometer (Phyto-PAM II; Heinz Walz GmbH, Effeltrich, Germany) measured *in situ* chl-*a* fluorescence to monitor biomass and identify the phytoplankton community structure in seven of BM lakes, weekly. The Phyto-PAM II uses five light-emitting diodes (440, 480, 540, 590, and 625 nm) to distinguish chl-*a* and auxiliary pigments. Community composition is characterized based on different accessory pigment assemblages, which are identified based on their absorption and fluorescence at specific regions of the visible spectrum. For instance, the pigment phycocyanin (PC) in cyanobacteria is excited by red light and not by blue light, whereas fucoxanthin and carotenoids in brown algae are excited by blue and green light. In order to classify phytoplankton taxa, measured spectrum of a water sample is deconvoluted into several components (via linear-unmixing) based on the reference fluorescence spectra of each group, or "fingerprints" (MacIntyre et. al 2010). This study utilized the references provided by the manufacturer, Heinz Walz GmbH, Germany. To obtain biomass and community composition data, chlorophyll fluorescence was measured immediately upon obtaining the composite subsample. Samples were measured in triplicate to ensure reproducibility. Approximately 4 mL of grab sample was loaded into the glass cuvette. The gain was adjusted to the fluorescence given off by the sample. A zero offset was performed on 0.22 µm-filtered lake water to eliminate fluorescence signal from natural organic matter. Biomass (indicated by chl-*a*) are reported as averages of the triplicate measurements. Chl-*a* data collected in this study are accessible from Environmental Data Initiative (https://doi.org/10.6073/pasta/e17b351933e981f369a058076f14c328).

At least 20% of chl-*a* fluorescence measurements were validated by chl-*a* extraction and spectrophotometric quantification. After chl-*a* fluorescence was measured, between 20 to 200 mL (depending on algal density) of the composite samples were collected onto 0.3 µm glass fiber filters (ADVANTEC, Japan) and frozen at -20°C until extraction. Three freeze-thaw cycles were applied to filters prior to being steeped in 15 mL of 90% acetone at -20°C overnight. Samples were then centrifuged at 8,000 rpm for 20 minutes. Next, 2 mL of supernatant was transferred into a quartz cuvette and absorbance was measured at 750 nm to ensure that the optical density was < 0.005. Supernatant with an optical density > 0.005 was passed through a 0.45 µm glass fiber filter (not often needed in this study). In order to determine chl-*a* concentrations, absorbance was measured at 664 nm (Genesys 30, ThermoScientific) and chl-*a* was quantified using an extinction coefficient with correction for phaeophytin at 665 nm after adding 60 µL of 0.1 N hydrochloric acid to the sample (Lorenzen 1967).

Dissolved Organic Carbon Analysis

Prior to sample collection for DOC analysis (defined as any carbon passing through a 0.45 µm filter) from BM lakes, amber glass vials and caps were acid-washed with 10% HCl overnight and rinsed with deionized water threefold (> 18.0 $M\Omega \cdot cm^{-1}$). Next, vials, caps, and glass fiber filters (Whatman, 0.45 µm) were wrapped in aluminum foil and pre-combusted at 400°C for at least 1 hour.

Water samples were collected from a subset of 30 lakes and filtered (0.22 µm, glass fiber membrane) into 25 mL acid-washed vials and acidified with phosphoric acid until pH < 2. Dissolved organic carbon was analyzed by persulfate digestion at the Iowa State University Environmental Engineering Research Laboratory (Shimadzu TOC).

Statistical Analysis

All statistical analyses were conducted in R (version 4.0.2; R Development Core Team). The Shapiro-Wilk test was used to assess for normality prior to analysis, which produced a p-value < 0.05. Therefore, normal distributions were achieved after applying a log transformation.

To determine whether DFe was different between artificial versus natural lakes, the Kruskal-Wallis test was applied to the ALM dataset. K-means hierarchical clustering was applied to the BM dataset to identify and group lakes with similar DFe patterns over the 15-week period. The optimal number of groups was determined by the elbow method. To further investigate variability in DFe, the effects of other variables (pH, temperature, turbidity, dissolved oxygen, and DOC) on DFe were determined by linear mixed modeling (R package "lme4"; Bates et al. 2015). Linear regression was used to determine whether chl-*a* also influenced DFe availability.

Results

Physical Parameters

Surface waters exhibited a temporal variability over the summer season. Surface water temperature and pH in BM lakes increased from May into June, with peak values in July (27°C and pH 8.49, respectively; Table 1). Surface water temperature dropped in August while pH increased slightly (Figure 3). Brushy Creek held the lowest temperature at 17°C in May and warmest surface water temperature was observed at Lake of Three Fires in July (33°C). Both minimum and maximum pH were observed in July, with the lowest pH in Lake Macbride in July (7.05) and peak pH at Lake Keomah (10.1).

Similarly, dissolved oxygen and turbidity in surface waters also varied spatially and from May through September (Table 1). Dissolved oxygen levels ranged between 0.7 to 19.9 mg L^{-1} and decreased from May to August (Supplemental Material Figure 1). Turbidity ranged from 0.8 to 84.0 FNU and increased from May to August, with higher levels observed at Green Valley Lake, Lake Keomah, and Union Grove Lake.

Dissolved Fe Variability

Dissolved Fe in lakes varied throughout Iowa, both spatially and temporally. Of the 124 ALM lakes, DFe concentrations in artificial lakes ranged from 5 to 1015 µg L^{-1}, with a mean at 41.3 µg L^{-1}.

Table 1. Temperature, pH, dissolved oxygen (DO), dissolved organic carbon (DOC), and turbidity measured in BM lakes (n = 30). The minimum and maximum values are in parenthesis. Surface water temperature, pH, DOC, and turbidity increased while DO declined throughout the summer season.

	May	June	July	August
Temperature (°C)	22 (17-27)	25 (18-31)	27 (23-33)	25 (18-31)
pH	8.36 (8-8.7)	8.3 (7.27-9.13)	8.49 (7.05-10.1)	8.53 (7.4-9.9)
DO (mg L^{-1})	9.9 (5.9-15.5)	8.0 (1.3-17.5)	8.9 (3.8-34.0)	7.5 (0.7-15.4)
DOC (mg L^{-1})	5.9 (2.8-8.3)	5.8 (1.5-25.4)	6.7 (1.5-39.1)	7.5 (1.5-38.1)
Turbidity (FNU)	8.5 (1.0-20.0)	10.5 (0.8-70.0)	15.2 (0.8-84.0)	16.7 (0.8-82.3)

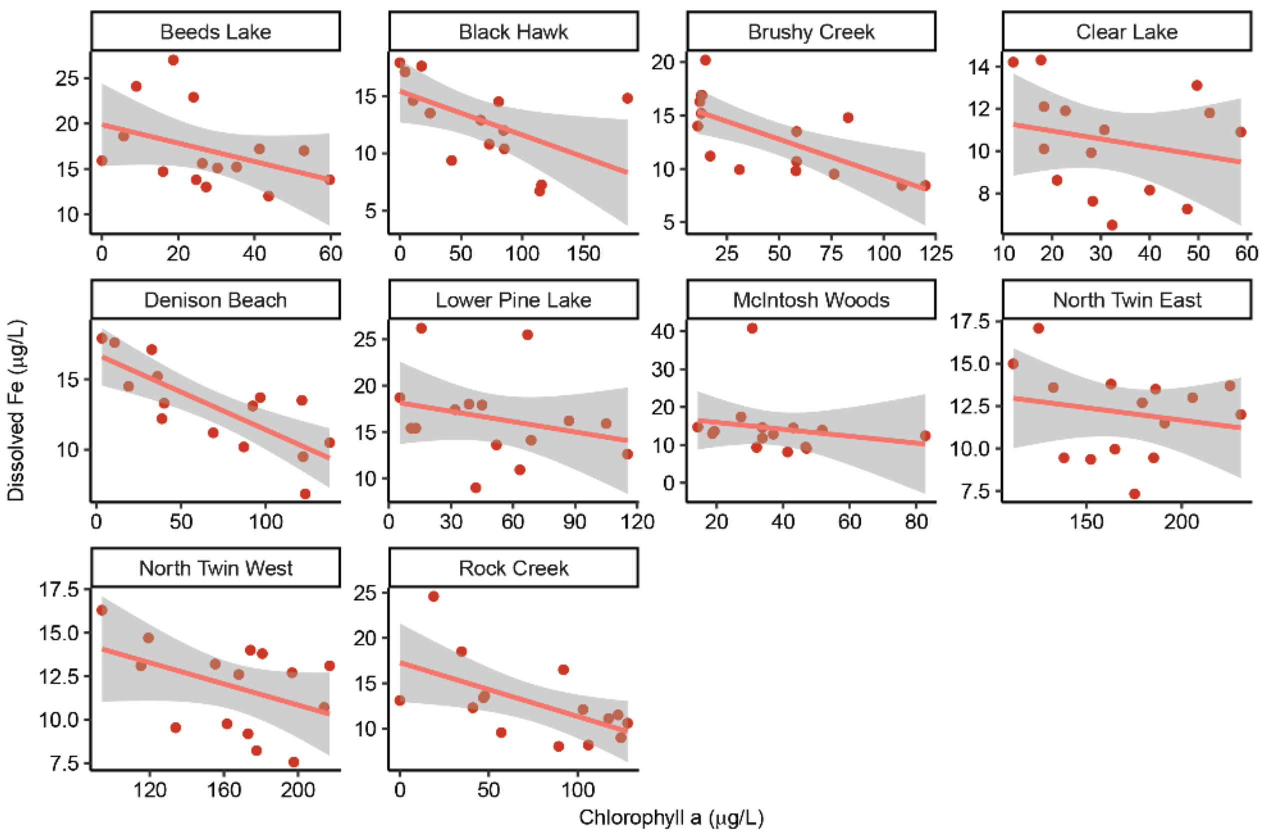


Figure 3. Linear regression of chlorophyll (measured by Phyto-PAM II) and DFe. The decline in DFe coincided with increased chl-*a* in some lakes. Measurements are representative of surface waters in the littoral zone, collected from seven BM lakes. Three lakes have multiple monitoring sites: Clear Lake-McIntosh Woods, North Twin Lake East-West, and Black Hawk-Denison.

Approximately 75% were below 40 µg L^{-1}, with 50% below 24 µg L^{-1} (Figure 1). Dissolved Fe in natural lakes ranged from 5 to 900 µg L^{-1}, with a mean at 46 µg L^{-1} (median DFe at 22 µg L^{-1}). Minimum DFe was observed at Greenfield Lake during August (5 µg L^{-1}) and the maximum DFe was at Bob White Lake during July (1015 µg L^{-1}), with both lakes located in southern Iowa. In fact, several lakes exhibiting DFe > 100 µg L^{-1} were in southern Iowa (Figure 1).

Weekly monitoring of the BM lakes revealed temporal variations in DFe, with elevated DFe in May (mean at 18.5 µg L^{-1}), declining DFe into June (13.2 µg L^{-1}) and July (9.3 µg L^{-1}), then rebounding DFe in August (11.2 µg L^{-1}; Figure 3). Each lake exhibited a similar concave trend even though the steepness of decline in June and July varied from lake to lake.

Phytoplankton Biomass

Total chl-*a* derived from PhytoPAM II varied across lakes in this study and ranged from 0 to > 200 µg L^{-1}. In general, chl-*a* was initially low in May, with total chl-*a* values as low as 3 µg L^{-1} at Black Hawk Lake. Chl-*a* continued to increase throughout the summer, with peak levels in August and September. Chl-*a* values were notably high in late summer, especially at North Twin Lake where values exceeded 200 µg L^{-1} (Figure 3).

Dissolved Organic Carbon Variability

Temporal trends in DOC were assessed for BM lakes. Both maximum and minimum values were observed in July, but at different sites. Maximum DOC was observed at Clear Lake (39 mg L^{-1}) and minimum DOC at Backbone (2 mg L^{-1}). Temporal DOC trends also showed a concave trend, with lower DOC in May (mean of 5.9 mg L^{-1}) and increased DOC into August (Supplemental Material Figure 2).

Statistical Analysis

The BM lakes were categorized into four groups based on their DFe trends over the summer season and k-means hierarchical clustering (Figure 4). The first group of BM lakes (Pink group) had only a slight variation of DFe with time compared to the other three groups (Figure 5). The second and third groups (Green and Orange groups) displayed a gradual decline in DFe during June and July, while the fourth group (Blue group) showed a sharp decline in DFe during June and July.

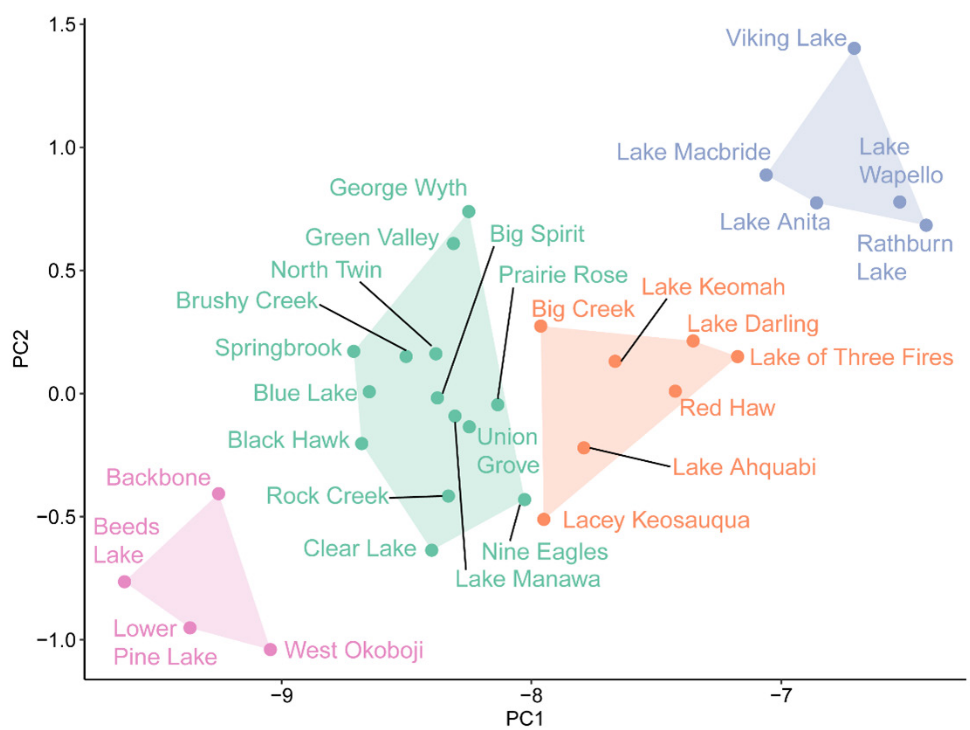


Figure 4. BM lakes were classified into four main groups based on DFe patterns over the summer (via k-means hierarchical clustering); Group 1 (pink), Group 2 (green), Group 3 (orange), and Group 4 (blue).

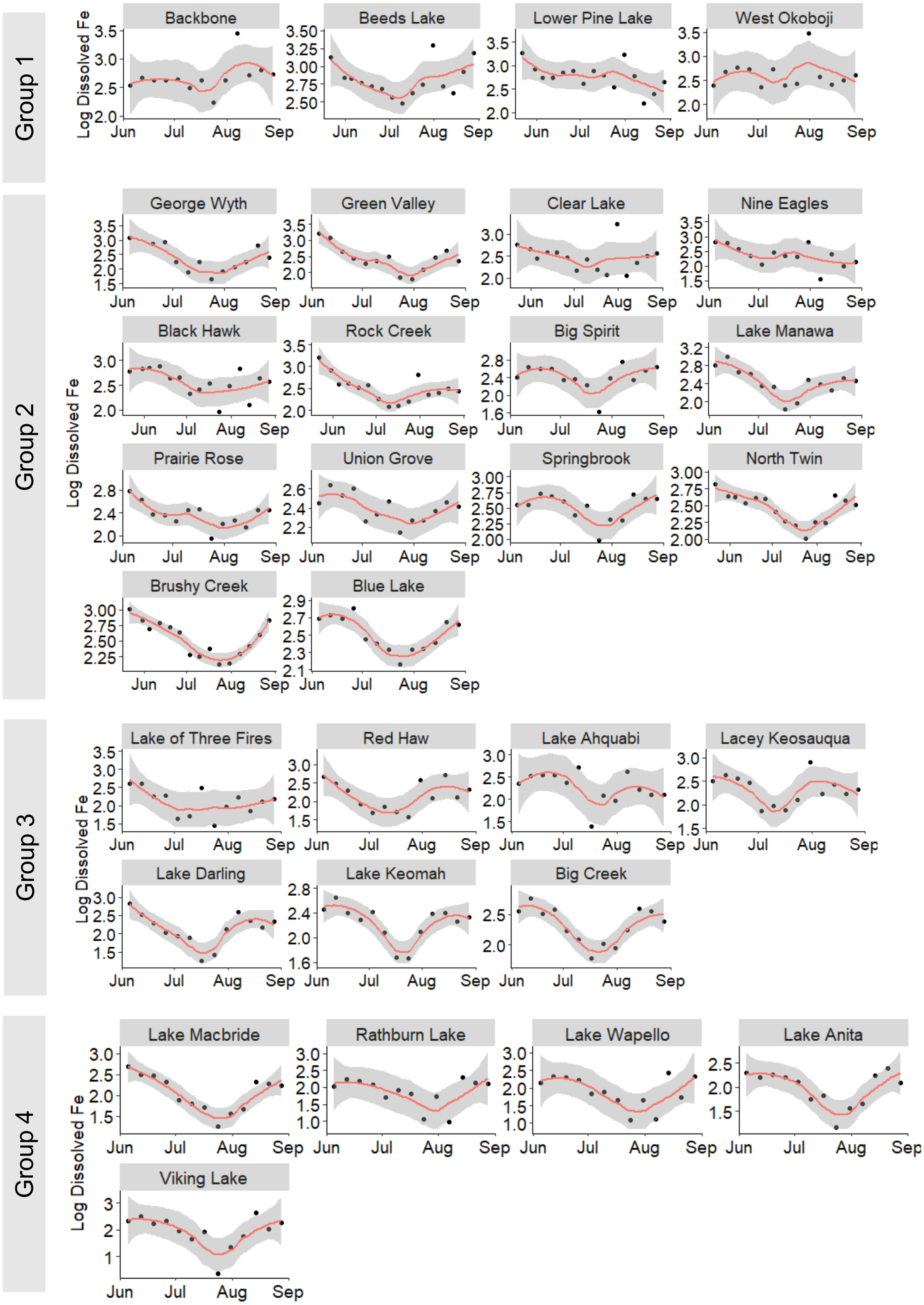


Figure 5. BM lakes are grouped into four categories (via k-means hierarchical clustering) based on DFe trends over time. In reference to Figure 4, Group 1 is the pink group, Group 2 is the green group, Group 3 is the orange group, and Group 4 is the blue group as defined in Figure 4.

To determine the effects of other variables on DFe, linear mixed modeling was applied to each group identified by k-means hierarchical clustering. Temperature was the main variable associated with DFe in all groups, while DOC was only negatively associated with lakes in Group 3 (Orange group; Table 2).

Discussion

This study conducted a statewide assessment of DFe in Iowa's lakes. Although there is limited literature regarding Fe in Iowa lakes, current DFe at West Okoboji Lake falls within the lower range of those previously measured (10 to 300 µg L^{-1}; Bachmann and Jones 1974). The wide range in DFe measured in this survey is similar to other lakes in the region. For example, lakes in Wisconsin also demonstrate a wide range, with mean Fe values from 10 to 400 µg L^{-1} (Magnuson et al. 2019).

Factors such as regional geology, watershed characteristics, and biological productivity, as well as mixing regime, influence the amount of DFe in freshwater systems (Gorham et al. 1983; Davison 1993; Nürnberg 1995; Dillon and Molot 2005; Leung et al. 2021). Dissolved Fe from ALM lakes was used to probe for spatial variability across lakes. Dissolved Fe was not significantly different between artificial and natural lakes ($p > 0.05$). In this study, lake max depth in artificial lakes (ranged from 3 to 33 m) was similar to those of natural lakes (5 to 40 m). Several lakes with DFe exceeding 100 µg L^{-1} are in southern Iowa, where regional bedrock is primarily Pennsylvanian aged (Figure 1). However, other lakes within the same region have DFe concentrations below 50 µg L^{-1}, indicating variables other than regional geology likely also contribute to DFe variability.

Dissolved Fe from BM lakes was used to assess for temporal variability, and DFe concentrations varied from May to August, with declining DFe in June and July and rebounding DFe in August (Figure 5). BM lakes were categorized into four groups based on the concave trend. It is unlikely that residence time affected the temporal variability in these lakes. In a prior study, there was a slight difference in the magnitude of DFe between individual monitoring sites on West Okoboji, although all sites displayed a similar temporal pattern (Leung et al.2021). The spatial variability in DFe within a lake is also seen in BM lakes with multiple monitoring sites (Black Hawk/Denison, Clear Lake/McIntosh Woods, and North Twin Lake East/West; Figure 5). The magnitude of DFe in Black Hawk Lake ranged from 1 to 3 µg L^{-1}, while the magnitude ranged up to 10 µg L^{-1} in West Okoboji (monitoring sites include Gull Point, Triboji Beach, and Emmerson Bay). Therefore, DFe variation within lakes suggests an alternative mechanism for DFe temporal trends.

Alternative mechanisms for temporal patterns in DFe could be Fe cycling within the lake due to biological uptake, decomposition, and complexation. Additionally, precipitation of Fe oxides and hydroxides, their deposition to the sediment, and reductive dissolution under anoxic conditions can also influence temporal DFe trends.

To explore whether temporal changes in DFe were due to biological uptake, a subset of BM lakes (n = 7) were monitored for chl-*a* (a proxy

Table 2. Based on the linear mixed model, temperature is potentially associated with DFe trends in Iowa's lakes. The table shows coefficient and standard error (SE) for each grouping identified by k-means hierarchical clustering. *Note: asterisk (*) denotes significance, with * indicating p-value < 0.05; ** indicating p-value < 0.01; *** indicating p-value < 0.001.

	Group 1		**Group 2**		**Group 3**		**Group 4**	
	Coefficient	**SE**	**Coefficient**	**SE**	**Coefficient**	**SE**	**Coefficient**	**SE**
Intercept	13.92	16.88	37.54	5.06	15.82	4.51	12.84	9.12
pH	0.94	2.04	-1.36	0.61*	1.37	0.53	0.61	0.93
Temperature	-0.33	0.31	-0.56	0.10***	-0.55	0.09**	-0.33	0.16*
DO	0.19	0.25	-0.14	0.09	0.04	0.09	-0.24	0.13
DOC	-0.05	0.13	-0.02	0.07	-0.20	0.05**	0.04	0.07
Turbidity	0.05	0.06	-0.04	0.02*	-0.05	0.01	0.02	0.03

for phytoplankton biomass). At Black Hawk and Brushy Creek, chl-*a* exhibited negative correlation with DFe ($r < 0.7$; Figure 3), suggesting that DFe availability may have enhanced growth in those lakes. The rebounding DFe in some lakes (e.g., Black Hawk, Brushy Creek) in August coincided with a decrease in chl-*a*. This potentially hints at an internal cycling of Fe from decomposition of phytoplankton biomass as a mechanism driving the rebounding in these lakes. However, this was not always the case for the other five lakes. The inverse correlation between DFe and phytoplankton is similar to lakes in Alberta, Canada, where Du et al. (2019) found that phytoplankton utilize Fe-binding ligands to acquire Fe.

Based on the linear mixed model, there is a negative correlation between temperature and DFe in the majority of lakes belonging to Groups 2 and 4 (Table 2). This relationship was more obvious in Brushy Creek, Blue Lake, and North Twin; the change in DFe coincided with warmer surface water temperatures. Although dissolved oxygen was not profiled in this study, it is also plausible that the increase in DFe during warmer temperatures may have led to Fe remobilization from anoxic sediments under a stronger stratification (Davison 1993; Saeed et al. 2018). Iron could be transported into the hypolimnion more efficiently at strongly thermally stratified lakes (Loh et al. 2013). As no data on stratification or mixing were collected in this study, we cannot further evaluate this hypothesis.

The presence of DOC in BM lakes potentially increases the solubility of DFe for biological uptake. In freshwater systems, DFe can bind with DOC to form complex ligands (Kikuchi et al. 2017; Qiu et al. 2020) and siderophores (Wilhelm and Trick 1994) . A positive correlation between Fe and DOC has been noted in lakes from Minnesota, Wisconsin, and Michigan (Björnerås et al. 2017; Lebret et al. 2018; Brezonik et al. 2019). This is generally attributable to allochthonous organic carbon. However, DOC was poorly correlated with DFe in this study, which may hint at the dominance of autochthonous DOC rather than allochthonous organic carbon. Dissolved organic carbon was a secondary factor for lakes categorized to Group 3 but it was not an important variable in Groups 1, 2, and 4. For group 3, the correlations of both temperature and DOC with DFe may suggest DFe assimilation by phytoplankton.

Conclusion

Biogeochemical cycling of Fe in aquatic systems regulates the global carbon cycle through its role as an important micronutrient for phytoplankton primary productivity (Smetacek et al. 2012). Furthermore, Fe bioavailability in aquatic systems may have far-reaching implications for ecosystem and public health. The survey of lake DFe conducted in this study serves as baseline for DFe concentrations in Iowa. Weekly monitoring revealed a concave DFe trend throughout the summer. While some lakes exhibit a correlation between DFe and chl-*a*, it is necessary to tease apart other factors that affect DFe in order to understand the mechanisms driving Fe variability in Iowa's lakes. Future work may distinguish the effects of regional bedrock from catchment-specific sources of Fe (e.g., wetlands and farmland soils) on Fe mobilization to lakes. A long-term survey of Fe in lakes would aid in developing an Fe budget for lakes, and provide insight into the biogeochemical cycle of Fe in lakes.

Acknowledgements

This research is partially funded as 2016IA267B ("Determining the effects of co-nutrient cycling on harmful phytoplankton blooms across water-bodies") under the provisions of section 104 of the Water Resources Research Act of 1984 annual base grants 104(b) distributed through the Iowa Water Center. This work was partially supported by the U.S. Environmental Protection Agency [EPA-G2017-STAR-A1]. Special thanks is given to Iowa Department of Natural Resources (Dan Kendall and Amy Buckendahl) for developing field sampling plans and to Kaoru Ikuma and Xuewei Liang for measuring dissolved organic carbon. We also thank Micah Fatka, Zackry Stevenson, Gabrielle Ledesma, and Kaleigh Block for their input in this manuscript. We are especially grateful to Grace Wilkinson, Yuyu Zhou, and Audrey McCombs for providing insight into data analysis.

Author Bio and Contact Information

Dr. Tania Leung completed her Ph.D. in Environmental Science and Geology at Iowa State University in 2021. Her research aims to adapt chlorophyll fluorescence to extensively monitor phytoplankton blooms across a suite of lakes. This allows her to explore the interaction between iron cycling and phytoplankton communities. She is currently a Research Associate at the University of California San Diego. She may be contacted at tleung@iastate.edu or by mail at Department of Geological & Atmospheric Sciences, Iowa State University, 2237 Osborn Drive, Ames, IA 50011, USA.

Dr. Elizabeth D. Swanner (corresponding author) is a biogeochemist investigating the geochemical imprint of microbial life through geological time. Her research studies microbial metabolisms and the behavior of metals and minerals under varying aqueous redox regimes. A major focus of her work aims to understand impacts of ferruginous conditions on regulating microbial activity and biogeochemical cycling. She finished her Ph.D. in Geology from the University of Colorado, Boulder (2011). She was an NSF postdoctoral scholar (2011-2013) and a Carl Zeiss Stiftung Postdoctoral Fellow (2014-2015) at the Center for Applied Geosciences, University of Tübingen, Germany. She joined Iowa State University in 2015. She may be contacted at eswanner@iastate.edu or by mail at Department of Geological & Atmospheric Sciences, Iowa State University, 2237 Osborn Drive, Ames, IA 50011, USA

References

Azzoni, R., G. Giordani, and P. Viaroli. 2005. Iron–sulphur–phosphorus interactions: Implications for sediment buffering capacity in a Mediterranean eutrophic lagoon (Sacca Di Goro, Italy). *Hydrobiologia* 550(1): 131-148. Available at: https://doi.org/10.1007/S10750-005-4369-X. Accessed March 15, 2023.

Bachmann, R.W. and J.R. Jones. 1974. *Water Quality in the Iowa Great Lakes: A Report to the Iowa Great Lakes Water Quality Control Plan*. Iowa State University, Ames, Iowa.

Bates, D., M. Mächler, B.M. Bolker, and S.C. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67(1): 1-48. Available at: https://doi.org/10.18637/jss.v067.i01. Accessed March 15, 2023.

Björnerås, C., G.A. Weyhenmeyer, C.D. Evans, M.O. Gessner, H.P. Grossart, K. Kangur, I. Kokorite, et al. 2017. Widespread increases in iron concentration in European and North American freshwaters. *Global Biogeochemical Cycles* 31(10): 1488-1500. Available at: https://doi.org/10.1002/2017GB005749. Accessed March 15, 2023.

Brezonik, P.L., J.C. Finlay, C.G. Griffin, W.A. Arnold, E.H. Boardman, N. Germolus, R.M. Hozalski, and L.G. Olmanson. 2019. Iron influence on dissolved color in lakes of the Upper Great Lakes States. *PLoS ONE* 14(2): e0211979. Available at: https://doi.org/10.1371/journal.pone.0211979. Accessed March 15, 2023.

Browning, T.J., E.P. Achterberg, J.C. Yong, I. Rapp, C. Utermann, A. Engel, and C.M. Moore. 2017. Iron limitation of microbial phosphorus acquisition in the tropical North Atlantic. *Nature Communications* 8(1): 1-7. Available at: https://doi.org/10.1038/ncomms15465. Accessed March 15, 2023.

Campbell, D., V. Hurry, A.K. Clarke, P. Gustafsson, and G. Öquist. 1998. Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. *Microbiology and Molecular Biology Reviews* 62(3): 667-683. Available at: http://mmbr.asm.org/content/62/3/667.abstract. Accessed March 15, 2023.

Davison, W. 1993. Iron and manganese in lakes. *Earth-Science Reviews* 34(2): 119-163. Available at: https://doi.org/10.1016/0012-8252(93)90029-7. Accessed March 15, 2023.

Dillon, P.J. and L.A. Molot. 2005. Long-term trends in catchment export and lake retention of dissolved organic carbon, dissolved organic nitrogen, total iron, and total phosphorus: The Dorset, Ontario, study, 1978–1998. *Journal of Geophysical Research- Biogeosciences* 110(G1): 1-7. Available at: https://doi.org/10.1029/2004jg000003. Accessed March 15, 2023.

Du, X.L., I.F. Creed, R.J. Sorichetti, and C.G. Trick. 2019. Cyanobacteria biomass in shallow eutrophic lakes is linked to the presence of iron-binding ligands. *Canadian Journal of Fisheries and Aquatic Sciences* 76(10): 1-12. Available at: https://doi.org/10.1139/cjfas-2018-0261. Accessed March 15, 2023.

Gorham, E., W.E. Dean, and J.E. Sanger. 1983. The chemical composition of lakes in the north-central United States. *Limnology and Oceanography* 28(2): 287-301. Available at: https://doi.org/10.4319/lo.1983.28.2.0287. Accessed March 15, 2023.

Hassler, C.S., S.M. Havens, G.S. Bullerjahn, R.M.L. McKay, and M.R. Twiss. 2009. An evaluation of iron bioavailability and speciation in western Lake Superior with the use of combined physical, chemical, and biological assessment. *Limnology and Oceanography* 54(3): 987-1001. Available at: https://doi.org/10.4319/lo.2009.54.3.0987. Accessed March 15, 2023.

Hoffman, A.R., D.E. Armstrong, and R.C. Lathrop. 2013. Influence of phosphorus scavenging by iron in contrasting dimictic lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 70(7): 941-952. Available at: https://doi.org/10.1139/CJFAS-2012-0391. Accessed March 15, 2023.

Iowa Department of Natural Resources. 2018. Water Quality Monitoring and Assessment- AQuIA. 2018. Available at: https://programs.iowadnr.gov/aquia/. Accessed March 15, 2023.

Kikuchi, T., M. Fujii, K. Terao, R. Jiwei, Y.P. Lee, and C. Yoshimura. 2017. Correlations between aromaticity of dissolved organic matter and trace metal concentrations in natural and effluent waters: A case study in the Sagami River Basin, Japan. *Science of the Total Environment* 576: 36-45. Available at: https://doi.org/10.1016/j.scitotenv.2016.10.068. Accessed March 15, 2023.

Klein, D.H. 1975. Fluxes, residence times, and sources of some elements to Lake Michigan. *Water, Air, and Soil Pollution* 4(1): 3-8. Available at: https://doi.org/10.1007/BF01794127. Accessed March 15, 2023.

Kranzler, C., H. Lis, Y. Shaked, and N. Keren. 2011. The role of reduction in iron uptake processes in a unicellular, planktonic cyanobacterium. *Environmental Microbiology* 13(11): 2990-2999. Available at: https://doi.org/10.1111/j.1462-2920.2011.02572.x. Accessed March 15, 2023.

Larson, C.A., B. Mirza, J.L. Mazza Rodrigues, and S.I. Passy. 2018. Iron limitation effects on nitrogen-fixing organisms with possible implications for cyanobacterial blooms. *FEMS Microbiology Ecology* 94(5). Available at: https://doi.org/10.1093/femsec/fiy046. Accessed March 15, 2023.

Lebret, K., Ö. Östman, S. Langenheder, S. Drakare, F. Guillemette, and E.S. Lindström. 2018. High abundances of the nuisance raphidophyte *Gonyostomum Semen* in brown water lakes are associated with high concentrations of iron. *Scientific Reports* 8(1): 13463. Available at: https://doi.org/10.1038/s41598-018-31892-7. Accessed March 15, 2023.

Leung, T., G.M. Wilkinson, and E.D. Swanner. 2021. Iron availability allows sustained cyanobacterial blooms: A dual-lake case study. *Inland Waters* 11(3): 417-429. Available at: https://doi.org/10.1080/20442041.2021.1904762. Accessed March 15, 2023.

Liu, X. and F.J Millero. 2002. The solubility of iron in seawater. *Marine Chemistry* 77(1): 43-54. Available at: https://doi.org/10.1016/S0304-4203(01)00074-3. Accessed March 15, 2023.

Loh, P.S., L.A. Molot, G.K. Nürnberg, S.B. Watson, and B. Ginn. 2013. Evaluating relationships between sediment chemistry and anoxic phosphorus and iron release across three different water bodies. *Inland Waters* 3:105-18. Available at: https://doi.org/10.5268/IW-3.1.533. Accessed March 15, 2023.

Lorenzen, C.J. 1967. Determination of chlorophyll and pheo-pigments: Spectrophotometric equations. *Limnology and Oceanography* 12(2): 343-346. Available at: https://doi.org/10.4319/lo.1967.12.2.0343. Accessed March 15, 2023.

MacIntyre, H.L., E. Lawrenz, and T.L. Richardson. 2010. Taxonomic discrimination of phytoplankton by spectral fluorescence. In: *Chlorophyll a Fluorescence in Aquatic Sciences: Methods and Applications.* Springer, Dordrecht, Netherlands, pp. 129-169. Available at: https://doi.org/10.1007/978-90-481-9268-7_7. Accessed March 16, 2023.

Magnuson, J., S. Carpenter, and E. Stanley. 2019. Chemical Limnology of Primary Study Lakes: Major Ions 1981 - Current Ver 34. Environmental Data Initiative. Available at: https://portal.edirepository.org/nis/mapbrowse?packageid=knb-lter-bes.3210.110. Accessed March 16, 2023.

McKay, R.M.L., G.S. Bullerjahn, D. Porta, E.T. Brown, R.M. Sherrell, T.M. Smutka, R.W. Sterner, M.R. Twiss, and S.W. Wilhelm. 2004. Consideration of the bioavailability of iron in the North American Great Lakes: Development of novel approaches toward understanding iron biogeochemistry. *Aquatic Ecosystem Health and Management* 7(4): 475-490. Available at: https://doi.org/10.1080/14634980490513364. Accessed March 16, 2023.

Morel, F.M.M., A.B. Kustka, and Y. Shaked. 2008. The role of unchelated Fe in the iron nutrition of phytoplankton. *Limnology and Oceanography* 53(1): 400-404. Available at: https://doi.org/10.4319/lo.2008.53.1.0400. Accessed March 16, 2023.

Nagai, T., A. Imai, K. Matsushige, K. Yokoi, and T. Fukushima. 2007. Dissolved iron and its speciation in a shallow eutrophic lake and its inflowing rivers. *Water Research* 41(4): 775-784. Available at: https://doi.org/10.1016/j.watres.2006.10.038. Accessed March 16, 2023.

North, R.L., S.J. Guildford, R.E.H. Smith, S.M. Havens, and M.R. Twiss. 2007. Evidence for phosphorus, nitrogen, and iron colimitation of phytoplankton communities in Lake Erie. *Limnology and Oceanography* 52(1): 315-328. Available at: https://doi.org/10.4319/lo.2007.52.1.0315. Accessed March 16, 2023.

Nürnberg, G.K. 1995. Quantifying anoxia in lakes. *Limnology and Oceanography* 40(6): 1100-1111. Available at: https://doi.org/10.4319/lo.1995.40.6.1100. Accessed March 16, 2023.

Qiu, Y., Z. Wang, Z. Huang, F. Liu, D. Tang, and J. Liu. 2020. Effect of four kinds of complexing iron on the process of iron uptake by *Anabaena flos-aquae. Microbiology* 166(4): 359-366. Available at: https://doi.org/10.1099/mic.0.000891. Accessed March 16, 2023.

Saeed, H., A. Hartland, N.J. Lehto, M. Baalousha, M. Sikder, D. Sandwell, M. Mucalo, and D.P. Hamilton. 2018. Regulation of phosphorus bioavailability by iron nanoparticles in a monomictic lake. *Scientific Reports* 8: 17736. Available at: https://doi.org/10.1038/s41598-018-36103-x. Accessed March 16, 2023.

Smetacek, V., C. Klaas, V.H. Strass, P. Assmy, M. Montresor, B. Cisewski, N. Savoye, et al. 2012. Deep carbon export from a southern ocean iron-fertilized diatom bloom. *Nature* 487: 313-319. Available at: https://doi.org/10.1038/nature11229. Accessed March 16, 2023.

Twiss, M.R., J-C. Auclair, and M.N. Charlton. 2000. An investigation into iron-stimulated phytoplankton productivity in epipelagic Lake Erie during thermal stratification using trace metal clean techniques. *Canadian Journal of Fisheries and Aquatic Sciences* 57(1): 86-95. Available at: https://doi.org/10.1139/f99-189. Accessed March 16, 2023.

Vassiliev, I.R., Z. Kolber, K.D. Wyman, D. Mauzerall, V.K. Shukla, and P.G. Falkowski. 1995. Effects of iron limitation on photosystem II composition and light utilization in dunaliella tertiolecta. *Plant Physiology* 109(3): 963-972. Available at: https://doi.org/10.1104/pp.109.3.963. Accessed March 16, 2023.

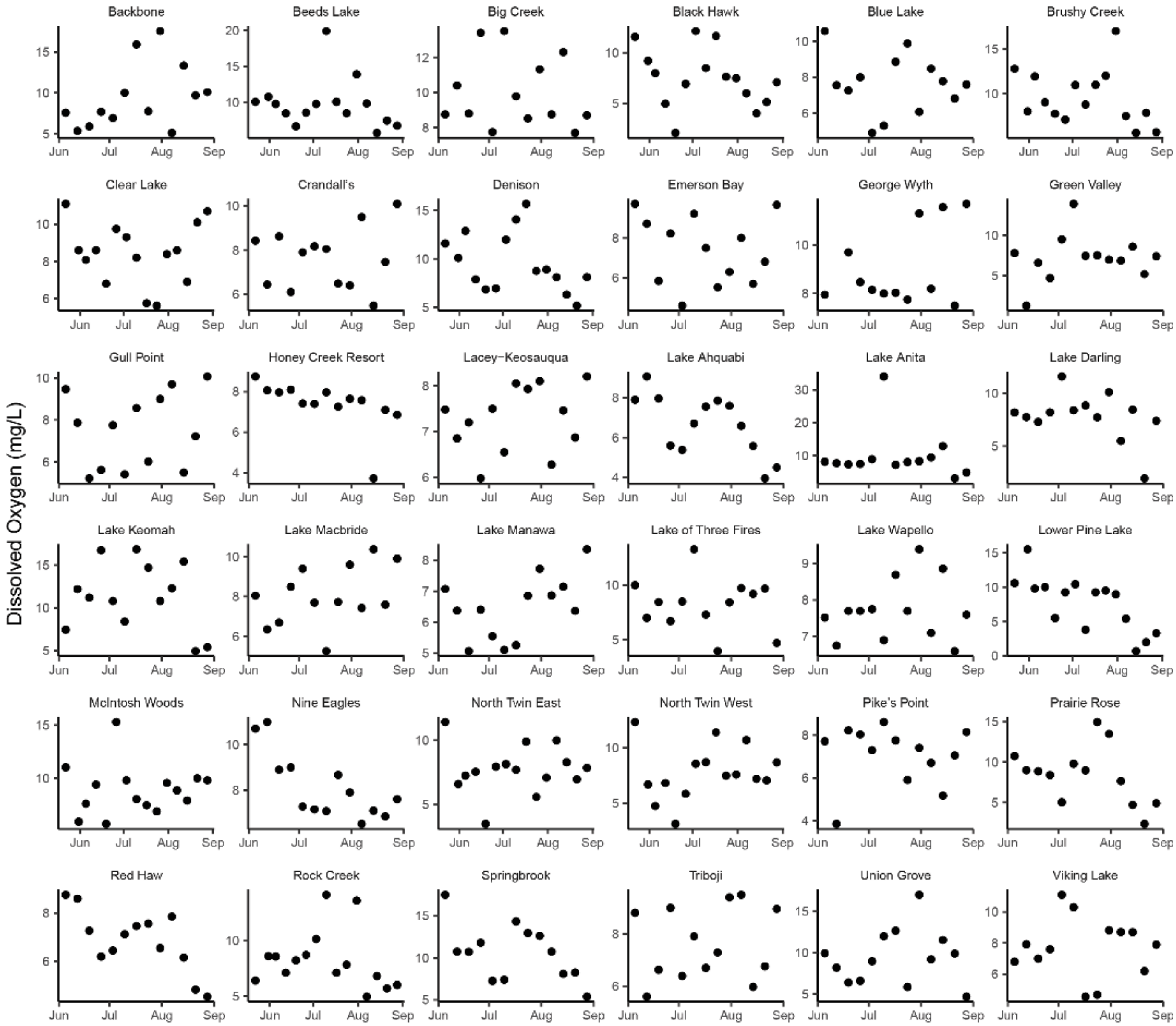
Wells, M.L., N.M. Price, and K.W. Bruland. 1995. Iron chemistry in seawater and its relationship to phytoplankton: A workshop report. *Marine Chemistry* 48(2): 157-182. Available at: https://doi.org/10.1016/0304-4203(94)00055-I. Accessed March 16, 2023.

Wilhelm, S.W. and C.G. Trick. 1994. Iron-limited growth of cyanobacteria: Multiple siderophore production is a common response. *Limnology and Oceanography* 39(8): 1979-1984. Available at: https://doi.org/10.4319/lo.1994.39.8.1979. Accessed March 16, 2023.

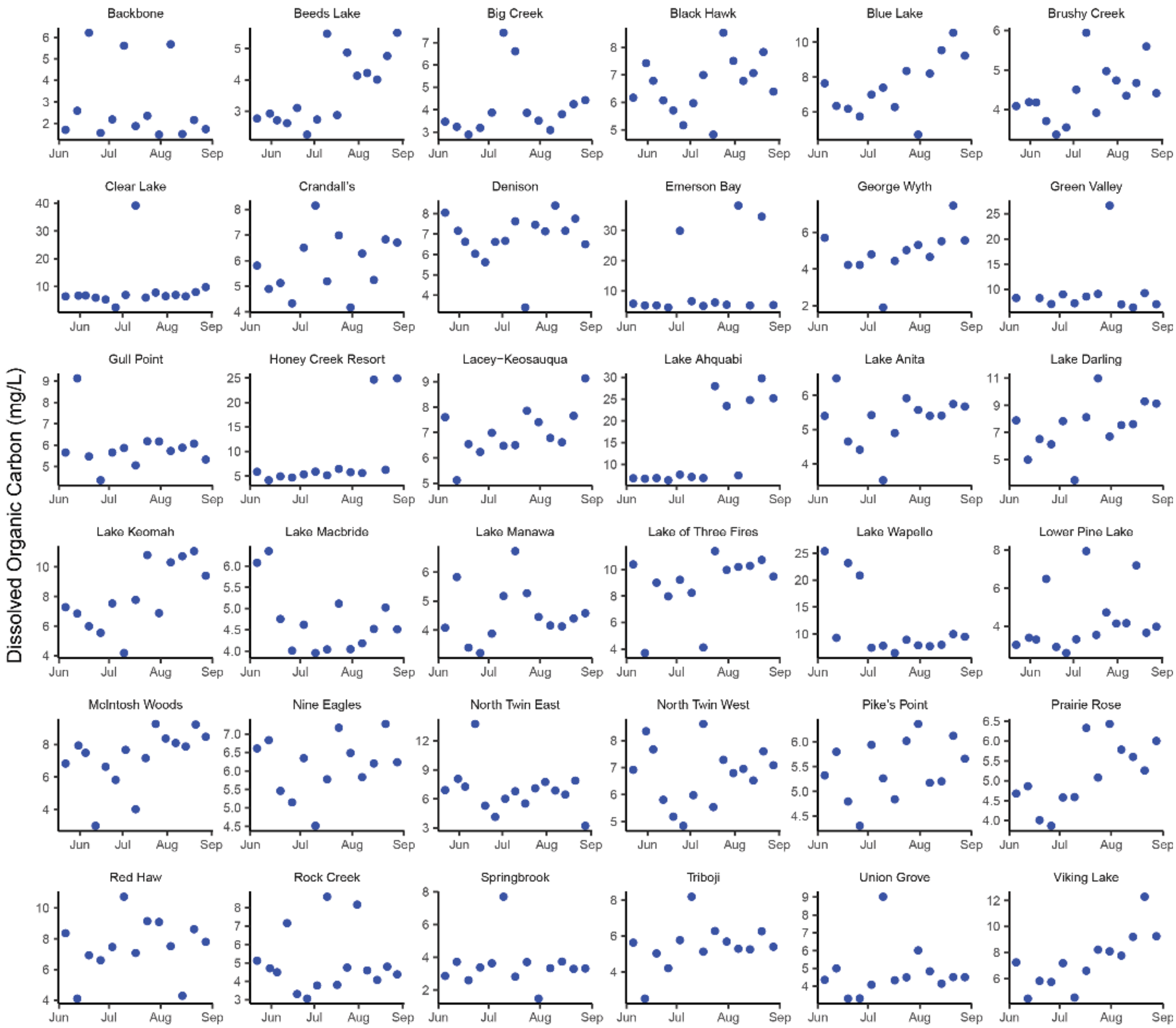
Xing, W., W-m. Huang, D-h. Li, and Y-d. Liu. 2007. Effects of iron on growth, pigment content, photosystem II efficiency, and siderophores production of *Microcystis aeruginosa* and *Microcystis wesenbergii*. *Current Microbiology* 55: 94-98. Available at: https://doi.org/10.1007/s00284-006-0470-2. Accessed March 16, 2023.

Xu, H., G. Zhu, B. Qin, and H.W. Paerl. 2013. Growth response of *Microcystis* spp. to iron enrichment in different regions of Lake Taihu, China. *Hydrobiologia* 700(1): 187-202. Available at: https://doi.org/10.1007/s10750-012-1229-3. Accessed March 16, 2023.

Supplemental Figures



Supplemental Material Figure 1. Dissolved oxygen in surface lake waters varied over the summer season.



Supplemental Material Figure 2. Dissolved organic carbon in surface lake waters varied over the summer season.

Universities Council on Water Resources
Journal of Contemporary Water Research & Education
Issue 177, Pages 17-30, April 2023

Remote-Sensing Method for Monitoring Suspended-Sediment Concentration on the Middle-Mississippi and Lower-Missouri Rivers

Megan J. Martinez[1] and *Amanda L. Cox[1,2]

[1]Civil Engineering, Saint Louis University, Saint Louis, Missouri
[2]WATER Institute, Saint Louis University, Saint Louis, Missouri
**Corresponding Author*

Abstract: Sediment transport, erosion, and deposition are primary drivers of river geomorphic processes and ecological services. Suspended-sediment concentration (SSC) is an important parameter for evaluating these processes and is accordingly of significant interest to engineers, scientists, and water resource managers. The United States Geological Survey (USGS) previously operated nine daily SSC gauging stations along the Mississippi River, with operating dates ranging from 1974 to 2018. Currently, there are no USGS gauging stations reporting daily SSC values along the Mississippi River. For this study, regression models were developed to compute the SSC along the Middle-Mississippi River (MMR) and Lower-Missouri River (LMOR) using publicly and freely available Landsat imagery. Surface reflectance data from Landsat satellites were used with USGS-measured SSC to develop regression models for three different Landsat sensors (Landsat 8 OLI/TIRS, Landsat 7 ETM+, and Landsat 4-5 TM). Previous models published for predicting SSC in the MMR and LMOR from Landsat images have a linear-regression form and have provided invalid negative values when extrapolated outside of the dataset used for development. The objectives of this study were to develop reflectance-SSC regression models using a power-function form and demonstrate their extrapolation performance using multiple novel applications in the MMR basin. The reflectance-SSC regression models were applied to the following conditions: 1) mixing at the Mississippi and Missouri River confluence, 2) point-source pollution, and 3) SSC changes along the entire MMR reach for a range of discharges. The regression models were also used to develop sediment rating curves for the four largest tributaries of the MMR.

Keywords: *suspended-sediment concentration, remote sensing, water quality, Landsat, Mississippi River*

Suspended sediments play a significant role in fluvial environments. Sediment is constantly being transported and deposited in a water system, therefore, affecting channel geomorphology and ecological services. These characteristics impact channel navigability and ecological health, and should be monitored frequently. Methods for estimating suspended-sediment concentration (SSC) in fluvial environments have evolved over several decades, from in-situ measurements to multiple surrogate methods. Laser diffraction instruments, such as the Laser In-Situ Scattering and Transmissometry (LISST), can be submerged in water to directly measure laser diffraction, and therefore indirectly measure SSC (e.g., Gray and Gartner 2010; Felix et al. 2017; Dos Santos et al. 2018). Acoustic instruments such as an acoustic Doppler current profiler (ADCP) can measure acoustic backscatter in water, which has been correlated to SSC and can therefore be converted to provide a surrogate measurement of SSC (e.g., Landers 2012; Guerrero et al. 2017).

Remote sensing can also be used as a surrogate method to monitor water quality parameters such as SSC, chlorophyll, and temperature because changes in these parameters alter the energy spectra

Research Implications

- Provides a surrogate method to monitor suspended-sediment concentration (SSC) along the Middle-Mississippi River where SSC is not currently being monitored by the United States Geological Survey (USGS).
- Reduces the need for in-situ collection of SSC, therefore reducing labor and laboratory needs.
- Provides a method that can be used to develop similar models with available historical SSC and Landsat data.

of reflected solar and/or emitting thermal radiation from surface waters (Ritchie et al. 2003; Pereira et al. 2018; Peterson et al. 2018). Remote-sensing techniques of measuring SSC use surface reflectance measured by multispectral sensors in satellites or cameras. Surface reflectance can be correlated to SSC to provide an indirect measurement of SSC by creating surface reflectance-SSC models. Publicly available remote-sensing satellite imagery, such as Landsat, can be used to obtain cost-free data for monitoring spatial and temporal trends in SSC. Remote sensing as a surrogate method for monitoring SSC is particularly valuable for the Mississippi River as the United States Geological Survey (USGS) currently does not monitor SSC at any Mississippi River gauge stations, with 2018 being the last year of record.

Pereira et al. (2018) developed an empirical relationship between surface reflectance from Landsat satellites and SSC for the Middle-Mississippi River (MMR). Three empirical SSC models were developed for the following satellites: Landsat 4-5 Thematic Mapper (TM), Landsat 7 Enhanced Thematic Mapper Plus (ETM+), and Landsat 8 Operational Land Imager (OLI)/Thermal Infrared Sensor (TIRS). The models were created to be used for further SSC studies along the MMR and its tributaries. However, when applied outside of the specific USGS gauge locations, several SSC values were predicted as negative values due to the linear form of the equations. The objectives of this study were to develop reflectance-SSC regression models using a power-function form and demonstrate their extrapolation performance using multiple novel applications in the MMR basin. The reflectance-SSC regression models were applied to the following conditions: 1) mixing at the Mississippi and Missouri River confluence, 2) point-source pollution, and 3) SSC changes along the entire MMR reach for a range of discharges. The regression models were also used to develop sediment rating curves for the four largest tributaries of the MMR.

Background

Several studies have investigated the relationship between Landsat surface reflectance and SSC (Richie et al. 1976; 1987; 1988; Topliss et al. 1990; Lathrop 1992; Mertes et al. 1993; Islam et al. 2001; Doxaran et al. 2003; Peterson et al. 2018). Ritchie et al. (1976) was one of the earliest studies to identify the optimum wavelength for quantitatively determining SSC of surface water from remote sensing. The study employed measured surface SSC data and spectrometer-measured reflected and incident solar radiation from six reservoirs in northern Mississippi, along with reflected solar radiation measurements from Landsat 1. The study found that the best spectral region to obtain a quantitative relationship between reflected solar radiation and SSC from surface water would be between 700 and 800 nm. Ritchie et al. (1987) developed multiple linear regression equations to estimate SSC in the surface water of Moon Lake from measured SSC data and reflectance measurements from Landsat Multispectral Scanner (MSS) data. Ritchie et al. (1988) tested the equations by comparing the predicted SSC with measured SSC from Moon Lake, an old oxbow lake off the Mississippi River in northwest Mississippi. The 1988 study found that when comparing single variable regressions with multiple variable linear regressions, the root mean squared error improved when adding up to three variables, but there was no improvement between three and four variables. The study also determined that the best equations for estimating SSC were based on Landsat MSS Near-Infrared (NIR) band (700 to 800 nm), but because of the linear form, all equations appeared to underestimate SSC at high concentrations.

Lathrop (1992) studied the relationship between Landsat 4-5 TM reflectance and measured

SSC from the Green Bay-Lake Michigan and Yellowstone Lake in Wyoming. The study found that the reflectance in the longer red and NIR wavelengths increased faster than in the shorter blue and green wavelengths. Lathrop (1992) also showed that the relationship between individual band reflectance and the ratio band combinations is nonlinear, approximating the general form of a power model. Long and Pavelsky (2013) compared 31 published empirical equations using a field dataset containing 147 observations of SSC and in-situ spectral reflectance to identify an appropriate reflectance-SSC model. Success of the reflectance-SSC models was contingent on the equation meeting the following criteria: 1) use of the NIR band in combination with at least one visible band, 2) development based on SSC like those in the observed region, and 3) use of a non-linear equation form (Long and Pavelsky 2013).

Regression Model Development

Data and Study Area

Landsat Satellite Data. The Landsat project is part of the Remote Sensing Missions component of the USGS Land Remote Sensing (LRS) Program. Landsat satellites have been collecting remote-sensing data for over 40 years with a temporal resolution of 16 days for each satellite. Landsat has the longest temporal record of moderate resolution multispectral data of the Earth's surface on a global basis. Data from Landsat 4-5 TM, 7 ETM+, and 8 OLI/TIRS were used for this study.

Landsat 4-5 TM has data available from July 1982 until January 2013, Landsat 7 ETM+ has data from April 1999 to April 2022, and Landsat 8 OLI/TIRS has data available from February 2013 until the present. Landsat 4-5 TM collection includes six, 30-m resolution spectral bands ranging from visible green to NIR wavelengths, two shortwave infrared (SWIR) bands, and a 120-m resolution thermal infrared (IR) band. Landsat 7 ETM+ collection includes 30-m resolution visible, NIR and SWIR bands, a 60-m resolution thermal band, and a 15-m panchromatic band. Landsat 8 OLI/TIRS collection includes 30-m resolution visible, NIR and SWIR bands, a 15-m resolution panchromatic band, two thermal bands, a coastal-aerosol band, and a band for cirrus cloud detection.

In 2016, the USGS started reorganizing the Landsat archive into a formal tiered data collection structure. Tier 1 (T1) data have the highest available geometric and radiometric quality. They include precision terrain processing and have been inter-calibrated across the Landsat sensors. The equations from Pereira et al. (2018) were developed using Landsat data before the application of this collection structure, and utilized all the images without the tier quality indicator. For this study, only T1 data were used for developing the regression models.

USGS Water Quality Gauge Stations. The USGS operates several gauge stations throughout the MMR, but only a small fraction of the stations has historical SSC data. This study used daily SSC data from four USGS gauge stations: i) Thebes, Illinois on the Mississippi River; ii) Hermann, Missouri on the Missouri River; iii) Chester, Illinois on the Mississippi River; and iv) St. Charles, Missouri on the Missouri River (Figure 1). These data were accessed through the USGS National Water Information System (NWIS) Web Interface. The beginning and end dates of the periods of record used in this study at each gage site are listed in Table 1. The Thebes, Hermann, Chester, and St. Charles gauging stations began their periods of record in 1982, 2009, 1982, and 2005, respectively. The period of record ended in 2017 for Thebes and Chester, and in 2008 for St. Charles. The Hermann station, located on the Missouri River, is currently continuing to provide daily SSC data.

Methods

Landsat Data Processing. Landsat T1 band surface reflectance values for blue, red, green, and NIR bands were used as independent variables in the regression model development. Surface reflectance values were obtained from delineated sampling areas at the four USGS gauge station locations. The rectangular sampling areas were 100 m wide by 330 m long. In MATLAB, each Landsat image was imported using an original MATLAB code, and the sampling area was delineated. The mean surface reflectance and standard deviation were calculated for each reflectance band of interest (green, blue, red, and NIR) within the sampling area.

The following chronological filters were used on each Landsat image to generate the final Landsat surface reflectance dataset: collection tier filter, pixel quality filter, blue band mean surface reflectance filter (removes images with cirrus cloud coverage in the sample area), and surface reflectance standard deviation filter (removes images with vessels in the sampling area). The collection tier filter only allows T1 Landsat images to be used, and the pixel quality filter only allows pixels to be used if they are defined as "low cloud confidence". The blue-band mean surface reflectance filter was then used to identify and remove images with values higher than 4.5% for Landsat 8 OLI/TIRS images, and 6.5% for Landsat 7 ETM+ and Landsat 4-5 TM images.

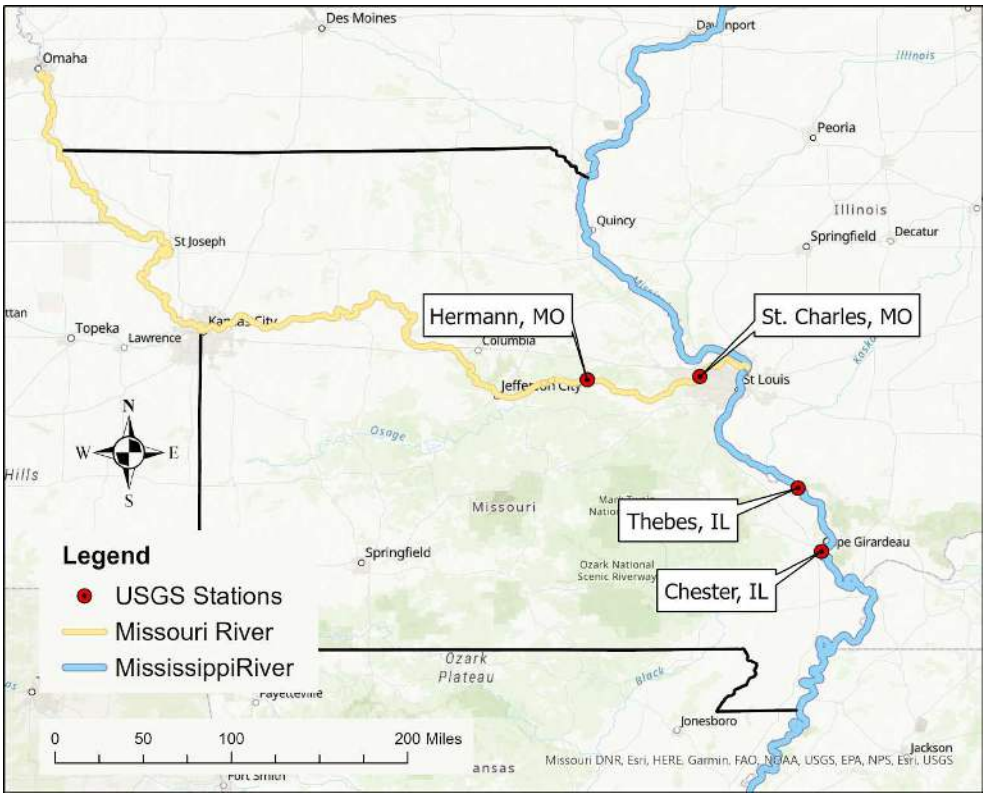


Figure 1. USGS gauge station locations used in regression equation analysis.

Table 1. Summary of development group and validation group USGS water quality gauge stations used in the development of the regression model.

Group	USGS Gauge Station (Location - Gauge No.)	Begin Date	End Date	------- No. of Data Points -------		
				L8 OLI/TIRS	L7 ETM+	L4-5 TM
Development	Thebes, IL – 07022000 Mississippi River	1982	2017	15	122	151
Development	Hermann, MO – 06934500 Missouri River	2009	2017	22	41	17
Validation	Chester, IL – 07020500 Mississippi River	1982	n/a	21	95	70
Validation	St. Charles, MO – 06935965 Missouri River	2005	2008	0	14	13

Lastly, the surface reflectance standard derivation filter identified and removed images with a surface reflectance standard deviation for any band greater than 0.5%. Details of the development of the blue-band and standard deviation filter methods are provided in Pereira et al. (2018). These steps ensured a high quality of Landsat data used in the development of the models.

Composite Dataset. The complete final dataset consisted of USGS daily SSC data and mean surface reflectance for the blue, green, red, and NIR bands. Each Landsat image product is representative of one date, and therefore dates in the Landsat dataset had to be matched to a date with a USGS SSC daily record. Each data point in the composite dataset is, therefore, representative of one date where there is both Landsat data and USGS data available. The temporal range of each final dataset varied among Landsat sensors. A summary of the Landsat data is provided in Table 1. Landsat 4-5 TM used data from almost 29 years, with a date range of January 1983 to November 2011; Landsat 7 ETM+ used data from nearly 18 years, with a date range of August 1999 to July 2017; and Landsat 8 OLI/TIRS used data from almost four and a half years, with a date range of March 2013 to July 2017.

Regression Analysis. The following power equation form was used for developing the regression model:

$$SSC = \alpha X_1^{\beta 1} X_2^{\beta 2} X_3^{\beta 3} + \varepsilon \quad (1)$$

where SSC is predicted in mgL^{-1}, α is the regression coefficient, ε is a constant term, X_1, X_2, and X_3 are band reflectance ratios Blue:NIR, Green:NIR, and Red:NIR, respectively, and β_1, β_2, and β_3 are exponents of band reflectance ratios X_1, X_2, and X_3, respectively. The least-squares fitting method was used to determine the optimal exponents and coefficients for Equation (1) for each of the regressions (i.e., Landsat 4/5 TM, Landsat 7 ETM+, and Landsat 8 OLI/TIRS).

For regression model development, the dataset was split into a development group (Thebes and Hermann) and a validation group (Chester and St. Charles). Regression coefficients and exponents were calibrated using data from the development group, and data from the validation group were used to independently assess the performance of the regression model. Splitting the development and validation datasets by location provided the best ability to assess the regional transferability of the regression models.

The St. Charles gauge station was not used in the validation group for Landsat 8 OLI/TIRS regression analysis because the gauge stopped reporting SSC data in 2008, before Landsat 8 OLI/TIRS was launched. Pereira et al. (2018) also included the St. Joseph gauge station in the validation group; however, when performing regression analyses, data from the St. Joseph station did not fit the regression trends for all Landsat sensors. Although St. Joseph is also on the Missouri River, the station is located 563 river kilometers upstream of the Herman station on the Missouri River. This finding reflects the significance of spatial transferability on reflectance-SSC empirical relationships.

Results and Discussion

A comparison between surface reflectance in visible and NIR Landsat bands and USGS daily SSC data showed that surface reflectance increases with increasing measured SSC. The peak surface reflectance within Landsat 8 OLI/TIRS visible and NIR bands alternated between the green (0.533 to 0.590 µm) and red (0.636 to 0.673 µm) bands for SSC values less than 155 mgL^{-1}. For SSC values greater than 155 mgL^{-1}, the peak reflectance switched to the red band. Landsat 7 ETM+ and Landsat 4-5 TM surface reflectance had peak reflectance in the green band (0.52 to 0.60 µm) when the SSC value was less than 140 mgL^{-1}. Peak reflectance alternated between green and red (0.63 to 0.69 µm) bands when the SSC value was between 140 to 160 mgL^{-1}, and at SSC values greater than 160 mgL^{-1} the peak was sustained in the red band. Pereira et al. (2018) show an example of the surface reflectance spectrum for Landsat 7 ETM+ and Landsat 4-5 TM.

Spectral sensitivity in Landsat bands was consistent when comparing Mississippi and Missouri River stations. The Mississippi River at Hermann and the Missouri River at Thebes showed similar spectral shapes (Figure 2). Lower SSC demonstrated peak reflectance in the green and red bands for both Hermann and Thebes. Concentrations higher than 155 mgL^{-1} had peak reflectance in the red band consistently for both

Hermann and Thebes, as well. This finding shows consistency in spectral sensitivity with spatial variability.

The calibrated reflectance-SSC regression models are provided in Table 2, and comparisons of observed versus predicted SSC values for the development and validation groups are shown in Figures 3 and 4, respectively. The regression exponents for all three models indicate the highest correlations with the green to NIR band ratio. The red to NIR ratio also had a relatively large exponent compared to the exponents for the blue to NIR band ratios. A summary of the statistical performance of the three regression models is shown in Table 3. For each regression model, the coefficient of determination (R^2) and the root mean square error (RMSE) statistics are reported for the development group, validation group, and the entire dataset.

For Landsat 4-5 TM, the development and validation groups included 168 and 83 records, respectively. The Landsat 4-5 TM regression model had development and validation R^2 values of 0.70 and 0.75, respectively. The increase in R^2 between the development and validation groups indicates that the regression model is not overfitting the development data and that the model is regionally transferable.

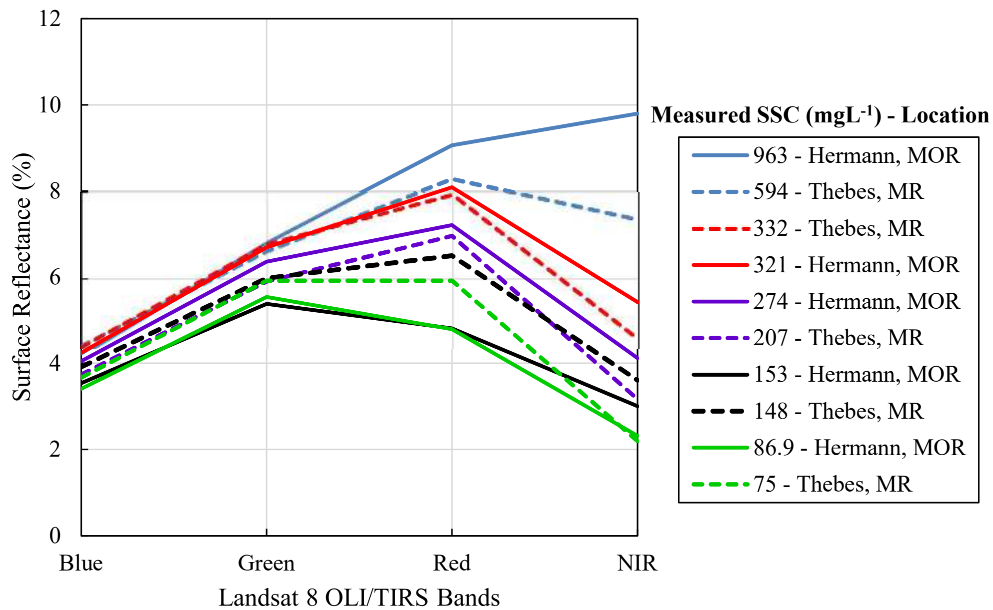


Figure 2. Comparison of spectral sensitivity for Mississippi and Missouri Rivers at similar SSC values.

Table 2. Reflectance-SSC empirical relationships for Landsat 8 OLI/TIRS, 7 ETM+, and 4-5 TM.

Landsat Sensor	Reflectance-SSC Empirical Relationship
8 OLI/TIRS	$SSC\ (\mathrm{mgL^{-1}}) = 159.9 \left(\frac{b2}{b5}\right)^{-0.1337} \left(\frac{b3}{b5}\right)^{-5.182} \left(\frac{b4}{b5}\right)^{3.663} + 87.67$
7 ETM+	$SSC\ (\mathrm{mgL^{-1}}) = 111.3 \left(\frac{b1}{b4}\right)^{-0.2684} \left(\frac{b2}{b4}\right)^{-6.033} \left(\frac{b3}{b4}\right)^{5.031} + 63.84$
4-5 TM	$SSC\ (\mathrm{mgL^{-1}}) = 74.80 \left(\frac{b1}{b4}\right)^{-1.387} \left(\frac{b2}{b4}\right)^{-4.639} \left(\frac{b3}{b4}\right)^{4.227} + 80.68$

Note. For Landsat 8 OLI/TIRS, *b*2, *b*3, *b*4, and *b*5 are blue, green, red, and NIR band surface reflectance, respectively; and for Landsat 7 ETM+ and 4-5 TM, *b*1, *b*2, *b*3, and *b*4 are blue, green, red, and NIR band surface reflectance, respectively.

For Landsat 7 ETM+, the development and validation groups included 163 and 109 records, respectively. The Landsat 7 ETM+ regression model had development and validation R^2 values of 0.74 and 0.71, respectively. Similar to the Landsat 4-5 TM regression, the minimal difference between the development and validation group R^2 values indicates a lack of model overfitting and regional transferability.

For Landsat 8 OLI/TIRS, the development and validation groups included 37 and 21 records, respectively. The Landsat 8 OLI/TIRS dataset included a total of 58 records which is 23% and 21% of the number of Landsat 4-5 TM and Landsat 7 ETM+ records, respectively. The Landsat 8 OLI/TIRS regression model had development and validation R^2 values of 0.95 and 0.72, respectively.

Table 3. Summary of R^2 and RMSE for the Reflectance-SSC regression models.

	8 OLI SSC	**7 ETM+ SSC**	**4-5 TM SSC**
Range (mgL^{-1})	49-963	41-961	44-863
No. of Samples	58	272	251
R^2_{Dev}	0.95	0.74	0.70
R^2_{Val}	0.72	0.71	0.75
R^2_{All}	0.87	0.73	0.72
$RMSE_{Dev}$	37	82	85
$RMSE_{Val}$	89	85	80
$RMSE_{All}$	61	83	83

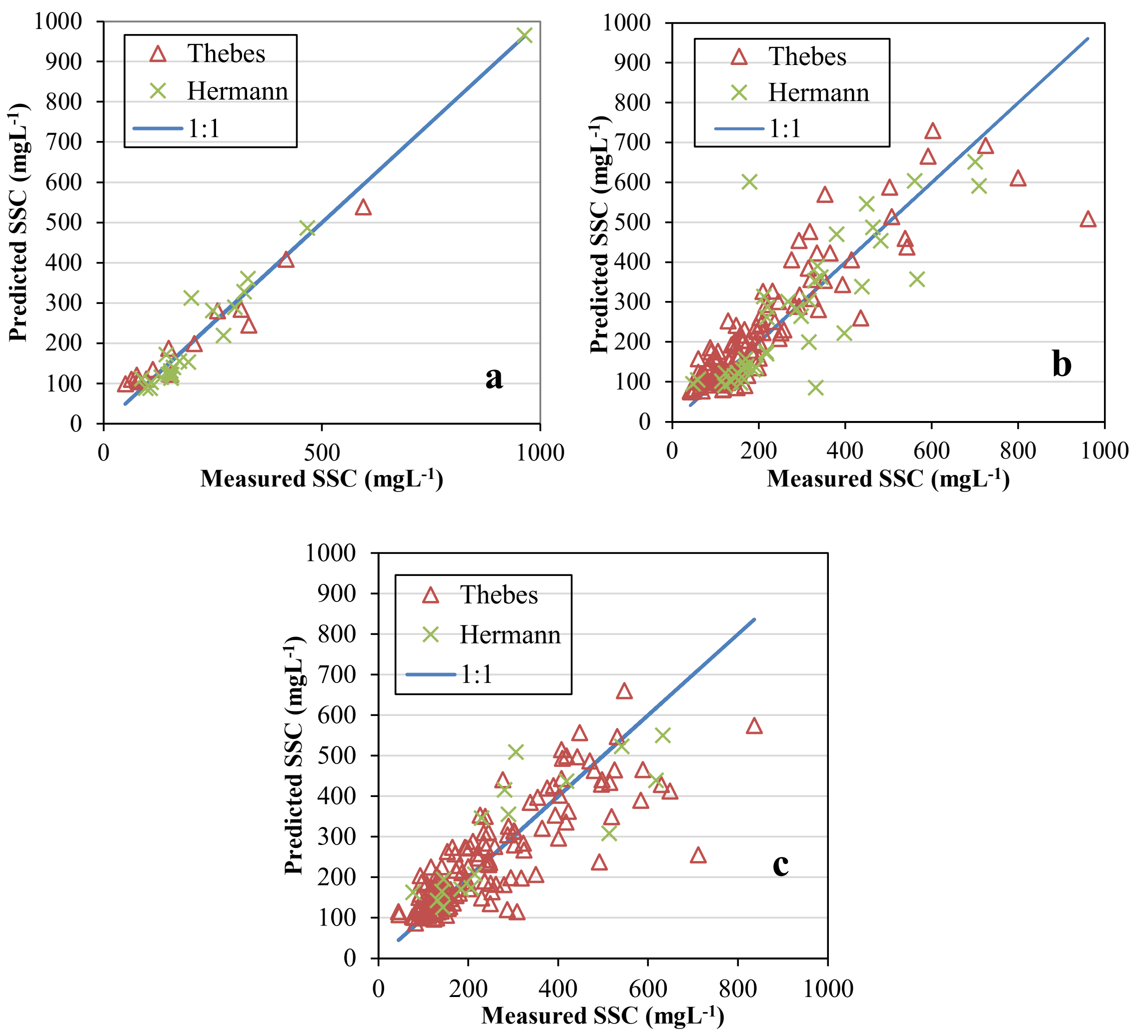


Figure 3. Development group relationship between predicted SSC and observed SSC for (a) Landsat 8 OLI/TIRS, (b) Landsat 7 ETM+, and (c) Landsat 4-5 TM.

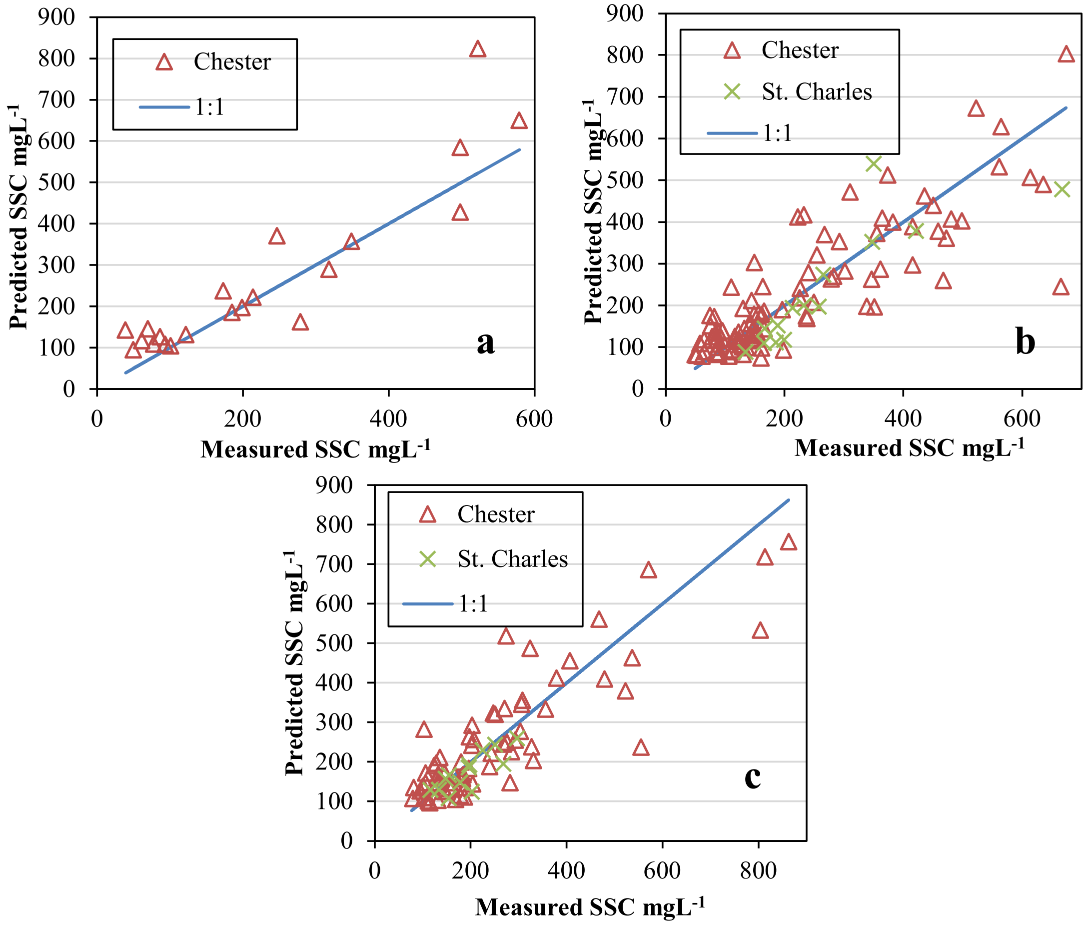


Figure 4. Validation group relationship between predicted SSC and observed SSC for (a) Landsat 8 OLI/TIRS, (b) Landsat 7 ETM+, and (c) Landsat 4-5 TM.

The high development R^2 value is a strong indication of the potential for the Landsat 8 OLI/TIRS sensor to predict SSC; however, the notably lower validation R^2 value suggests that the model is overfitting and that more records are needed to develop a robust regression model.

The power-regression form of the reflectance-SSC model has several advantages over the model provided by Pereira et al. (2018):

- Power-regression model does not allow for any negative estimated SSC values which were observed during the application of the Pereira et al. (2018) model;
- Approximately two additional years of period of record were used in the combined development and validation dataset;
- The revised filtering methodology is consistent with the updated Landsat product formats (i.e., Tier quality classifications); and
- R^2 values (0.62 to 0.72 for the validation group) were notably improved for the Landsat 8 model (negligible differences in Landsat 4/5 and Landsat 7 models) likely due to the increased number of data points used in the model development.

Reflectance-SSC Regression Applications

Confluence Mixing

The Landsat 8 OLI/TIRS regression model was applied at the confluence of the Mississippi and Missouri Rivers to analyze the difference in SSC between the two rivers and evaluate the downstream mixing length. The Landsat real-color image of the Mississippi–Missouri River confluence from September 12, 2016, shown in Figure 5, illustrates a visible color difference between the flow from the Mississippi River and the Missouri River. On this date, discharges in the Mississippi River and Missouri River were 9,940 cubic meters per second (cms) and 4,191 cms, respectively, with corresponding exceedance probabilities of 15% and 25%, respectively. The SSC distribution computed from the Landsat 8 OLI/TIRS regression model is also shown in Figure 5. The computed SSC values for the Mississippi River and Missouri River were 700 mgL^{-1} and 200 mgL^{-1}, respectively. For this image, the computed and visible mixing divide extended approximately 161 river kilometers downstream.

Point-Source Pollution

Along the MMR, areas of abnormally high SSC can be identified and quantified using reflectance-SSC regression models. Figure 6 shows an area of high SSC at the same location on four different dates between 2014 and 2017. The SSC data in Figure 6 were developed using the Landsat 8 OLI/TIRS regression model. Figure 6-f shows the same point in 2013, but the area has no noticeably higher concentration. The discharge on the 2013 date was 1087 cms (observed at St. Louis Gage Station 0701000), which was significantly lower than the discharges on the other dates, which ranged from 5,692 cms to 7,108 cms. This reduced flow rate is likely the cause of the lack of increased SSC in the area of concern.

Using the reflectance-SSC regression models, an automated algorithm could be developed to process the entire reach of the MMR and quantify local regions of increased SSC. Applying this automated method with images from several dates could then be used to identify zones with consistently higher SSC values. This application could be a powerful tool for environmentalists or government agencies to ensure that regulations are being properly followed.

Analysis across Large River Reaches

The three regression models were used to investigate profile distributions of SSC along the main channel of the Mississippi River. Landsat-predicted SSC values were obtained from 33,000-m^2 sampling areas along the MMR every 16 river

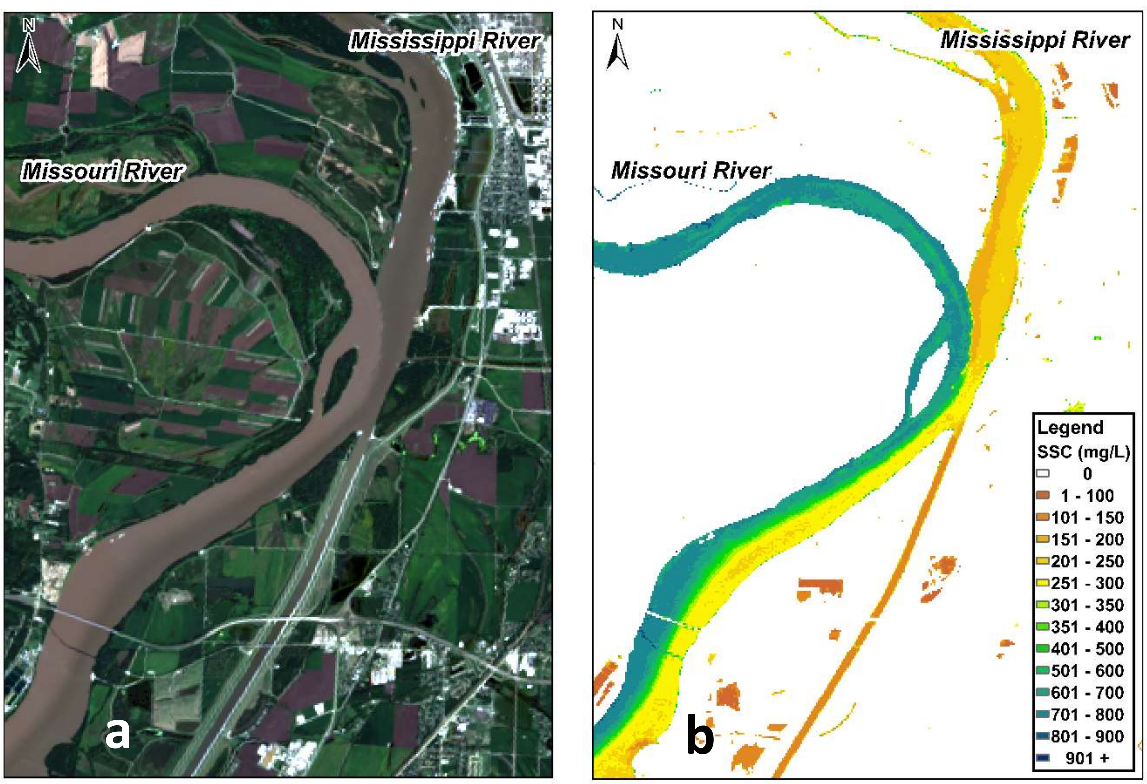


Figure 5. Mississippi–Missouri River confluence from Landsat 8 Surface Reflectance Image on September 12, 2016 (a) Landsat Real Color Image and (b) Landsat-Predicted SSC Image.

kilometers for the following dates: September 29, 1993 (Landsat 4-5 TM); September 4, 2010 (Landsat 7 ETM+); and November 13, 2015 (Landsat 8 OLI/TIRS). These dates were selected to include a low, medium, and high discharge condition. The Mississippi River discharges on these dates, extracted from the St. Louis USGS Gage (07010000), were 19,539, 8,948, and 3,766 cms, respectively, with corresponding exceedance probabilities of 1%, 19%, and 72%, respectively. The Missouri River discharges on these dates were 13,168, 3,710, and 1,487 cms, respectively, with corresponding exceedance probabilities of 0.12%, 21%, and 67%, respectively.

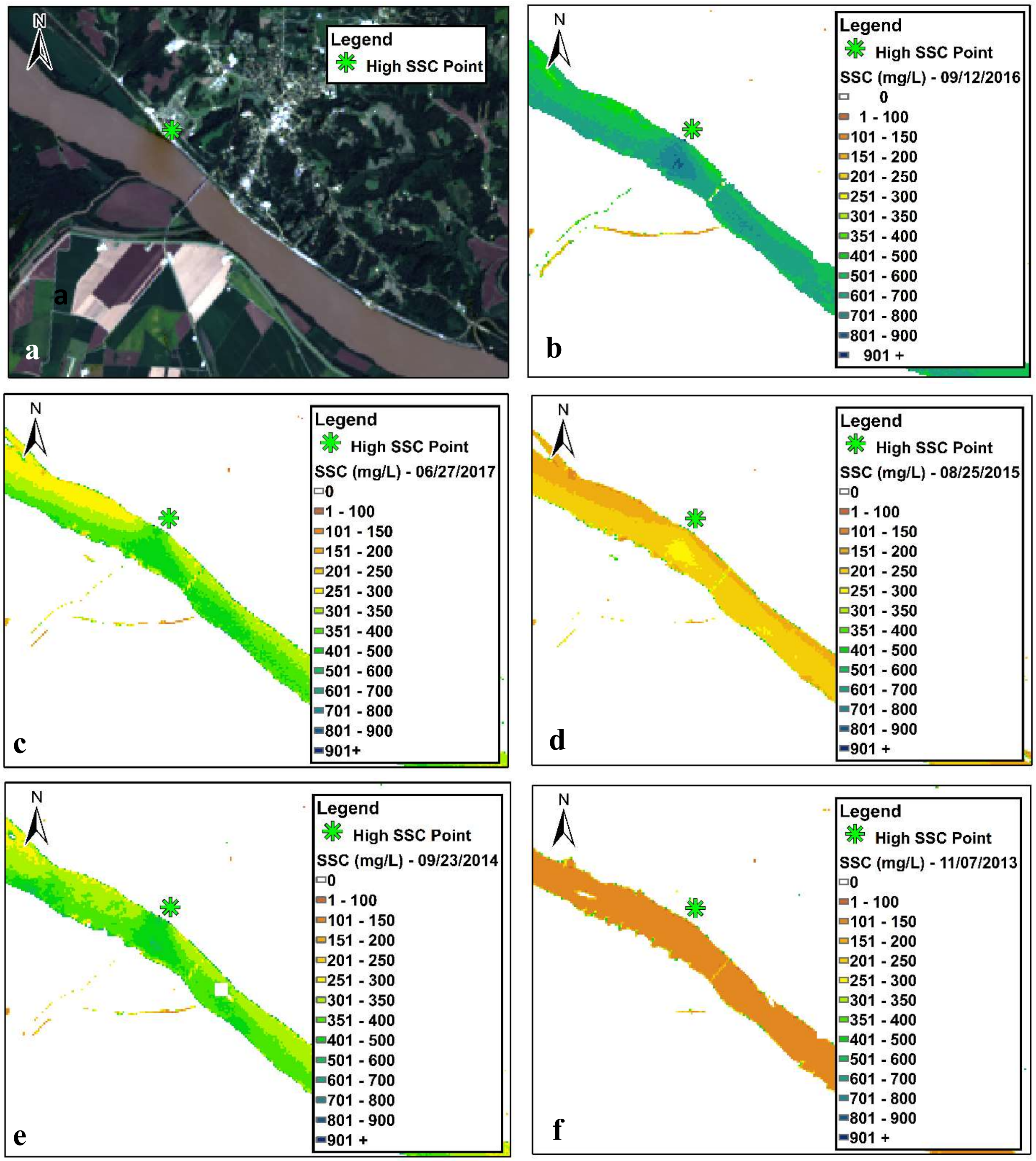


Figure 6. High SSC point along the MMR showed on (a) Landsat Real Color Image - 09/12/2016, Landsat-Predicted SSC Image, (b) 09/12/2016, (c) 06/27/2017, (d) 08/25/2015, (e) 09/23/2014, and (f) 11/07/2013.

Computed SSC values as a function of river kilometer are shown in Figure 7. The results show that SSC values generally increased with increasing downstream distance and increased with increasing water discharges. The September 29, 1993 data show a drastic spike downstream of the Missouri River confluence. This date occurred during the great Mississippi River and Missouri River flood, which lasted from April to October 1993, and had disproportionally high-water discharges in the Missouri River relative to the Mississippi River.

Sediment Rating Curves

The final demonstrated application of the reflectance-SSC regression models is the development of sediment rating curves for the following MMR tributaries: the Missouri, Meramec, Kaskaskia, and Big Muddy Rivers. Due to the 30-m resolution of Landsat imagery, only the tributaries that had a median channel width of 30 m or greater were used in this application. SSC data for each tributary were obtained using all available Landsat 4-5 TM, Landsat 7 TM+, and Landsat 8 OLI/TIRS, the image filtering techniques described in the Methods Section, and the reflectance-SSC regression models. All sampling areas were 33,000 m^2 rectangular areas located immediately upstream of each confluence. The median channel width of each tributary varied; therefore, sample area dimensions for each tributary were as follows: 100 m wide by 330 m long for the Missouri River, 60 m wide by 550 m long for the Meramec and Kaskaskia, and 30 m wide by 1,100 m long for the Big Muddy River. Daily mean discharge data were taken from the gauge station that was nearest to each tributary's confluence with the Mississippi River. The gauge station at Hermann, MO (Gauge No. 06924500) was used for the Missouri River; the gauge station at Eureka, MO (Gauge No. 07019000) was used for the Meramec River; the gauge stations at Venedy, IL (Gauge No. 05594100) and Freeburg, IL (Gauge No. 05594800) were used for the Kaskaskia River; and the gauge station at Murphysboro, IL (Gauge No. 05599490) was used for the Big Muddy River. The least-squares method was used to find the best-fit form of the rating curve equations for each site. The following non-linear, power-regression equation was used:

$$SSC = \alpha Q^{\beta} + \varepsilon \tag{2}$$

where SSC is predicted in mgL^{-1}, α is the regression coefficient, Q is discharge in cubic feet per second,

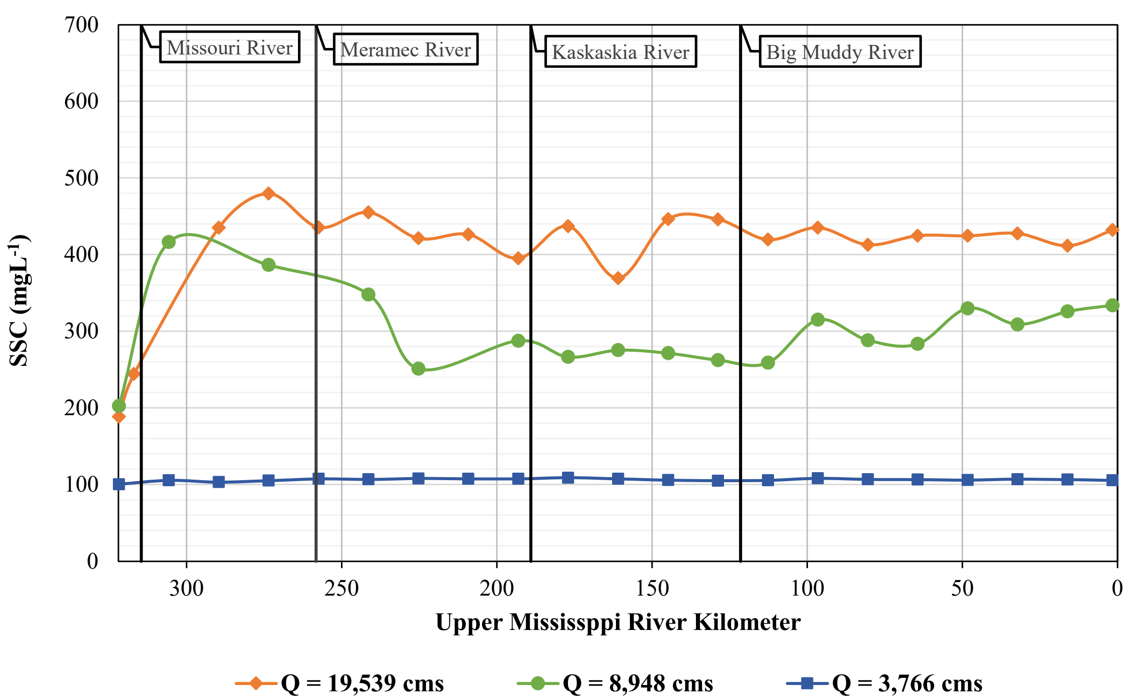


Figure 7. Graph showing SSC along the Middle-Mississippi River (Upper-Mississippi River from River Kilometer 197 to 1) for different flow frequencies.

β is the power term for the discharge, and ε is the constant term.

Observed versus predicted SSC plots for each site, along with the calibrated rating equations, are shown in Figure 8. The Missouri River had an R^2 value of 0.433, the Meramec River had the highest rating curve R^2 value of 0.747, Kaskaskia had an R^2 value of 0.360, and the Big Muddy River had an R^2 value of 0.519. Estimates of sediment yield based on rating-curve calculations will have greater errors than those obtained from direct measurements; however, a sediment rating curve can be valuable in the absence of direct measurements. Asselman (2000) stated that scatter about the rating curve regression line is caused by variations in sediment supply due to seasonal effects, antecedent conditions in the river basin, and differences in sediment availability at the beginning and the ending of a flood, which are not accounted for in rating curves.

Summary and Conclusions

Reflectance-SSC regression models for the

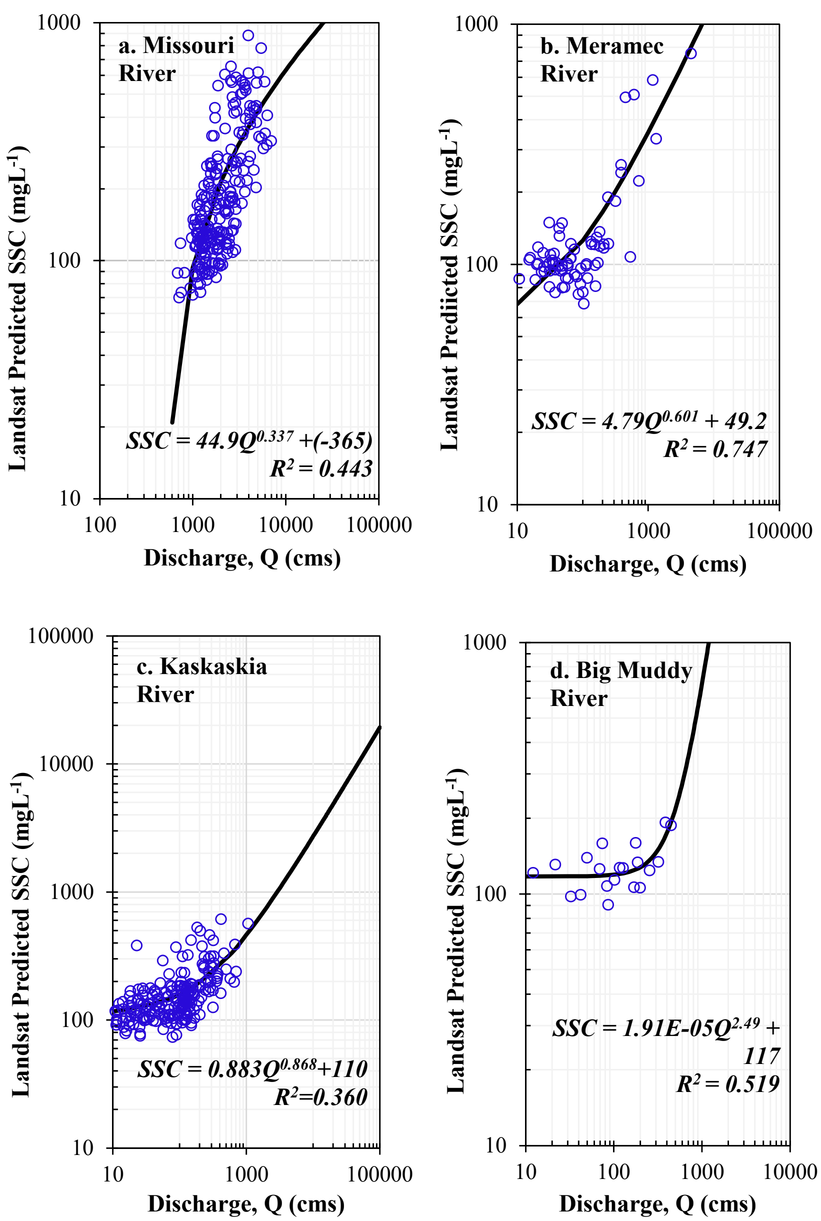


Figure 8. Sediment rating curves obtained from empirical relationship between Landsat predicted SSC and observed discharge for (a) Missouri River, (b) Meramec River, (c) Kaskaskia River, and (d) Big Muddy River.

MMR and the LMOR were developed from Landsat measured surface reflectance and USGS SSC records. The calibrated reflectance-SSC models had validation R^2 values of 0.72, 0.71, and 0.75 for Landsat 8 OLI/TIRS, Landsat 7 ETM+, and Landsat 4-5 TM, respectively. Landsat satellites have been collecting data for 36 years, with a temporal resolution of 16 days. These reflectance-SSC relationships enable researchers to study spatial and temporal trends in SSC for dates of available Landsat data.

Three applications of the reflectance-SSC regression models were demonstrated: 1) mixing at the Mississippi and Missouri River confluence, 2) point-source pollution, and 3) SSC changes along the entire MMR reach for a range of discharges. The following conclusions were made from these analyses:

Analysis of SSC distributions at the Mississippi and Missouri River confluence for September 12, 2016, showed the mixing field extended approximately 161 kilometers downstream of the confluence.

Using the regression models, a point-source pollution location along the MMR was identified with elevated SSC values on dates in five different years. This type of application can be used to identify local areas with consistently elevated SSC that may be causing negative impacts on the MMR.

Longitudinal profile distributions of SSC in the MMR for a range of flow rates revealed that SSC values generally increased with increasing downstream distance and increased with increasing discharges.

The regression models were also used to develop sediment rating curves for the Missouri, Meramec, Kaskaskia, and Big Muddy Rivers. The R^2 values for these rating curves were 0.433, 0.747, 0.360, and 0.519, respectively. The Missouri River is monitored for SSC, but there are gaps in the period of record which can be supplemented with data derived from the sediment rating curves. The remaining three tributaries are unmonitored for SSC and the sediment rating curves can provide an estimate of the sediment load contributions from each tributary to the MMR. In future studies, the sediment rating curves could be used to create a sediment budget for the MMR.

Acknowledgements

Funding for this project came from the USGS Grant G16AP00066 awarded by the Missouri Water Resources Research Center.

Author Bio and Contact Information

Megan Martinez was a graduate research assistant at Saint Louis University from August 2017 to May 2019. She graduated in May 2019 with her Master of Science in Civil Engineering and is currently employed as a Civil Engineer/Site Engineer at M&M Engineering Consultants Limited in Belize. She may be contacted at meganjosephinemartinez@gmail.com or by mail at 3450 Lindell Blvd, St. Louis, Missouri, 63103.

Dr. Amanda L. Cox (corresponding author) is an associate professor of civil engineering and Director of the WATER Institute at Saint Louis University. Her research interests are focused on hydraulic engineering and include hydraulic modeling, sediment transport, hydraulic structures, river engineering and stream restoration, urban drainage, and stormwater erosion. She has a Ph.D. in civil engineering from Colorado State University and is a licensed professional engineer in the states of Missouri and Colorado. She may be contacted at amanda.cox@slu.edu or by mail at 3450 Lindell Blvd, St. Louis, Missouri, 63103.

References

Asselman, N.E.M. 2000. Fitting and interpretation of sediment rating curves. *Journal of Hydrology* 234(3): 228-248.

Dos Santos, B.B., R.B. De Miranda, M.V. Estigoni, J.M. Villela, and F.F. Mauad. 2018. Evaluation of the laser diffraction method for the measurement of suspended sediment concentration in the Mogi-Guacų reservoir (São Paulo, Brazil). *International Journal of River Basin* 17(1): 89-99.

Doxaran, D., J. Froidefond, and P. Castaing. 2003. Remote-sensing reflectance of turbid sediment-dominated waters. Reduction of sediment type variations and changing illumination conditions effects by use of reflectance ratios. *Applied Optics* 42(15): 2623-2634.

Felix, D., I. Albayrak, and R.M. Boes. 2017. In-situ investigation on real-time suspended sediment measurement techniques: Turbidimetry, acoustic attenuation, laser diffraction (LISST) and vibrating tube densimetry. *International Journal of Sediment Research* 33(1): 3-17.

Gray, J.R. and J.W. Gartner. 2010. Overview of selected surrogate technologies for high-temporal resolution suspended-sediment monitoring. In: *2nd Joint Sedimentation and Hydrologic Modeling Federal Interagency Conference*, Las Vegas, NV, 27 June - 1 July 2010.

Guerrero, M., N. Ruther, S. Huan, and S. Baranya. 2017. A combined use of acoustic and optical devices to investigate suspended sediment in rivers. *Advances in Water Resources* 102: 1-12.

Islam, M.R., Y. Yamaguchi, and K. Ogawa. 2001. Suspended sediment in the Ganges and Brahmaputra Rivers in Bangladesh: Observation from TM and AVHRR data. *Hydrological Processes* 15: 493-509. Available at: https://onlinelibrary.wiley.com/doi/10.1002/hyp.165. Accessed March 8, 2023.

Landers, M.N. 2012. Fluvial suspended sediment characteristics by high-resolution, surrogate metrics of turbidity, laser-diffraction, acoustic backscatter, and acoustic attenuation. Doctoral Dissertation. Georgia Institute of Technology, Atlanta, Georgia, USA.

Lathrop, Jr., R.G. 1992. Landsat Thematic Mapper monitoring of turbid inland water quality. *Photogrammetric Engineering & Remote Sensing* 58(4): 465-470.

Long, C.M. and T.M. Pavelsky. 2013. Remote sensing of suspended sediment concentration and hydrologic connectivity in a complex wetland environment. *Remote Sensing of Environment* 129: 197-209. Available at: https://doi.org/10.1016/j.rse.2012.10.019. Accessed March 8, 2023.

Mertes, L.A.K., M.O. Smith, and J.B. Adams. 1993. Estimating suspended sediment concentrations in surface waters of the Amazon River wetlands from Landsat images. *Remote Sensing of Environment* 43(3): 281-301. Available at: http://dx.doi.org/10.1016/0034-4257(93)90071-5. Accessed March 8, 2023.

Pereira, L.S.F., L.C. Andes, A.L. Cox, and A. Ghulam. 2018. Measuring suspended-sediment concentration and turbidity in the Middle Mississippi and Lower Missouri Rivers using Landsat data. *Journal of the American Water Resources Association* 54(3): 440-450. Available at: https://dx.doi.org/10.1111/1752-1688.12616. Accessed March 8, 2023.

Peterson, K.T., V. Sagan, P. Sidike, A.L. Cox, and M.J. Martinez. 2018. Suspended sediment concentration estimation from Landsat imagery along the Lower Missouri and Middle Mississippi Rivers using an extreme learning machine. *Remote Sensing* 10(10): 1503. Available at: https://doi.org/10.3390/rs10101503. Accessed March 8, 2023.

Ritchie, J.C., F.R. Schiebe, and J.R. McHenry. 1976. Remote sensing of suspended sediments in surface water. *Photogrammetric Engineering & Remote Sensing* 42(12): 1539-1545.

Ritchie, J.C., C.M. Cooper, and J. Yongqing. 1987. Using Landsat multispectral scanner data to estimate suspended sediments in Moon Lake, Mississippi. *Remote Sensing of Environment* 23: 65-81.

Ritchie, J.C. and C.M. Cooper. 1988. Comparison of measured suspended sediment concentrations with suspended sediment concentrations estimated from Landsat MSS data. *International Journal of Remote Sensing* 9(3): 379-387. Available at: https://dx.doi.org/10.1080/01431168808954861. Accessed March 8, 2023.

Ritchie, J.C., P.V. Zimba, and J.H. Everitt. 2003. Remote sensing techniques to assess water quality. *Photogrammetry and Remote Sensing* 69(6): 695-704.

Topliss, B.J., C.L. Almos, and P.R. Hill. 1990. Algorithms for remote sensing of high concentration, inorganic suspended sediment. *International Journal of Remote Sensing* 11(6): 947-966. Available at: https://dx.doi.org/10.1080/01431169008955069. Accessed March 8, 2023.

Universities Council on Water Resources
Journal of Contemporary Water Research & Education
Issue 177, Pages 31-45, April 2023

Prevalence and Distribution of Microplastics in Oysters from the Mississippi Sound

Kendall Wontor[1], *James V. Cizdziel[1], Austin Scircle[2], Deborah J. Gochfeld[3,4], and Ann Fairly Pandelides[4]

[1]*Department of Chemistry and Biochemistry, University of Mississippi, University, MS*
[2]*U.S. Army Corps of Engineers, Engineer Research and Development Center, Environmental Laboratory, Vicksburg, MS*
[3]*National Center for Natural Products Research, University of Mississippi, University, MS*
[4]*Division of Environmental Toxicology, Department of BioMolecular Sciences, University of Mississippi, University, MS*
**Corresponding Author*

Abstract: Oysters are a foundational part of their ecosystem and research has shown they are negatively impacted by exposure to microplastics (MPs). High MP levels have been documented in waters surrounding oyster reefs, and as filter feeders, oysters can ingest MPs along with their food. Here, we determined MPs (>30 μm) in oysters (*Crassostrea virginica*) from ten sites across the Mississippi Gulf Coast. Further, a subset of these samples was dissected to quantify MPs within specific tissues. Average concentrations ranged from 30.7± 11.5 to 4.7 ± 0.25 putative MPs/g wet weight (ww) of whole tissue, with sites inside bays near population centers displaying higher levels of MPs than those exposed directly to the Gulf. Mantle, gill, and adductor muscle tissues had similar concentrations of putative MPs (15.9 ± 13.4, 11.5 ± 8.6 and 12.8 ± 6.7 MPs/g, respectively), whereas digestive system tissues had lower concentrations (6.8 ± 6.1 MPs/g of tissue). This suggests that most MPs in an oyster likely adhere to external tissues and are not actually ingested. Most of the MPs retained were in the smallest size fraction of 30-90 μm (80%), followed by 125-250 μm (9%), 90-125 μm (8%), and >250 μm (3%). Analysis of samples from Biloxi Bay by μ-FTIR to assess MP composition shows that polyurethane, polyethylene, and polyamide are common, but additional analyses are needed to fully characterize the MP profile across sites. Overall, this work provides much-needed empirical data on the abundances and sizes of MPs in oysters from the Mississippi Sound, as well as the tissues where they reside.

Keywords: *microplastics, oyster condition index, fluorescence microscopy, Gulf of Mexico, Mississippi*

The global nature of microplastics (MPs) as an environmental contaminant has been well documented (Ding et al. 2019; Dodson et al. 2020; Shen et al. 2020; Suaria et al. 2020). The northern Gulf of Mexico is of particular interest for MP contamination, being the outlet of several major rivers including the Mississippi River system. The load of MPs tends to increase as the rivers flow toward the Gulf of Mexico, which acts as a sink for these particles (Scircle et al. 2020a). Thus, it is not surprising that Gulf Coast waters and beaches have high levels of MP contamination compared to many other coastlines worldwide (Wessel et al. 2016; Di Mauro et al. 2017).

While all marine animals are exposed to the MPs in ocean waters, oysters and other filter feeders are particularly vulnerable. This is especially concerning because oysters are foundation species (Ridlon et al. 2021), responsible for the structure and function of oyster reef ecosystems. Oyster reefs serve as nurseries and habitats for other species, act as barriers to protect the shoreline from erosion, and clean the surrounding waters by virtue of the oysters' filter feeding behavior (Beck et al. 2011). Loss of an oyster reef is typically followed by a decrease in biodiversity in the area, causing both environmental and economic damage in areas dependent on commercial fishing, such as the Mississippi Gulf Coast (Beck et al. 2011). Thus, assessing the risk posed to native oyster reefs by MPs is crucial.

Risk assessment is important because some

Research Implications

- Oysters from locations inside bays near population centers had higher average concentrations of MPs.
- Oysters accumulate more MPs on their external tissues than in their digestive system, though the latter gives a snapshot of recently consumed MPs.
- MP concentration in oysters was not correlated with oyster condition index.
- Characterizing the types of MPs present in oysters may provide insight into likely sources of contamination at different sites.
- Lawmakers need to consider federal legislation to address MP pollution in river systems at a national level.

types of MPs can have adverse impacts on oysters. Previous studies have shown that MPs can negatively affect oyster reproduction and energy uptake (Sussarellu et al. 2016; Gardon et al. 2018). More troubling, long term exposure to polystyrene MPs may also result in increased mortality rates (Thomas et al. 2019). Given that previous work has shown high levels of MPs in waters surrounding Mississippi Gulf Coast oyster reefs (Scircle et al. 2020b), this study sought to quantify and characterize the MPs that Mississippi Gulf Coast oysters (*Crassostrea virginica)* ingest and accumulate in their tissues.

While several other studies have assessed MP concentrations in oysters (Li et al. 2018; Keisling et al. 2020; Cho et al. 2021), such studies have been primarily concerned with using oysters for environmental monitoring purposes. As a result, their analyses were focused on whole oysters in order to assess MP levels across a variety of sites. However, such data do not provide information on where those MPs are located inside the oysters, which is crucial to assess potential health risks to both oysters and the humans that eat them. Therefore, another goal of this study was to analyze oyster tissues separately in order to assess whether they contained different concentrations and types of MPs.

Methods

Study Site and Oyster Sampling

Oysters were sampled from ten sites along the Mississippi Gulf Coast (Figure 1), with two of the sites associated with Mississippi Based RESTORE (Resources and Ecosystem Sustainability, Tourist Opportunities, and Revived Economies) Act Center of Excellence (MBRACE) sensor platforms (hereafter called *landers*) and the remaining eight associated with the Mississippi Oyster Gardening Program (MSOGP). GPS coordinates for each site are given in Table 1.

Landers. Oysters were obtained from the Thad Cochran Marine Aquaculture Center in Ocean

Figure 1. Map of oyster collection sites along the Mississippi Gulf Coast. A description of sampling sites is given in Table 1. Sites 1 and 10 correspond to MBRACE landers.

Table 1. Oyster sampling sites include those from the Mississippi Oyster Gardening Program (MSOGP) and MBRACE lander sites, with the latter designated by *.

Site	Description	Nearest City	Coordinates
1*	St. Stanislaus High School	Bay St. Louis	30.305025, -89.325315
2	St. Stanislaus High School	Bay St. Louis	30.304968, -89.325304
3	MSOGP site	Bay St. Louis	30.334387, -89.331403
4	MSOGP site	Pass Christian	30.331054, -89.283668
5	Biloxi Maritime Museum	Biloxi	30.392968, -88.857867
6	MSOGP site	Biloxi	30.416986, -88.908997
7	MSOGP site	Ocean Springs	30.418516, -88.836062
8	MSOGP site	Ocean Springs	30.343711, -88.722355
9	MSOGP site	Gautier	30.363593, -88.637757
10*	Grand Bay	Moss Point	30.369654, -88.420534

Springs, MS. Each lander was deployed with about 20-25 oysters on 13 October 2020 along the Mississippi Gulf Coast. Each lander consists of a metal frame resting on top of a rubber tire, which prevents the lander from sinking into the surrounding sediment when deployed (Gledhill et al. 2020). Inside the lander, a milk crate and several trays are used to hold the oysters. Each lander also contains dissolved oxygen, temperature, and conductivity sensors, which continuously monitor environmental conditions. Initially, landers were deployed at ten sites. Unfortunately, the majority of these landers were destroyed in Hurricane Zeta. Only two landers remained following hurricane season, those located at St. Stanislaus High School and in Grand Bay. Thus, we only report lander data from these two sites. Upon collection on 8 December 2020, average oyster wet tissue weights were 21.2 ± 5.4 g at St. Stanislaus and 10.2 ± 1.0 g at Grand Bay.

MSOGP Sites. Additional oysters were sourced from eight MSOGP on 8 December 2020. Briefly, this program helps restore Mississippi's oyster reefs by providing juvenile hatchery-reared oysters for volunteers to raise in cages on private docks until they are old enough to be planted onto oyster reefs. Although they cannot be harvested themselves, the goal is for them to spawn and produce larvae that will re-seed harvestable reefs. Oyster gardening programs also increase public awareness of how oysters improve the water quality and their economic role in Gulf Coast communities ("Mississippi – Oyster Gardening on the Gulf Coast" n.d.). Whereas these oysters were in the Gulf over a longer period (July to December 2020), they tended to be smaller than the lander oysters because they were deployed as oyster spat (newly attached larvae). Upon collection, their average wet tissue weight for each site ranged from 1.93 g to 7.85 g.

Oyster Condition Index Measurements

Condition index (CI) measurements were used to assess the oysters' condition at each site (n = 6-13 per site) following methods from Abbe and Albright (2003). Whole oysters were weighed intact to determine the total wet weight (ww). Each oyster was then shucked, and the wet tissue was separated, and empty shells were weighed alone. The wet shell cavity volume was then calculated by subtracting the weight of the wet oyster shells from the total wet weight. Following this, the wet oyster tissue was freeze-dried and weighed again to determine the dry tissue weight. The following equation was used to determine the CI for each oyster (Abbe and Albright 2003):

$$CI = \frac{dry\ tissue\ weight}{wet\ shell\ cavity\ volume}$$

Oyster Dissection

In the lab, oysters were assessed based on size to determine which oysters would be dissected and which would be analyzed whole. Due to differences in oyster size between sites, oysters from the two landers and Site 5 were dissected (n = 5 for each site, 15 total), while the smaller oysters from the other sites were analyzed whole (n = 5 for each site; 35 total). Each oyster was shucked with a shucking knife and the mantle pulled back with tweezers to expose the gills (Figure 2). Using tweezers and dissecting scissors, the gills were removed and placed in a labeled 20 mL glass scintillation vial with a foil-lined cap. The mantle was placed in a separate labeled glass vial. A knife was then used to separate the adductor muscle from the shell. Finally, the adductor muscle and heart were separated from the digestive system tissue. The digestive system was placed in one glass vial while the adductor muscle and heart were placed in a separate vial. If the oyster was too small to ensure a clean separation of tissues, it was shucked and placed in a glass vial whole.

Contamination Mitigation Protocols

Sample preparation occurred in a laminar flow hood (AirClean 6000 Workstation) within a HEPA-filtered clean room to reduce the risk of contamination by MPs. Plastic tools were avoided wherever possible in favor of glass and metal tools. All glassware was heat cleaned at 450°C for three hours before use. Additionally, glassware and metal tools were rinsed three times with milliQ water between samples. Reagents were pre-filtered through a 25 µm pore size Monel filter to remove any MPs. Analysts also wore 100% cotton lab coats and nitrile gloves to further reduce contamination risk. Finally, two methodological blanks for each sample run were prepared and used to quantify any contamination that might have occurred despite these precautions. All data reported consist of blank subtracted values.

Sample Preparation

Samples were prepared using a modified version of the single pot method previously described (Scircle et al. 2020a). Briefly, each whole oyster

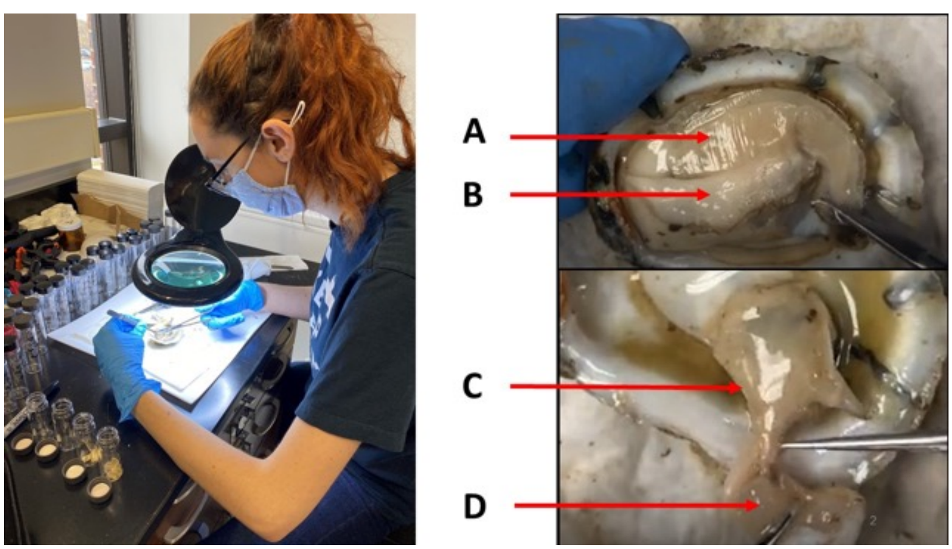


Figure 2. Oyster dissection with mantle (A) peeled back to expose gills (B). Following removal of gills and mantle, the digestive system (C) is separated from the adductor muscle (D) and heart (not visible).

or dissected tissue was weighed and placed in a Mason jar along with 150 mL of 10% w/v KOH solution to digest the biological tissue. A lid was used to cover each jar but not screwed down to allow gases to escape. Samples were then placed in a vacuum oven at 40°C for 24 hours and stirred twice daily. Digestion was performed at 40°C as studies have shown that higher temperatures can cause damage to some polymer types (Thiele et al. 2019). Fully digested samples were removed from the oven while undigested samples were heated for an additional 24 hours. In general, samples with higher masses (>3 g) needed longer digestion times. Once samples had been digested, the solid lids were exchanged for lids with a 57 mm diameter hole. The new lids were placed into the screw band and an 84 mm diameter 30 μm pore Monel filter was placed on top. These were then screwed onto the tops of the jars. Each jar was swirled and turned upside down over a waste bucket and a stream of clean air was applied to the filter to help break the surface tension. After removal of the lids, milliQ water was used to rinse any solids left on the filter back into the Mason jar. A glass vacuum filtration apparatus was used to filter the samples onto 25 mm diameter 30 μm pore Monel filters. During filtration, each jar was rinsed twice with milliQ water to ensure transfer of all MPs. These smaller Monel filters were then rinsed with a 1.63 g/cm^3 density $ZnCl_2$ solution into a 40 mL glass scintillation vial. Each vial was then filled to 30 mL with $ZnCl_2$ solution. The vials were capped and centrifuged at ~1610 G for 12 minutes to separate shell fragments and other inorganic materials. The supernatant was filtered through a 25 mm diameter 10 μm pore polycarbonate filter. The filters were then rinsed with 1 mL of 2% HCl, followed by 5 mL of milliQ water to remove any $ZnCl_2$ precipitate.

Microplastic Analysis by Fluorescence Microscopy

Filters were placed on labeled glass slides and allowed to dry in a laminar flow clean bench. A 10 μg/mL Nile red in methanol solution was used to stain the samples by pipetting 3-4 drops of dye onto each filter. The filters were allowed to dry for ~5 minutes before being covered with a glass cover slip and taped shut. A Nikon Ti2 Eclipse Fluorescence Microscope along with the NIS-Elements application was used to analyze these samples. Filters were imaged in their entirety and the software's object count feature was used to automatically count the number of fluorescing particles above a defined threshold (i = 15000). Each counted object was then manually inspected to ensure that it was a putative MP. Objects with biological features such as striations or intracellular patterning were excluded from the count. Each sample count was then subtracted by the average blank counts of the run to yield the blank-subtracted data.

It is important to note that although fluorescence microscopy is frequently used in MP studies due to its relative low cost and fast analysis times, it does not yield any chemical data about the particles imaged. Although the digestion process, density separation, use of Nile red (a lipophilic dye that preferentially stains plastics), and particle examination (only objects lacking biological features such as cellular structure or striations are counted) minimize false positives, it is still possible to overestimate the number of MPs in a sample. Thus, herein we use the term putative MPs when referring to fluorescence microscopy data.

Determination of MP Compositions by μ-FTIR

Since fluorescence microscopy does not yield information about the chemical identity of the MPs, five samples from two Sites (6 and 7) were prepared for analysis using micro-Fourier transform infrared spectroscopy (μ-FTIR). Polycarbonate filters containing the putative MPs were sonicated for 2 minutes in 30 mL of 50% ethanol. The resulting solution was filtered through a 25 mm aluminum oxide filter (Anodisc). Filters were then dried in a laminar flow clean bench before being analyzed with a Bruker LUMOS II FTIR microscope. Samples were imaged in transmission mode using the FPA detector. A 4-mm square of each filter was analyzed using a resolution of 4 cm^{-1}, 6 scans, and 4 x 4 binning. Data were processed using the OPUS v8.5 and Purency v4.07 software.

Statistics

In order to assess whether statistically significant differences existed between sample sites, one-way ANOVA was utilized. If significant differences were found ($p < 0.05$), post hoc tests were used to determine which groups gave rise to these differences. Due to having unequal groups in the CI data, Dunn's post hoc test was used for this purpose. Tukey's honestly significant difference (HSD) was used for the MP concentration data as there was an equal number of samples in each group. In order to assess statistical differences between average MP concentration in different types of oyster tissue, a two-way ANOVA analysis followed by Tukey's HSD post hoc test was used. This made it possible to determine differences due to both site and tissue type, as not all dissected oysters came from the same location.

Results and Discussion

Condition Index

The average CI of oysters for sites in this study ranged from 9.3 ± 3.0 to 15.6 ± 2.4, and differed significantly among sites (ANOVA, df = 9, $p < 0.001$), with the lowest values at Site 3 (Bay St. Louis) and the highest at Site 5 (Biloxi) (Figure 3). These values are similar to those of oysters in Alabama and Louisiana Gulf Coast waters (Casas et al. 2017; Leonhardt et al. 2017). We observed no correlation between CI and MP concentration (Pearson's correlation coefficient = -0.13, $p = 0.73$). One limitation to our analysis is that unlike fresh (wet) oyster tissue the freeze-dried oyster tissue used to calculate CI could not be fully digested. As a result, both the CI and MP concentrations could not be determined for the same individual oyster. Instead, we compared the average CI values (n = 6-13) and the average MP concentrations (based on wet weight, n = 5) for each site.

The lack of correlation between CI and MP concentration may be related to the duration of exposure, as MPs have long-term effects. One study found that CI values of oysters continuously exposed to high concentrations of polystyrene MPs increased within the first 10-20 days, but decreased as time went on (Thomas et al. 2019). However, unlike in that study, oysters in this study were not kept in tanks. As such, they were exposed to a variety of environmental conditions, which also may have affected their CI. For example, oysters in areas of low salinity tend to have lower CI values (Leonhardt et al. 2017). This may account for some variability in CI between the sites, as previous work showed much lower salinity levels within Bay St. Louis than at sites on more exposed coastline (Scircle et al. 2020b). This could explain the lower CI values for Sites 3 and 4, which are located inside the bay, compared to Sites 1 and 2 at the bay's entrance. A similar trend, albeit less pronounced, is seen in the Biloxi Bay sites, with

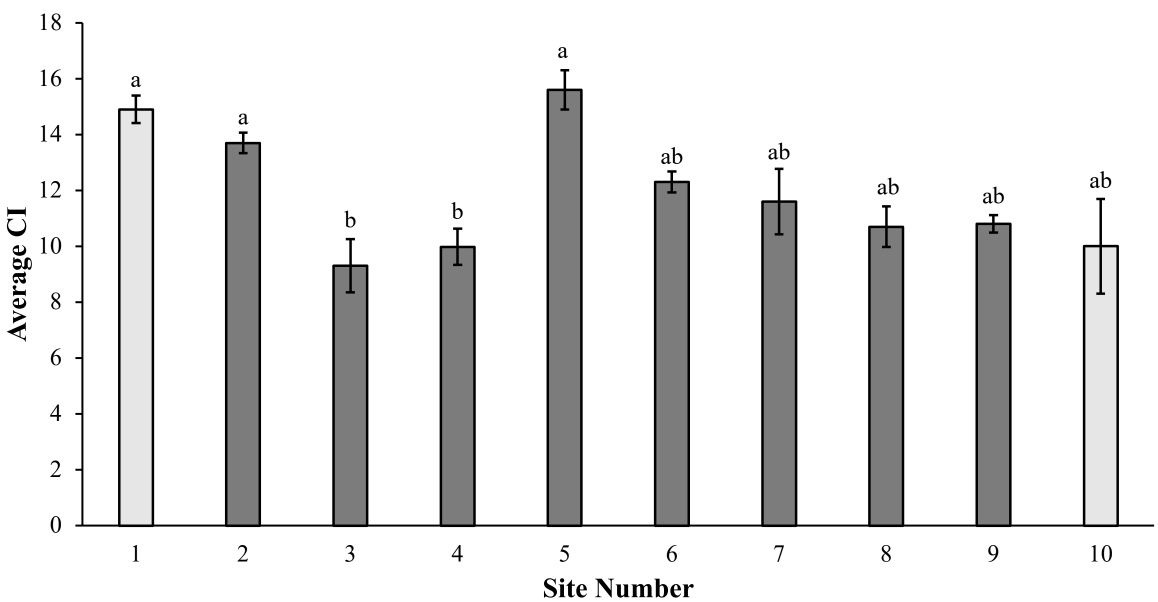


Figure 3. Mean (± SE) condition index of oysters (n = 6-13) from each site (lander sites in light gray and Mississippi Oyster Gardening Program sites in dark gray). Error bars = ± one standard error. Different letters denote means that are significantly different determined via one-way ANOVA followed by Dunn's post hoc test ($p < 0.05$).

Sites 6 and 7 located further in the bay having lower CI values than Site 5.

Abundance of MPs by Location on the Mississippi Gulf Coast

Oysters from the ten sites ranged from a high of 30.7 ± 11.5 to a low of 4.7 ± 0.25 putative MPs/g of oyster tissue (Figure 4). Previous studies have shown that the proximity of oyster reefs to urban areas increases the abundance of MPs retained (Li et al. 2018; Cho et al. 2021). Though the most urban sites near Biloxi did have higher MP concentrations, there was only a moderate correlation (Pearson's coefficient = 0.61, $p = 0.059$) between the number of putative MPs/g of tissue and city population observed in this study. However, there are many factors that influence the circulation inside bays that may also influence MP concentrations and residence time in the water column. Further, both Bay St. Louis (Sites 1-4) and Biloxi Bay (Sites 5-6) had collection sites located inside the bay and at the mouth of the bay, where they would be more exposed to open waters of the Gulf of Mexico. For Bay St. Louis, Sites 1 and 2 at the mouth of the bay did not have statistically significant differences in putative MP concentrations compared to Sites 3 and 4, but they did have lower concentrations (Table 2). Although this seemingly contrasts with previous work, which showed that the waters inside Bay St. Louis had lower MP concentrations than sites located directly on the Gulf, the prior work was conducted during an historic flooding event when freshwater from the Mississippi River was diverted through Lake Pontchartrain into the western Mississippi Sound, including Bay St. Louis (Gledhill et al. 2020; Scircle et al. 2020b).

Our data include an anomalously low concentration at Site 7 located within Biloxi Bay. Site 6, located deep within the bay had the highest MP concentration in this study (30.7 ± 11.5 putative MPs/g of tissue), while Site 5, near open water at the mouth of the bay, had an average putative MP concentration of approximately half that (13.7 ± 1.82 putative MPs/g of tissue). However, Site 7 is also located within the bay but had a significantly ($p = 0.007$) lower MP concentration (5.8 ± 2.2 putative MPs/g of tissue) compared to Site 6. Unlike Site 5, Site 7 is located within the Old Fort Bayou Coastal Preserve that runs into Biloxi Bay and is likely exposed to lower salinity and less polluted water, probably resulting in this site's low average MP concentration.

While not all of the sites had statistically significant differences in their average MP concentration, a one-way ANOVA analysis (df =

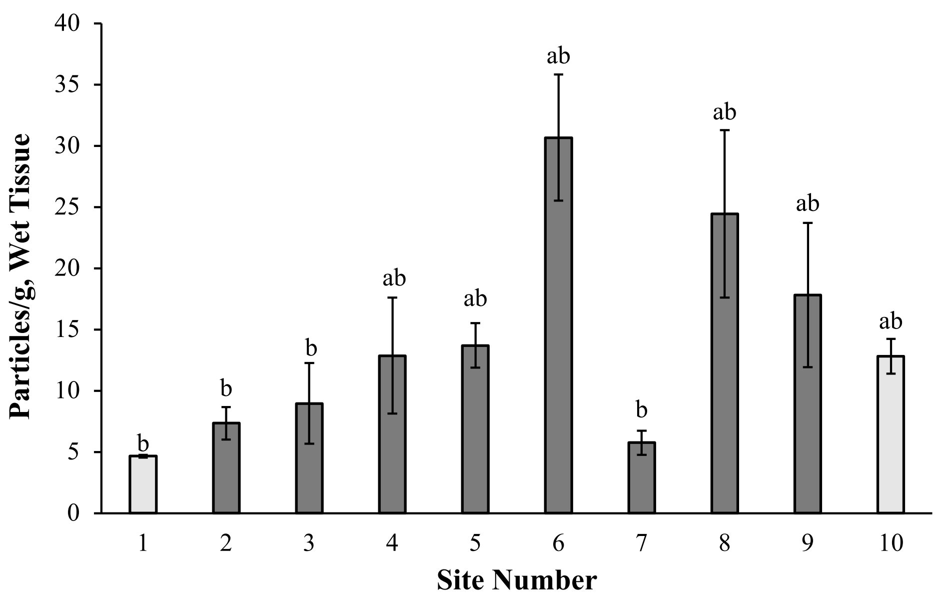


Figure 4. Mean number of putative microplastics per gram of whole oyster tissue by location (n = 5 for each location). Lander sites depicted in light gray, MSOGP in dark gray. Error bars = ± one standard error. Different letters denote means that are significantly different determined via one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$).

40, $p = 0.0113$) followed by Tukey's HSD post-hoc tests showed that some did. Specifically, Site 6 was significantly different than Sites 1, 2, 3, and 7 ($p = 0.018$, 0.005, 0.036, and 0.009, respectively). This suggests that Biloxi Bay and Bay St. Louis do represent distinct environments when it comes to MP concentrations in oysters, potentially because the Bay St. Louis area has a population of roughly one third of the population of Biloxi. Larger populations usually result in a larger amount of plastic waste. When such waste is mismanaged, MPs can find their way into water systems due to stormwater runoff and both household and industrial wastewater. As the Biloxi area has both a larger population and more roadways than the Bay St. Louis area, it is not surprising to see higher MP concentrations in oysters from those sites.

As shown in Figure 5, a two-way ANOVA and Tukey's HSD on the dissected oyster tissue samples showed statistically significant differences when used to assess the effect of both location and tissue type on MP concentration in oysters (tissue type: $df = 3$, $p = 0.0009$; site: $df = 2$, $p = 0.005$; interaction between tissue type and site: $df = 6$, $p < 0.001$). This indicates that there is a large interaction between the tissue in which the MPs localize and the site at which the oyster was located. Post hoc testing showed that Site 2 was significantly different from Sites 5 and 10 ($p = 0.0007$ and 0.031). However, because Sites 2 and 10 represent MBRACE lander samples and Site 5 is a MSOGP site, these differences could possibly stem from the different durations in the field or oyster age instead of true site differences.

A two-way ANOVA did show statistically significant differences in the interaction between MP sizes and sampling sites (sites: $df = 6$, $p < 0.001$; size range: $df = 3$, $p = < 0.001$; interaction between size range and site: $df = 18$, $p < 0.001$). Consistent with most MP studies, most of the putative MPs retained were in the smallest size fraction of 30-90 µm (80%) (Figure 6) (Li et al. 2018; Cho et al. 2021; Dehm et al. 2022). The larger size fractions of 90-125 µm, 125-250 µm, and >250 µm, contained 8%, 9%, and 3% of the putative MPs, respectively. While MPs of

Table 2. Oyster condition index and putative microplastic concentrations. * = lander sites.

Site	------------ Condition Index ------------			---- Concentration (MPs/g tissue, ww) ----		
	Average	Standard Error	n	Average	Standard Error	n
1*	14.9	0.49	6	4.66	0.11	5
2	13.7	0.37	11	7.35	1.32	5
3	9.30	0.95	10	8.97	3.30	5
4	9.98	0.65	11	12.9	4.73	5
5	15.6	0.70	12	13.7	1.82	5
6	12.3	0.37	13	30.7	5.14	5
7	11.6	1.17	11	5.76	0.98	5
8	10.7	0.73	11	24.5	6.83	5
9	10.8	0.31	6	17.8	5.90	5
10*	10.0	1.69	7	12.8	1.42	5

all of these sizes are believed to be too large to translocate through tissue, it is worrying that the number of smaller MPs is so much higher than the larger size classes. It is likely that there are even more MP particles in the <30 μm range. While the methodology utilized in this study was not able to measure them, <10 μm MPs are of special concern as they can translocate and may cause damage to oyster tissue (Teng et al. 2021).

Abundances of MPs by Tissue

To assess the risk of MPs to oyster health, it is necessary to determine whether MPs localize in specific tissues, and if so, which ones. To that end, oysters from Sites 2 (St. Stanislaus), 5 (Biloxi Bay), and 10 (Grand Bay) were dissected and their gills, mantles, digestive systems, and adductor muscles/hearts were analyzed separately (Figure 5). The mantle showed the highest average number of MPs (15.9 ± 13.4 putative MPs/g of tissue). The gills and adductor muscle/heart tissues exhibited very similar levels of MPs, with 11.5 ± 8.6 and 12.8 ± 6.7 putative MPs/g of tissue, respectively. The digestive system had much lower levels of MPs, with an average of 6.8 ± 6.1 putative MPs/g of tissue. As these samples had come from multiple

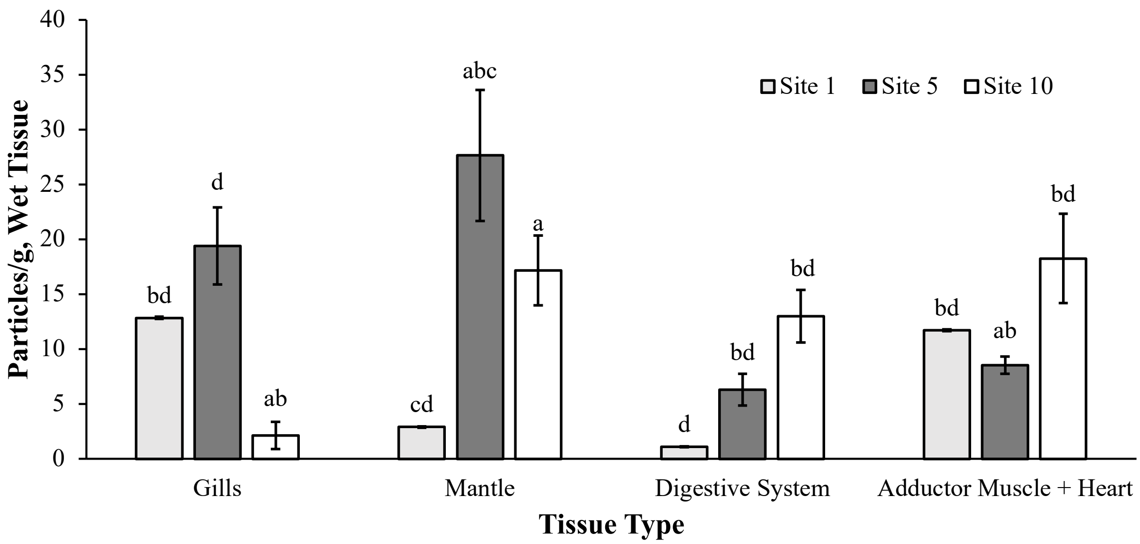


Figure 5. Average number of microplastics per gram of wet tissue by type of tissue (n = 5 oysters from each site, n = 15 for combined data). Each oyster was dissected and analyzed as four separate tissues. Landers data depicted in light gray and white; MSOGP site data in dark gray. Error bars = ± one standard error.

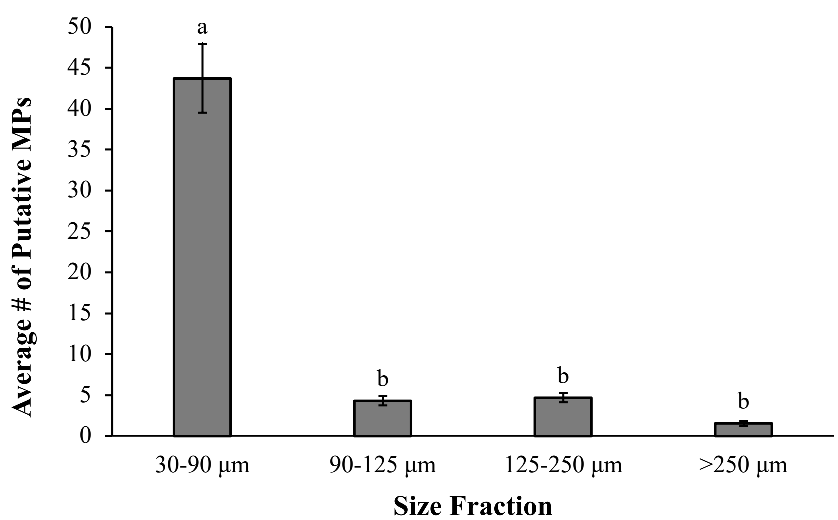


Figure 6. Size distribution of microplastics in oysters (n = 2278 putative MPs). Error bars = ± one standard error.

sites, a two-way ANOVA analysis followed by Tukey's HSD was used to identify the effects of site and tissue type on MP concentrations. Results showed that only the differences between the mantle and digestive system means were statistically significant ($p = 0.0025$). Interestingly, these results also indicated a significant interaction in MP concentrations between the oyster's site of collection and tissue type. At first glance this may seem odd, as we hypothesized that contaminants localize in the same tissue regardless of where an organism is located. However, to understand these results, one must contend with the fundamental nature of MPs as contaminants.

Unlike more traditional contaminants, MPs are not a single element or compound but rather a diverse suite of contaminants. MPs may be made up of many different sizes, shapes, and polymer types, as well as having a variety of chemical additives. Each of these factors could contribute to which tissue the particle ultimately associates with. Moreover, as each site presumably has its own composition of MP particles present in the surrounding waters (Scircle et al. 2020b), it is not surprising that oysters from different locations have putative MPs localizing in different tissues dependent on local MP composition.

As the gills, mantle, and adductor muscle are all exposed to the surrounding water to varying degrees, it is perhaps to be expected that they exhibit higher levels of MPs than the internal digestive system. While it has been shown that smaller (<10 μm) particles can be translocated across tissues in mussels (Browne et al. 2008), this study targeted larger (>30 μm) MPs that are unlikely to translocate. Thus, MPs associated with the gills, mantle, and adductor muscle are likely adhering to the outside of the tissue instead of being embedded within them. Moreover, because the oysters were rinsed with site water in the field, these MPs appear to adhere relatively strongly. Thus, the digestive system tissue represents the best choice for studies targeting MPs consumed by the oysters. Such samples offer a "snapshot" of the particles the oyster had consumed at harvest. Targeting MPs in the digestive system is also important because the MPs enter an environment with substantially different conditions (pH, enzymes, etc.) that may promote desorption and leaching of chemical contaminants from the MPs and that may cause fragmentation, further reducing the size of the MPs. Average MP concentrations in the digestive system were only slightly lower than those reported for whole oysters in China (Li et al. 2018) and were much higher than concentrations reported in oysters and mussels off the coast of Korea (Cho et al. 2021), suggesting that Mississippi oysters have higher overall concentrations than those previously studied.

MP Compositions and Study Limitations

A limitation with observing MPs by fluorescence microscopy is that it does not yield any chemical information that can be used to definitively identify the MP particle. One study comparing results from fluorescence microscopy and μ-FTIR found that fluorescence microscopy overestimates MP abundance by 18-75% (de Guzman et al. 2022). While we sought to address this issue through an automated counting method and a conservative selection approach, it is possible that our counts may still represent overestimates of MP abundances.

Thus, we are currently analyzing the samples used for this work by FTIR microscopy to confirm the particle counts and identify the polymers comprising the MPs. Whereas this will be the subject of a future report, five oysters from two Sites (6 and 7) were analyzed at the time of writing. Our results show that polyurethane, polyethylene, and polyamide are the most common types of MPs in the oysters. Figure 7 depicts a representative sample from this set. A two-way ANOVA did not reveal any statistical differences in polymer types between the two sites (site: $df = 1$, $p = 0.323$; polymer type: $df = 20$, $p = 0.065$; interaction between polymer type and site: $df = 20$, $p = 0.331$). However, as only a portion of each filter was scanned, these results should be considered preliminary.

Because MPs may be unevenly distributed on the filter and because we were unable to scan the entire filter, we cannot yet compare the number of MPs detected by the two methods (fluorescence and μ-FTIR) or make true comparisons between the two sites. Future work will widen the scan area and determine the full MPs profile (abundance, type, size, and shape) across sample sites and for individual tissues.

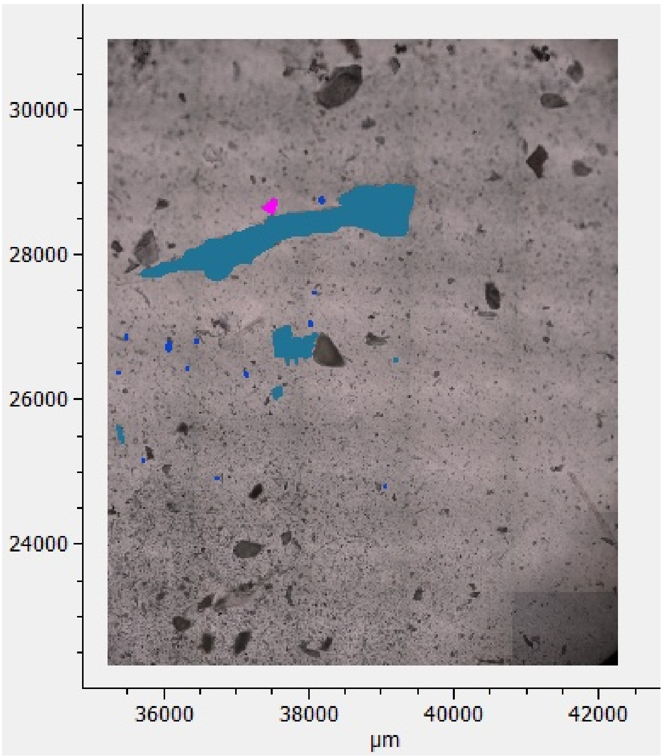


Figure 7. Portion of an Anodisc filter containing microplastics extracted from a Site 6 oyster. Color overlay denotes polymer identity, with light blue signifying polyurethane, dark blue denoting polyamide, and pink representing polyester. Other particles were not identified as plastics. Image created using Purency v4.07.

Conclusions

This study demonstrated that MPs (>30 µm) are retained in relatively high concentrations by Gulf of Mexico oysters along the Mississippi Coast. This is concerning due to the negative impact MPs are known to have on oysters, which are a foundational species for oyster reef ecosystems. Oysters from locations inside bays nearer population centers showed higher average numbers of MPs than those outside of bays, with average concentrations ranging from 30.7 ± 11.5 to 4.7 ± 0.25 putative MPs/g ww of whole tissue. Due to the relatively low concentrations of MPs in the digestive system tissues (6.8 ± 6.1 MPs/g ww of tissue), it appears that most MPs in the oysters are likely adhering to tissues exposed directly to the surrounding water, with lower numbers being ingested. However, given that predators and humans often consume the entire oyster, where MPs are located may not make a difference from a risk standpoint. It remains to be seen if more rigorous washing of oysters can dislodge adhering MPs and decrease MP loads in oysters destined for human consumption. Most of the putative MPs belong to the smallest size fraction studied (30-90 µm). This result is similar to most other MP studies but is still concerning due to potentially higher toxicities of smaller particles. Results from micro-spectroscopy of the extracted MPs indicate that polyester, polyethylene, and polystyrene are the most common types of MPs in the oysters, which is not surprising given their widespread occurrence in the environment. However, more study is needed to fully characterize the MP composition across all sites. Overall, this study demonstrates that MPs are accumulating in the tissues of Gulf Coast oysters, which are consumed by both humans and wildlife.

Recommendations and Policy Implications

While the state of Mississippi does have regulations covering plastic waste disposal in marine waters (Mississippi Code R 2006), this research shows that the current legislation is not sufficient to protect oysters in those waters from MP pollution. One reason for this is that the code only targets plastic disposal from water-going vessels and nearby access areas. Our previous research has shown that the Mississippi River system acts as a funnel for MPs, concentrating and transporting them into nearshore waters of the Gulf of Mexico (Scircle et al. 2020a). As such, current regulations neglect other key sources of MPs and are insufficient to reduce MP pollution in Gulf of Mexico waters.

In order to reduce exposure of Mississippi's oysters to MPs, additional legislation would need to both account for additional sources of MP pollution, such as residential and commercial wastewater and storm-water runoff, as well as be broad enough to encompass all waters flowing into the northern Gulf of Mexico. This brings up two major issues from a legislative perspective. The first is that such legislation may be difficult to enact at a local level. For example, while only covering one potential source of plastic pollution, current Mississippi state law prevents local municipalities from instituting bans or fees on the use of plastic bags (Mississippi State Legislature 2018). Additionally, Mississippi legislation can only regulate the waters within the state itself. While further legislation may be needed to address MP pollution in Mississippi, such legislation will be ineffective if similar regulations do not govern MP pollution in states upstream of the Mississippi watershed, particularly those on the Mississippi River and its tributaries. While Mississippi may pose an interesting example of this problem due to the impact of MPs on the state's oyster populations, it is far from the only state facing this issue. As such, lawmakers need to consider federal legislation to address both macro- and MP pollution in river systems at a national and global level.

Whereas the problem of MP pollution is ever growing, so too is the awareness of this issue and willingness to address plastic pollution. Recently, Mississippi passed legislation encouraging growth in its recycling sector by recognizing it as a business, not as solid waste disposal (Mississippi State Legislature 2022). While it is far too early to assess what impact this will have on MP concentrations in the Gulf of Mexico, one would hope that an increased focus on recycling could help decrease the number of plastics and ultimately MPs reaching Gulf Coast waters. Further study is needed to evaluate how shifting attitudes and new laws regarding plastic disposal affect MP concentrations in Mississippi oysters.

Acknowledgements

Sincere thanks to P.J. Waters, Rayne Palmer, and volunteer gardeners in the MSOGP, the Mississippi Department of Marine Resources, Megan Gima at the Thad Cochran Marine Aquaculture Center, and Letha Boudreaux and interns at the Saint Stanislaus High School Marine Science Program for help with oyster sampling. We are also grateful to Greg Easson and Alex Warren for access to the landers; Nicole Ashpole at the University of Mississippi for the use of the fluorescence microscope; and Klara Missling for assistance in developing the oyster digestion method.

Funding

This project was supported by USGS 104g Grant #G16AP00065 and NSF Grant MRI-2116597. The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the USGS. Mention of trade names or commercial products does not constitute their endorsement by the USGS.

Additional support was provided by a Disaster Resilience Constellation Seed Grant from the University of Mississippi, and with federal funding from the U.S. Department of the Treasury and the Mississippi Department of Environmental Quality, and the Mississippi Based RESTORE Act Center of Excellence under the Resources and Ecosystems Sustainability, Tourist Opportunities, and Revived Economies of the Gulf Coast States Act of 2012 (RESTORE Act). The statements, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect the views of either the Department of the Treasury or the Mississippi Department of Environmental Quality, or the Mississippi Based RESTORE Act Center of Excellence.

Disclaimer

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Author Bio and Contact Information

Kendall Wontor is currently pursuing her Ph.D. in Chemistry through the Department of Chemistry and Biochemistry at the University of Mississippi. Her research focuses on method development for analyses of microplastics in environmental and biological samples. She can be reached at 377 Coulter Hall, University, MS 38677 or kwontor@go.olemiss.edu.

Dr. James Cizdziel (corresponding author) is a professor in the Department of Chemistry and Biochemistry at the University of Mississippi. His research interests include analytical, environmental, and forensic chemistry. He has authored >75 papers and has been funded by the EPA, DOE, NSF, and DOJ. He teaches courses in analytical chemistry, instrumental analysis, and spectroscopy, and enjoys working with students to develop new analytical methods or applying methods in novel ways. He can be reached at 380 Coulter Hall, University, MS 38677 or cizdziel@olemiss.edu.

Dr. Deborah Gochfeld is a Principal Scientist in the National Center for Natural Products Research and Research Professor in Environmental Toxicology in the Department of BioMolecular Sciences at the University of Mississippi. Trained as a marine ecologist, her research interests include the effects of environmental stressors on marine organisms and communities. She has authored numerous papers and has been funded by MBRACE, NSF, NOAA, NASA, and NIH. Her research combines lab and fieldwork, and has taken her and her students all over the world. She can be reached at 2014 Thad Cochran Research Center, University, MS 38677 or gochfeld@olemiss.edu.

Ann Fairly Pandelides is currently LO-SPAT Project Manager in the Department of Biology at the University of Louisiana at Lafayette, and previously worked as R&D Biologist in the Environmental Toxicology Research Program in the Department of BioMolecular Sciences at the University of Mississippi. Her prior research at UM focused on how climate change-related stressors affect the health and survival of oysters at varying life history stages, both in the lab and the field. She can be reached at 244 Billeaud Hall, Lafayette, LA 70504 or ann.pandelides@louisiana.edu.

Austin Scircle is a research chemist with the U.S. Army Corps of Engineers at the Engineer Research and Development Center (ERDC) in Vicksburg, Mississippi. He received his Ph.D. in analytical chemistry from the University of Mississippi where his research focused on microplastic pollution in the Mississippi River and the Mississippi Sound Estuary. He can be reached at U.S. Army Corps of Engineers, Engineer Research and Development Center, Environmental Laboratory, 3909 Halls Ferry Rd., Vicksburg, MS 39180 or austin.r.scircle@usace.army.mil.

References

Abbe, G.R. and B.W. Albright. 2003. An improvement to the determination of meat condition index for the eastern oyster *Crassostrea virginica* (Gmelin 1791). *Journal of Shellfish Research* 22(3): 747-752.

Beck, M.W., R.D. Brumbaugh, L. Airoldi, A. Carranza, L.D. Coen, C. Crawford, O. Defeo, G.J. Edgar, B. Hancock, M.C. Kay, H.S. Lenihan, M.W. Luckenbach, C.L. Toropova, G. Zhang, and X. Guo. 2011. Oyster reefs at risk and recommendations for conservation, restoration, and management. *Bioscience* 61(2): 107-116. Available at: https://doi.org/10.1525/bio.2011.61.2.5. Accessed December 7, 2022.

Browne, M.A., A. Dissanayake, T.S. Galloway, D.M. Lowe, and R.C. Thompson. 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environmental Science & Technology* 42(13): 5026-5031. Available at: https://doi.org/10.1021/es800249a. Accessed December 7, 2022.

Casas, S., W. Walton, G. Chaplin, S. Rikard, J. Supan, and J. La Peyre. 2017. Performance of oysters selected for dermo resistance compared to wild oysters in northern Gulf of Mexico estuaries. *Aquaculture Environment Interaction* 9: 169-180. Available at: https://doi.org/10.3354/aei00222. Accessed December 7, 2022.

Cho, Y., W.J. Shim, M. Jang, G. Han, and S. Hong. 2021. Nationwide monitoring of microplastics in bivalves from the coastal environment of Korea. *Environmental Pollution* 270: 116175. Available at: https://doi.org/10.1016/j.envpol.2020.116175. Accessed December 7, 2022.

De Guzman, M.K., M. Andjelković, V. Jovanović, J. Jung, J. Kim, L.A. Dailey, A. Rajković, B. De Meulenaer, and T.Ć. Veličković. 2022. Comparative profiling and exposure assessment of microplastics in differently sized Manila clams from South Korea by µFTIR and Nile Red Staining. *Marine Pollution Bulletin* 181: 113846. Available at: https://doi.org/10.1016/j.marpolbul.2022.113846. Accessed December 7, 2022.

Dehm, J., M. Volau, E. Ledua, and C. Hewavitharane. 2022. Occurrence of microplastics within a freshwater aquaculture system in the Pacific Islands, Viti Levu, Fiji. *Environmental Monitoring and Assessment* 194: 624. Available at: https://doi.org/10.1007/s10661-022-10305-3. Accessed December 7, 2022.

Di Mauro, R., M.J. Kupchik, and M.C. Benfield. 2017. Abundant plankton-sized microplastic particles in shelf waters of the northern Gulf of Mexico. *Environmental Pollution* 230: 798-809. Available at: https://doi.org/10.1016/j.envpol.2017.07.030. Accessed December 7, 2022.

Ding, J., F. Jiang, J. Li, Z. Wang, C. Sun, Z. Wang, L. Fu, N.X. Ding, and C. He. 2019. Microplastics in the coral reef systems from Xisha Islands of South China Sea. *Environmental Science & Technology* 53(14): 8036-8046. Available at: https://doi.org/10.1021/acs.est.9b01452. Accessed December 7, 2022.

Dodson, G.Z., A.K. Shotorban, P.G. Hatcher, D.C. Waggoner, S. Ghosal, and N. Noffke. 2020. Microplastic fragment and fiber contamination of beach sediments from selected sites in Virginia and North Carolina, USA. *Marine Pollution Bulletin* 151: 110869. Available at: https://doi.org/10.1016/j.marpolbul.2019.110869. Accessed December 7, 2022.

Gardon, T., C. Reisser, C. Soyez, V. Quillien, and G. Le Moullac. 2018. Microplastics affect energy balance and gametogenesis in the pearl oyster *Pinctada margaritifera*. *Environmental Science & Technology* 52(9): 5277-5286. Available at: https://doi.org/10.1021/acs.est.8b00168. Accessed December 7, 2022.

Gledhill, J.H., A.F. Barnett, M. Slattery, K.L. Willett, G.L. Easson, S.S. Otts, and D.J. Gochfeld. 2020. Mass mortality of the eastern oyster *Crassostrea virginica* in the western Mississippi Sound following unprecedented Mississippi River flooding in 2019. *Journal of Shellfish Research* 39(2): 235-244. Available at: https://doi.org/10.2983/035.039.0205. Accessed December 7, 2022.

Keisling, C., R.D. Harris, J. Blaze, J. Coffin, and J.E. Byers. 2020. Low concentrations and low spatial variability of marine microplastics in oysters (*Crassostrea virginica*) in a rural Georgia estuary. *Marine Pollution Bulletin* 150: 110672. Available at: https://doi.org/10.1016/j.marpolbul.2019.110672. Accessed December 7, 2022.

Leonhardt, J., S. Casas, J. Supan, and J. La Peyre. 2017. Stock assessment for eastern oyster seed production and field grow-out in Louisiana. *Aquaculture* 466: 9-19. Available at: https://doi.org/10.1016/j.aquaculture.2016.09.034. Accessed December 7, 2022.

Li, H.-X., L.-S. Ma, L. Lin, Z.-X. Ni, X.-R. Xu, H.-H. Shi, Y. Yan, G.-M. Zheng, and D. Rittschof. 2018. Microplastics in oysters *Saccostrea cucullata* along the Pearl River Estuary, China. *Environmental Pollution* 236: 619-625. Available at: https://doi.org/10.1016/j.envpol.2018.01.083. Accessed December 7, 2022.

"Mississippi – Oyster Gardening on the Gulf Coast." n.d. Available at: https://oystergardening.org/mississippi/. Accessed December 7, 2022.

Mississippi Code R. 2006. §22-10-04-100 Rules and Regulations to Prohibit the Disposal of Plastics and Other Garbage in Marine Waters of the State of Mississippi.

Mississippi State Legislature. 2018. Senate Bill 2570 An Act to Prohibit Local Governments from Adopting or Enforcing an Ordinance that Regulates the Use, Disposition or Sale of, or Prohibits, Restricts or Imposes any Fee, Charge or Tax on, Certain Auxiliary Containers; and for Related Purposes.

Mississippi State Legislature. 2022. House Bill 1135 An Act to Amend Section 17-17-3, Mississippi Code Of 1972.

Ridlon, A.D., A. Marks, C.J. Zabin, D. Zacherl, B. Allen, J. Crooks, G. Fleener, E. Grosholz, B. Peabody, J. Toft, and K. Wasson. 2021. Conservation of marine foundation species: Learning from native oyster restoration from California to British Columbia. *Estuaries and Coasts* 44: 1723-1743. Available at: https://doi.org/10.1007/s12237-021-00920-7. Accessed December 7, 2022.

Scircle, A., J.V. Cizdziel, K. Missling, L. Li, and A. Vianello. 2020a. Single-pot method for the collection and preparation of natural water for microplastic analyses: Microplastics in the Mississippi River System during and after historic flooding. *Environmental Toxicology and Chemistry* 39(5): 986-995. Available at: https://doi.org/10.1002/etc.4698. Accessed December 7, 2022.

Scircle, A., J.V. Cizdziel, L. Tisinger, T. Anumol, and D. Robey. 2020b. Occurrence of microplastic pollution at oyster reefs and other coastal sites in the Mississippi Sound, USA: Impacts of freshwater inflows from flooding. *Toxics* 8(2): 35. Available at: https://doi.org/10.3390/toxics8020035. Accessed December 7, 2022.

Shen, M., W. Huang, M. Chen, B. Song, G. Zeng, and Y. Zhang. 2020. (Micro)plastic crisis: Un-ignorable contribution to global greenhouse gas emissions and climate change. *Journal of Cleaner Production* 254: 120138. Available at: https://doi.org/10.1016/j.jclepro.2020.120138. Accessed December 7, 2022.

Suaria, G., V. Perold, J.R. Lee, F. Lebouard, S. Aliani, and P.G. Ryan. 2020. Floating macro- and microplastics around the Southern Ocean: Results from the Antarctic Circumnavigation Expedition. *Environment International* 136. Available at: http://www.sciencedirect.com/science/article/pii/S0160412019335962. Accessed December 7, 2022.

Sussarellu, R., M. Suquet, Y. Thomas, C. Lambert, C. Fabioux, M.E.J. Pernet, N. Le Goïc, V. Quillien, C. Mingant, Y. Epelboin, C. Corporeau, J. Guyomarch, J. Robbens, I. Paul-Pont, P. Soudant, and A. Huvet. 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. *Proceedings of the National Academy of Sciences* 113(9): 2430-2435. Available at: https://doi.org/10.1073/pnas.1519019113. Accessed December 7, 2022.

Teng, J., J. Zhao, X. Zhu, E. Shan, C. Zhang, W. Zhang, and Q. Wang. 2021. Toxic effects of exposure to microplastics with environmentally relevant shapes and concentrations: Accumulation, energy metabolism and tissue damage in oyster *Crassostrea gigas*. *Environmental Pollution* 269: 116169. Available at: https://doi.org/10.1016/j.envpol.2020.116169. Accessed December 7, 2022.

Thiele, C.J., M.D. Hudson, and A.E. Russell. 2019. Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size. *Marine Pollution Bulletin* 142: 384-393. Available at: https://doi.org/10.1016/j.marpolbul.2019.03.003. Accessed December 7, 2022.

Thomas, M., B. Jon, S. Craig, R. Edward, H. Ruth, B. John, V.A. Dick, L.A. Heather, and S. Matthew. 2019. The world is your oyster: Low-dose, long-term microplastic exposure of juvenile oysters. *Heliyon* 6(1): e03103. Available at: https://doi.org/10.1016/j.heliyon.2019.e03103. Accessed December 7, 2022.

Wessel, C., G. Lockridge, D. Battiste, and J. Cebrian. 2016. Abundance and characteristics of microplastics in beach sediment: Insights into microplastic accumulation in northern Gulf of Mexico estuaries. *Marine Pollution Bulletin* 109(1): 178-183. Available at: https://doi.org/10.1016/j.marpolbul.2016.06.002. Accessed December 7, 2022.

Universities Council on Water Resources
Journal of Contemporary Water Research & Education
Issue 177, Pages 46-62, April 2023

Case Study Article

Informing Volunteer Water Quality Monitoring Program Design and Watershed Planning: Case Study of StreamSmart Data Analysis in the Upper White River Basin, Arkansas

*Erin Grantz[1], Brian E. Haggard[1,2], and BENG 4973/5973[2]

[1]*Arkansas Water Resources Center, University of Arkansas Division of Agriculture, Fayetteville, AR*
[2]*Biological and Agricultural Engineering Department, University of Arkansas, Fayetteville, AR*
**Corresponding Author*

Abstract: The watershed group H_2Ozarks founded the StreamSmart Citizen Science Program to establish baseline and long-term water quality data for the Upper White River Basin, Arkansas. StreamSmart volunteers collect water samples and conduct habitat and macroinvertebrate community assessments at >20 sites across a land use-land cover (LULC) gradient. Since 2020, H_2Ozarks has adaptively assessed the program to ensure that the investment in water quality data meets core goals, with particular interest in planning tools and aligning expectations of volunteer effort with the level of training and support. Study objectives were to use StreamSmart data to 1) facilitate understanding of water quality response to stressors in the basin using a range of methods (Spearman rank correlation, non-parametric changepoint analysis, and categorical and regression tree analysis) and 2) explore implications for program design and watershed planning. Water chemistry-LULC relationships were in-line with prior regional studies, as well as global patterns. Detected thresholds and hierarchy provide potential targets for managing LULC change to protect water quality, but further analysis is warranted to refine these relationships. Macroinvertebrate stressor-response was most detectable for sensitive and less sensitive taxa and for habitat index components, suggesting potential to streamline these programmatic elements. Study findings for StreamSmart should also be informative for other small-scale volunteer monitoring programs with limited resources, but which actively evaluate the types of data and program activities that yield a maximum scientific return on investment.

Keywords: *Volunteer monitoring, water quality, watershed management*

Human activities in watersheds influence the quality of adjacent and downstream water resources for beneficial uses like drinking water, aquatic life habitat, and recreation. At both global and local scales, greater extent of anthropogenic land use-land cover (LULC) types like urban and agriculture in watersheds is correlated with greater levels of nutrients, sediments, and salts in connected water bodies (Giovanetti et al. 2013; Lintern et al. 2017). Point sources, such as industrial or municipal wastewater discharges, also play a role, though these inputs are more easily quantified, regulated, and mitigated compared to non-point sources (Haggard 2010; Scott et al. 2011).

The water quality effects of human activities in watersheds extend beyond water chemistry, with in-stream and riparian habitat quality often becoming less stable and complex as watershed disturbance increases, supporting fewer sensitive species (White and Walsh 2020). The combined effects of water chemistry changes and habitat quality loss compound in the biological community response, with shifts to increased densities of individuals from pollution-tolerant taxa and overall reduced taxonomic richness (Xu et al. 2013).

It is increasingly recognized that a broad array of stakeholders must be mobilized to effectively address the effects of human activities on water

Research Implications

- StreamSmart water chemistry data responded to land use-land cover (LULC) gradients, most notably human development index thresholds and hierarchy that may provide useful targets for watershed planning.
- Highly predictive water chemistry-LULC relationships suggest that StreamSmart data can be combined with other datasets in knowledge "co-creation" around watershed management and planning.
- Macroinvertebrate and habitat stressor-response relationships were most detectable when considering sensitive groups and habitat components, like epifaunal substrate/cover, riffle/bend frequency, and channel flow status.
- Relationships between sensitive groups and habitat components may reflect volunteer biases, but also present an opportunity for StreamSmart to collect the same information with less volunteer time and effort.

quality (USEPA 2005). Watershed management planning is a stakeholder-driven process that takes a holistic approach to water quality protection and restoration in a specific watershed (USEPA 2008). Water quality monitoring is a core component of a watershed management plan, both to establish baseline conditions and to collect real-time information as watersheds evolve. Citizen science programs have an established history in water resources research and management, including water quality monitoring (Buytaert et al. 2014). These programs can be an entry point for stakeholders to community involvement and education around watershed management (Savan et al. 2003; Storey et al. 2016), and have been shown to produce water quality data of comparable quality to professionally collected datasets (Hoyer and Canfield 2021).

In Northwest Arkansas, the watershed group H_2Ozarks (formerly Ozark Water Watch) seeks to increase stakeholder awareness of water quality and watershed function by engaging the public in the StreamSmart Citizen Science Program. StreamSmart leverages volunteer monitoring to establish a baseline water quality database for the Upper White River Basin. The volunteers collect water samples and assess habitat quality and the aquatic macroinvertebrate community at more than 20 sites. The Upper White River Basin is rapidly urbanizing (NWARPC 2016) and is also the source water area for Beaver Lake, which provides drinking water for ~1 in 6 Arkansans. StreamSmart complements monitoring by other entities by providing more granular coverage of the basin.

Since 2020, StreamSmart has been adaptively assessing the program to ensure that the investment in water quality data meets core goals. The primary goal is to inform stakeholders about current water quality and any potential changes in the basin. But, H_2Ozarks and its partners, the Beaver Water District's source water protection program and the Beaver Watershed Alliance, also want to use StreamSmart data to inform nutrient reduction strategies, such as siting best management practices. Further, program changes have focused on making sure the expected volunteer time and effort investment matches the level of support that the group can provide. Training is an essential component of citizen science program success (Nerbonne and Vondracek 2003; Lewandoski and Specht 2015; San Llorente Capdevila et al. 2020), and the current StreamSmart training and staffing levels may not be sufficient for reliably generating complex data such as macroinvertebrate or habitat assessments (Fore et al. 2001). Volunteer interests and desired time investment are also considerations. Future changes may include scaling back macroinvertebrate work, and habitat assessments have already been discontinued.

The Arkansas Water Resources Center has provided lab services funded through Section 104(b) of the Water Resources Research Act of 1984 and technical support of volunteer training since StreamSmart began. In this study, we conducted a comprehensive stressor-response analysis of the StreamSmart volunteer water quality monitoring database. The study objectives were to 1) facilitate understanding water quality response to stressors in the basin using a range of stressor-response methods and 2) explore potential implications of study findings for the StreamSmart program design and watershed planning. Study findings for StreamSmart should also be informative for other volunteer monitoring programs of similar size, scale, and resource availability in evaluating, or re-evaluating, the types of data to collect for maximum return on investment.

Methods

Study Location and Site Characteristics

StreamSmart was founded in 2012 to monitor water quality in the Upper White River Basin (Figure 1). The basin is primarily forested (>60%), and pasture agriculture is the predominant anthropogenic LULC. However, rapid urbanization is also occurring, typically on prior pasture lands. StreamSmart volunteers have collected water samples, conducted habitat assessments, and collected information on aquatic macroinvertebrates at 23 sites since the program began (Table 1), with 14 sites currently active. StreamSmart selects and maintains core monitoring locations to encompass gradients of LULC types. Volunteers are required to attend a half-day comprehensive training covering best

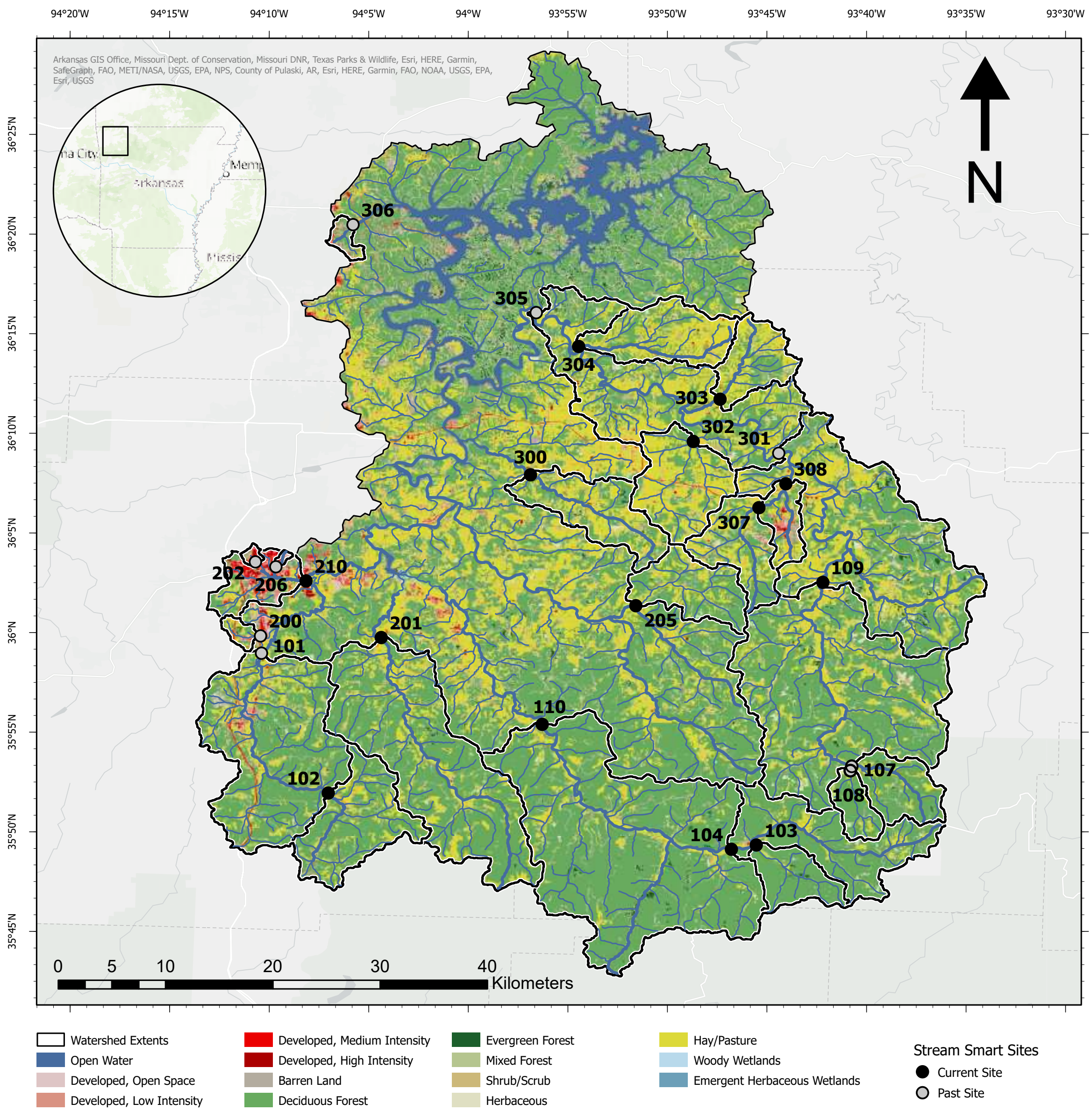


Figure 1. Map of the study area, the Upper White River Basin located in Northwest Arkansas, showing distribution of land use-land cover (LULC) characteristics in the watershed (Dewitz and USGS 2021), as well as the locations of StreamSmart volunteer monitoring sites (2012 – present).

practices for collecting water samples, kicking for macroinvertebrates, and habitat assessment terminology. The training includes a brief on-site demonstration by water quality professionals.

This analysis used data from 21 sites and focused on the period 2012 – 2020. Two sites were not included; site 110 was not established until 2020, and site 308 has a point-source discharger in the watershed (City of Huntsville municipal wastewater treatment plant). Signals of point-source pollution were expected to confound analysis, as it is recognized that non-point sources are dominant in the watershed, including channel erosion and runoff from farms, unpaved roads, and urban or urbanizing areas (Perez et al. 2015).

StreamSmart site sub-watershed areas and LULC data (MRLC 2018) were obtained from https://modelmywatershed.org/. Summary LULC categories were calculated as the percentage of each site's sub-watershed area (Table 1). Agricultural land (%) was the sum of pasture/hay, grassland/herbaceous, and cultivated crops; forest land (%) was the sum of deciduous, evergreen, and mixed forest categories, as well as shrub/scrub; and urban land (%) was the sum of all developed and barren land categories. A human development index (%) for each site was calculated as the sum of agricultural and urban land. Locations of poultry houses were obtained from the Arkansas Highway and Transportation Department cultural features GIS database (Arkansas GIS Office 2014), and poultry house density (houses/km^2) was calculated as number of houses divided by sub-watershed area, for each site.

Table 1. StreamSmart monitoring site information and watershed land use-land cover (LULC) characteristics, including poultry house density, agricultural, forest, and urban land, as well as the human development index (HDI).

Site #	Hydrologic Unit Code 10 Name	Latitude	Longitude	Site sub-watershed area (km^2)	Poultry house density (houses/km^2)	Agriculture (%)	Forest (%)	Urban (%)	HDI (%)
101	West Fork	35.982714	-94.173129	215	0.49	25.3	66.9	7.0	32.3
102	West Fork	35.865723	-94.117257	65	0.92	26.3	68.1	5.1	31.5
103	Headwaters	35.822256	-93.758937	29	0.17	4.0	92.6	1.7	5.7
104	Headwaters	35.818676	-93.779774	106	0.26	10.9	84.7	3.7	14.6
107	War Eagle	35.888319	-93.679017	50	0.04	15.5	81.1	2.7	18.2
108	War Eagle	35.887989	-93.678974	17	0	13.5	84.3	1.5	15.1
109	War Eagle	36.041958	-93.703225	273	0.29	22.2	73.6	3.3	25.5
200	West Fork	35.997178	-94.173949	5	1.20	40.0	36.8	29.6	69.6
201	Middle Fork	35.995825	-94.072894	174	0.67	27.3	68.8	2.8	30.1
202	West Fork	36.059103	-94.178209	1.63	0	1.2	19.0	79.8	81.0
205	Richland	36.022453	-93.859784	43	0.61	33.1	62.7	3.1	36.2
206	West Fork	36.055019	-94.161107	1.13	0	0	0.9	99.1	99.1
210	West Fork	36.043179	-94.135852	31	0	15.5	33.4	49.2	64.7
300	Beaver Reservoir	36.131947	-93.947956	52	1.00	51.0	42.6	5.5	56.5
301	War Eagle	36.149997	-93.740137	525	0.46	30.7	63.6	4.8	35.6
302	War Eagle	36.159851	-93.81169	56	1.18	66.8	27.9	5.3	72.1
303	War Eagle	36.195153	-93.789276	32	1.28	59.8	34.3	5.3	65.0
304	War Eagle	36.239342	-93.907653	50	1.00	61.7	31.8	5.8	67.4
305	War Eagle	36.267597	-93.94313	808	0.68	39.5	54.7	4.9	44.4
306	Beaver Reservoir	36.341208	-94.096513	7	0	15.1	55.4	31.0	46.1
307	War Eagle	36.104418	-93.75675	42	0.74	43.2	48.6	7.2	50.4
308	War Eagle	36.124453	-93.734211	61	0.62	43.4	44.6	11.3	54.8

Sample Collection and Analysis

Water Chemistry. Volunteer teams collected grab water samples quarterly (February, May, August, and November) at each site. Each sampling event was carried out at all sites within a two-week timeframe during base flow conditions. Samples were collected from the thalweg while facing upstream from the access point and taking care not to capture any disturbed sediments. Clean and acid-washed sample bottles were provided by the Arkansas Water Resources Center Water Quality Lab and were triple rinsed at the stream by volunteers prior to sample collection. Samples were stored on ice and in the dark until being returned to the lab within 36 hours to allow processing within 48 hours. Chain of custody was documented at each step.

Water samples were analyzed at the lab using standard procedures for the following water chemistry variables: alkalinity (mg/L $CaCO_3$), conductivity (uS/cm), pH, total dissolved solids (TDS, mg/L), nitrate+nitrite-nitrogen (NO_x-N, mg/L), total nitrogen (TN, mg/L), total phosphorus (TP, mg/L), and total suspended solids (TSS, mg/L). Analytical methods, detection and reporting limits, preservation, holding times, and quality assurance details are available at https://awrc.uada.edu/water-quality-lab/certification-and-quality-assurance/. The lab is certified under the State Environmental Laboratory Certification Program by the Arkansas Department of Energy and Environment – Environmental Quality Division.

Habitat Quality Assessment. At each site visit, StreamSmart volunteers completed the USEPA Rapid Bioassessment Protocols for Habitat Assessment rubric (Barbour et al. 1999), which uses visual assessment of habitat quality for aquatic life use. The rubric includes ten components: 1) epifaunal substrate/available cover, 2) embeddedness, 3) velocity/depth regime, 4) sediment deposition, 5) channel flow status, 6) channel alteration, 7) frequency of riffles (or bends), 8) bank stability, 9) vegetative protection, and 10) riparian vegetative zone width. Descriptions were provided for 5-point intervals to score each component (0 – 20); component scores were summed into a habitat quality index score.

Aquatic Macroinvertebrate Community Index. During May and August site visits, volunteers collected aquatic macroinvertebrates for identification and community assessment. Stream riffle cross sections were sampled at an angle moving upstream at three locations, avoiding bridges and road crossings. Riffle locations were sampled by kicking into a D-frame net within a 1 m^2 area for one minute after first setting aside any large substrate in a collection tub. The net was rinsed with stream water into a container after each kick. Large substrate and net contents were examined for macroinvertebrates, which were removed for identification using StreamSmart's simplified flow chart.

The macroinvertebrate community index was designed by StreamSmart specifically for non-expert volunteers. Pre-defined taxonomic units, approximately at the Family level, were marked as present (1) or absent (0) in a rubric. Taxa were grouped into categories based on relative sensitivity to habitat and water quality degradation (i.e., sensitive, less sensitive, and tolerant). Sensitive taxa included caddisfly larvae, hellgrammites, mayfly nymphs, gilled snails, riffle beetle adult, stonefly nymphs, and water penny larvae. Less sensitive taxa were beetle larvae, clams, crane fly larvae, crayfish, damselfly nymphs, dragonfly nymphs, scuds, sowbugs, fishfly larvae, alderfly larvae, and watersnipe fly larvae. Tolerant taxa were aquatic worms, blackfly larvae, leeches, midge larvae, and pouch snails. The macroinvertebrate community index was the sum of the count of present taxa after weighting each sensitivity group using multipliers of 3, 2, and 1 for sensitive, less sensitive, and tolerant taxa, respectively. Site water quality was classified as excellent (≥22), good (17-22), fair (11-16), or poor (<11) based on the index score.

Data Analysis

Site medians were calculated for all water chemistry, habitat, and macroinvertebrate variables, including habitat components and macroinvertebrate sensitivity groups, for use in stressor-response analysis. Site median calculation and all subsequently described analyses were carried out using R 4.1.2 (R Core Team 2021). We explored stressor-response relationships using Spearman rank-order correlation and non-parametric changepoint analysis (nCPA) (King and Richardson 2003; Qian et al. 2003). Correlation is commonly used to describe monotonic water chemistry-LULC relationships, facilitating comparison with preceding studies in the basin (Giovanetti et al. 2013). However, these relationships can also be non-linear, such as thresholds stressor values associated with disproportionate water quality response. Non-parametric changepoint analysis divides data at a threshold value in the explanatory variable by minimizing deviance within groups. For a changepoint to be detected, groups on both sides of the threshold had to have at least three observations. For water chemistry, stressor-response analysis focused on LULC. For macroinvertebrates, potential stressors included water chemistry, habitat quality index and component scores, as well as LULC. Correlations and changepoints were considered significant if $p<0.10$.

Water quality can also relate to watershed stressors in a hierarchy, where a relationship may only be observed, or is much stronger, if other primary conditions are met. We explored potential hierarchy in water chemistry responses to LULC using categorical and regression tree analysis (CART; De'Ath and Fabricius 2000) with the rpart package in R (Therneau and Atkinson 2019). Data were insufficient to explore stressor hierarchy and structure for macroinvertebrates. Similar to nCPA, CART divides and groups data to minimize deviance. However, CART can consider multiple variables simultaneously and recursively partitions data into subsets based on identified thresholds. These data subsets may then be split again based on secondary or tertiary thresholds. Control parameters in CART were tuned to require groups to contain at least three observations. Splits in final models were required to reduce deviance by at least 5% (i.e., complexity parameter ≥0.05). We used urban, agriculture, and human development index (but not forest) as model inputs to simplify results interpretation.

Results

StreamSmart Site Medians

Site medians for alkalinity, conductivity, and TDS each spanned an order of magnitude (6 – 150 mg/L $CaCO_3$, 24 – 542 µS/cm, and 30 – 303 mg/L, respectively) (Table 2). Auto-correlation among these three variables was evident, with the least and greatest medians aligning across sites. Site median pH ranged from slightly less than neutral (6.6) to alkaline (8.0). For nutrients, TP varied within a narrow range (0.010 – 0.038 mg/L), except for site 308, where municipal wastewater treatment plant influence was evident (0.11 mg/L). TN medians, in contrast, varied over an order of magnitude (0.12 – 3.8 mg/L). All TSS medians were less than the lab's reporting limit of 10 mg/L. The minimum habitat quality index (99) and macroinvertebrate community index (7.5) medians were both observed at site 210, while site 301 had the greatest median habitat quality index (145), and site 205 had the greatest median macroinvertebrate community index (20).

Water Chemistry-LULC Relationships

All water chemistry variables, except TSS ($p > 0.10$), were correlated with the level of total anthropogenic watershed disturbance (Table 3; Figure 2A-F), increasing with increasing human development index ($p < 0.001$, rho = 0.61 – 0.95) and decreasing with increasing forest ($p < 0.001$, rho = -0.66 – -0.94). All water chemistry analytes were significantly (positively) correlated with urban land (p = < 0.001 – 0.080, rho = 0.39 – 0.87), but correlation analysis was not effective for describing water chemistry-LULC relationships with agricultural land, with the exception of TN ($p = 0.014$, rho = 0.53). Water quality medians were highly variable among sites with the least agriculture, which diluted otherwise linear signals above ~10% agriculture. As with agriculture, only TN was significantly correlated with poultry house density ($p = 0.097$, rho = 0.37). Pasture land and poultry houses are often spatially paired in the basin.

Changepoints in the human development index and forest land were found for all analytes (p < 0.001 – 0.034, R^2 = 0.37 – 0.82), except TSS ($p > 0.10$), suggesting that water chemistry values tended to be greater above a human development index threshold range = 27.8 – 45.3% and tended to be less above a forest land threshold range = 29.9 – 71.2%. Thresholds ranging from 4 – 5% urban land were also detected for all variables (p < 0.001 – 0.068, R^2 = 0.31 – 0.75), except TSS.

In contrast to correlation analysis, agricultural land thresholds were identified for both TN ($p < 0.001$, $R^2 = 0.56$) and TP ($p = 0.070$, $R^2 = 0.36$), estimated as 43.2% (CI = 16.0 – 49.9%) and 4.0% (CI = 1.2 – 45.3%), respectively. The nCPA models for alkalinity, conductivity, pH, and TDS tended to have greater explanatory power ($R^2 = 0.67 – 0.82$) relative to nutrients ($R^2 = 0.31 – 0.56$), as well as greater confidence in the threshold estimate (i.e., narrower CI).

Macroinvertebrate Community Relationships

Total macroinvertebrate community index scores were correlated with urban land ($p = 0.047$, rho = -0.56) and three components of the habitat quality index, but not the cumulative index (Table 4; Figure 3A-F). These components were channel flow status ($p = 0.032$, rho = 0.60), epifaunal substrate/available cover ($p = 0.035$, rho = 0.59), and frequency of riffles/bends ($p = 0.045$, rho = 0.56). Changepoints were also identified, suggesting greater macroinvertebrate community scores occurring above thresholds of 11 in both channel flow status sub-scores (CI = 10.0 – 13.3) and epifaunal substrate/available cover sub-scores (CI = 9.5 – 14.0). The sub-score of 11 ranks just above the mid-point in the possible range (i.e., 0 – 20), and is the lowest value considered to represent "sub-optimal" conditions.

These same relationships were also observed when sub-scores for the sensitive (epifaunal substrate/available cover and frequency of

Table 2. Sample counts for total sampling events (n_{events}) and macroinvertebrate collections (n_{MI}) at StreamSmart monitoring sites. Site medians for each water chemistry variable, as well as habitat quality index (HQI) assessment and macroinvertebrate community index scores.

Site	n_{events}	Alk (mg/L $CaCO_3$)	Cond (µS/cm)	pH	TDS (mg/L)	TN (mg/L)	TP (mg/L)	TSS (mg/L)	HQI	n_{MI}	Macro Index
101	22	64	193	7.8	99	0.34	0.012	2.1	125	0	-
102	24	32	103	7.7	54	0.34	0.014	2.2	124	6	15
103	20	6	24	6.6	30	0.12	0.014	1.2	133	8	19
104	19	10	32	6.8	31	0.28	0.014	1.8	120	8	12
107	8	20	55	7.0	39	0.13	0.013	0.6	135	0	-
108	7	12	40	6.8	32	0.12	0.012	0.7	135	0	-
109	7	30	81	7.0	60	0.53	0.015	1.7	141	2	14
200	14	138	527	7.8	312	0.29	0.012	3.0	108	0	-
201	23	43	118	7.5	65	0.47	0.010	1.4	129	6	15
202	10	143	542	7.7	303	1.2	0.023	1.9	134	0	-
205	13	32	105	7.3	65	0.93	0.012	1.1	143	3	20
206	26	150	502	8.0	284	2.9	0.038	1.3	105	0	-
210	21	132	476	7.8	254	0.92	0.020	2.7	99	6	8
300	29	135	396	7.7	218	3.5	0.026	1.2	128	8	21
301	24	64	194	7.7	97	1.3	0.026	4.5	146	0	-
302	28	134	352	8.0	200	3.4	0.025	1.3	145	9	15
303	30	100	260	7.4	153	3.4	0.020	0.4	120	10	14
304	28	139	348	7.3	207	3.8	0.020	1.1	145	10	9
305	27	84	227	7.7	126	1.9	0.019	3.9	140	0	-
306	29	140	337	7.9	181	1.8	0.016	3.2	111	1	14
307	21	76	239	7.6	127	1.0	0.020	1.4	125	2	12
308	21	100	436	7.8	233	2.7	0.11	2.1	136	2	10

Table 3. Water chemistry and land use-land cover (LULC) relationships based on results of Spearman rank correlation analysis and non-parametric changepoint analysis (nCPA) on StreamSmart site medians. For both tests, a result is significant if $p<0.10$. The Spearman rank correlation coefficient (rho) describes the relationship strength and ranges from -1 to 1, with positive and negative values denoting positive and inverse correlations, respectively. The results of nCPA include a changepoint (CP) value with a confidence interval (CI) encompassing the lower (5%) and upper (95%) confidence estimates around the threshold values. The mean of the water chemistry variable values distributed below (left) and above (right) the LULC threshold are also provided.

		--- Spearman ---		------------------------ nCPA ------------------------				
Water Chemistry	Geospatial	p	rho	p	CP (CI)	R^2	mean left	mean right
Alk (mg/L $CaCO_3$)	% Agriculture	0.36	-	0.11	-	-	-	-
Alk (mg/L $CaCO_3$)	% Forest	<0.001	-0.92	<0.001	59.1 (42.7-61.2)	0.82	125	31
Alk (mg/L $CaCO_3$)	% HDI	<0.001	0.93	<0.001	40.3 (38.4-54.6)	0.82	31	125
Alk (mg/L $CaCO_3$)	% Urban	<0.001	0.87	<0.001	5.2 (4.3-5.4)	0.75	33	123
Alk (mg/L $CaCO_3$)	PHD (house/km^2)	0.60	-	0.100	-	-	-	-
Cond (µS/cm)	% Agriculture	0.28	-	0.24	-	-	-	-
Cond (µS/cm)	% Forest	<0.001	-0.94	<0.001	59.1 (41.0-61.2)	0.72	382	94
Cond (µS/cm)	% HDI	<0.001	0.95	<0.001	45.3 (39.2-57.7)	0.74	107	398
Cond (µS/cm)	% Urban	<0.001	0.87	<0.001	5.2 (4.3-18.4)	0.69	98	379
Cond (µS/cm)	PHD (house/km^2)	0.46	-	0.28	-	-	-	-
pH	% Agriculture	0.66	-	0.40	-	-	-	-
pH	% Forest	<0.001	-0.70	<0.001	71.2 (58.6-74.9)	0.77	7.67	6.84
pH	% HDI	<0.001	0.72	<0.001	27.8 (22.6-33.2)	0.77	6.84	7.67
pH	% Urban	<0.001	0.79	<0.001	4.3 (2.7-4.5)	0.69	7.00	7.71
pH	PHD (house/km^2)	0.80	-	0.32	-	-	-	-
TDS (mg/L)	% Agriculture	0.26	-	0.22	-	-	-	-
TDS (mg/L)	% Forest	<0.001	-0.93	<0.001	45.6 (41.0-61.2)	0.74	241	77
TDS (mg/L)	% HDI	<0.001	0.95	<0.001	45.3 (40.3-61.0)	0.75	63	224
TDS (mg/L)	% Urban	<0.001	0.88	<0.001	5.2 (4.5-19.0)	0.67	60	212
TDS (mg/L)	PHD (house/km^2)	0.43	-	0.19	-	-	-	-
TN (mg/L)	% Agriculture	0.014	0.53	<0.001	43.2 (16.0-49.9)	0.56	0.86	3.53
TN (mg/L)	% Forest	<0.001	-0.77	0.008	59.1 (32.6-65.2)	0.49	2.20	0.45
TN (mg/L)	% HDI	<0.001	0.73	0.005	40.3 (33.5-64.9)	0.49	0.45	2.20
TN (mg/L)	% Urban	0.018	0.51	0.047	5.2 (4.0-5.4)	0.34	0.61	2.06
TN (mg/L)	PHD (house/km^2)	0.097	0.37	0.019	1.0 (0.1-1.0)	0.46	0.89	2.88
TP (mg/L)	% Agriculture	0.60	-	0.070	4.0 (1.2-45.3)	0.36	0.025	0.017
TP (mg/L)	% Forest	0.001	-0.66	0.015	29.9 (10.0-65.8)	0.42	0.029	0.016
TP (mg/L)	% HDI	0.003	0.61	0.034	33.9 (33.5-72.1)	0.37	0.013	0.021
TP (mg/L)	% Urban	0.013	0.53	0.068	4.3 (4.0-49.2)	0.31	0.013	0.021
TP (mg/L)	PHD (house/km^2)	0.99	-	0.66	-	-	-	-
TSS (mg/L)	% Agriculture	0.58	-	0.26	-	-	-	-
TSS (mg/L)	% Forest	0.77	-	0.45	-	-	-	-
TSS (mg/L)	% HDI	0.71	-	0.42	-	-	-	-
TSS (mg/L)	% Urban	0.080	0.39	0.21	-	-	-	-
TSS (mg/L)	PHD (house/km^2)	0.61	-	0.81	-	-	-	-

riffles/bends) or less sensitive macroinvertebrate group (channel flow status) were the response variable (Table 4; Figure 3A-F). The strength of relationships, variability explained, and value of changepoints for macroinvertebrate group sub-

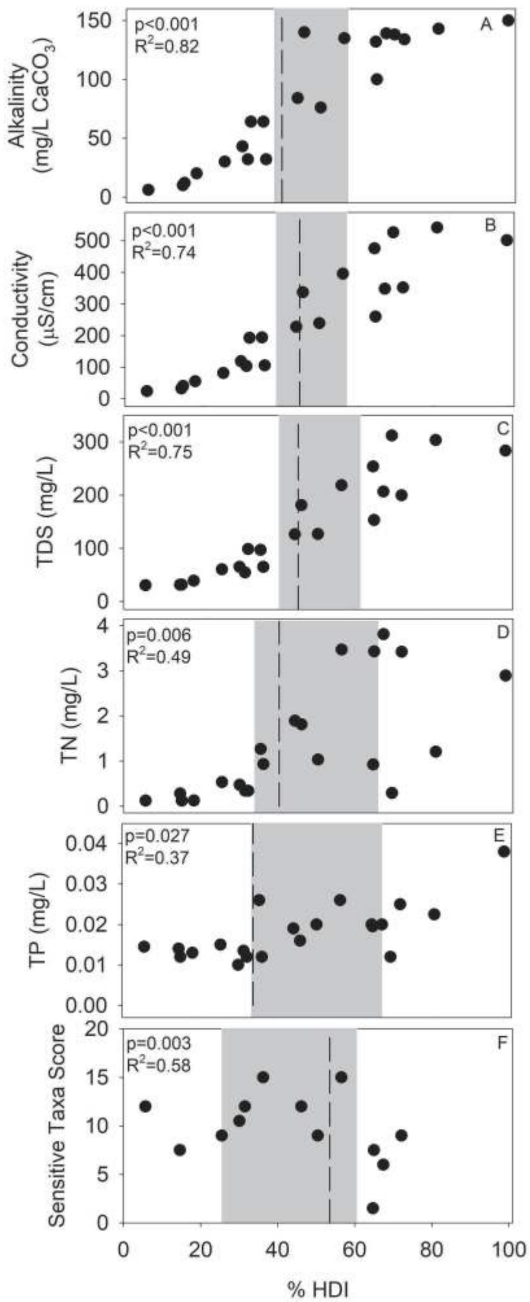


Figure 2. Non-parametric changepoint analysis results for A) alkalinity, B) conductivity, C) total dissolved solids, D) total nitrogen (TN), E) total phosphorus (TP), and F) macroinvertebrate community sensitive taxa showing thresholds and associated confidence interval in the human development index (HDI, %) as dashed lines and gray shaded areas, respectively.

scores was in range with the values observed using the total community index. Further, additional stressors were identified using group sub-scores. These included thresholds in forest land ($p = 0.033$, $R^2 = 0.48$) of 38.5% (CI = 33.8 – 57.1%) and the human development index ($p = 0.099$, $R^2 = 0.19$) of 53.5% (CI = 35.5 – 60.6%), which suggested greater sensitive taxa presence when forest was greatest and the human development index was least. For less sensitive taxa, correlations with agricultural land ($p = 0.098$, rho = 0.48) and TSS ($p = 0.051$, rho = -0.55) were identified, as well as thresholds in bank stability scores ($p = 0.091$, $R^2 = 0.39$) of 11.5 (CI = 10.5 – 15.0) and the total habitat quality index ($p = 0.098$, $R^2 = 0.46$) of 126.5 (CI = 115.5 – 131.0). Both changepoints suggested greater less sensitive taxa presence when habitat quality was greater. For tolerant taxa, a changepoint in sediment deposition scores of 14.5 (CI = 11.0 – 15.0) was detected that suggested greater presence with less sediment deposition.

Categorical and Regression Tree Models

Hierarchy in LULC characteristics was detected for TN, TP, conductivity, and TDS (Figure 4A-C; TDS not shown). For the remaining variables, secondary splits in the data were either not identified or did not reduce relative error beyond the primary split. For TN, the primary LULC predictor was agricultural land, with all of the greatest TN concentrations (n=4, 3.5 mg/L, on average) observed above a threshold of 47% (Figure 4A). For sites with less than 47% agriculture, a secondary split was observed in the human development index, explaining an additional 15% of dataset variability, with the least TN concentrations (n=8; 0.29 mg/L, on average) occurring below 34% human development. A tertiary split in agricultural land = 15.3% was observed in TN concentrations at sites with ≥ 34% human development, but no more than 47% agriculture, that divided intermediate TN concentrations into two groups based on the relative contribution of urban versus agricultural land to the human development index.

For TP, CART identified two thresholds in the human development index, with 71% and 34% as the primary and secondary thresholds, respectively (Figure 4B). This result differed from nCPA, which identified 34% as the most meaningful changepoint, with 71% as the upper bounds of the confidence interval. The CART model suggested that the greatest TP concentrations (n=3, TP = 0.028 mg/L on average) were associated with human development ≥ 71%, accounting for 42% of variability in the dataset. The secondary split associated the smallest TP concentrations (n=8, TP = 0.013 mg/L, on average) with human development < 34% and intermediate TP concentrations (n=10, TP = 0.019 mg/L, on average) with a human development index range of 34 – 71%.

For conductivity, the primary split was at 45% human development (Figure 4C). A secondary split in the human development index = 32% was also observed, suggesting that the least conductivity (n=7, 65 µs/cm, on average) was associated with human development < 32%, while intermediate conductivities (n=4, 180 µs/cm, on average) occurred within a range of 32 – 45%. For sites with a human development index ≥ 45%, a secondary split was also observed at 18% urban. Similar to the CART model for TN, this threshold separated median conductivities for sites above a human development threshold based on relative contributions of agricultural and urban lands to the overall index. The greatest conductivities (n=5, 477 µS/cm, on average) were observed at sites where urban land was > 18% (of at least 45% human development), while conductivities at sites with urban land below that threshold were about 1/3 less (n=5, 319 µS/cm, on average).

Discussion

Implications for StreamSmart and Other Volunteer Monitoring Programs

Our synthesis of stressor-response approaches showed a number of relationships in the StreamSmart database, including thresholds and hierarchy. These findings show the importance of considering multiple types of relationships in stressor-response analysis, and this process could

Table 4. Select macroinvertebrate community index and sensitivity group sub-score relationships with potential biological stressors, including the habitat quality index and components, land use-land cover (LULC), and water chemistry based on results of Spearman rank correlation analysis and non-parametric changepoint analysis (nCPA) on StreamSmart site medians. For both tests, a result is significant if p<0.10, and only statistically significant relationships are shown due to the large number of stressor-response pairs. The Spearman rank correlation coefficient (rho) describes the relationship strength and ranges from -1 to 1, with positive and negative values denoting positive and inverse correlations, respectively. The results of nCPA include a changepoint (CP) value with a confidence interval (CI) encompassing the lower (5%) and upper (95%) confidence estimates around the threshold values. The mean of the water chemistry variable values distributed below (left) and above (right) the LULC threshold are also provided.

Macro metric	Stressor	--- Spearman ---		----------------- nCPA -----------------		
		p	rho	p	R^2	CP (CI)
Total	% Urban	0.047	-0.56	-	-	-
Total	Channel flow status	0.032	0.60	0.032	0.49	11.0 (10.0-13.3)
Total	Epifaunal substrate/available cover	0.035	0.59	0.011	0.48	11.0 (9.5-14.0)
Total	Frequency of riffles/bends	0.045	0.56	-	-	-
Sensitive	% Forest	0.21	-	0.033	0.48	38.5 (33.8-57.1)
Sensitive	% HDI	0.23	-	0.099	0.19	53.5 (25.5-60.6)
Sensitive	Epifaunal substrate/available cover	0.011	0.68	0.003	0.58	12.3 (9.5-14.0)
Sensitive	Frequency of riffles/bends	0.085	0.50	-	-	-
Less Sensitive	% Agriculture	0.098	0.48	-	-	-
Less Sensitive	Bank stability	0.13	-	0.091	0.39	11.5 (10.5-15.0)
Less Sensitive	Channel flow status	0.052	0.55	0.083	0.32	11.5 (10.0-12.8)
Less Sensitive	HQI	0.10	-	0.098	0.46	126.5 (115.5-131.0)
Less Sensitive	TSS	0.051	-0.55	-	-	-
Tolerant	Sediment deposition	0.008	0.70	0.008	0.52	14.5 (11.0-15.0)

be applied to data exploration by other volunteer monitoring groups. We found that it was possible to detect water quality dynamics with only 21 sites for water chemistry and as few as 14 sites for macroinvertebrate data. However, the small number of StreamSmart sites is a limitation, as evidenced by large confidence intervals around many of the threshold estimates (such as in Figure 2D-F). The StreamSmart program can increase statistical power and return on investment by joining program data with datasets from other local, state, or national entities for further analysis and refinement (Stepenuck and Genskow 2018). StreamSmart would also benefit by introducing scientific knowledge "co-creation" to the volunteer experience, which promotes stakeholder buy-in to watershed planning (Buytaert et al. 2014).

Thresholds and CART results may be especially useful for planning tools, as they not only show that water chemistry and LULC are related, but also offer potential target values for managing LULC change to protect water quality. Detected water-chemistry-LULC relationships were consistent with prior studies in the region (Giovanetti et al. 2013; McCarty et al. 2018), as well as global patterns (Lintern et al. 2017), and showed greater nutrients, salts, and sediments with greater human activity in the basin.

Stressor-response relationships were detected for the StreamSmart macroinvertebrate data, but fewer than for water chemistry. The total community index decreased with increasing urban land and decreases in several individual habitat components. Many preceding studies have observed biodiversity loss and community shifts related to habitat quality (Santucci et al. 2005; Stone et al. 2005; Liao et al. 2018), watershed LULC (Weijters et al. 2009; Kuemmerlen et al. 2015), and nutrients (Evans-White et al. 2013). These include volunteer monitoring studies, which also observed relationships with urban land (Fore et al. 2001). The small number of sites

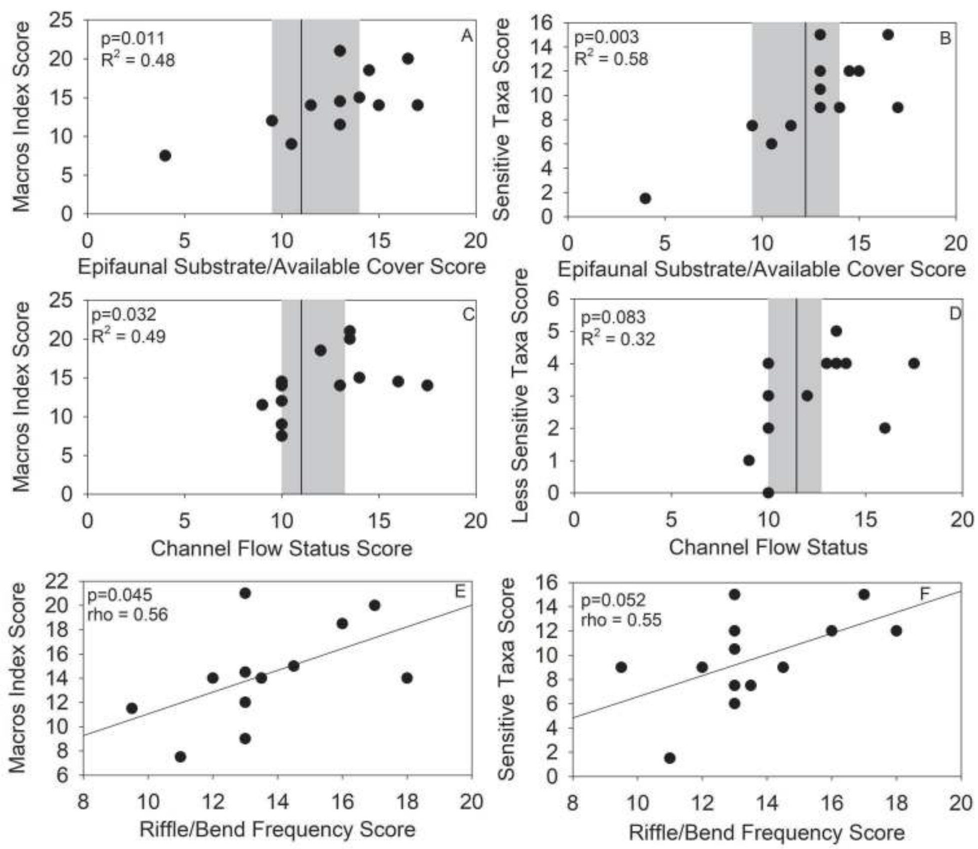


Figure 3. Side-by-side comparison of stressor-response relationships using the full macroinvertebrate community index versus sensitive or less sensitive taxa groups, for habitat component stressors A-B) epifaunal substrate/available cover, C-D) channel flow status, and E-F) riffle/bend frequency score.

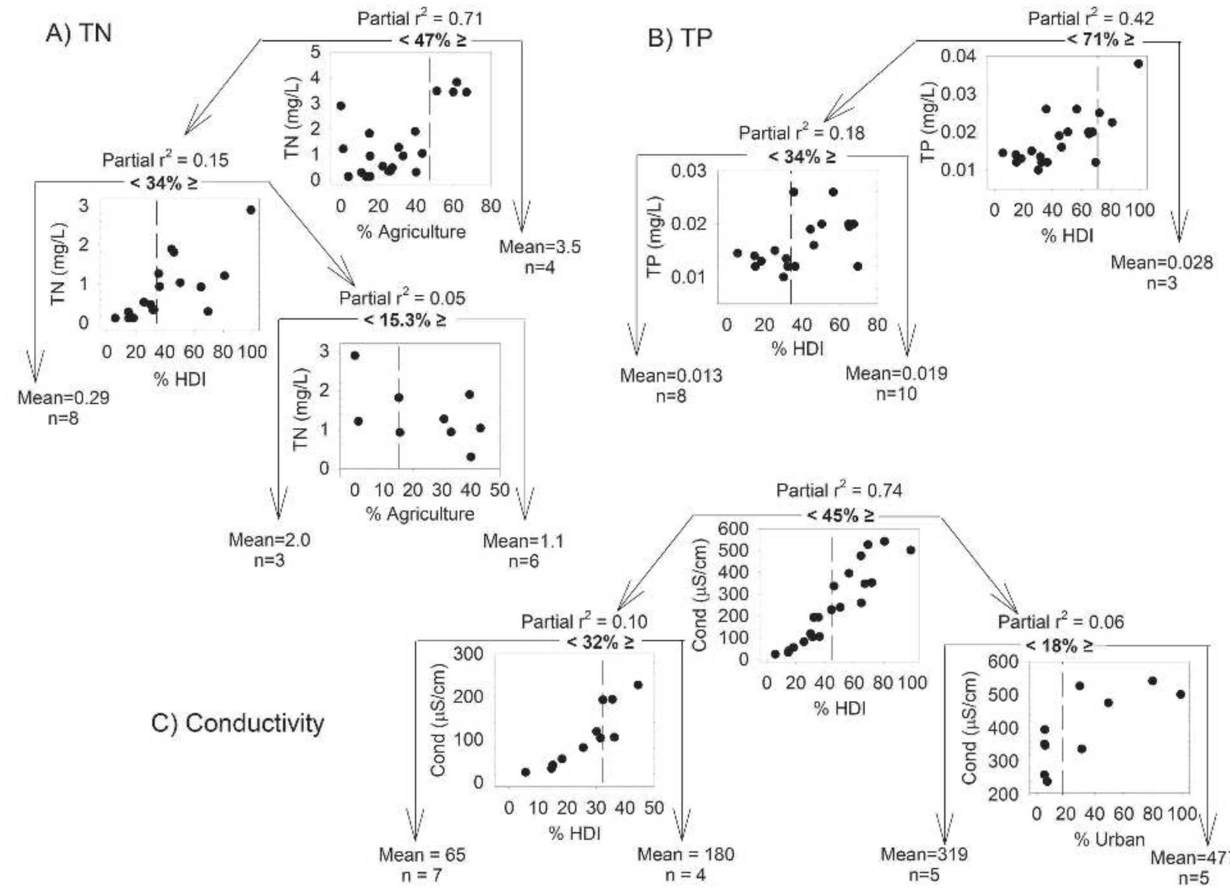


Figure 4. Categorical and regression tree models for A) TN, B) TP, and C) conductivity based on the watershed land use-land cover characteristics agriculture land (%), urban land (%), and agricultural and urban land combined in a human development index (HDI, %).

with macroinvertebrate data likely limited study findings. StreamSmart's unique index also presents a challenge for increasing statistical power and scope by incorporating outside datasets. More traditional monitoring programs use more complex, but standard metrics, such as the USEPA Rapid Bioassessment Protocols Macroinvertebrate Index of Biotic Integrity (RBIBI; Barbour et al. 1999). Validating the StreamSmart index to the RBIBI or other professional indices may be possible (Engel and Voshell, Jr. 2002). Alternately, StreamSmart could explore partnerships with other groups that also want to use a simplified index.

StreamSmart could also go in a different direction and use study findings to streamline the macroinvertebrate and habitat assessments by including just a few key pieces of information. Analysis showed the same meaningful stressor-response relationships were detected when macroinvertebrate sensitivity groups were the response variable, as well as when habitat components were the stressors. These findings suggest that StreamSmart could obtain the same information by assessing only the components that showed meaningful stressor-response relationships. This approach could also be applied by other volunteer monitoring groups with concerns about providing adequate training for more complex assessments, or about keeping the volunteer experience focused on fun and a minimal time investment.

It is not known why the sensitive macroinvertebrate groups or specific habitat components were especially predictive. But, a possible explanation is natural biases (Nerbonne et al. 2008) and inherent properties of the different macroinvertebrates (Nerbonne and Vondracek 2003) that mean volunteers do a better job with these variables. Many sensitive and less sensitive taxa (e.g., mayflies, stoneflies, crayfish) have traits, such as high motility or large bodies, that make them easier to detect, as well as anthropomorphically charismatic features such as visible eyes, legs, gills, and pincers. Even those that are smaller or lack as many charismatic features, such as caddisfly larvae, display engaging and highly identifiable behaviors, such as constructing casings from gravel or leaf litter. By contrast, tolerant taxa tend to have less motility and smaller bodies, as well as association with detritus, that can make both detection and identification more difficult, tedious, and unappealing (Peeters et al. 2022).

Only sedimentation was identified as a stressor for tolerant taxa, and this result was the opposite of the expected relationship, suggesting greater tolerant taxa presence with less sedimentation (i.e., greater scores). However, other studies have shown that community tolerance increases with greater TSS and turbidity (Chase et al. 2017). The StreamSmart data relationship makes the valid point that tolerant taxa are part of the diverse communities associated with good habitat quality. But, it also shows that the function of tolerant taxa in the StreamSmart index is redundant. In general, it may not be possible to capture community tolerance dynamics with simplified identification protocols because information on community tolerance is tied up in metrics using counts and percentages of individuals (Xu et al. 2013), or identification below the Family level is needed (Dusabe et al. 2022).

It is also possible that some habitat components may have a disproportionately large effect in the river networks of the Upper White River Basin. Components identified as stressors were epifaunal substrate/available cover, riffle/bend frequency, and channel flow status, which do have commonality as descriptors of the stream channel itself, rather than bank or riparian attributes. However, another explanation is differences in volunteers' relative understanding of the different components. For example, the strongest habitat predictor was epifaunal substrate/available cover, which may already be encompassed by local knowledge of ideal in-stream habitat for game fishing.

Implications for Watershed Management Planning

Water chemistry-LULC relationships identified in this study can be used to inform programming decisions by H_2Ozarks, Beaver Watershed Alliance, and the Beaver Water District source water protection program, with the caveat that these findings would ideally be refined and strengthened by bringing in additional data sources. For all water chemistry variables, the most meaningful anthropogenic LULC relationship was with the human development index, which combines agricultural and urban lands. Attempts to separately describe agricultural effects showed the advantages of basing management tools for mixed urban-agriculture watersheds on the human development index. Watershed LULC characteristics are interrelated and agricultural land was inversely auto-correlated with both urban and forest land. The opposite effects of these LULC characteristics on water chemistry created noise for low-range agriculture sites that prevented detection of an exclusively agricultural effect on water chemistry, with the exception of TN.

Similarly, uniform nCPA results suggesting consistent and highly predictive thresholds at minimal levels of urban land (4 – 5%) likely reflect that urbanization in the basin tends to occur on prior pasture, where the watershed human development index may already be near or above thresholds for greater water chemistry effects. Indeed, water chemistry-human development index relationships showed little scatter that would evidence such a disproportionate effect of urban land. The CART models for TN and conductivity, however, add nuance to this interpretation, suggesting that a disproportionate effect of urban and agricultural lands may be present, but only in watersheds with human development at or above thresholds of 34 – 45%. Further, urban land thresholds associated with disproportionate effects in CART models were closer to 20% than the 4 – 5% suggested by nCPA.

Thresholds in the human development index may be especially useful watershed management and planning tools because they delineate a level, or range, that suggests an increase in potential risks to water quality due to human activities. Watershed organizations in the basin can prioritize among candidate sites for best management practices by screening for watershed human development index greater than thresholds to determine both the greatest need for mitigation and the greatest potential for return on investment. Human development index thresholds differed between water chemistry variables, though not substantially, ranging from approximately 30 – 45%. These differences could reflect different controls on salt, nutrient, or sediment concentrations, but also data limitations related to a small sample size.

The CART models provide further context for prioritization, with both similarities and differences between variables. For the salts that contribute to conductivity, forest land maintenance of at least 55% (i.e., human development index = 45%) is a potential path to keeping levels low in least-developed watersheds. Additional benefits may accrue if forest is maintained >70%. For more developed watersheds (human development index ≥45%), mitigations may have the greatest efficacy by targeting urban and urbanizing areas with low-impact development or green infrastructure that reduces or slows runoff from impervious surfaces (Carey et al. 2013). However, agricultural conservation practices should also provide benefit.

For nutrients, multiple TP response thresholds to the human development provide forest maintenance targets for both the least and most developed watersheds (~70% and ~30% forest, respectively), as well as multiple human development index ranges to delineate best management practice candidate sites. The CART model for TN differed from other analytes by having a primary split in agricultural land. All sites having median TN concentration > 3.0 mg/L also had > 50% agriculture, making implementation of agricultural conservation practices, specifically in areas where agriculture is greatest, a potential priority route to TN reduction, with the primary goal of reducing runoff and leaching of nutrients from animal manures (Quinn and Stroud 2002). However, a similar disproportionate urban versus agricultural effect to the conductivity model was observed for TN among sites with the greatest human development index, after screening out the sites with the most agriculture. Thus, investment in urban-oriented practices also has potential to make a difference for watershed areas with this LULC profile.

Sediment-LULC relationships were less clear than for other water chemistry variables, with a monotonic TSS response to urban land, but no responses to agriculture or the human development index. The overall low-range of TSS site medians may limit potential to observe LULC effects on in-stream sediment concentrations. StreamSmart data are collected, by design, only under base flow conditions, which has been shown to be effective for identifying sub-watersheds with greater nutrient concentrations, while also allowing for broadening monitoring coverage (McCarty and Haggard 2016). This analysis suggests potential limits to this approach for TSS; a more pronounced TSS gradient, as well as LULC linkages, might better be detected under storm flow conditions.

Conclusions

Study results show that StreamSmart volunteers are providing a valuable water quality monitoring service. The value of a high-quality water chemistry baseline dataset and LULC relationships for the Upper White River Basin accrues return on investment by StreamSmart volunteers, H_2Ozarks, and partner organizations, with the potential to inform watershed management planning through "co-creation" of decision-making support tools with other monitoring entities. The volunteer monitoring macroinvertebrate community and habitat quality data were also informative about water quality dynamics in the basin, though potentially limited by sample size and complexities around combining with outside data. Stressor-response relationships detected for sensitive and less sensitive macroinvertebrate groups, as well as habitat components, suggest potential bias in how the StreamSmart volunteers conduct these assessments, but also offer an approach to streamlining these complex tasks. StreamSmart, as well as similar volunteer monitoring programs, can leverage this information to improve the volunteer experience and find the best avenues for communication with stakeholders through the aspects of watershed science, habitat quality, and biodiversity that are already encompassed by local knowledge.

Acknowledgements

The following student authors, listed alphabetically, contributed to preliminary data analysis and manuscript development: Lillie Haddock, Tarah Inema, Solomon Isu, Mahmood Jebur, Ian Kennedy, Kyle Lawrence, Marguerita Leavitt, Oliva Liedel, Marret Lineberry, John Richins, and Holly Wren. We thank our partners at H_2Ozarks, Beaver Water District, and Beaver Watershed Alliance, especially the StreamSmart program coordinator Erin Scott. Funding for this project was provided through Section 104(b) of the Water Resources Research Act of 1984 administered by the United States Geological Survey, as well as the University of Arkansas System Division of Agriculture.

Author Bio and Contact Information

Mrs. Erin Grantz (corresponding author) is a program manager and data scientist with the Arkansas Water Resources Center, and she is currently working on her Ph.D. in Environmental Dynamics at the University of Arkansas. She drafted the manuscript and helped manage the data analysis for BENG 4973/5973, including the development of the classification and regression trees. She has an M.S. and B.S. in Crop, Soil and Environmental Sciences from the University of Arkansas, and her graduate research focuses on water quality and watershed stoichiometry. She may be contacted at egrantz@uark.edu or 1371 West Altheimer Drive, Don Tyson Center for Agricultural Sciences, Fayetteville, AR 72704, USA.

Dr. Brian Haggard has been focused on water quality issues since his graduate studies. He is currently the director of the Arkansas Water Resources Center and a professor in the Biological and Agricultural Engineering Department with the University of Arkansas System Division of Agriculture. Prior to his current positions, he worked for the US Geological Survey and USDA Agricultural Research Service in hydrology and water quality. He conceptualized using this data in BENG 4973/5973 and the statistical analysis. He has a Ph.D. in Biosystems Engineering from Oklahoma State University, a M.S. in Agronomy from the University of Arkansas, and a B.S. in Life Sciences from the Missouri University of Science and Technology. He may be contacted at haggard@uark.edu or Engineering Hall 203, University of Arkansas, Fayetteville, AR 72701, USA.

References

Arkansas GIS Office. 2014. All Cultural Feature (point). Available at: https://gis.arkansas.gov/prodcut/all-cultural-feature-point/. Accessed March 3, 2023.

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

Buytaert, W., Z. Zulkafli, S. Grainger, L. Acosta, C.A. Tilashwork, J. Bastiaensen, B. De Bievre, J. Bhusal, J. Clark, A. Dewulf, M. Foggin, D.M. Hannah, C. Hergarten, A. Isaeva, T. Karpouzoglou, B. Pandeya, D. Paudel, K. Sharma, T. Steenhuis, S. Tilahun, G. Van Hecken, and M. Zhumanova. 2014. Citizen science in hydrology and water resources: Opportunities for knowledge generation, ecosystem service management, and sustainability development. *Frontiers in Earth Science* 2. Available at: https://www.frontiersin.org/articles/10.3389/feart.2014.00026/full. Accessed February 22, 2023.

Carey, R.O., G.J. Hochmuth, C.J. Martinez, T.H. Boyer, M.D. Dukes, G.S. Toor, and J.L. Cisar. 2013. Evaluating nutrient impacts in urban watersheds: Challenges and research opportunities. *Environmental Pollution* 173: 138-149. Available at: https://doi.org/10.1016/j.envpol.2012.10.004. Accessed February 22, 2023.

Chase, J.W., G.A. Benoy, and J.M. Culp. 2017. Combined effects of nutrient enrichment and inorganic sedimentation on benthic biota in an experimental stream. *Water Quality Research Journal* 52(3): 151-165.

De'Ath, G. and K.E. Fabricius. 2000. Classification and regression trees: A powerful yet simple technique for ecological data analysis. *Ecology* 81(11): 3178-3192. DOI: 10.1890/0012-9658(2000)081[3178:CARTAP]2.0.CO;2.

Dewitz, J. and U.S. Geological Survey. 2021. National Land Cover Database (NLCD) 2019 Products (ver. 2.0, June 2021): U.S. Geological Survey data release. Available at: https://doi.org/10.5066/P9KZCM54. Accessed February 22, 2023.

Dusabe, M.C., T.A. Neubauer, F.A. Muvundja, B.L. Hyangya, and C. Albrecht. 2022. Family-level bio-indication does not detect the impacts of dams on macroinvertebrate communities in a low-diversity tropical river. *Frontiers in Environmental Science* 10: 902246. DOI: 10.3389/fenvs.2022.902246.

Engel, S.R. and J.R. Voshell Jr. 2002. Volunteer biological monitoring: Can it accurately assess the ecological condition of streams? *American Entomologist* 48: 164-177.

Evans-White, M.A., B.E. Haggard, and J.T. Scott. 2013. A review of stream nutrient criteria development in the United States. *Journal of Environmental Quality* 42(4): 1002-1014. DOI: 10.2134/jeq2012.0491.

Fore, L.S., K. Paulsen, and K. O'Laughin. 2001. Assessing the performance of volunteers in monitoring streams. *Freshwater Biology* 46: 109-123.

Giovannetti, J., L.B. Massey, B.E. Haggard, and R.A. Morgan. 2013. Land use effects on stream nutrients at Beaver Lake Watershed. *Journal of the American Water Works Association* 105(1): E1-E10. Available at: https://awwa.onlinelibrary.wiley.com/doi/abs/10.5942/jawwa.2013.105.0005. Accessed February 22, 2023.

Haggard, B.E. 2010. Phosphorus concentrations, loads, and sources within the Illinois River drainage area, Northwest Arkansas, 1997 – 2008. *Journal of Environmental Quality* 39: 2113-2120. DOI: 10.234/jeq2010.0049.

Hoyer, M.V. and D.E. Canfield, Jr. 2021. Volunteer-collected water quality data can be used for science and management. *Lake and Reservoir Management* 37: 235-245. DOI: 10.1080/10402381.2021.1876190.

King, R.S. and C.J. Richardson. 2003. Integrating bioassessment and ecological risk assessment: An approach to developing numerical water-quality criteria. *Environmental Management* 31(6): 795-809. DOI: 10.1007/s00267-002-0036-4.

Kuemmerlen, M., B. Schmalz, Q. Cai, P. Haase, N. Fohrer, and S.C. Jähnig. 2015. An attack on two fronts: Predicting how changes in land use and climate affect the distribution of stream macroinvertebrates. *Freshwater Biology* 60(7): 1443-1458. DOI: 10.1111/fwb.12580.

Lewandowski, E. and H. Specht. 2015. Influence of volunteer and project characteristics on data quality of biological surveys. *Conservation Biology* 29(3): 713-723. Available at: https://doi.org/10.1111/cobi.12481. Accessed February 22, 2023.

Liao, H., E. Sarver, and L.-A.H. Krometis. 2018. Interactive effects of water quality, physical habitat, and watershed anthropogenic activities on stream ecosystem health. *Water Research* 130: 69-78. Available at: https://www.sciencedirect.com/science/article/pii/S0043135417309934?via%3Dihub. Accessed February 22, 2023.

Lintern, A., J.A. Webb, D. Ryu, S. Liu, U. Bende-Michl, D. Water, P. Leahy, P. Wilson, and A.W. Western. 2017. Key factors influencing differences in stream water quality across space. *WIRES Water* 5: e1260. Available at: https://doi.org/10.1002/wat2.1260. Accessed February 22, 2023.

McCarty, J.A. and B.E. Haggard. 2016. Can we manage non-point source pollution using nutrient concentrations during seasonal baseflow? *Agricultural and Environmental Letters* 1(1): 160015. DOI: 10.2134/ael2016.03.0015.

McCarty, J.A., M.D. Matlock, J.T. Scott, and B.E. Haggard. 2018. Risk indicators for identifying critical source areas in five Arkansas watersheds. *Transactions of the ASABE* 61(3): 1025-1032. DOI: 10.13031/trans.12699.

Multi-Resolution Land Characteristics Consortium (MRLC). 2018. National Land Cover Database 2011 (NLCD 2011). Multi-Resolution Land Characteristics Consortium (MRLC). Available at: https://data.nal.usda.gov/dataset/national-land-cover-database-2011-nlcd-2011. Accessed February 22, 2023.

Nerbonne, J.F. and B. Vondracek. 2003. Volunteer macroinvertebrate monitoring: Assessing training needs through examining error and bias in untrained volunteers. *Journal of the North American Benthological Society* 22: 152-163.

Nerbonne, J.F., B. Ward, A. Ollila, M. Williams, and B. Vondracek. 2008. Effect of sampling protocol and volunteer bias when sampling for macroinvertebrates. *Journal of the North American Benthological Society* 27: 640-646. DOI: 10.1899/07-101.1.

Northwest Arkansas Regional Planning Commission (NWARPC). 2016. 2040 Northwest Arkansas Metropolitan Transportation Plan. Available at: https://www.nwarpc.org/datacatalog/2040-northwest-arkansas-metropolitan-transportation-plan/. Accessed February 22, 2023.

Peeters, E.T.H.M., A.A.M. Gerritsen, L.M.S. Seelen, M. Begheyn, F. Rienks, and S. Teurlincx. 2022. Monitoring biological water quality by volunteers complements professional assessments. *PLoS ONE* 17: e0263899. DOI: 10.1371/journal.pone.0263899.

Perez, A.P., K. Higgins, and M. Freyaldenhoven. 2015. Nonpoint Source Pollution in the Beaver Reservoir/Upper White River Watershed. FSPPC125. University of Arkansas Cooperative Extension Service. Available at: https://www.uaex.uada.edu/publications/pdf/FSPPC125.pdf. Accessed February 23, 2023.

Qian, S.S., R.S. King, and C.J. Richardson. 2003. Two statistical methods for the detection of environmental thresholds. *Ecological Modelling* 166(1-2): 87-97. Available at: https://doi.org/10.1016/S0304-3800(03)00097-8. Accessed February 22, 2023.

Quinn, J.M. and M.J. Stroud. 2002. Water quality and sediment and nutrient export from New Zealand hill-land catchments of contrasting land use. *New Zealand Journal of Marine and Freshwater Research* 36(2): 409-429.

R Core Team. 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/. Accessed February 23, 2023.

San Llorente Capdevila, A., A. Kokimova, S.S. Ray, T. Avellán, J. Kim, and S. Kirschke. 2020. Success factors for citizen science projects in water quality monitoring. *Science of the Total Environment* 728: 137843

Santucci, Jr., V.J., S.R. Gephard, and S.M. Pescitelli. 2005. Effects of multiple low-head dams on fish, macroinvertebrates, habitat, and water quality in the Fox River, Illinois. *North American Journal of Fisheries Management* 25: 975-992. DOI: 10.1577/M03-216.1.

Savan, B., A.J. Morgan, and C. Gore. 2003. Volunteer environmental monitoring and the role of the universities: The case of Citizens' Environment Watch. *Environmental Management* 31(5): 561-568. Available at: https://doi.org/10.1007/s00267-002-2897-y. Accessed February 23, 2023.

Scott, J.T., B.E. Haggard, A.N. Sharpley, and J.J. Romeis. 2011. Change point analysis of phosphorus trends in the Illinois River (Oklahoma) demonstrates the effects of watershed management. *Journal of Environmental Quality* 40: 1249-1256. DOI: 10.2134/jeq2010.0476.

Stepenuck, K.F. and K.D. Genskow. 2018. Characterizing the breadth and depth of volunteer water monitoring programs in the United States. *Environmental Management* 61: 46-57. DOI: 10.1007/s00267-017-0956-7.

Stone, M.L., M.R. Whiles, J.A. Webber, K.W.J. Williard, and J.D. Reeve. 2005. Macroinvertebrate communities in agriculturally impacted Southern Illinois streams: Patterns with riparian vegetation, water quality, and in-stream habitat quality. *Journal of Environmental Quality* 34: 907-917. DOI: 10.2134/jeq2004.0305.

Storey, R.G., A. Wright-Stow, E. Kin, R. Davies-Colley, and R. Stott. 2016. Volunteer stream monitoring: Do the data quality and monitoring experience support increased community involvement in freshwater decision making? *Ecology and Society* 21(4): 32. Available at: https://doi.org/10.5751/ES-08934-210432. Accessed February 23, 2023.

Therneau, T. and B. Atkinson. 2019. rpart: Recursive Partitioning and Regression Trees. R package version 4.1-15. Available at: https://CRAN.R-project.org/package=rpart. Accessed February 23, 2023.

United States Environmental Protection Agency (USEPA). 2005. Community-Based Watershed Management: Lessons from the National Estuary Program. EPA-842-B-05-003. Available at: https://www.epa.gov/nep/community-based-watershed-management-handbook. Accessed February 23, 2023.

United States Environmental Protection Agency (USEPA). 2008. Handbook for Developing Watershed Plans to Restore and Protect Our Waters. EPA-841-B-08-002. Available at: https://www.epa.gov/nps/handbook-developing-watershed-plans-restore-and-protect-our-waters. Accessed February 23, 2023.

Weijters, M.J., J.H. Janse, R. Alkemade, and J.T. Verhoeven. 2009. Quantifying the effect of catchment land use and water nutrient concentrations on freshwater river and stream biodiversity. *Aquatic Conservation: Marine and Freshwater Ecosystems* 19(1): 104-112. DOI: 10.1002/aqc.989.

White, J.Y. and C.J. Walsh. 2020. Catchment-scale urbanization diminishes effects of habitat complexity on instream macroinvertebrate assemblages. *Ecological Applications* 30(8): e02199. DOI: 10.1002/eap.2199.

Xu, M., Z. Wang, X. Duan, and B. Pan. 2013. Effects of pollution on macroinvertebrates and water quality bio-assessment. *Hydrobiologia* 729(1): 247-259. DOI: 10.1007/s10750-013-1504-y.

Universities Council on Water Resources
Journal of Contemporary Water Research & Education
Issue 177, Pages 63-71, April 2023

Case Study Article

Chlorophyll and Phycocyanin Raw Fluorescence May Inform Recreational Lake Managers on Cyanobacterial HABs and Toxins: Lake Fayetteville Case Study

*Brian E. Haggard[1,2], Erin Grantz[1], Bradley J. Austin[1], Nicole D. Wagner[3], and J. Thad Scott[4,5]

*[1]Arkansas Water Resources Center, University of Arkansas Division of Agriculture, Fayetteville, AR; [2]Biological and Agricultural Engineering Department, University of Arkansas, Fayetteville, AR; [3]Department of Biological Sciences, Oakland University, Rochester, MI; [4]Center for Reservoir and Aquatic System Research, Baylor University, Waco, TX; [5]Department of Biology, Baylor University, Waco, TX; *Corresponding Author*

Abstract: Cyanobacterial harmful algal blooms (cyanoHABs) have been observed across the USA and worldwide, and even locally in Lake Fayetteville (Arkansas, USA) once we started monitoring for total microcystin. The goal of this research note was to present a framework that might help guide cyanoHAB and toxin public health advisories at Lake Fayetteville. We evaluated nonparametric change points (i.e., thresholds) and hierarchical structure (using classification and regression trees) between total microcystin concentrations, chlorophyll, and phycocyanin; chlorophyll-a is a pigment in all algae, while phycocyanin is specific to cyanobacteria. Pigment concentrations and raw fluorescence units (RFUs) all showed significant thresholds with total microcystin concentrations, basically showing that as concentration or RFUs increased above the thresholds that total microcystin was greater at Lake Fayetteville. The regression tree with total microcystin concentrations showed a first split with phycocyanin RFUs at 4524, and then when phycocyanin RFUs were greater there was an optimal range for the phycocyanin to chlorophyll RFU ratio (0.64-1.5). At this recreational lake, total microcystin concentrations were greatest when water samples met these criteria, providing a possible framework for when lake managers might suggest an increased risk for elevated cyanobacterial toxins.

Keywords: *cyanobacterial blooms, total microcystin, pigment raw fluorescence, lake management*

Cyanobacterial harmful algal blooms (cyanoHABs) are being observed across the USA and globally, and these present a threat to inland freshwaters and challenges for lake users and managers (Brooks et al. 2016). These cyanoHABs are often dominated by species capable of producing toxins under the right environmental conditions, including *Aphanizomenon*, *Cylindrospermopsis*, *Dolichospermum*, *Microcystis*, and *Oscillatoria* (*Planktothrix*) among others (see Carmichael 2001). The toxicity of these cyanoHABs and dominance of individual cyanobacterial species are driven by water temperatures, nutrient availability and ratios, and likely global climate change (Paerl and Otten 2016).

Cyanobacteria in freshwaters can produce several different types of toxins, which can result in everything from mild skin irritation to basic gastrointestinal issues and even acute pneumonia during recreational exposures (Falconer 1999). However, many studies focus on microcystin and its various forms in lakes and reservoirs because microcystins generally occur in lake waters when other classes of cyanobacterial toxins are present (Graham et al. 2010). Microcystin and its physicochemical and environmental drivers in lakes and reservoirs have been a research focus for the last couple decades, showing the importance of water temperature (Walls et al. 2018), nitrogen (N) supply (Wagner et al. 2021), and cyanobacterial

Research Implications

- Total microcystin concentrations were predicted using easily measured raw fluorescence of chlorophyll and phycocyanin in water samples from a recreational lake.
- Total microcystin concentrations were greatest when raw fluorescence units (RFUs) of phycocyanin exceeded 4254 and the ratio of chlorophyll and phycocyanin RFUs was between 0.64 and 1.5; these values could be rounded for management purposes.
- Public health advisories for cyanobacterial harmful algal blooms (cyanoHABs) and total microcystin concentrations could be issued based on RFUs of chlorophyll and phycocyanin, which is less expensive than toxin analysis.

biomass (Haggard et al. 2023). Recently, the Environmental Protection Agency (EPA) (2019) has released guidance on cyanobacterial toxins in recreational lakes and reservoirs for states and tribes, providing 8 µg L^{-1} as the target guideline for total microcystin concentrations.

When recreational lakes and reservoirs exceed guidelines for cyanobacterial toxins (i.e., 8 µg L^{-1} total microcystin) in Arkansas, the entity responsible for the lake deals with public health advisories and notices. The Arkansas HAB Response Program provides guidance on this topic (Arkansas HAB Workgroup 2019), but the episodic nature of toxins exceeding these guidelines makes it challenging to issue and retract public health advisories and notices. Plus, the general public is not well informed on the differences between nuisance algal blooms in freshwaters and those that might be toxic (i.e., cyanoHABs). Given this issue, the Arkansas Water Resources Center (AWRC), in collaboration with the Arkansas HAB Workgroup, published an educational fact sheet to help inform the general public on nuisance algal blooms and HABs in streams, ponds, and lakes (Austin et al. 2018).

The goal of this study is to present a management framework for HAB risk in recreational waters, based on preliminary analysis of relatively easy to measure water quality data or physicochemical properties measured in the recreational Lake Fayetteville, and how these relate to total microcystin concentrations. The specific objectives are to 1) describe total microcystin concentrations over time during the cyanoHAB events in 2020, 2) evaluate threshold responses and hierarchical structure between total microcystin and cyanobacterial or algal variables related to fluorescence, and 3) present a framework to help lake managers determine when the chances of cyanobacterial HABs with elevated toxins are greatest. This analysis focuses on sustained and prevalent HABs at Lake Fayetteville, where total microcystin concentrations varied more than an order of magnitude (Haggard et al. 2023).

Methods

Lake Fayetteville is a small lake used for secondary contact recreational purposes (boating, fishing, and kayaking) in Fayetteville, Arkansas city limits, and the lake and surrounding park are managed by the municipality; for more information, see: https://www.fayetteville-ar.gov/3130/Lake-Fayetteville. The lake is relatively small with a surface of ~0.6 km^2 and a watershed area of 24 km^2. In 2019, the first lake advisory for cyanobacterial toxins (i.e., microcystin) was issued after the AWRC observed total microcystin concentrations exceeding recreational guidelines (8 µg L^{-1}; EPA 2019). The lake has been a research focus for watershed and lake nutrient budgets (Grantz et al. 2014), sediment phosphorus (P) sources (Haggard et al. 2023), and cyanobacterial HABs (Wagner et al. 2021; Haggard et al. 2023). However, cyanobacteria have dominated the phytoplankton community since 1968 or since initial impoundment (Meyer 1971).

Water samples have been collected since 2019; however, this study focused on water samples collected during calendar year 2020. Water samples were collected at three sites accessible by foot along the north shore, where the public has relatively easy access to the lake (see Haggard et al. 2023). Upon return to the AWRC water quality lab, water samples were analyzed for raw fluorescence of chlorophyll and phycocyanin using a CyanoFlour (hand-held fluorometer, Turner Designs, San Jose, CA, USA). At the lab, water was filtered and analyzed for chlorophyll-a pigment analysis (Method APHA 10200 H3, Turner Designs Fluorometer). A subsample of lake water (20-30 mL) was stored in an amber glass vial and put through three freeze thaw cycles before being analyzed for total microcystin concentration, using the enzyme linked immunoassay technique (Method EPA 546). The subsample volume for total microcystin was determined based on a study by Austin and Haggard (2023), which suggests that the minimum volume needed to reduce sampling variability is at least 20 mL.

We used these data to evaluate correlations between parameters (particularly chlorophyll-a pigment and chlorophyll/phycocyanin raw fluorescence units (RFUs)), thresholds between microcystin and algal parameters (nCPA; King and Richardson 2003; Qian et al. 2003), and any hierarchical structure in the microcystin relationships (CART; De'Ath and Fabricius 2000). The nCPA and CART analyses were performed in R 4.1.2 using the rpart package for CART analysis (Therneau and Atkinson 2019), requiring a minimum of five observations per terminal node and that each split increase the complexity parameter by at least 0.05. We used the deviance explained by each split relative to the total deviance of the model to approximate an R^2 for each split within the CART models (R. King, personal communication, 5 April 2022).

Results

Total microcystin concentrations revealed an interesting pattern at Lake Fayetteville (Figure 1), showing little measurable microcystin (<0.200 µg L^{-1}) from March through early June 2020; the one exception was 0.277 µg L^{-1} at the mid-lake site on May 19. After this period, total microcystin concentrations increased to an average of 4.692 µg L^{-1} across all three sites on June 29, 2020. Total microcystin concentrations remained elevated (~1 µg L^{-1} or greater) through late summer, August 18, 2020. After this time, total microcystin concentrations decreased to less than 0.200 µg L^{-1} on September 14, 2020, and then a secondary peak in microcystin occurred in late fall, where mean total microcystin concentration reached 1.619 µg L^{-1} on October 19, 2020. Microcystin remained elevated in November and then decreased to a mean less than 0.200 µg L^{-1} in December 2020.

Chlorophyll-a pigment concentrations varied in 2020 from 2.6 to 96.4 µg L^{-1}, averaging 33.3 µg L^{-1} across all sampling sites and dates in 2020; chlorophyll and phycocyanin RFUs were variable over time too (Figure 1). Chlorophyll-a concentration showed a bimodal peak in 2020, where chlorophyll-a pigment concentrations were greatest in late April through May and then increased again in mid-October through early November following lake turnover. Phycocyanin RFUs (R^2=0.54) were more strongly related to chlorophyll-a pigment concentrations than chlorophyll RFUs (R^2=0.10), and neither chlorophyll-a pigment concentration nor the RFUs of either phycocyanin or chlorophyll showed a linear relation to total microcystin concentrations.

The cyanobacterial or algal fluorescence properties showed significant thresholds with total microcystin concentrations at Lake Fayetteville (Figure 2), including:

- 23.4 chlorophyll-a pigment mg L^{-1} (R^2=0.10, P=0.013), where mean total microcystin was 0.30 µg L^{-1} to the left (less than) of the threshold and 1.07 µg L^{-1} to the right (greater than);
- 4172 chlorophyll RFUs (R^2=0.14, P=0.005), where mean total microcystin was 0.25 µg L^{-1} to the left of the threshold and 1.14 µg L^{-1} to the right;
- 4524 phycocyanin RFUs (R^2=0.18, P=0.001), where mean total microcystin was 0.27 µg L^{-1} to the left of the threshold and 1.25 µg L^{-1} to the right;
- 0.37 phycocyanin to chlorophyll-a (PC:CHL) RFU ratio (R^2=0.14, P=0.002), where mean total microcystin was 0.13 µg L^{-1} to the left of the threshold and 1.09 µg L^{-1} to the right; and
- pheophytin did not show a significant nonparametric change point with microcystin (P=0.273).

More information about physicochemical thresholds with total microcystin concentrations at Lake Fayetteville using this cyanoHAB database from March through September 2020 is available in Haggard et al. (2023).

When we considered hierarchical structure in the relation between these cyanobacterial or algal fluorescence properties and total microcystin concentrations, an interesting pattern emerged (Figure 3). The first split in this relation was with phycocyanin RFU and total microcystin concentrations using the change point defined above, and then water samples with phycocyanin RFUs above the threshold split twice with the PC:CHL RFU ratio. The least mean total microcystin concentration was 0.27 μg L^{-1} when phycocyanin was less than 4524 RFUs. The next grouping of the water samples was when phycocyanin exceeded 4524 RFUs but had a PC:CHL RFU ratio greater than or equal to 1.5; the mean total microcystin concentration was 0.43 μg L^{-1}. When the PC:CHL RFU ratio was less than 1.5, the data split again with this ratio. So, water samples with phycocyanin greater than 4524 RFUs but a PC:CHL RFU ratio less than 0.64 had a mean total microcystin concentration of 1.1 μg L^{-1}. The greatest mean total microcystin concentration (2.6 μg L^{-1}) was observed when phycocyanin RFUs exceeded the first threshold and the PC:CHL RFU ratio was between 0.64 and 1.5. These multiple, sequential thresholds or this hierarchical structure

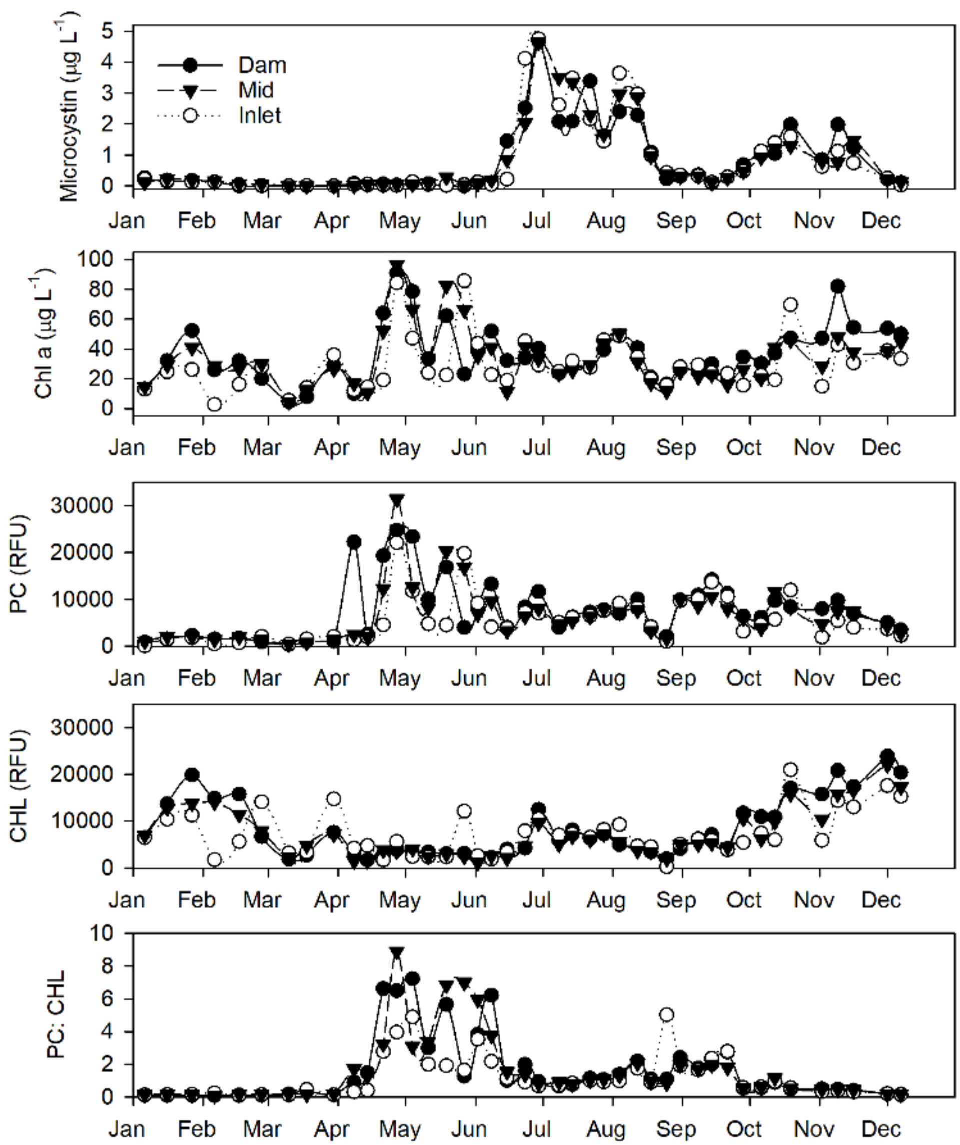


Figure 1. Time series graph of total microcystin, chlorophyll-a pigment and raw fluorescence of chlorophyll and phycocyanin at Lake Fayetteville, Arkansas, calendar year 2020.

explained more than half of the deviation in total microcystin concentrations at Lake Fayetteville during calendar year 2020.

Discussion

When lakes have elevated microcystin concentrations and exceed recreational guidelines (8 µg L^{-1}; EPA 2019), the next step is public notice by the authority in charge of the water body. At Lake Fayetteville, the City of Fayetteville has issued public health and contact advisories, and the signage went up in 2019 when total microcystin concentrations hit 11 µg L^{-1} in one lake sample on May 7, 2019 (mean of two samples that day shown in Wagner et al. 2021). However, the next week total microcystin concentrations dropped below EPA guidance levels at Lake Fayetteville, and then total microcystin concentrations (15 µg L^{-1}, June 4, 2019; Wagner et al. 2021) once again exceeded EPA guidance levels. Total microcystin concentrations were highly variable in the 2019 growing season, but that could be due to the variability in the cyanoHABs that year or due to the smaller volumes (~2 mL) used in the freeze thaw cycles for analysis (Austin and Haggard 2023).

The cyanoHABs in 2020 were sustained for a longer period during the growing season, but lake water below the water surface was always less than EPA guidance for total microcystin concentrations. The year 2020 was also the first year where we had a complete database of total microcystin data paired with RFUs for chlorophyll and phycocyanin, and total microcystin was measured using at least 20 mL following the three freeze thaw cycles (Austin and Haggard 2023). While microcystin below the water surface never exceeded EPA guidelines, we did observe surface scums of these cyanoHABs, especially at the marina on the northwest corner of Lake Fayetteville. These surface scums concentrate

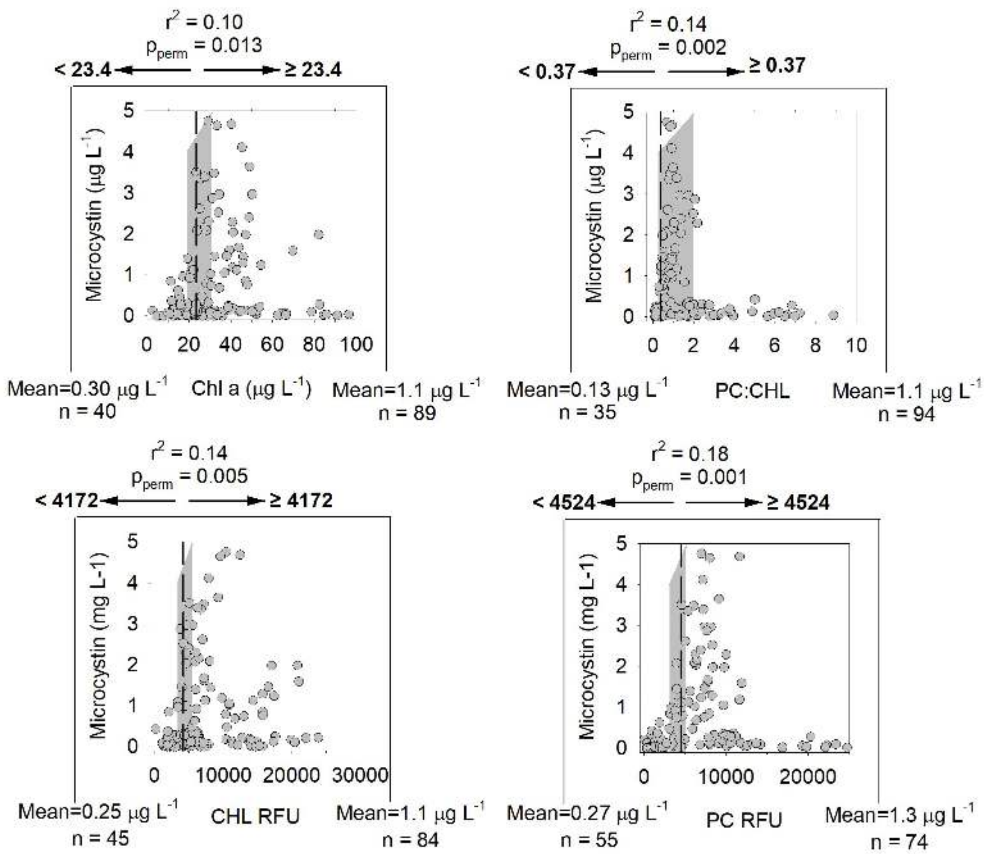


Figure 2. Plots showing significant thresholds (vertical dashed line) based on nonparametric change point analysis between total microcystin concentrations and cyanobacterial and algal fluorescence properties, including chlorophyll-a pigment concentrations, chlorophyll (CHL) raw fluorescence units (RFUs), phycocyanin (PC) RFUs, and the ratio of CHL to PC RFUs; the grey shaded area represents the 90% confidence interval about the threshold.

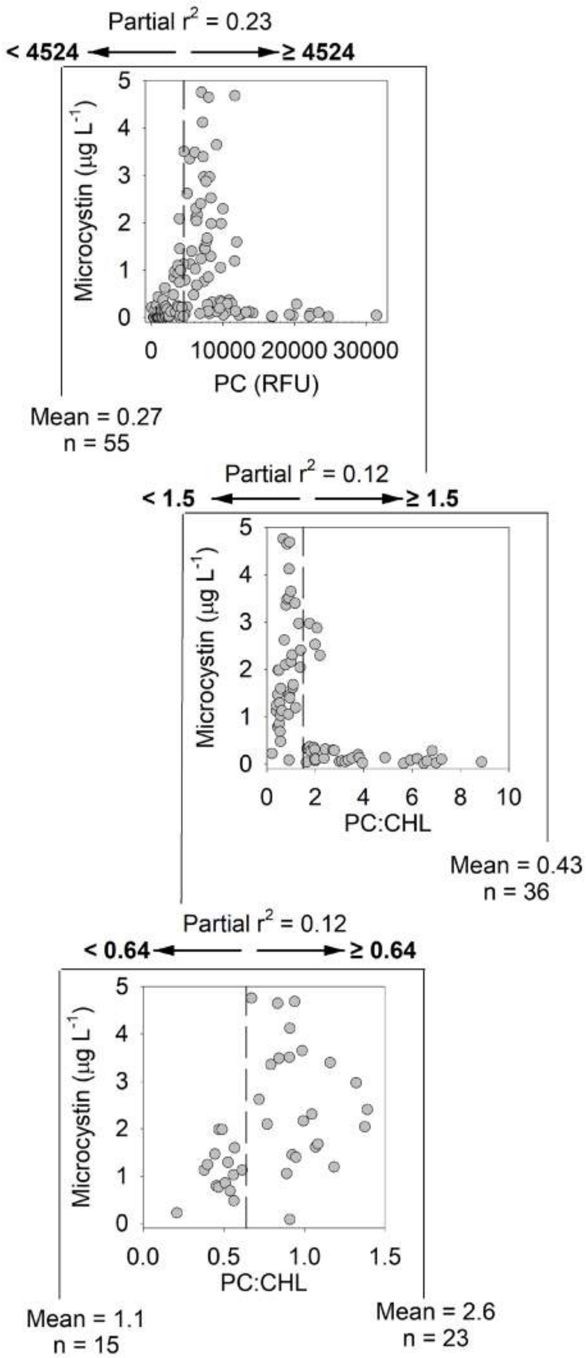


Figure 3. Thresholds and hierarchical structure in splits with total microcystin concentrations at Lake Fayetteville using only raw fluorescence of chlorophyll (CHL) and phycocyanin (PC) at all three sites during calendar year 2020; the top graph shows the first threshold between total microcystin and PC RFUs, the middle graph shows data exceeding PC 4524 RFUs and its threshold with PC:CHL, and the bottom graph shows data less than a PC:CHL of 1.5 and its final thresholds with PC:CHL of 0.64.

the cyanobacteria which can produce cyanotoxins such as microcystins (Plaas and Paerl 2021).

Ideally, lake managers would have access to total microcystin concentrations in the lake waters. While microcystin can be measured by laboratory services, it can be costly for municipalities, and the data are not necessarily quickly available to make decisions on public advisories. The turnaround time for many commercial water labs is up to eight days for total microcystin analysis (based on web search), and field test strips and or kits have their own challenges to accurately estimate total microcystin concentrations (Aranda-Rodriguez et al. 2015). Therefore, lake managers need access to a decision matrix based on more easily measurable and readily available data. These data could include phycocyanin and chlorophyll RFUs, which could be measured in grab samples like this study, or provided continuously by deployed data sondes (e.g., see Izydorczyk et al. 2005). However, these data sondes, when deployed near the surface in lakes, can have light-induced quenching of fluorescence, resulting in errors in measurement of phycocyanin and chlorophyll (Rousso et al. 2021).

The regression tree splits in microcystin, phycocyanin, and PC:CHL RFU ratios might help provide some guidance on when to expect the greatest microcystin concentrations in the lake water, especially at Lake Fayetteville. Microcystin in below-surface lake water at Fayetteville was consistently less than recreational guidelines in 2020, but it showed significant thresholds and hierarchical structure with cyanobacterial fluorescence properties. When phycocyanin RFUs were greater than 4524 and PC:CHL was between 0.64 and 1.5, microcystin was greatest at Lake Fayetteville. Alternatively, total microcystin is likely to be elevated or of potential concern when phycocyanin RFU exceeds 4524 and the PC:CHL RFU ratio is between 0.64 and 1.5. These parameters can be [relatively] easily and rapidly measured using the CyanoFlour (Turner Designs, San Jose, CA) or other handheld fluorometers, providing lake managers an opportunity to issue initial advisories or even rate the risk of elevated microcystin in recreational lakes dominated throughout the growing season by cyanobacteria.

The use of RFUs for chlorophyll and phycocyanin to forecast total microcystin concentrations and cyanoHABs might be advantageous for recreational lakes because phycocyanin RFU and cyanobacterial biovolumes show a strong positive correlation (Thomson-Laing et al. 2020). While other studies have suggested that chlorophyll and phycocyanin RFUs do not necessarily correlate with pigment analysis for chlorophyll-a and phycocyanin, respectively (e.g., see Chaffin et al. 2018), we observed a relatively strong correlation between phycocyanin RFU and chlorophyll-a pigment concentrations. This suggests that phycocyanin RFU is likely a good proxy for cyanobacterial biomass at Lake Fayetteville. The regression tree observed with these parameters at Lake Fayetteville may be a potential management or decision tool for HAB advisories. However, we need to expand this database to see if these thresholds vary across years at this lake and across lakes. These data could also be paired with other parameters that are relatively easy to measure, including conductivity, dissolved oxygen, pH, and water temperature.

Since Lake Fayetteville is used for recreational purposes including fishing, the potential bioaccumulation of microcystin would be a concern with fish consumption. Microcystin in its various forms has been detected in fish tissues in other lakes, where these tissues likely exceeded total daily intake guidelines (Gurbuz et al. 2016). Globally, microcystin concentrations in the water column and fish tissues show a positive relation (Flores et al. 2018), suggesting that elevated concentrations in the lake water would correlate with increased concentrations in the fish at Lake Fayetteville. It is also likely that microcystin accumulates in the bottom and shoreline sediments at Lake Fayetteville, and it often persists in sediments for many days following algal blooms and toxin production (Preece et al. 2021). Benthic cyanobacteria are also an emerging concern in cyanoHABs (Burford et al. 2020), potentially requiring consideration in recreational lakes. We do not know what the microcystin concentrations are in benthic cyanobacteria, sediments, and fish tissues from Lake Fayetteville, but the widespread occurrence of this toxin in the water and likely accumulation of this toxin in the sediments and fish should give rise to potential public health concerns.

Conclusions

At Lake Fayetteville, total microcystin concentrations were variable across the calendar year, but sustained above 1 μg L^{-1} in the early and late periods of the growing season in 2020. The algal or cyanobacterial fluorescence variables were not linearly related to total microcystin concentrations at this lake, but each variable did show a significant threshold or non-parametric change point. The hierarchical structure between total microcystin concentrations and phycocyanin RFU and the PC:CHL RFU ratio provides guidance on when elevated microcystin might be present at Lake Fayetteville. When lake water exceeds 4524 RFUs for phycocyanin and has a PC:CHL RFU ratio between 0.64 and 1.5, lake managers might want to consider issuing public health advisories for cyanoHABs and elevated toxins relative to recreational contact guidelines.

Acknowledgements

Funding for this project was provided through Section 104(b) of the Water Resources Research Act of 1984 administered by the US Geological Survey, as well as the University of Arkansas System Division of Agriculture. The ability of the AWRC to use US Geological Survey 104(b) based funding to tackle emerging water issues in Arkansas was a key driver in the success of this project, leading to this publication and now four years (2019-2022) of data collection on cyanoHABs and microcystin concentrations.

Author Bio and Contact Information

Dr. Brian Haggard (corresponding author) has been focused on water quality issues since his graduate studies, and he has a keen interest in cyanoHABs. He is currently the director of the Arkansas Water Resources Center and a professor in the Biological and Agricultural Engineering Department with the University of Arkansas System Division of Agriculture. Prior to his current positions, he worked for the US Geological Survey and USDA Agricultural Research service in hydrology and water quality. He conceptualized the data collection and this sampling and analytical variability research project at Lake Fayetteville, as well as drafted the manuscripts. He has a Ph.D. in Biosystems Engineering from Oklahoma State University, a M.S. in Agronomy from the University of Arkansas, and a B.S. in Life Sciences from the Missouri University of Science and Technology. He may be contacted at

haggard@uark.edu or Engineering Hall 203, University of Arkansas, Fayetteville, AR 72701, USA.

Mrs. Erin Grantz is a program manager and data scientist with the Arkansas Water Resources Center, and she is currently working on her Ph.D. in Environmental Dynamics at the University of Arkansas. She helped manage the data analysis for this project at Lake Fayetteville, including the development of the classification and regression trees. She has a M.S. and B.S. in Crop, Soil and Environmental Sciences from the University of Arkansas, and her graduate research includes nutrient mass balances at Lake Fayetteville. She may be contacted at egrantz@uark.edu or 1371 West Altheimer Drive, Don Tyson Center for Agricultural Sciences, Fayetteville, AR 72704, USA.

Dr. Brad Austin is a research scientist with the Arkansas Water Resources Center, where he provides and helps manage field and lab services for all research projects. Specifically, he managed the field work, lab analysis including microcystins, and statistical analysis for this research project at Lake Fayetteville. He has a Ph.D. in Biology from the University of Arkansas, a M.S. in Aquatic Biology and Limnology from Fort Hays State University, and a B.S. in Biological Sciences from Ottawa University. He may be contacted at bjaustin@uark.edu or 1371 West Altheimer Drive, Don Tyson Center for Agricultural Sciences, Fayetteville, AR 72704, USA.

Dr. Nicole Wagner is an assistant professor in the Biological Sciences Department at Oakland University, and she helped conceptualize this research project and has been involved with research at Lake Fayetteville since 2020. Her research examines how aquatic organisms, including cyanobacteria, are influenced by nutrients and anthropogenic stressors. She has a M.S. and Ph.D. in Environmental and Life Sciences from Trent University, and a B.S. in Biology from Trent University. She may be contacted at nicolewagner@oakland.edu or Dodge Hall Room 375, 118 Library Drive, Rochester, MI 48309, USA.

Dr. Thad Scott is an aquatic ecologist focused on the interactions of cyanobacteria, nutrients, and environmental triggers. He is a professor in the Biology Department at Baylor University, and a former professor in the Crops, Soils and Environmental Sciences Department at the University of Arkansas. He has several past publications on nutrient dynamics at Lake Fayetteville, and he helped conceptualize the management applications within this manuscript. He has a Ph.D. in Biology from Baylor University, a M.S. in Environmental Sciences from Tarleton State University, and a B.S. in Biology from Howard Payne University. He may be contacted at thad_scott@baylor.edu or B.207 Baylor Science Building, One Bear Place, Waco, TX 76798, USA.

References

Aranda-Rodriguez, R., Z. Jin, J. Harvie, and A. Cabecinha. 2015. Evaluation of three field test kits to detect microcystins from a public health perspective. *Harmful Algae* 42: 34-42.

Arkansas HAB Workgroup. 2019. Harmful Algal Bloom Management Plan. Arkansas State Waters, Arkansas Department of Energy and Environment – Division of Environmental Quality. Available at: https://www.adeq.state.ar.us/water/pdfs/HAB-ResponsePlan-Manual-bookmarks-2019-12-12-Final.pdf. Accessed February 1, 2023.

Austin, B.J., B. Olsen, T. Wentz, and B.E. Haggard. 2018. Algal Blooms in Arkansas Streams, Ponds, and Lakes. Arkansas Water Resources Center FS-2018-02. Available at: https://scholarworks.uark.edu/awrcfs/9?utm_source=scholarworks.uark.edu%2Fawrcfs%2F9&utm_medium=PDF&utm_campaign=PDFCoverPages. Accessed February 1, 2023.

Austin, B.J. and B.E. Haggard. 2023. Total microcystin concentration variability in water samples and recommended minimum volume for freeze thaw cycles. *Journal of Contemporary Water Research and Education* (this Issue).

Brooks, B.W., J.M. Lazorchak, M.D.A. Howard, M.V. Johnson, S.L. Morton, D.A.K. Perkins, E.D. Reavie, G.I. Scott, S.A. Smith, and J.A. Steevens. 2016. Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems? *Environmental Toxicology and Chemistry* 35: 6-13.

Burford, M.A., C.C. Carey, D.P. Hamilton, J. Huisman, H.W. Paerl, S.A. Wood, and A. Wulff. 2020. Perspective: Advancing the research agenda for improving understanding of cyanobacteria in a future of global change. *Harmful Algae* 91: 101601.

Carmichael, C.W. 2001. Health effects of toxin-producing cyanobacteria: "The CyanoHABs". *Human and Ecological Risk Assessment: An International Journal* 7(5): 1393-1407.

Chaffin, J.D., T.W. Davis, D.J. Smith, M.M. Baer, and G.J. Dick. 2018. Interactions between nitrogen form, loading rate, and light intensity on Microcystis and Planktothrix growth and microcystin production. *Harmful Algae* 73: 84-97.

De'Ath, G. and K.E. Fabricius. 2000. Classification and regression trees: A powerful yet simple technique for ecological data analysis. *Ecology* 81(11): 3178-3192.

Environmental Protection Agency (EPA). 2019. Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. Office of Water, Health and Ecological Criteria Division, EPA 822-R-19-001, Washington, D.C. Available at: https://www.epa.gov/sites/default/files/2019-05/documents/hh-rec-criteria-habs-document-2019.pdf. Accessed February 1, 2023.

Falconer, I.R. 1999. An overview of problems caused by toxic blue-green algae (cyanobacteria) in drinking and recreational water. *Environmental Toxicology* 14(1): 5-12.

Flores, N.M., T.R. Miller, and J.D. Stockwell. 2018. A global analysis of the relationship between concentrations of microcystins in water and fish. *Frontiers in Marine Science* 5: 00030.

Graham, J.L., K.A. Loftin, M.T. Meyer, and A.C. Ziegler. 2010. Cyanotoxin mixtures and taste-and-odor compounds in cyanobacterial blooms from the Midwestern United States. *Environmental Science and Technology* 44(19): 7361-7368.

Grantz, E.M., B.E. Haggard, and J.T. Scott. 2014. Stoichiometric imbalance in rates of nitrogen and phosphorus retention, storage, and recycling can perpetuate nitrogen deficiency in highly-productive reservoirs. *Limnology and Oceanography* 59(6): 2203-2216.

Gurbuz, F., O.Y. Uzunmehmetoğlu, Ö. Diler, J.S. Metcalf, and G.A. Codd. 2016. Occurrence of microcystins in water, bloom, sediment and fish from a public water supply. *Science of the Total Environment* 562: 860-868.

Haggard, B.E., E. Grantz, B.J. Austin, A.L. Lasater, L. Haddock, A. Ferri, N.D. Wagner, and J.T. Scott. 2023. Microcystin shows thresholds and hierarchical structure with physiochemical properties at Lake Fayetteville, Arkansas, May through September 2020. *Journal of the ASABE* 66(2): 307-317.

Izydorczyk, K., M. Tarczynska, T. Jurczak, J. Mrowczynski, and M. Zalewski. 2005. Measurement of phycocyanin fluorescence as an online early warning system for cyanobacteria in reservoir intake water. *Environmental Toxicology* 20(4): 425-430.

King, R.S. and C.J. Richardson. 2003. Integrating bioassessment and ecological risk assessment: An approach to developing numerical water-quality criteria. *Environmental Management* 31: 795-809.

Meyer, R.L. 1971. *A Study of Phytoplankton Dynamics in Lake Fayetteville as a Means of Assessing Water Quality*. Arkansas Water Resources Center Technical Report Number PUB010A, Fayetteville, AR.

Paerl, H.W. and T.G. Otten. 2016. Duelling 'CaynoHABs': Unravelling the environmental drivers controlling dominance and succession among diazotrophic and non-N2-fixing harmful cyanobacteria. *Environmental Microbiology* 18(2): 316-324.

Plaas, H.E. and H.W. Paerl. 2021. Toxic cyanobacteria: A growing threat to water and air quality. *Environmental Science and Technology* 55(1): 44-64.

Preece, E.P., W. Hobbs, F.J. Hardy, L. O'Garro, E. Frame, and F. Sweeney. 2021. Prevalence and persistence of microcystin in shoreline lake sediments and porewater, and associated potential for human health risk. *Chemosphere* 272: 129581.

Qian, S.S., R.S. King, and C.J. Richardson. 2003. Two statistical methods for the detection of environmental thresholds. *Ecological Modelling* 166: 87-97.

Rousso, B.Z., E. Bertone, R.A. Stewart, K. Rinke, and D.P. Hamilton. 2021. Light-induced fluorescence quenching leads to errors in sensor measurement of phytoplankton chlorophyll and phycocyanin. *Water Research* 198: 117133.

Therneau, T. and B. Atkinson. 2019. rpart: Recursive Partitioning and Regression Trees. R Package version 4.1-19. Available at: https://rdrr.io/cran/rpart/. Accessed February 1, 2023.

Thomson-Laing, G., J. Puddick, and S.A. Wood. 2020. Predicting cyanobacterial biovolumes from phycocyanin fluorescence using a handheld fluorometer in the field. *Harmful Algae* 97: 101869.

Wagner, N.D., E. Quach, S. Buscho, A. Ricciardelli, A. Kannan, S.W. Naung, G. Phillip, B. Sheppard, L. Ferguson, A. Allen, C. Sharon, J.R. Duke, R.B. Taylor, B.J. Austin, J.K. Stovall, B.E. Haggard, C.K. Chambliss, B.W. Brooks, and J.T. Scott. 2021. Nitrogen form, concentration, and micronutrient availability affect microcystin production in cyanobacterial blooms. *Harmful Algae* 103: 102002.

Walls, J.T., K.H. Wyatt, J.C. Doll, E.M. Rubenstein, and A.R. Rober. 2018. Hot and toxic: Temperature regulates microcystin release from cyanobacteria. *Science of The Total Environment* 610-611: 786-795.

Universities Council on Water Resources
Journal of Contemporary Water Research & Education
Issue 177, Pages 72-82, April 2023

Case Study Article

Continuous Hydrologic Modeling of a Parking Lot and Related Best Management Practices with PCSWMM

Robson L. Pachaly[1], Don Guy V.V. Biessan[1], Jose G. Vasconcelos[1], *Frances C. O'Donnell[1], and Benjamin F. Bowers[1]

[1]Department of Civil and Environmental Engineering, Auburn University, Auburn, Alabama
**Corresponding Author*

Abstract: Permeable pavements are a green infrastructure stormwater management practice that can serve as a functional component of the site design. However, previous field studies suggest high uncertainty in the parameters used for performing hydrologic calculations for permeable pavements. The Environmental Protection Agency (EPA) Storm Water Management Model (SWMM) within the PCSWMM software package was used to simulate the hydrologic dynamics of a parking lot that is 25% covered with permeable interlocking concrete pavers in Auburn, AL. The model was calibrated to field observations of water level at two points where the pavement system outflows to a bioretention basin and rainfall data from a nearby weather station. The use of the Curve Number (CN) method within SWMM resulted in good prediction of pavement outflow by the calibrated model, with R^2 and Nash-Sutcliffe model efficiency both greater than 0.8, except where issues with precipitation data coverage occurred. This demonstrates that permeable pavements can be modeled as a land cover type rather than as detention storage. The calibrated value of the runoff CN for permeable pavement was 60, much lower than what is recommended in many design guidelines for the underlying soil type at the research site, which is hydrologic soil group B. Based on evaluation of alternative model scenarios, the permeable pavement reduced runoff by 11-38% across contrasting rain events.

Keywords: *stormwater, permeable pavement, hydrologic modeling, model calibration, curve numbers*

Stormwater management is integral to mitigating the impacts of urban development on water resources. The sponsors of a development or redevelopment project that exceeds a certain size are typically required by local, state, or federal law to have a stormwater management plan to maintain or restore to the maximum extent technically feasible the pre-development hydrology of the property (US EPA 2009). This is achieved with stormwater best management practices (BMPs), which are structures and functional site components that store and treat stormwater before it is released from the watershed.

The conventional design of stormwater systems does not perform well in terms of reducing peak flows (NRCS 1986) and removing runoff contaminants (Roseen et al. 2006). Also, land uses with high levels of imperviousness are known for contributing to higher peak flows (NRCS 1986) and contaminant loadings (US EPA 1983). Permeable pavement is a stormwater BMP that also serves as a functional component of the site, such as a roadway or parking area, with the benefits of reducing peak flows by flattening flow-duration curves (Hood, Clausen, and Warner 2007), lowering contaminant loads (Dietz and Clausen 2008), and decreasing temperature in downstream water bodies (Van Dam et al. 2015). Examples of permeable pavements include interlocking pavers, pervious concrete, and porous asphalt; they have an open void structure that

Research Implications

- The Environmental Protection Agency (EPA) Stormwater Management Model (SWMM) is a useful tool for performing continuous hydrological modeling of permeable pavements in urban settings.
- Implementation of interlocking concrete pavers substantially reduced runoff volumes and this can be quantifiable through modeling.
- The curve numbers recommended for site design with permeable paver systems may be too high, resulting in overdesign of detention storage in systems that include both permeable paver systems and storage-based stormwater practices.

allows infiltration of water through the pavement and into an underlying drainage basin made of crushed stone or high permeability soil.

The rainfall-runoff dynamics of permeable pavements must be calculated as part of the site design process. The most common approach for rainfall-runoff calculations for stormwater management for small catchments is the Natural Resources Conservation Service (NRCS) Curve Number (CN) approach (NRCS 1986). Other infiltration calculation methods, including Horton, Modified Horton, Green-Ampt, and Modified Green-Ampt, are available in most modeling software but require field data that can be difficult to obtain. The advantage of the CN method is that it is based on a dimensionless parameter that is related to land use, hydrological soil group, and antecedent soil moisture. The CN method was developed for small agricultural watersheds (< 4 ha) and was originally developed as an event-based simulation approach. Within the Storm Water Management Model (SWMM), the CN method is implemented with time as a variable (continuous simulation) and the initial abstraction can be used as a calibration parameter. Due to the ease of calibration and simulation in SWMM and the minimal input data requirements, the CN method was chosen for this study.

To apply this approach to a catchment containing permeable pavements, a CN must be assigned to the permeable pavement surface so that runoff from each rainfall event can be calculated as it would be for a pervious or impervious land cover, such as grass or conventional pavement. However, simulation of unsaturated flow through permeable pavements suggests that runoff is only produced when the permeable pavement subgrade becomes fully saturated from below (Chai et al. 2012). Therefore, mass balance routing approaches that treat permeable pavements as detention storage rather than as a land cover type have been proposed as more appropriate than the CN approach, particularly for systems with underdrains (Martin and Kaye 2014; 2015).

One advantage of the CN approach is that the parameters can be readily obtained with the knowledge of hydrological soil group and land use type. Thus, it is widely used as a means to compute runoff abstractions in hydrological models such as the EPA SWMM. SWMM is a dynamic hydrologic-hydraulic model that is used to simulate runoff quantity and quality for single or continuous events. The model estimates the runoff generated by subcatchments, transporting it through collection systems and computing the flow rate, flow depth, and water quality in each component of the collection system (Rossman 2015). The implementation of CN into SWMM is relatively new. The original NRCS CN method was an event-based methodology, which was adapted for continuous simulation as outlined in Rossman and Huber (2016). Software packages such as PCSWMM, developed by Computational Hydraulics International (CHI, Guelph, ON, Canada), are often used for this type of modeling. The computational engine of the PCSWMM software package is SWMM. SWMM was selected for use in this study because it was designed for stormwater management in urban watersheds and simulates both hydrologic and hydraulic dynamics. Other models, such as the United States Department of Agriculture (USDA) Soil Water Assessment Tool (SWAT) (Arnold et al. 2012), are more appropriate for agriculture and forested watersheds, while models such as the U.S. Army Corps of Engineers Hydrologic Engineering Center's Hydrological Modeling System (HEC-HMS) (Feldman 2000) and River Analysis System (HEC-RAS) (Brunner 2010) are more appropriate for focused hydrologic or hydraulic analysis, respectively.

Recent research has made progress in representing permeable pavements within SWMM. Zhang and Guo (2015) performed an initial investigation into permeable pavement modeling and provided recommendations for setting time steps and other model parameters for these systems. Later work found that SWMM produced representative hydrographs for permeable interlocking concrete paver systems using the porous paver module (Randall et al. 2020). Madrazo-Uribeetxebarria et al. (2023) linked porous pavers and CN, focusing on creating an equivalency between the CN model in SWMM and the approach based on green infrastructure practices (GIP).

A major challenge associated with using the CN approach for permeable pavements is the broad range of hydrologic behavior observed in the literature (Bean, Hunt, and Bidelspach 2007; Beisch and Foraste 2011; Eger, Chandler, and Driscoll 2017), which leads to uncertainty in the parameters of the calculation. The CN values recommended in many design manuals for permeable pavement surfaces are high. For example, a value of 85 is recommended by the City of Auburn, Alabama, which has relatively high permeability soils (primarily hydrologic soil group B) (e.g., City of Auburn 2019). However, the limited empirical studies on the topic have reported a wide range of values between 6 and 89 (Bean, Hunt, and Bidelspach 2007; Beisch and Foraste 2011), with much of the variability attributed to differences in design and underlying soil type. The use of a CN that is too large could result in an overly conservative design of downstream detention storage and higher construction costs (Ellis et al. 2022).

Few studies have combined field data collection with hydrologic modeling to evaluate the hydrologic behavior of permeable pavements. In this study, hydrologic monitoring data were collected for a municipal parking lot in Auburn, AL, that includes permeable interlocking concrete pavements (PICP) and these data were used to develop a calibrated model in SWMM. The following research objectives were addressed.

1. Determine the calibrated value of the runoff CN for PICP and compare it to recommended values for design.
2. Evaluate the ability of SWMM with its CN method to accurately model a small catchment with PICP for an extended period.
3. Use the calibrated model to assess how increasing or decreasing the area of PICP would affect runoff.

Methods

Study Site

This study was conducted at a municipal parking lot in Auburn, AL (32°36'32.06"N, 85°28'37.01"W). The climate is humid subtropical with mean annual precipitation of 1340 mm and mean annual temperature of 18°C. Soils are classified as well-drained loamy sands and sandy loams in hydrologic soil group B (NRCS 2020). The parking lot consists of a 0.29 ha paved area, of which 25% is PICP and 75% is impervious asphalt (Figure 1). The PICP are underlain by a 15 cm aggregate choker course (5 cm of #89 stone above 10 cm of #57 stone) and a 60 cm aggregate recharge bed consisting of #2 stone. The permeable pavement system drains to an onsite bioretention basin through an underdrain. There is also a grass channel to convey excess surface runoff from the asphalt and PICP area to the bioretention basin. In addition to the asphalt and PICP, 0.015 ha of greenspace drains to the bioretention basin, making the total catchment area 0.31 ha.

Field Data Collection

Field data were collected from April to July 2021, to allow for calibration of PCSWMM. Five-minute precipitation data recorded with a HOBO RX2100 tipping bucket rain gauge (Onset Computer Corporation, Bourne, MA) were obtained from the City of Auburn from a station that is 1.3 km from the research site, which introduced some uncertainty in the timing of rain events. Two HOBO U20L-04 (Onset Computer Corporation, Bourne, MA) water level loggers were used to measure water depth above the bioretention basin media at the entrance to and within the bioretention basin. The sensors have an accuracy of 0.6 mm and measured at a 5-minute interval. A third logger was installed outside of the bioretention basin to measure barometric pressure for atmospheric compensation. The water level logger at the entrance of the bioretention basin was installed behind a two-stage hydraulic control to allow for the calculation of flow rate (Figure 2). The structure included a 75 mm diameter circular sharp-crest orifice to enable a depth-discharge relationship for low flow computations within SWMM, and a trapezoidal crest weir with a base of 1.6 m and 30-degree side slopes that was used for the discharge of large flows. The assessment of these control structures was made through a comparison between the observed depths and the modeled results.

SWMM Model Development

Because of the simple geometry of the system, each different land cover type could be explicitly represented as subcatchments in SWMM. Thirteen

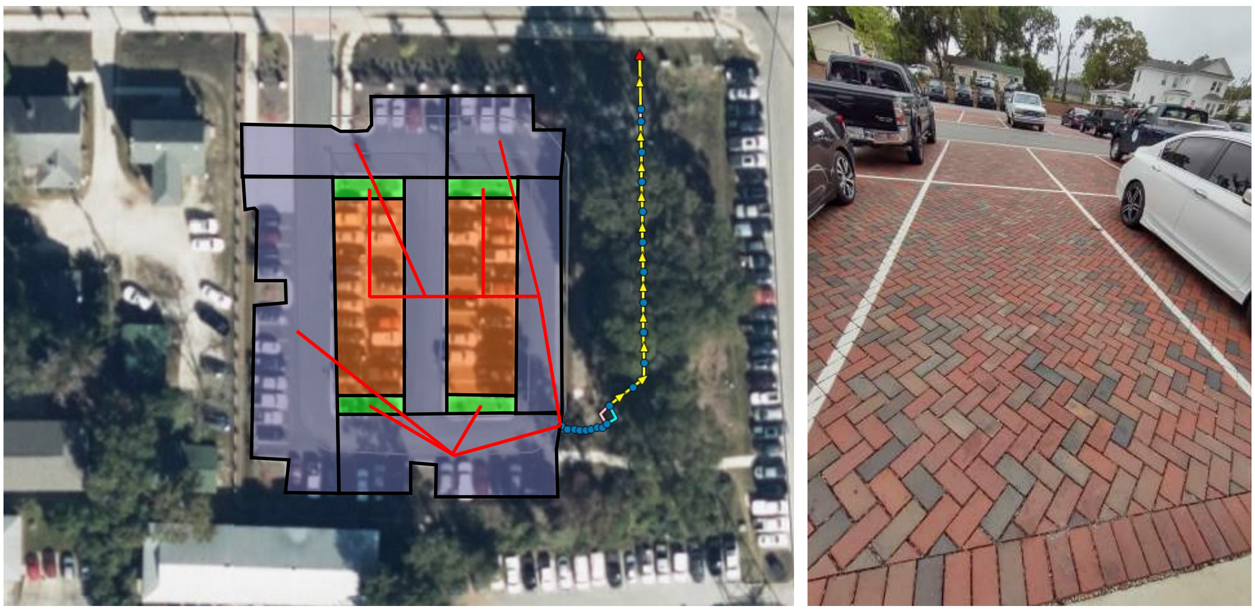


Figure 1. Left: Parking lot catchment draining to the bioretention basin on the east side of the parking lot including asphalt (purple), PICP (orange), and greenspace (green). The flow paths to the bioretention basin (blue dots) and through the bioretention basin (yellow arrows) are also shown. Right: PICP installed at the site.

Figure 2. Hydraulic structure at the entrance to the bioretention basin with an orifice for low-flow measurement and a weir for high-flow measurement.

subcatchments were represented, all draining to the channel that leads into the bioretention basin (Figure 3). The bioretention basin was represented as a trapezoidal channel with an outlet structure at the downstream end. The infiltration in the bioretention basin was represented by setting the property seepage loss rate for the channel reaches with values calibrated from measured water level data.

The groundwater/aquifer module in SWMM was not used to represent exfiltration in the model. The exfiltration was represented within the model as a seepage rate of 5 mm/hr in each link. Changes in vegetation growth, which would influence the surface roughness for flows in the bioretention basin and greenspace, were not considered in this study since SWMM does not consider this process in its calculations. Evaporation was represented using a daily evaporation rate based on temperature obtained through the Global Historical Climatology Network - Daily (GHCN-Daily). The CN value for dense graded asphalt pavement (98) was obtained from the NRCS TR-55 (NRCS 1986). The small areas of constructed greenspace within the parking lot are pine straw over a high-permeability soil draining to the permeable pavement recharge bed and were assigned a low CN value of 40. Model calibration was performed using the Sensitivity-based Radio Tuning Calibration (SRTC) tool in PCSWMM Version 5.1. The calibrated parameters were the following:

- CN value for PICP;
- Manning's roughness of overland flow for pervious subcatchments (PICP and greenspace);
- depression storage for each subcatchment;
- drying time parameter for each land cover type;
- Manning's roughness of the bioretention basin;
- seepage rate in the bioretention basin; and
- discharge coefficients for the weir and orifice.

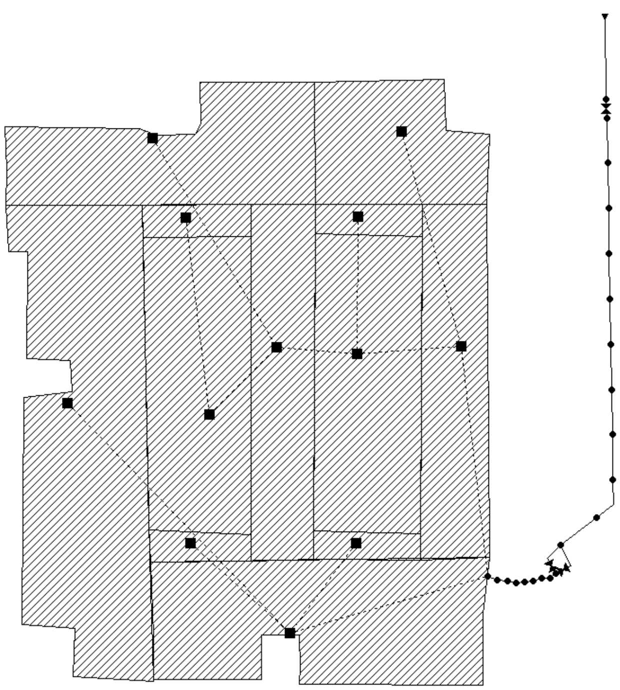


Figure 3. SWMM model representation of the parking lot. The subcatchments (hatched areas) converge to the bioretention basin, which is represented as a channel (dotted lines).

The model was run continuously at a sub-daily routing time step of 0.1 s from April to July of 2021, which included 15 rain events. The routing time step obeys the Courant-Friedrichs-Lewy (CFL) condition and a small routing time step was selected because of the unsteady characteristics of the system and the way that the bioretention was modeled. This period includes the period of active convective thunderstorms that typically represents the highest annual rainfall intensities in the study region. A select group of rain events that represent the range of outflows from the parking lot were used for model calibration and validation. The coefficient of determination (R^2) and Nash-Sutcliffe model efficiency (NSME) were used to evaluate model performance. There was some variation in model performance statistics across rain events, which is represented by the selected rain events. Additionally, a visual inspection of the rising and falling limbs of the hydrograph was used to determine which modeling conditions were most representative of the field measurements.

Scenario Analysis

Within the calibrated model, it was possible to create alternate scenarios to study the effect of permeable pavements on runoff. Two alternative scenarios were considered and the total runoff volume for the full simulation period (April to July 2021) was compared across the scenarios. First, a scenario was considered in which the entire parking lot was constructed from impermeable dense graded asphalt pavement. Second, a scenario was considered in which the entire parking lot was constructed from PICP. These scenarios were developed to consider the full range of design possibilities for a parking lot built with a combination of impermeable pavement and PICP.

Results and Discussion

Model Calibration

Within the period of the hydrological modeling, four rain events in 2021 were selected for SWMM calibration that span the range of conditions observed in the study area (Figure 4).

1. April 10: Total depth 27 mm, duration 6 h.
2. April 24: Total depth 63 mm, duration 19 h.
3. May 3: Total depth 68 mm, duration 45 h.
4. June 19: Total depth 48 mm, duration 72 h.

The site-specific calibrated values of the model parameters are given in Table 1. All calibrated values were within the range typically found in the literature except for the discharge coefficients. The weir coefficient was lower than SWMM's traditional value of 1.8 (Brater et al. 1996), likely due to a lack of perfect horizontal alignment of the weir crest. The orifice coefficient was larger than SWMM's recommended value of 0.65. At this study site, there were frequent problems with debris, such as leaves and twigs, blocking discharge through the orifice. This reduced the area

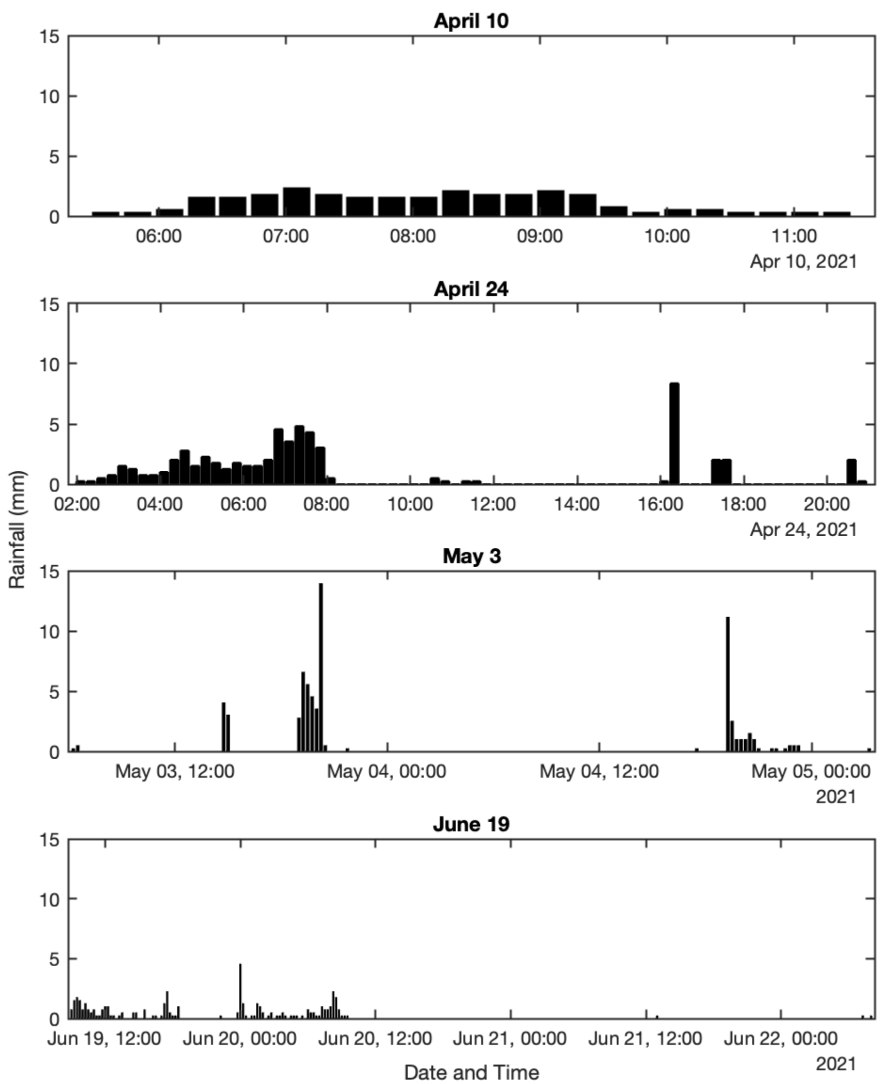


Figure 4. Fifteen-minute hyetographs of the four rain events selected for SWMM calibration. Note that the time range (x axis) is different for each subplot.

Table 1. Calibrated SWMM parameters. The range of values tested in the calibration procedure and the final calibrated value are shown.

Parameter	Range	Calibrated Value
PICP CN	40-80	60
Manning's Roughness (Overland Flow)	0.011-0.031	0.021
Depression Storage (mm)	0.6-2.4	1.2
Drying Time (days)	2.5-7.5	5.0
Bioretention Basin Seepage Rate (mm/h)	2.3-9.0	4.5
Manning's Roughness (Bioretention Basin)	0.022-0.090	0.045
Weir Discharge Coefficient	0.6-1.2	0.8
Orifice Discharge Coefficient	0.2-0.5	0.3

of flow through the orifice, leading the calibrated value of the discharge coefficient to be larger. In this case, it was easier to correct for this problem by calibrating the discharge coefficient to a larger value than to adjust the discharge area.

Observed and Modeled Water Depths

Figure 5 presents a comparison of the observed water depths with calibrated model results above the media in the bioretention basin and at the hydraulic structure. The response at the hydraulic structure was very flashy in response to rain, as it captures the surface drainage from the parking lot, a small and largely impervious catchment. The water depth in the bioretention basin rises quickly in response to the rain events, due to its small volume. However, the process of drainage through infiltration was much slower, taking at least four days.

Based on R^2 and NSE, the model predictions of bioretention basin water level were satisfactory, with values consistently above 0.8 for both statistics. The exception was the June rain event (Figure 5g-h), in which the observed onset of water level rise did not match the model. This may be because the rain gauge is not located immediately at the study site and rain may have started earlier at the rain gauge site. The calibrated model also performed reasonably well at representing the dynamics of runoff from the parking lot, though R^2 and NSE were lower for both the May (Figure 5e-f) and June (Figure 5g-h) rain events. In the May rain event, there was a small early spike in the observed data that is not captured in the model. It is likely that this is also due to issues with the rain gauge location. The amount of runoff in this early spike was not large enough to cause a change in the water level in the bioretention basin. The falling limb of the bioretention basin hydrograph was longer in the model simulation than in the observed data, which is likely due to an underestimation of infiltration rate. However, representing peak flows correctly was the primary goal, and these show good agreement.

Some studies have suggested that a modeling approach that treats permeable pavements as a detention reservoir rather than a catchment is more appropriate (Martin and Kaye 2015). The calibrated SWMM results demonstrated that the CN approach is adequate for modeling permeable pavements, though a very low depression storage value (> 2 mm) must be used. Further, the calibrated values of CN (60) indicated that the values recommended in many design guidance are too high. One important remark is the importance of the field data acquisition to ensure the calibration of the hydrological modeling. Finally, the ability to perform continuous hydrological modeling provides more confidence that previous rain events are properly incorporated in the predictions and that the CN values obtained here are representative for the test site.

Alternative Pavement Scenarios

For the alternative scenarios, a complete replacement of dense graded asphalt pavement by

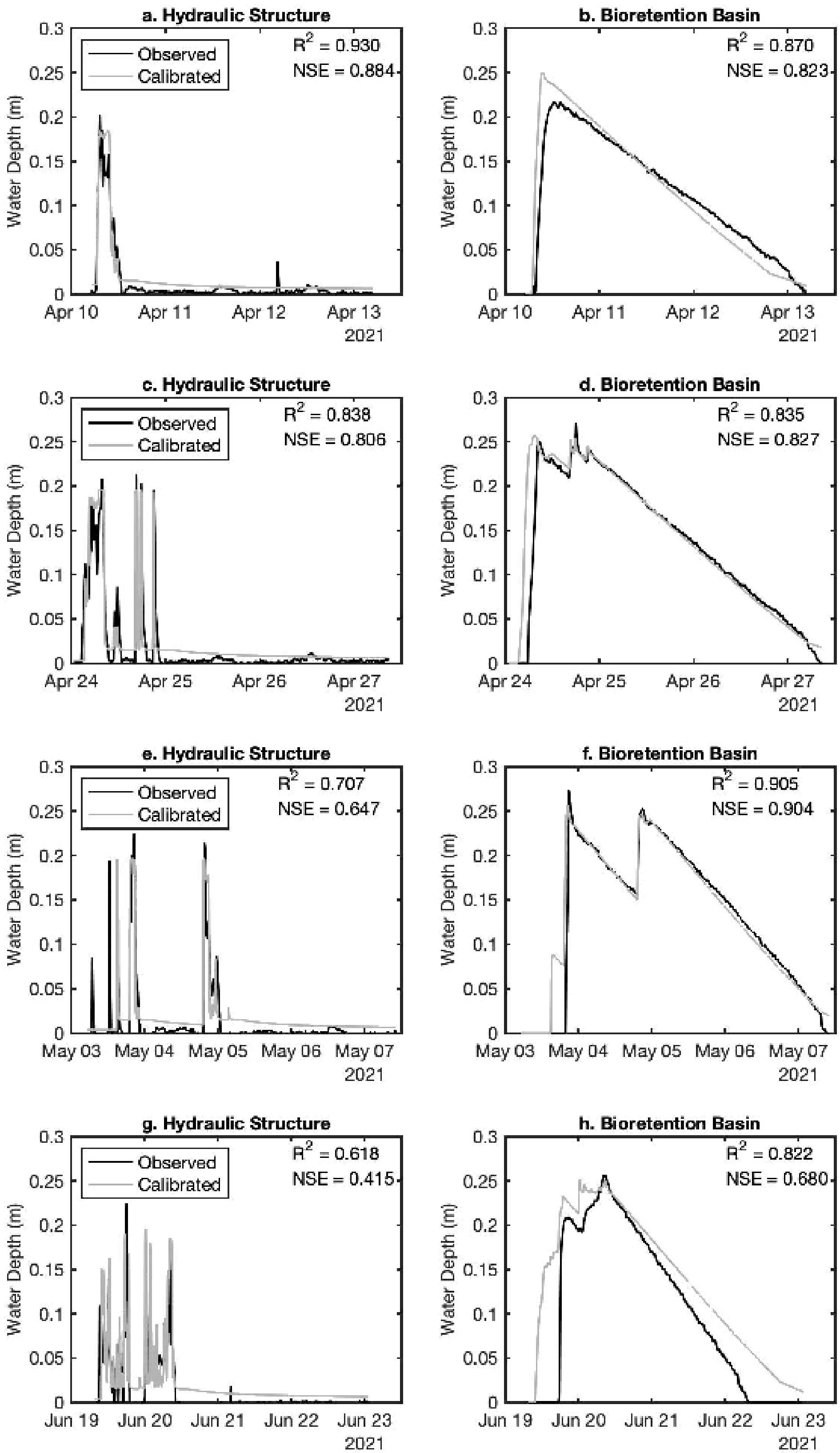


Figure 5. Observed (black line) and modeled (gray line) changes in water depth in (a, c, e, g) the hydraulic structure and (b, d, f, h) the bioretention basin for the rain event on (a-b) April 10, 2021, (c-d) April 24, 2021, (e-f) May 3, 2021, and (g-h) June 19, 2021.

PICP substantially reduced the runoff volume. The decrease in volume from the as-built design ranged from 86% for the smallest rain event to 60% for the largest rain event (Figure 6). The difference between the as-built design and a parking lot built entirely from impermeable dense graded asphalt pavement was much smaller, ranging from 11-38%. Expanding the area of PICP in the lot could reduce runoff volume, which reduces the required size and cost of detention storage (Ellis et al. 2022). However, this must be weighed against other considerations, such as the greater durability of impermeable pavement for high-traffic areas.

Conclusions

This work, consisting of continuous hydrologic modeling supported by field monitoring, concluded that a calibrated CN of 60 yielded a representative description of the parking lot hydrology. This is much lower than the value of 85 that is currently recommended by the City of Auburn stormwater design guidelines. While some parts of the city have less permeable soils and may require a more conservative CN, the value of 85 is likely too high for the large parts on the city with hydrologic soil group B. This finding indicates that using a lower CN for permeable pavements at this site will more accurately represent the hydrologic benefits of using this type of green infrastructure practice. Further studies of this nature at other sites could encourage wider application of permeable pavements in Auburn and across Alabama. The scenario analysis in this study demonstrated that PICP can reduce runoff volume from a parking lot by up to 86% depending on the percentage of area covered by PICP and the type of rain event.

The results also highlight the benefits of using SWMM as a tool for designing stormwater management for sites that include green infrastructure practices, as the predictions of the model showed good agreement with measured values following calibration. Within this model, and considering this parking lot, the modeling parameter depression storage (which is very important for computing hydrological abstractions) was found to be in the range of 1 to 2 mm. Future work should consider the application of extended-period SWMM to other types of green infrastructure practices to precisely quantify their benefits. Another possible future direction is to perform similar studies in other sites with permeable pavements but different hydrological characteristics, to understand how these can impact the values of CN for hydrological modeling.

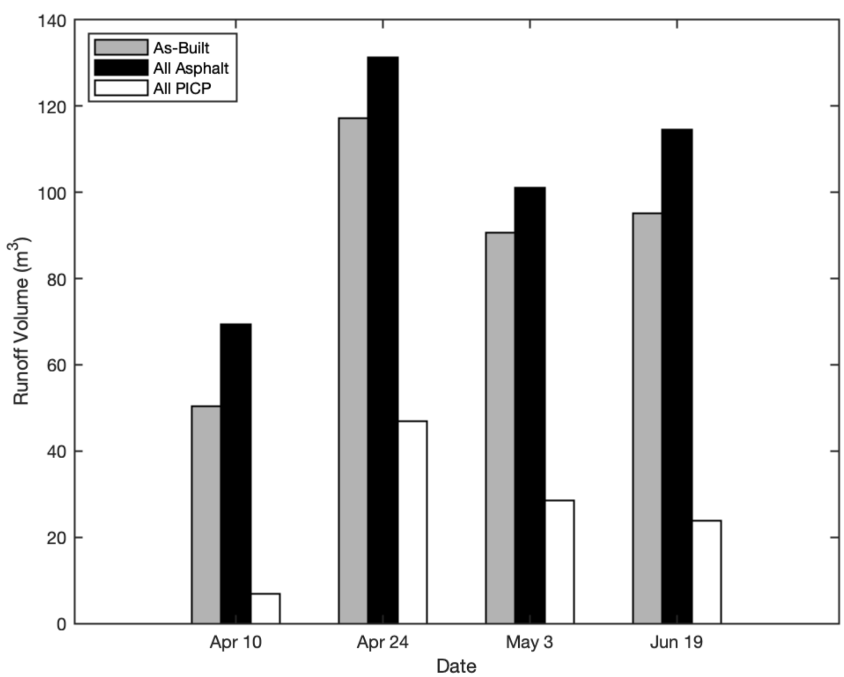


Figure 6. Total runoff volume generation for the as-built design and alternative scenarios where the parking lot is entirely impervious asphalt or entirely permeable pavement.

Acknowledgements

The authors thank Dan Ballard and Marla Smith from the City of Auburn for providing site access, precipitation data, and design information. We also would like to acknowledge the support of Computational Hydraulics International (CHI) that allowed access to PCSWMM through a CHI educational grant. This research was funded by a USGS 104(b) grant awarded by the Alabama Water Resources Research Institute, grant #G16AP00037-2020AL354B.

Author Bio and Contact Information

Robson L. Pachaly holds a Ph.D. in Civil Engineering, area of concentration in water resources. He obtained his Ph.D. in Civil and Environmental Engineering from the Department of Civil and Environmental Engineering at Auburn University in 2021. His research was focused on the numerical modeling of stormwater systems. He holds a bachelor's degree in Sanitary and Environmental Engineering and a master's degree in Environmental Engineering, both degrees obtained at the University of Santa Maria, Brazil. He may be contacted at rlp0046@auburn.edu.

Don Guy Biessan is a Ph.D. student in Civil and Environmental Engineering at Auburn University. His research interest is in pavement geotechnics. His research projects focus on evaluating the effects of moisture on limerock/limestone used as pavement base materials and the effects of sea level rise on surface transportation infrastructures. For his masters' thesis, he developed a practical tool for the design and cost optimization of permeable pavements. He may be contacted at dvb0007@auburn.edu or by mail at 238 Harbert Engineering Center, Auburn, AL 36849.

Jose G. Vasconcelos is a Water Resources engineer and associate professor of Civil and Environmental Engineering at Auburn University. His research involves applications of computational hydraulics and hydrology, including how urban water infrastructure responds to extreme hydrological events. He teaches graduate and undergraduate courses in introduction to civil engineering, hydraulics, and numerical modeling. He also advises the Auburn University chapter of Engineers Without Borders in projects in Rwanda and Guatemala. He may be contacted at jgv@auburn.edu or by mail at 238 Harbert Engineering Center, Auburn, AL 36849.

Frances C. O'Donnell (corresponding author) is an ecohydrologist and assistant professor of Civil and Environmental Engineering at Auburn University. Her research focuses on the use of green infrastructure and ecosystem restoration to improve the sustainability and climate resilience of water resources systems. She also teaches graduate and undergraduate courses in statistics, hydraulics, and hydrology and works with students and colleagues in northern Haiti through a partnership with the Henri Christophe Campus of the State University of Haiti. She may be contacted at fco0002@auburn.edu or by mail at 238 Harbert Engineering Center, Auburn, AL 36849.

Benjamin F. Bowers is an assistant professor in the Department of Civil and Environmental Engineering at Auburn University. His expertise is in sustainable and resilient pavements. He teaches graduate classes in civil engineering materials, advanced asphalt pavement mixture design, and sustainable transportation infrastructure. He may be contacted at bfbowers@auburn.edu.

References

Arnold, J.G., D.N. Moriasi, P.W. Gassman, K.C. Abbaspour, M.J. White, R. Srinivasan, et al. 2012. SWAT: Model use, calibration, and validation. *Transactions of the ASABE* 55(4): 1491-1508.

Bean, E.Z., W.F. Hunt, and D.A. Bidelspach. 2007. Evaluation of four permeable pavement sites in eastern North Carolina for runoff reduction and water quality impacts. *Journal of Irrigation and Drainage Engineering* 133(6): 583-592.

Beisch, D. and A. Foraste. 2011. Memorandum: 3rd Party Peer Review of I-66 and Route 234 Bypass Park & Ride Facility. SWM Comparative Study of Porous Asphalt in Prince William County, Virginia. WEG Project # 4905.

Brater, E.F., H.W. King, J.E. Lindell, and C.Y. Wei. 1996. *Handbook of Hydraulics,* 7th Edition. McGraw-Hill, New York, NY.

Brunner, G.W. 2010. *HEC-RAS Hydraulic Reference Manual- Hydrologic Engineering*. U.S. Army Corps of Engineers, Hydrologic Engineering Center, Davis, CA.

Chai, L., M. Kayhanian, B. Givens, J.T. Harvey, and D. Jones. 2012. Hydraulic performance of fully permeable highway shoulder for storm water runoff management. *Journal of Environmental Engineering* 138(7): 711-722.

City of Auburn. 2019. Guidance Document for the Integration of Green Infrastructure. Auburn, AL. Available at: https://www.auburnalabama.org/water-resource-management/watershed/green-infrastructure-master-plan/City%20of%20Auburn%20GI%20Master%20Plan%20FINAL%20COMPLETE%20Sept%202019.pdf. Accessed March 17, 2023.

Dietz, M.E. and J.C. Clausen. 2008. Stormwater runoff and export changes with development in a traditional and low impact subdivision. *Journal of Environmental Management* 87(4): 560-566. Available at: https://doi.org/10.1016/j.jenvman.2007.03.026. Accessed March 17, 2023.

Eger, C.G., D.G. Chandler, and C.T. Driscoll. 2017. Hydrologic processes that govern stormwater infrastructure behaviour. *Hydrological Processes* 31(25): 4492-4506. Available at: https://doi.org/10.1002/hyp.11353. Accessed March 17, 2023.

Ellis, J.R., D.G. Biessan, F.C. O'Donnell, J.G. Vasconcelos, and B.F. Bowers. 2022. Developing a practical tool for integrating green infrastructure into cost-effective stormwater management plans. *Journal of Hydrologic Engineering* 27(2): 04021045. Available at: https://doi.org/10.1061/(ASCE)HE.1943-5584.0002144. Accessed March 17, 2023.

Feldman, A.D. 2000. *Hydrologic Modeling System HEC-HMS: Technical Reference Manual*. U.S. Army Corps of Engineers, Hydrologic Engineering Center, Davis, CA.

Hood, M.J., J.C. Clausen, and G.S. Warner. 2007. Comparison of stormwater lag times for low impact and traditional residential development. *Journal of the American Water Resources Association* 43(4): 1036-1046. Available at: https://doi.org/10.1111/j.1752-1688.2007.00085.x. Accessed March 17, 2023.

Madrazo-Uribeetxebarria, E., M.G. Antín, J.A. Berrondo, and I. Andrés-Doménech. 2023. Modelling runoff from permeable pavements: A link to the curve number method. *Water* 15(1): 160. Available at: https://doi.org/10.3390/w15010160. Accessed March 17, 2023.

Martin, W.D. and N.B. Kaye. 2014. Hydrologic characterization of undrained porous pavements. *Journal of Hydrologic Engineering* 19(6): 1069-1079. Available at: https://doi.org/10.1061/(ASCE)HE.1943-5584.0000873. Accessed March 17, 2023.

Martin W.D. and N.B. Kaye. 2015. Characterization of undrained porous pavement systems using a broken-line model. *Journal of Hydrologic Engineering* 20(2): 04014043. Available at: https://doi.org/10.1061/(ASCE)HE.1943-5584.0001017. Accessed March 17, 2023.

Natural Resources Conservation Service (NRCS). 1986. TR-55: Urban Hydrology for Small Watersheds. Available at: https://www.hydrocad.net/tr-55.htm. Accessed March 17, 2023.

Natural Resources Conservation Service (NRCS). 2020. Web Soil Survey. Available at: https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm. Accessed March 17, 2023.

Randall, M., J. Støvring, M. Henrichs, and M.B. Jensen. 2020. Comparison of SWMM evaporation and discharge to in-field observations from lined permeable pavements. *Urban Water Journal* 17(6): 491-502. Available at: https://doi.org/10.1080/1573062X.2020.1776737. Accessed March 17, 2023.

Roseen, R.M., T.P. Ballestero, J.J. Houle, P.Avelleneda, R. Wildey, and J.F. Briggs. 2006. Performance Evaluations for a Range of Stormwater LID, Conventional Structural, and Manufactured Treatment Strategies for Parking Lot Runoff under Varied Mass Loading Conditions. *Transportation Research Record* 1984: 135–47.

Rossman, L.A. 2015. Storm Water Management Model (SWMM) User's Manual Version 5.1. U.S. Environmental Protection Agency, Cincinnati, OH.

Rossman L.A. and W.C. Huber. 2016. *Storm Water Management Model Reference Manual Volume I — Hydrology*. U.S. Environmental Protection Agency, Office of Research and Development, EPA/600/R-15/162A, Washington, D.C.

U.S. Environmental Protection Agency (US EPA). 1983. *Results of the Nationwide Urban Runoff Program (NURP), Volume 1- Final Report*. Water Planning Division, Publication PB84-185545, Washington, D.C. Available at: https://www3.epa.gov/npdes/pubs/sw_nurp_vol_1_finalreport.pdf. Accessed March 17, 2023.

U.S. Environmental Protection Agency (US EPA). 2009. Technical Guidance on Implementing the Stormwater Runoff Requirements for Federal Projects under Section 438 of the Energy Independence and Security Act. Office of Water, EPA 841-B-09-001, Washington, D.C. Available at: https://19january2017snapshot.epa.gov/sites/production/files/2015-08/documents/epa_swm_guidance.pdf. Accessed March 17, 2023.

Van Dam, T.J., J. Harvey, S.T. Muench, K.D. Smith, M.B. Snyder, I.L. Al-Qadi, H. Ozer, et al. 2015. *Towards Sustainable Pavement Systems: A Reference Document*. U.S. Department of Transportation, Report number FHWA-HIF-15-002, Washington, D.C. Available at: https://rosap.ntl.bts.gov/view/dot/38541. Accessed March 17, 2023.

Zhang, S. and Y. Guo. 2015. SWMM simulation of the storm water volume control performance of permeable pavement systems. *Journal of Hydrologic Engineering* 20(8): 06014010. Available at: https://doi.org/10.1061/(ASCE)HE.1943-5584.0001092. Accessed March 17, 2023.

Universities Council on Water Resources
Journal of Contemporary Water Research & Education
Issue 177, Pages 83-93, April 2023

Case Study Article

Monitoring Algal Blooms in Small Lakes Using Drones: A Case Study in Southern Illinois

Di Wu[1], *Ruopu Li[1], Jia Liu[2], Nafeesa Khan[2]

[1]*School of Earth Systems and Sustainability, Southern Illinois University, Carbondale, IL*
[2]*School of Civil, Environmental and Infrastructure Engineering, Southern Illinois University, Carbondale, IL*
**Corresponding Author*

Abstract: Harmful Algal Blooms (HABs) persist in many water bodies around the world and pose adverse health and economic impacts to the affected communities. Small Unmanned Aerial Vehicles (UAVs) have recently been applied as a cost-effective tool for HABs monitoring. In this study, HABs in two small lakes in Southern Illinois (Carbondale Reservoir and the Campus Lake of Southern Illinois University) were monitored using UAVs and biomass concentrations in lake waters. By analyzing vegetation indices derived from multispectral UAV images and chlorophyll-*a* concentrations in the two lakes, statistical regression models were established for each waterbody. The model relates spectral characteristics of the lake water to its algae biomass. It was found that normalized difference vegetation index (NDVI) and blue-to-green band ratio are the best-fit indices to the variation in chlorophyll-*a* in Carbondale Reservoir and the Campus Lake, respectively. The findings in this study can be used for monitoring HABs using UAVs in these lakes in the future.

Keywords: *HABs, drone, UAV, NDVI, spectral analysis*

As a severe water quality problem, Harmful Algal Blooms (HABs) pose serious threats to human health, aquatic ecosystem health, and recreational activities (Brooks et al. 2016; Le Moal et al. 2019). They are commonly linked to eutrophication, a process resulting from increasing accumulation of Nitrogen (N) and Phosphorus (P) from anthropogenic activities (Beusen et al. 2016). These nutrients sometimes trigger excessive growth of cyanobacteria or cyanophyceae that produce cyanotoxins such as cylindrospermopsin and microcystin (Paerl 2009). Consumption of HAB-contaminated fish or direct contacts can cause harmful health implications to community residents, especially children (Heil and Muni-Morgan 2021).

Because of health and environmental concerns, monitoring HABs outbreaks and their dynamics are imperative for managing water quality. However, the current HABs monitoring programs largely rely on regular funding from governmental agencies and/or volunteering efforts from the communities for collecting water samples and conducting biochemical testing. Such programs are often constrained by limited spatial coverage, sample size, and sampling frequency due to formidable financial and labor costs (Lomax et al. 2005; Palmer et al. 2015). Therefore, monitoring the conditions of HABs using cost-effective tools

Research Implications

- A UAV-based workflow was developed to monitor harmful algae blooms in two small lakes in Southern Illinois.
- Spectral indices such as NDVI and blue-to-green band ratio have proven to be useful indicators for HABs monitoring.
- The relationships between chlorophyll-*a* and spectral indices vary by waterbodies.
- The flexibility and low cost of UAVs allow cost-effective community-based HABs monitoring programs.

is critical to develop coping strategies to mitigate and manage their outbreaks.

Remote sensing has proven to be an effective tool for monitoring toxic algal blooms over large water bodies (Anderson 2009). As chlorophyll-*a* (Chl-*a*) is a typical estimator of phytoplankton biomass (Huot et al. 2007; Kasprzak et al. 2008), remote sensing provides a cost-effective solution to monitor HABs conditions by evaluating spectral signatures of Chl-*a* (Kubiak et al. 2016). Conventionally, the monitoring approach was mainly implemented using satellite remote sensing imagery (Matthews 2014). Wolny et al. (2020) detected HABs in the Chesapeake Bay using multispectral data products from Sentinel-3 satellites and identified HABs species based on *in-situ* phytoplankton data and ecological characteristics including salinity/temperature regimes, nutrient preferences, time of year, and locations within the Chesapeake Bay. Ma et al. (2021) propose a multi-source remote sensing approach for HABs monitoring in Chaohu Lake, China, which integrates MODIS, Landsat 8 OLI, and Sentinel-2A/B. However, the reliability of most satellite-based models is subject to a few limitations, including low spatial resolution (e.g., 30-meter for Landsat and 500-meter for MODIS images), relatively long revisiting flyover periods (e.g., ~15 days for Landsat), uncertainties of image quality associated with clouds, and formidable costs of high-quality images (Lomax et al. 2005).

Unmanned Aerial Vehicles (UAVs), also known as drones, or unmanned aircraft system (UAS), have been increasingly utilized in HABs monitoring in the recent decade (Wu et al. 2019). Compared with satellite platforms, they have demonstrated a few unique advantages for HABs monitoring including high spatial resolution (in the scale of centimeters), flexible scheduling, and customizable combined spectral properties (e.g., coupling different optical and/or thermal sensors) (Kislik et al. 2018; Manfreda et al. 2018). As an emerging alternative to satellite-based monitoring, UAVs, equipped with multispectral sensors, have demonstrated successful cases in monitoring algal blooms. Fráter et al. (2015) found that UAVs allow identification of gradual growth of algae that is hard to observe onshore. Lyu et al. (2017) established a HABs monitoring framework using a UAV, which allows responsive monitoring of algae blooms. Kim et al. (2021) used a UAV to successfully develop three spectral indices for monitoring algae in a stream. However, most of these projects were conducted in a single water body without intercomparing spectral responses from different waters.

In this context, the objective of this project is to develop UAV-based monitoring models that can be used to monitor the HABs in two different lakes in Southern Illinois. We developed vegetation indices based on spectrum bands collected by a UAV and established a remote sensing inversion model that can statistically relate the spectral characteristics of UAV images to algae biomass. By comparing models with different vegetation indices, we determine the best-fit models for monitoring HABs.

Methodology

General Workflow

Figure 1 shows the general workflow of UAV-based HABs monitoring. First, we collected water samples from locations near the shorelines of the proposed water bodies. Those water sampling locations were clearly marked using permanent marks that were recognizable from drone images. Second, a DJI Phantom Matrice-100 with an onboard MicaSense RedEdge-MX multispectral sensor was used to capture the images of water bodies in five bands that can be used to derive spectral indices. Third, linear regression models were used to establish the relationships between the Chl-*a* biomass indicators and spectral indices.

Study Area

Carbondale Reservoir and the Campus Lake, both located near or at Southern Illinois University (SIU), were selected for developing the UAV-based monitoring procedure. Two sampling points from each water body were selected and labeled as Site 1 and 2. Table 1 and Figure 2 show the locations of these water sampling sites for statistical analysis.

Data Collection

Our datasets include (1) 5-band UAV images collected from May to October 2021 (Bands: Red, Green, Blue, Near Infrared, Red Edge), and (2) water samples for testing Chl-*a* (unit μg/L) that

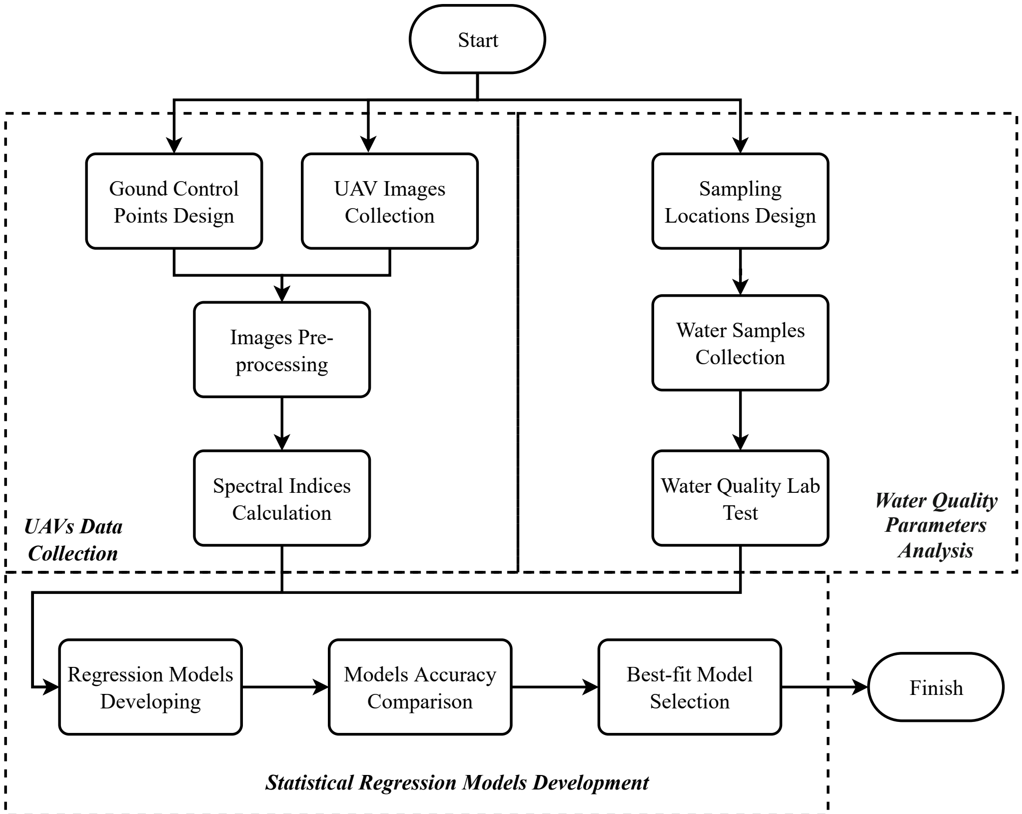


Figure 1. General workflow for developing statistical HABs predictive models.

were collected at the same time with the UAV images collection.

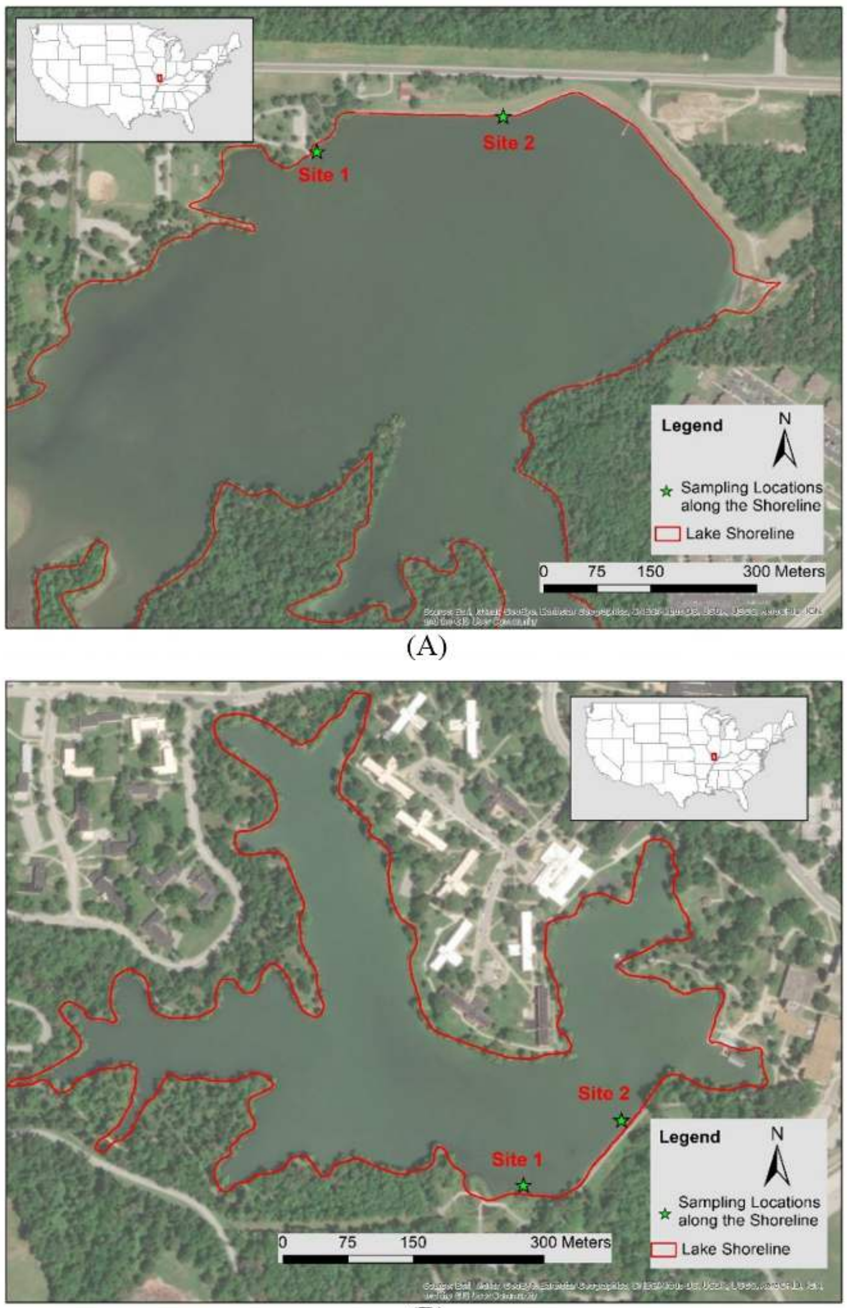
UAV Images Collection. A DJI Phantom UAS equipped with a multispectral sensor, RedEdge-MX, was used to collect aerial images in five spectral bands (Figure 3). As a professional multispectral camera, RedEdge-MX is capable of simultaneously capturing Blue (B, 475 nm ± 20 nm), Green (G, 560 nm ± 20 nm), Red (R, 668 nm ± 10 nm), Near infrared (NIR, 840 nm ± 40 nm), and Red Edge (RE, 717 nm ± 10 nm) bands with 1280 x 960 pixels. Figure 4 shows the spectral reflectance of five image bands at those water sampling sites.

Determination of Chl-a Concentration. Water samples were collected at each labelled location. Water sampler (dipper) and glass bottles autoclaved at 121°C were used to collect samples from the shoreline of the lake at ~ 0.5 m depth. Sampling was conducted once per month from March 2021 to October 2021. After collecting samples, they were stored at 4°C in glass bottles for lab tests. Chl-*a* concentrations of the water samples were measured to determine the biomass of algal species.

The Chl-*a* concentration was determined by a UV-vis spectrophotometer (Thermo BioMate 3S) based on a method from literature (Sartory 1982). Briefly, water samples of varying volumes (volume ranging from 20 mL to 400 mL) were filtered in 0.45 µm nitrocellulose membrane filters to collect wet algae samples. Filter paper was then rolled and placed into a 15 mL centrifuge tube. Freshly prepared 95% ethanol of 10 mL was added to each tube. The tube was then kept in a water bath at 78°C (boiling point of ethanol) and was boiled for 5 min at that temperature. The tube was then removed from the water bath and kept in the dark for 24 h at room temperature. After 24 h, it was centrifuged at 4000 rpm for 5 min. From the tube, 4 mL of the supernatant was taken into a 1 cm pathlength cuvette and was placed in a UV-vis spectrophotometer. Solvent (95% ethanol) was used as the blank. Peak absorbance reading was taken at 665 nm. Then the sample was acidified in the cuvette by adding 120 µL of 0.1 mol/L HCl solution. The cuvette was

Table 1. Sampling locations for developing statistical models.

Location		Latitude	Longitude
Carbondale Reservoir	Site 1	37° 41' 58.5" N	89° 13' 45.5" W
	Site 2	37°42' 00.5" N	89°13' 32.7" W
Campus Lake	Site 1	37°42' 27.5" N	89°13' 29.1" W
	Site 2	37°42' 29.1" N	89°13' 24.7" W



(A)

(B)

Figure 2. Location maps for Carbondale Reservoir (A) and the Campus Lake (B).

shaken and after 4 min, the absorbance was re-read by scanning for the peak between 664 and 666 nm.

The following equation was used to calculate the concentration of Chl-*a* from the absorbance reading:

$$\textit{Chlorophyll-a concentration}\ (\text{mgL}^{-1}) = (\mathcal{E}_{665,0} - \mathcal{E}_{665,a}) \times \frac{R}{R-1} \times \frac{K}{L} \times \frac{V_{extract}}{V_{sample}}$$

where:

- $\mathcal{E}_{665,0}$ is the absorbance at 665 nm before acidification;
- $\mathcal{E}_{665,a}$ is the absorbance at 665 nm after acidification;
- R is acid ratio;
- K is absorbance coefficient of Chl-*a* in ethanol, which equals to 1000/(*specific absorption coefficient*);
- L is pathlength of cuvette (1 cm);
- $V_{extract}$ is volume of extract used as solvent in liters (10 mL, i.e., 0.01 L); and
- V_{sample} is volume of water sample filtered in liters.

An acid ratio of 1.72 and a specific absorption coefficient of 83.6 L(g·cm)$^{-1}$ have been used for ethanol extraction.

UAV Image Processing and Spectral Indices Calculation

Pix4Dmapper image processing software was used to automatically conduct the image stitching,

Figure 3. The UAV equipment used for data collection.

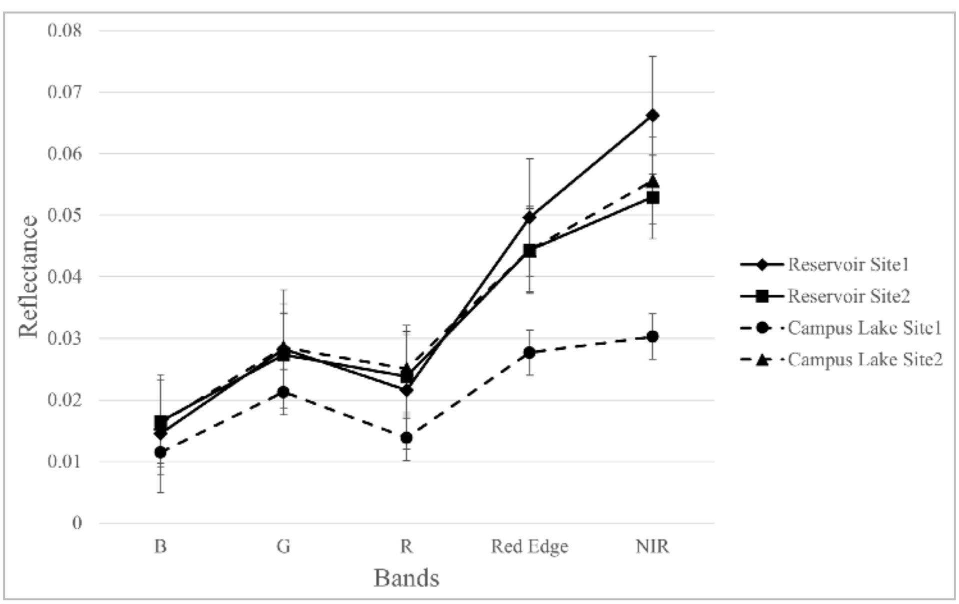


Figure 4. Spectral reflectance from each UAV image band.

radiometric calibration, and orthoimage generation. To fix the potential geometric distortion and topographic displacement, eight Ground Control Points (GCPs) for each lake were set up and applied for polynomial image correction. The original calibration panel accompanying the multispectral sensor was used for radiometric correction. The examples of stitched images from both lakes are shown in Figure 5.

Then ArcGIS georeferencing tool was used to assign the ground truth coordinates to matched pixels on stitched UAV images. The spectral values of image pixels at water sampling sites were extracted using the ArcGIS Spatial Analyst toolset. Seven indices were used to estimate the relationships between Chl-*a* and spectral indices. As shown in Table 2, these UAV-derived spectral indices have been applied in different HABs monitoring projects based on reports in the literature.

Statistical Regression Models Development

Linear regression models describe a continuous response variable as a function of one or more predictor variables, which are commonly applied to analyze environmental data and predict the dynamics of an ecosystem. In this paper, linear regression models were used to establish the relationships between Chl-*a* and the spectral indices. For each index, simple linear regression was applied with its values as independent variables to estimate the correlation with concentrations of Chl-*a*. The performance of each model was evaluated by Coefficient of Determination (R^2). A best-fit model was identified to determine the best-fit spectral indices for HABs monitoring. Based on

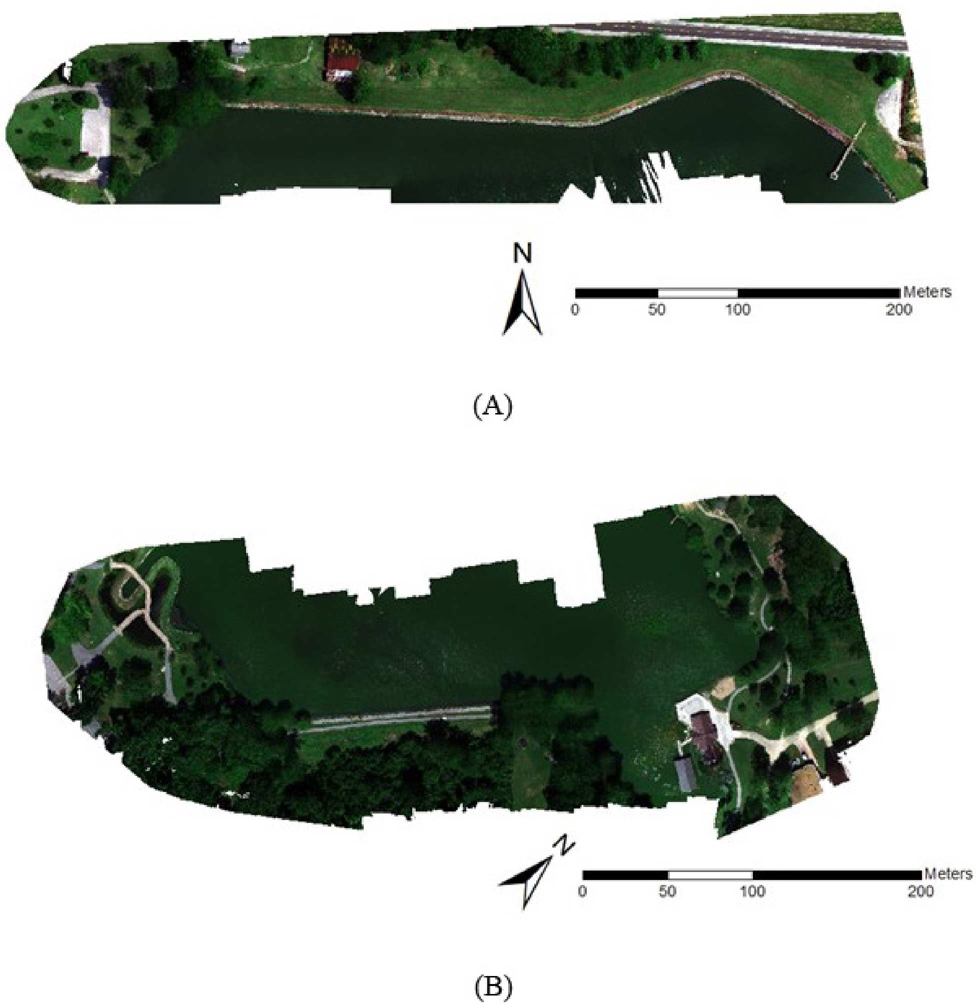


Figure 5. Stitched UAV images from Carbondale Reservoir (A) and the Campus Lake (B).

Table 2. Spectral indices used to estimate Chl-*a* according to relevant literature.

Index	Name	Formula	Reference
NDVI	Normalized Difference Vegetation Index	$\frac{NIR - Red}{NIR + Red}$	Cai et al. 2021
BNDVI	Blue Normalized Difference Vegetation Index	$\frac{NIR - Blue}{NIR + Blue}$	Van der Merwe and Price 2015
GNDVI	Green Normalized Difference Vegetation Index	$\frac{NIR - Green}{NIR + Green}$	Goldberg et al. 2016
NDRE	Normalized Difference Red Edge	$\frac{NIR - Red\ Edge}{NIR + Red\ Edge}$	Song and Park 2020
RVI	Ration Vegetation Index	$\frac{NIR}{Red}$	Han and Rundquist 1997
CVI	Chlorophyll Vegetation Index	$\frac{NIR * Red}{Green^2}$	Balogun et al. 2020
B/G	Band Ratio	$\frac{Blue}{Green}$	Zeng et al. 2016

the best-fit model, the Chl-*a* concentration can be predicted from the UAV-based spectral indices.

Results and Discussion

Table 3 shows a summary of goodness-of-fit for linear regression models with different spectral indices. It was found that NDVI, BNDVI, GNDVI, and RVI exhibit significant positive relationships with the concentrations of Chl-*a* in Carbondale Reservoir. In particular, NDVI was used to establish the best-fit model based on the coefficients of determination (R^2 = 0.4881, Figure 6a). As high chlorophyll concentration reflects more near-infrared light but absorbs more red light, NDVI, measuring the contrast between NIR and red light, would increase when the algae (chlorophyll) density increases. That makes NDVI become a sensitive indicator of lake algae greenness. For the Campus Lake, most spectral indices exhibit no statistically significant relationship except for NDRE (R^2 = 0.4674) and B/G (R^2 = 0.3915), which present significant inverse relationships with Chl-*a* (Figure 6b).

The positive relationship between NDVI and Chl-*a* identified in Carbondale Reservoir is consistent with most literature (Zhang et al. 2011; Goldberg et al. 2016; Salarux and Kaewplang 2020; Ma et al. 2021). The inverse relationship between B/G and Chl-*a* found in the Campus Lake is consistent with Piech et al. (1978), Woźniak and Stramski (2004), and Zeng et al. (2016). Kim et al. (2021) show that the spectral index NDRE had an insignificant statistical relationship for water quality monitoring. Song and Park (2020) did not see the significant change of NDRE when aquatic plants had proliferated. However, Che et al. (2021) proved that the strong and positive correlations existed between *Pyropia yezoensis* (a type of red macroalgae) biomass and NDRE. Therefore, blue-to-green reflectance ratio is deemed as the best-fit model predictor of Chl-*a* for the Campus Lake (Figure 6b).

In addition, Figure 6 indicates that the relationships between spectral indices and Chl-*a* vary at different lakes. Piech et al. (1978) attributed such inter-lake variations to different trophic status. Different phytoplankton community and water constituents like chromophoric dissolved organic matter (CDOM) or mineral particles could also be the potential factors as they could modulate specific

Table 3. Statistical relationships between each spectral index and Chl-*a* (**p*-value < 0.05).

Lake	Spectral Index	R Square	*p*-value (α = 0.05)
Carbondale Reservoir	NDVI	0.4881	0.0115*
	BNDVI	0.4262	0.0214*
	GNDVI	0.3932	0.0291*
	NDRE	0.1809	0.2204
	RVI	0.4137	0.0241*
	CVI	0.1871	0.1602
	B/G	0.0280	0.6033
Campus Lake	NDVI	0.1009	0.3143
	BNDVI	0.0873	0.3511
	GNDVI	0.2190	0.1249
	NDRE	0.4674	0.0142*
	RVI	0.0970	0.3243
	CVI	0.1434	0.2247
	B/G	0.3915	0.0295*

spectral reflectance patterns and refractive indices (Woźniak and Stramski 2004; Zeng et al. 2016).

There are a few limitations to note in this project. First, we only collected water samples from two near-shore locations from each lake due to budget constraints in this concept-proof project. In this case, water sampling sites do not necessarily represent the conditions in the entire water bodies. Rather, the variabilities of tested and modeled Chl-*a* more reflect the temporal patterns of water quality. The small sample size could also limit the accuracy of regression models, since it may not have sufficient statistical power to detect significant relationships between Chl-*a* and spectral indices. To overcome the limitations, future research will be designed to expand to larger sample sizes and more sampling locations.

Conclusions and Policy Implications

UAVs, as an emerging remote sensing technique, have proven to be cost-efficient, flexible, and reliable tools for environmental monitoring in open waterbodies. This study shows the effectiveness and robustness of a UAV and its onboard multispectral sensor for monitoring HABs in two waterbodies from Southern Illinois. Seven vegetation indices were tested for estimating algae biomass in Carbondale Reservoir and the Campus Lake of SIU. Results show that the specific relationships between algae biomass (Chl-*a*) and vegetation indices vary by different waterbodies, which is likely due to the complex compositions of each lake. NDVI was found to be the best-fit spectral index for Carbondale Reservoir, while Blue-Green ratio was the best predictor for the Campus Lake of SIU. In our future work, we plan to substantially expand the number of water sampling locations and increase the sample size in each location. We expect to further examine and verify these statistical relationships that may be directly applied for UVA-based HABs monitoring.

With increasing anthropogenic activities, HABs have become one of the major water quality problems that harass communities around the world. There are critical needs for monitoring the onset and progress of HABs for public safety. However, due to funding limitations, many small water bodies that have been intensively used for drinking and recreation by communities were largely uncovered

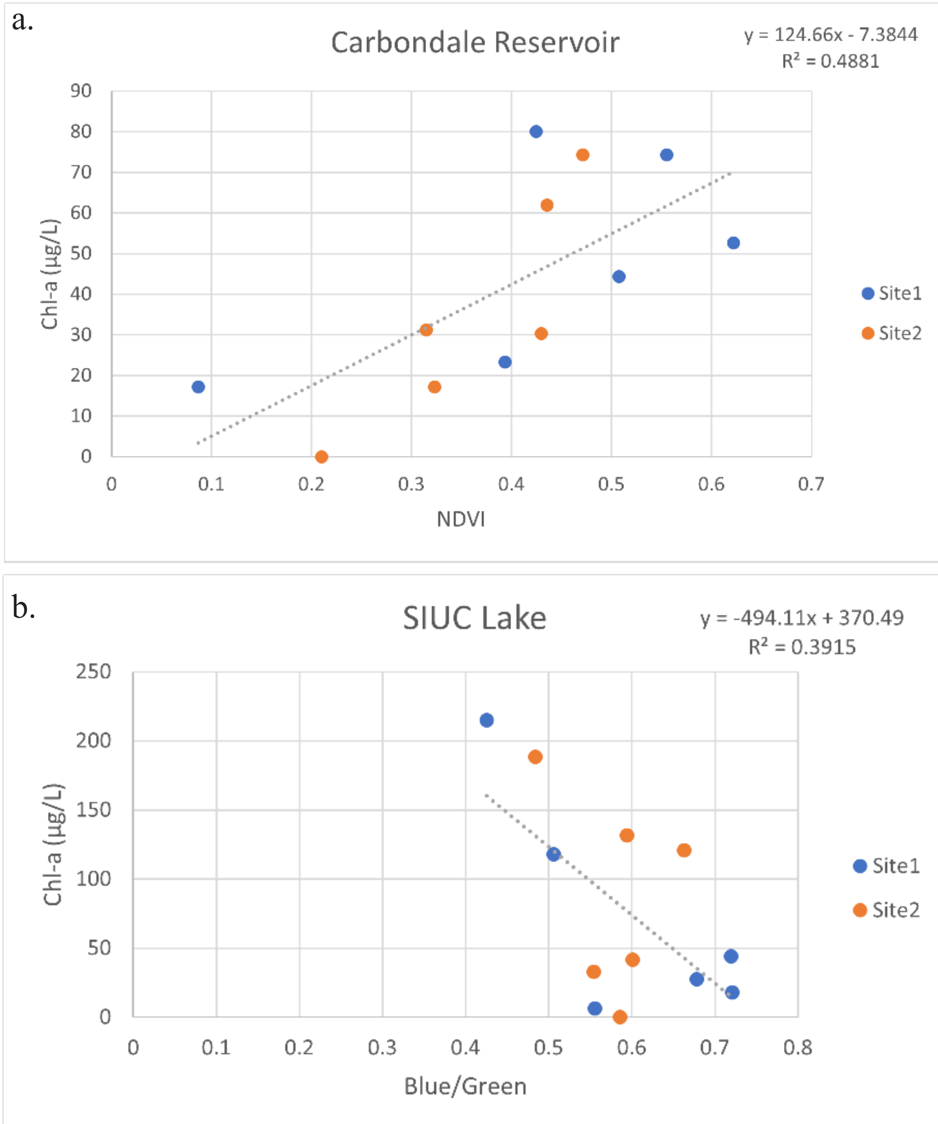


Figure 6. Selected linear regression models that characterize the relationships between spectral indices and Chl-*a* in two lakes.

by government-initiative monitoring programs. Due to its flexibility and lower operational cost, multispectral UAVs have shown tremendous potential for developing community-based HABs monitoring programs that may be operated by local municipalities or even homeowner organizations. It may serve as one of the promising solutions to the 'last mile' problem of broader policies for ensuring public water safety (Cheng 2015; Da Mata et al. 2021). In combination with emerging water technologies such as water treatment using magnetic nanomaterials under solar light (Madany et al. 2021), a UAV-based monitoring program may be used to guide *in-situ*, low-cost treatment of areas with high HABs concentrations. In addition, drone-based HABs monitoring programs can be potentially integrated with existing governmental funding programs as a supplemental monitoring effort. For example, such a program may be integrated with the Harmful Algal Bloom Program and the Volunteer Lake Monitoring Program in Illinois.

Acknowledgements

This research was sponsored by a 104b grant from the DOI USGS Water Resources Research Act program via Illinois Water Resources Center. It was also partially funded by the U.S. Environmental Protection Agency (SU84017401). The views expressed in this paper are solely those of the authors and do not necessarily reflect those of the funding agencies.

Author Bio and Contact Information

Dr. Ruopu Li (corresponding author) is an Associate Professor in Geography and Environmental Resources at Southern Illinois University Carbondale. His research areas include Water Resources Planning and Management, GIS Analysis and Modeling, Energy Geography, and Sustainability Education. In recent years, he focuses on interdisciplinary and emerging technologies, such as geospatial artificial intelligence, social media data mining, and environmental monitoring using UAVs. He may be contacted at ruopu.li@siu.edu, or 4543 Faner Hall, Carbondale, IL 62901.

Di Wu is a Ph.D. student in the Environmental Resources and Policy Program at Southern Illinois University Carbondale, focusing on using geospatial artificial intelligence and high-resolution DEMs to improve hydrologic connectivity delineation. She may be contacted at di.wu@siu.edu, or 4544 Faner Hall, Carbondale, IL 62901.

Dr. Jia Liu is an Associate Professor in the School of Civil, Environmental and Infrastructure Engineering at Southern Illinois University Carbondale. Dr. Liu received her Ph.D. degree in Environmental Engineering from the University of Houston in 2014. Her research focuses on developing cost-effective environmental materials used in water treatment including the remediation of harmful algal blooms. She may be contacted at jialiu@siu.edu.

Nafeesa Khan is a M.S. research assistant in the School of Civil, Environmental and Infrastructure Engineering at Southern Illinois University Carbondale. She may be contacted at nafeesa.khan@siu.edu.

References

Anderson, D.M. 2009. Approaches to monitoring, control and management of harmful algal blooms (HABs). *Ocean & Coastal Management* 52(7): 342-347.

Balogun, A.L., S.T. Yekeen, B. Pradhan, and O.F. Althuwaynee. 2020. Spatio-temporal analysis of oil spill impact and recovery pattern of coastal vegetation and wetland using multispectral satellite landsat 8-OLI imagery and machine learning models. *Remote Sensing* 12(7): 1225.

Beusen, A.H., A.F. Bouwman, L.P. Van Beek, J.M. Mogollón, and J.J. Middelburg. 2016. Global riverine N and P transport to ocean increased during the 20th century despite increased retention along the aquatic continuum. *Biogeosciences* 13(8): 2441-2451.

Brooks, B.W., J.M. Lazorchak, M.D.A. Howard, M.-V.V. Johnson, S.L. Morton, D.A.K. Perkins, et al. 2016. Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems? *Environmental Toxicology and Chemistry* 35(1): 6-13.

Cai, L., Z. Cao, M. Xu, H. Sheng, and J. Wan. 2021. Spatial-temporal distribution analysis based on multiyear HAB extraction in the Yellow Sea of China. In: *2021 IEEE International Geoscience and Remote Sensing Symposium IGARSS,* 11-16 July 2021, Brussels, Belgium, pp. 4794-4797.

Che, S., G. Du, N. Wang, K. He, Z. Mo, B. Sun, et al. 2021. Biomass estimation of cultivated red algae Pyropia using unmanned aerial platform based multispectral imaging. *Plant Methods* 17(1): 1-13.

Cheng, D. 2015. Contestations at the last mile: The corporate–community delivery of water in Manila. *Geoforum* 59: 240-247.

Da Mata, D., L. Emanuel, V. Pereira, and B. Sampaio. 2021. Climate adaptation policies and infant health: Evidence from a water policy in Brazil. *IZA Discussion Paper* No. 14295. Available at: https://ssrn.com/abstract=3833227. Accessed January 23, 2023.

Fráter, T., T. Juzsakova, J. Lauer, L. Dióssy, and A. Rédey. 2015. Unmanned aerial vehicles in environmental monitoring—An efficient way for remote sensing. *Journal of Environmental Science and Engineering A* 4: 85-91.

Goldberg, S.J., J.T. Kirby, and S.C. Licht. 2016. *Applications of Aerial Multi-spectral Imagery for Algal Bloom Monitoring in Rhode Island*. SURFO Technical Report No. 16-01, Narragansett, Rhode Island, pp. 28-32.

Han, L. and D.C. Rundquist. 1997. Comparison of NIR/RED ratio and first derivative of reflectance in estimating algal-chlorophyll concentration: A case study in a turbid reservoir. *Remote Sensing of Environment* 62(3): 253-261.

Heil, C.A. and A. Muni-Morgan. 2021. Florida's harmful algal bloom (HAB) problem: Escalating risks to human, environmental and economic health with climate change. *Frontiers in Ecology and Evolution* 9: 299.

Huot, Y., M. Babin, F. Bruyant, C. Grob, M.S. Twardowski, and H. Claustre. 2007. Does chlorophyll a provide the best index of phytoplankton biomass for primary productivity studies? *Biogeosciences Discussions* 4(2): 707-745.

Kasprzak, P., J. Padisák, R. Koschel, L. Krienitz, and F. Gervais. 2008. Chlorophyll a concentration across a trophic gradient of lakes: An estimator of phytoplankton biomass? *Limnologica* 38(3-4): 327-338.

Kim, E.-J., S.-H. Nam, J.-W. Koo, and T.-M. Hwang. 2021. Hybrid approach of unmanned aerial vehicle and unmanned surface vehicle for assessment of Chlorophyll-a imagery using spectral indices in Stream, South Korea. *Water* 13(14): 1930.

Kislik, C., I. Dronova, and M. Kelly. 2018. UAVs in support of algal bloom research: A review of current applications and future opportunities. *Drones* 2(4): 35.

Kubiak, K.A., J. Kotlarz, P. Czapski, and A.M. Mazur. 2016. Monitoring cyanobacteria blooms in freshwater lakes using remote sensing methods. *Polish Journal of Environmental Studies* 25(1): 27-35.

Le Moal, M., C. Gascuel-Odoux, A. Ménesguen, Y. Souchon, C. Étrillard, A. Levain, et al. 2019. Eutrophication: A new wine in an old bottle? *Science of the Total Environment* 651(Pt. 1): 1-11.

Lomax, A.S., W. Corso, and J.F. Etro. 2005. Employing unmanned aerial vehicles (UAVs) as an element of the Integrated Ocean Observing System. In: *Proceedings of OCEANS 2005 MTS/IEEE*, 17-23 September 2005, Washington, D.C., pp. 184-190.

Lyu, P., Y. Malang, H.H. Liu, J. Lai, J. Liu, B. Jiang, et al. 2017. Autonomous cyanobacterial harmful algal blooms monitoring using multirotor UAS. *International Journal of Remote Sensing* 38(8-10): 2818-2843.

Ma, J., S. Jin, J. Li, H. Yang, and W. Shang. 2021. Spatio-temporal variations and driving forces of harmful algal blooms in Chaohu Lake: A multi-source remote sensing approach. *Remote Sensing* 13(3): 427.

Madany, P., C. Xia, L. Bhattacharjee, N. Khan, R. Li, and J. Liu. 2021. Antibacterial activity of γFe2O3/TiO2 nanoparticles on toxic cyanobacteria from a lake in Southern Illinois. *Water Environment Research* 93(11): 2807-2818.

Manfreda, S., M.F. McCabe, P.E. Miller, R. Lucas, V. Pajuelo Madrigal, G. Mallinis, et al. 2018. On the use of unmanned aerial systems for environmental monitoring. *Remote Sensing* 10(4): 641.

Matthews, M.W. 2014. Eutrophication and cyanobacterial blooms in South African inland waters: 10 years of MERIS observations. *Remote Sensing of Environment* 155: 161-177.

Paerl, H.W. 2009. Controlling eutrophication along the freshwater–marine continuum: Dual nutrient (N and P) reductions are essential. *Estuaries and Coasts* 32(4): 593-601.

Palmer, S.C.J., T. Kutser, and P.D. Hunter. 2015. Remote sensing of inland waters: Challenges, progress and future directions. *Remote Sensing of Environment* 157: 1-8.

Piech, K.R., J.R. Schott, and K.M. Stewart. 1978. The blue-to-green reflectance ratio and lake water quality. *Photogrammetric Engineering and Remote Sensing* 44(10): 1303-1310.

Salarux, C. and S. Kaewplang, 2020. Estimation of algal bloom biomass using UAV-based remote sensing with NDVI and GRVI. *Mahasarakham International Journal of Engineering Technology* 6(1): 1-6.

Sartory, D.P. 1982. *Spectrophotometric Analysis of Chlorophyll a in Freshwater Phytoplankton*. Republic of South Africa, Department of Water Affairs, Technical Report TR 115.

Song, B. and K. Park. 2020. Detection of aquatic plants using multispectral UAV imagery and vegetation index. *Remote Sensing* 12(3): 387.

Van der Merwe, D. and K.P. Price. 2015. Harmful algal bloom characterization at ultra-high spatial and temporal resolution using small unmanned aircraft systems. *Toxins* 7(4): 1065-1078.

Wolny, J.L., M.C. Tomlinson, S. Schollaert Uz, T.A. Egerton, J.R. McKay, A. Meredith, et al. 2020. Current and future remote sensing of harmful algal blooms in the Chesapeake Bay to support the shellfish industry. *Frontiers in Marine Science* 7: 337.

Woźniak, S.B. and D. Stramski. 2004. Modeling the optical properties of mineral particles suspended in seawater and their influence on ocean reflectance and chlorophyll estimation from remote sensing algorithms. *Applied Optics* 43(17): 3489-3503.

Wu, D., R. Li, F. Zhang, and J. Liu. 2019. A review on drone-based harmful algae blooms monitoring. *Environmental Monitoring and Assessment* 191(4): 1-11.

Zeng, C., H. Xu, and A.M. Fischer. 2016. Chlorophyll-a estimation around the Antarctica peninsula using satellite algorithms: Hints from field water leaving reflectance. *Sensors* 16(12): 2075.

Zhang, Y., X. Feng, X. Cheng, and C. Wang. 2011. Remote estimation of chlorophyll-a concentrations in Taihu Lake during cyanobacterial algae bloom outbreak. In: *2011 19th International Conference on Geoinformatics*, 24-26 June 2011, Shanghai, China, pp. 1-6.

Universities Council on Water Resources
Journal of Contemporary Water Research & Education
Issue 177, Pages 94-102, April 2023

Research Note

Spatiotemporal Variability Comparisons of Water Quality and *Escherichia coli* in an Oklahoma Stream

*Grant M. Graves and Jason R. Vogel

University of Oklahoma, School of Civil Engineering and Environmental Science, Norman, OK
**Corresponding Author*

Abstract: Fecal indicator bacteria, *Escherichia coli*, for primary body contact recreation (PBCR) in Oklahoma waterbodies, is defined as the geometric mean of 10 samples from the recreation season, May 1 to September 30, with an impairment threshold of 126 colony forming units (cfu) per 100 mL. However, the water quality standards provide limited guidance on spatiotemporal and environmental factors that could influence samples collected and analyzed. In this study, two stream cross sections under baseflow conditions in a central Oklahoma urban perennial stream, Spring Creek, were densely sampled to investigate temporal and spatial variability of *E. coli* concentrations and water quality parameters across the stream channel. Water quality parameters (specific conductivity, temperature, dissolved oxygen, pH, turbidity, and total suspended solids (TSS)), stream discharge, and bacteria samples were collected simultaneously at equal intervals across the two cross sections in the morning and afternoon during one summer day with sunny, dry, and hot weather conditions. Results indicate a significant difference between time-of-day samples and water quality parameters and *E. coli* concentrations. Strong correlations between temperature, dissolved oxygen, and time versus *E. coli* concentrations were observed, while location, turbidity, and TSS were not significant or correlated to measured values. Furthermore, *E. coli* concentrations were highly variable spatially across each stream cross section, regardless of time of day or location. Results from this study provide an initial indication that stream water quality, spatial cross section sample location, and diurnal variations may be influencing factors on bacteria concentrations.

Keywords: *Escherichia coli, fecal indicator bacteria, sampling, freshwater, stream*

Fecal indicator bacteria (FIB) such as *Escherichia coli* in freshwater waterbodies are frequently monitored to assess potential human health risk from pathogen contact in recreational waters. The State of Oklahoma and U.S. Environmental Protection Agency water quality standard criteria for FIB for primary body contact recreation (PBCR) in waterbodies is defined as the geometric mean of 10 samples from the recreation season, May 1 to September 30, with an impairment threshold of 126 colony forming units (cfu) per 100 mL for *E. coli* (OWRB 2017). Thresholds were derived from epidemiology studies in freshwater and marine swimming beach areas in lakes and oceans where subjects contacted potential contaminated water and incidents of gastrointestinal illness occurred (USEPA 1986; 2012).

Escherichia coli has been studied extensively for fecal source tracking, pathogenic strains, waterbody conditions, and other associated research questions related to human health risk and fecal water quality indicators for PBCR (Gitter et al. 2020). However, water quality standards provide limited guidance of how samples should be collected during the recreation season. State agencies and other entities that collect samples and make assessments often develop their own sampling metrics, but are not standardized to sampling protocols (USEPA 2012). The U.S. Environmental Protection Agency and others recognize that temporal and spatial factors could play significant roles in bacteria concentrations within a stream (USEPA 2010; Muirhead and Meenken 2018). Recent studies found that sampling location and frequency were significant factors when developing a

Research Implications

- Sample location and time can influence *E. coli* concentrations in streams and rivers.
- Environmental parameters can be used to develop relationships to predict bacteria concentrations.
- Monitoring approaches should consider sampling location, time, and other environmental conditions when sampling for fecal indicator bacteria.
- An improved understanding on how, when, where, and why to sample fecal indicator bacteria is needed to ensure representativeness of stream conditions for impairment determination for recreational waters.

monitoring plan to obtain representative samples for the evaluation of potential fecal contamination (Crosby et al. 2019; Stocker et al. 2019). In addition, previous research has indicated that sample type and technique when monitoring a stream should be considered to reduce uncertainty in analyses (Harmel et al. 2016). Gregory et al. (2019) determined there was a significant difference between streamflow thresholds (i.e., baseflow, floods) and *E. coli* concentrations, and indicated that specific hydrologic factors may provide stronger relationships to FIB stream concentrations and associated human health risk. Therefore, given the number of temporal and spatial factors within a stream sample reach, determining sample representativeness could be an important consideration for waterbody impairment designation.

Stream characteristics and environmental conditions have been shown to influence FIB and have been used to develop relationships between parameters and FIB concentrations (Dwivedi et al. 2013). Particularly, suspended solids, turbidity, water temperature, and habitat have previously been used as predictors for *E. coli* densities (Desai and Rifai 2010; Petersen and Hubbart 2020). Others have found significant relationships between nutrients, turbidity, and FIB in streams that can be used to predict bacteria concentrations (Christensen et al. 2002). Furthermore, discharge and precipitation, along with turbidity, have been found to strongly correlate with *E. coli* concentrations in streams (Hamilton and Luffman 2009). Comparison of stream reaches within similar land use segments has been explored with differentiating results for variable fecal indicator concentrations and environmental conditions (Stocker et al. 2016). Results indicated that there were significant differences between stream sampling locations, and that more research is needed to understand stream dynamics that may affect FIB. Diurnal variation and sunlight are also important considerations for evaluating FIB in streams and rivers (Desai and Rifai 2013). Previous research has indicated that FIB concentrations in waterbodies are cyclical, with decay shown during high sunlight periods and increases in bacteria concentrations during low light periods (Whitman et al. 2004; Schultz-Fademrecht et al. 2008). Hydrologic extremes such as floods and droughts can increase variability within stream reaches due to external bacterial inputs from stormwater conveyance, wastewater overflows, and non-point sources (Vogel et al. 2009; McKergow and Davies-Colley 2010; Sanders et al. 2013; Verhougstraete et al. 2015; Rochelle-Newall et al. 2016; Stocker et al. 2018). Furthermore, Piorkowski et al. (2014) showed a variable spatial distribution of FIB in stream sediments under different flow conditions and sampling location. Sediment type and stream habitats have also shown to be *E. coli* reservoirs within streams (Brinkmeyer et al. 2015; Devane et al. 2020). Stream bed sediments have the potential to provide a consistent source of resuspended FIB in the stream water column due to dynamic hydrologic conditions and can create variable sampling conditions (Jamieson et al. 2005; Haller et al. 2009; Bradshaw et al. 2016).

While environmental and hydrologic conditions have been extensively studied to develop relationships between these factors and *E. coli* within streams and rivers, limited information exists to understand the variability of bacteria concentrations within the longitudinal and cross-section profiles of streams. The objectives of this study were to 1) investigate spatial and temporal variability in two stream cross sections, 2) evaluate physical and chemical factors for correlations between variables and evaluate statistical trends, and 3) provide preliminary information for future research targeting specific environmental and spatiotemporal factors that may influence bacteria concentrations in streams and rivers, and ultimately, drive impairment criteria for water quality monitoring.

Methods

Two stream cross sections in a central Oklahoma urban perennial stream, Spring Creek, under baseflow conditions (less than 2.54 mm precipitation in previous seven days) were densely sampled during a seasonally average dry and hot, central Oklahoma summer day (Figure 1). Additionally, in-situ water quality parameters were collected across the stream channel sections at sampling points. Spring Creek is located in northwest Oklahoma City, OK at 35° 36' 18.7" N and -97° 36' 29.3" W, and the site location has an approximate drainage area of 30 km^2 as calculated in StreamStats (Smith and Esralew 2010). The land use category of the watershed is highly urban (>90%) with silty clay to clay loam soil types (USDA NRCS 2023). Potential bacteria inputs are primarily from non-point sources from urban runoff, as no septic tanks, wastewater discharges, or agriculture are located in the watershed. Stream cross sections were evaluated at two daily time periods, morning (0800) and afternoon (1500), at two locations. The two measured cross section stream feature morphologies were a pool (upstream) and a run (downstream) and were separated by 200 m of a series of riffles, glides, pools, and runs. The upstream cross section had a width of 6.7 m and downstream location had a cross section width of 7.3 m.

Factors investigated included *E. coli* concentration, dissolved oxygen (DO), specific conductivity (SC), total suspended solids (TSS), turbidity, water temperature (T), stream velocity and flow, channel depth, stream location and cross section, and time. Water quality samples and parameters were collected across the cross section simultaneously by our sampling team for evaluation of spatial variability (Figure 2). Grab samples were collected at evenly spaced 1.2 m cross section locations (minimum of six sampling locations) at mid-depth in sterile 1 L polypropylene bottles and split into respective subsamples for bacteria (*E. coli*), water quality parameters (turbidity, pH, conductivity), and sediment (TSS) analyses (Figure 2). Sampling protocols adhered to the U.S. Geological Survey sampling methods (USGS 2014). Discharge measurements were collected using a Sontek Flowtracker2® handheld-ADV (acoustic Doppler velocimeter) at each cross section, following collection of water quality samples. At each time period, samples were first collected at the downstream location to

Figure 1. Site sampling locations at Spring Creek in central Oklahoma.

Figure 2. Cross-section water quality sampling at the "run" location at Spring Creek.

minimize disturbance of the water column from the upstream location. *E. coli* concentrations in water were analyzed using IDEXX Quantitray Colilert (SM9223-B) to determine most probable number (MPN) per 100 ml (Baird and Bridgewater 2017). TSS analyses were completed using SM 2540-D and turbidity was measured using a Hach® portable turbidity meter. Water temperature, pH, DO, and SC were measured using a ThermoFisher Scientific Orion Star A329 multiparameter meter.

Data Analysis

Data were analyzed using Microsoft Excel® and R statistical software. Differences in means were evaluated using a two-sample t-test with unequal variances. A Pearson correlation test with a two-sample t-test with unequal variances was performed to determine significant linear relationships between variables. An F-test was used to evaluate variance of water quality data collected from each stream section. All statistical figures were generated using R and Excel®.

Results and Discussion

Stream flow characteristics were measured at both the morning and afternoon sampling periods. Stream locations had mean column depths of 0.35 m at the pool and 0.15 m at the run. Discharge during the morning and afternoon periods (measurement was within ± 0.01 m^3s^{-1} at both the upstream and downstream locations) was 0.08 m^3s^{-1} and 0.04 m^3s^{-1}, respectively, which is within range of the estimated 50% flow-duration for Spring Creek in July (0.05 m^3s^{-1}) (Smith and Esralew 2010). The drainage area is characterized as highly urban, silty clay soils (hydrologic soil group D), which could increase the potential for anthropogenic influences and explain the higher discharge in the morning period when lawn irrigation is most common. No measurable precipitation (>2.54 mm) was recorded at the nearest Oklahoma City East Mesonet station for the preceding seven days (Brock et al. 1995; McPherson et al. 2007).

From a two-sample t-test with unequal variances, *E. coli* concentrations between the upstream (pool) and downstream (run) were not significantly different between the means for each location for all time periods ($p=0.23$). However, a significant difference ($p<0.001$) between time periods (morning and afternoon) was shown between each location for *E. coli* densities. The geometric mean in the morning for *E. coli* was 664 MPN/100 ml (SD ± 116) and was 137 MPN/100 ml (SD ± 108) in the afternoon. Results from a t-test comparing Pearson correlation coefficients between factors indicate that time, DO, SC, and T were significant ($p<0.05$) for *E. coli* concentrations. Furthermore, DO was significantly higher ($p<0.01$) in the morning than afternoon and displayed a strong positive correlation of 0.69 to *E. coli* concentrations. Conversely, a very strong negative correlation (-0.93) of T was shown and a strong positive relationship with SC (0.78) was found versus *E. coli* concentrations ($p<0.01$). The mean DO and T for both locations was 9.05 mg/L (SD ± 0.12) and 26.63°C in the morning, and 7.02 mg/L (SD ± 0.42) and 29.14°C in the afternoon. When comparing SC to *E. coli* concentrations, a significant difference was statistically determined, however, the means of SC for the morning and afternoon were 1167 (SD ± 1.72) and 1171 µS/cm (SD ± 4.59), respectively, which provides limited inference for interpretation given the minute difference between time points. However, the flow was a factor of two higher in the morning than in the afternoon and could suggest that more flow slightly altered the water chemistry through dilution. Significant differences ($p<0.01$) were found from the Pearson correlation coefficient t-test when comparing *E. coli* concentrations from both sampling locations to water quality parameters (DO, T), water column depth, and time. However, no significant differences were shown ($p>0.05$) for TSS, turbidity, and stream velocity. Boxplots of water quality parameters are shown in Figure 3.

Previous research has indicated that sediment parameters are strong predictors for FIB sampling (Stocker et al. 2019). However, our results from the Pearson correlation indicated high variability and no significant relationship between turbidity, TSS, and *E. coli* for each cross section and location. Stream cross section versus TSS is presented in Figure 4, and visually demonstrates the variability of suspended sediments at time points and cross section location. Stream cross sections at both locations were evaluated using a two-sample F-test to determine if variability exists across the lateral

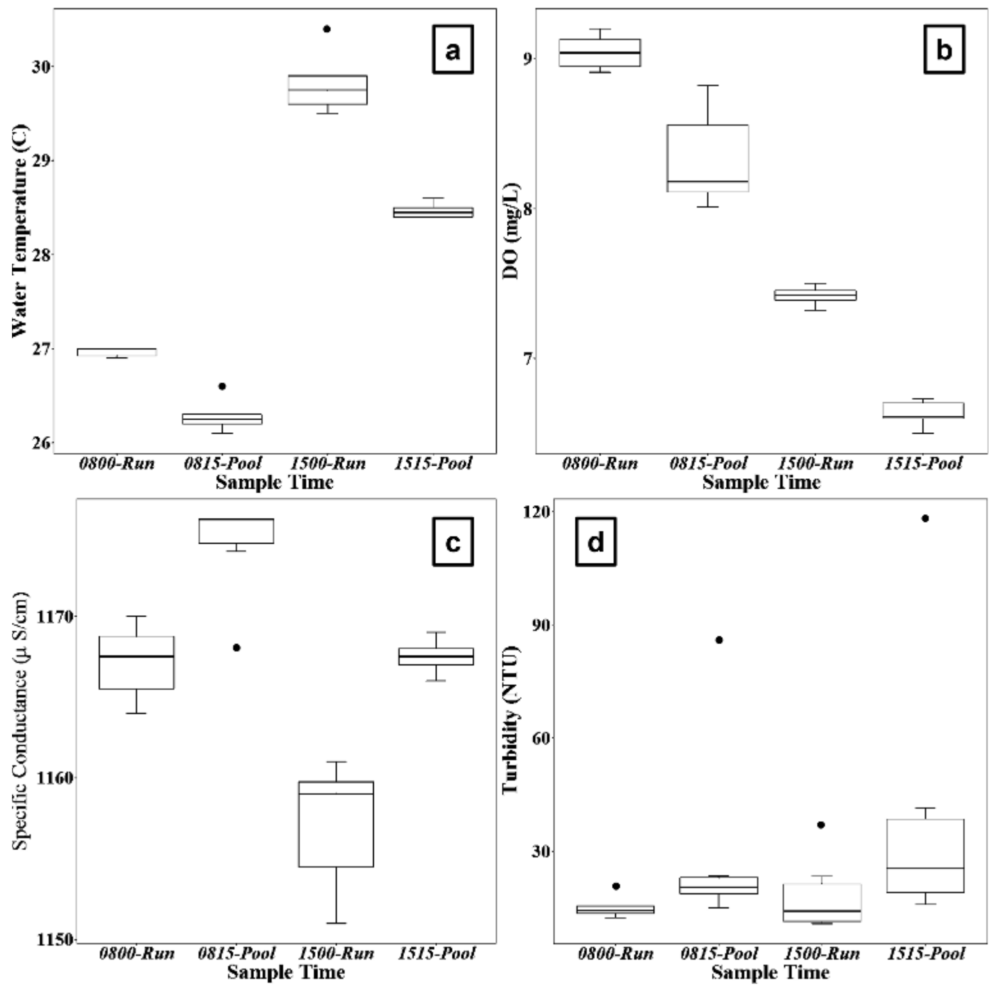


Figure 3. Standard box plots of a) Water Temperature, b) Dissolved Oxygen (DO), c) Specific Conductance (SC), and d) Turbidity, showing the median (line in box), lower (Q1) and upper (Q3) (T bars outside of box) and outlier values (points) grouped by sample time at each of the two Spring Creek sampling locations.

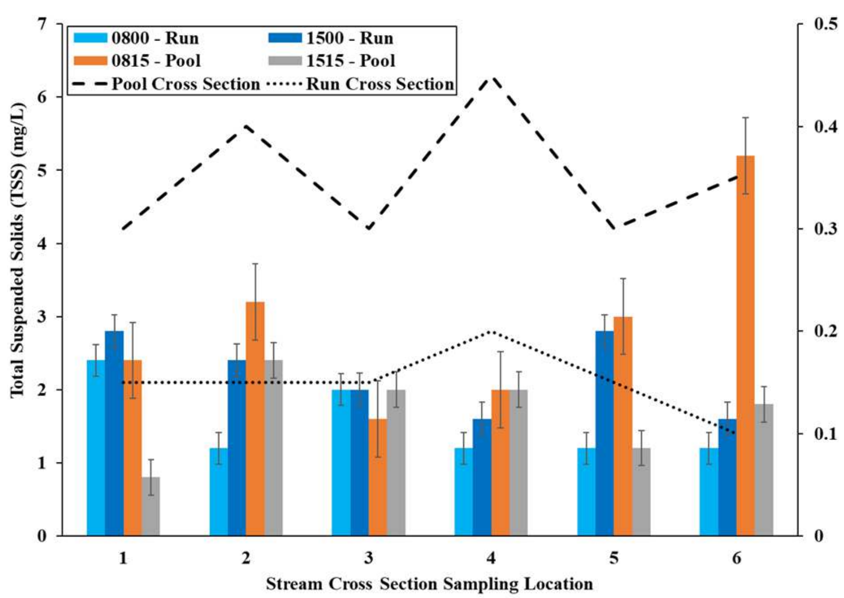


Figure 4. Combination of plot of morning and afternoon total suspended solids (TSS) at the pool and run cross sections. Cross section depth for each location is indicated by the dashed lines. Standard error is represented by the error bars.

profile of the stream for *E. coli* concentrations. Results show significant high variability between the pool and run locations (p<0.01) at both times, where the standard deviation was approximately a factor of three lower in the run location than the pool location. No significant difference in variability was found when comparing two time periods for the pool location (p=0.44), whereas a significant difference was indicated for the run location (p=0.038) when comparing two different time periods. *E. coli* stream cross section concentrations for two time periods and locations are displayed in Figure 5.

While sediment is generally highly correlated to *E. coli* concentrations, variability between sample times has been shown to skew results while monitoring (Crosby et al. 2019). Results from our cross-section study comparing stream location indicate that variability of FIB concentrations, specifically *E. coli*, can be reduced if samples are collected in a well-mixed stream reach, such as from the stream run location, with consideration that variability can occur across the cross section even when hydrologic conditions and other factors are considered. Others have indicated that composite samples may be a better representation of stream water quality parameters when compared to other sample types (e.g., grab samples) (Harmel et al. 2016). In our preliminary research, water quality parameters (DO, T, and SC) were better predictors for *E. coli* than sediment, which may be related to time-of-day conditions within the stream since T can influence DO, SC, and *E. coli* concentrations. Diurnal variation and percent sunlight at each location were not measured for this study, but when comparing to previous research, this variable may be an important consideration of where and when to sample. More research is needed in various stream types, geographic locations, and spatial and temporal resolutions to validate the variability within stream cross sections and longitudinal segments.

Conclusions

Sampling FIB for water quality impairment determination is important to evaluate recreational waterbodies for potential pathogen presence that can affect human health. However, the water quality standards do not provide detailed guidance of the spatial and temporal distribution of water samples at a point of interest in a waterbody. Our research provides initial evidence that sampling methods should be investigated further to

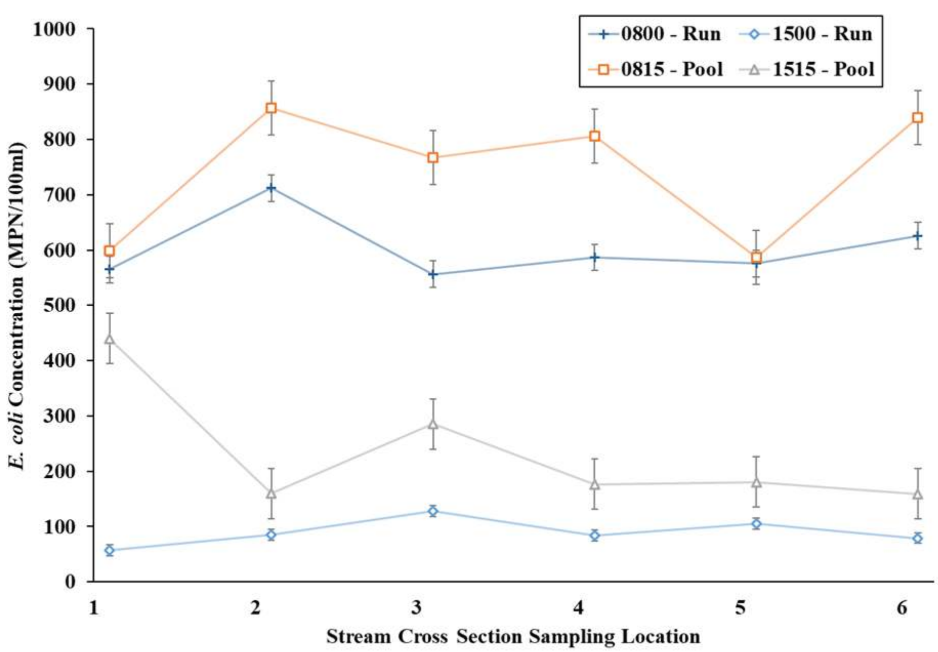


Figure 5. *Escherichia coli* concentrations at two cross section locations (pool and run) at the Spring Creek study site for two time periods, morning (0800 and 0815) and afternoon (1500 and 1515). Standard error is represented by the error bars.

properly evaluate streams for water quality fecal indicators. We demonstrated that high spatial variability of bacteria concentrations across both stream reaches was shown regardless of time of day or other waterbody conditions. Furthermore, basic water quality parameters (DO, T, and SC), time of day, and stream section locations may be useful predictors when selecting a representative location. This proof-of-concept study indicates that more emphasis should be placed on selecting site conditions that are representative (e.g., sampling reach) of the waterbody being sampled, with spatial and temporal considerations. Furthermore, other water quality and hydrologic factors could potentially be used to target stream reaches that are impaired and improve sampling protocols by understanding stream dynamics to obtain quality samples. Future work in this research area is needed to improve the water science community's approaches to enhance our understanding of streams and rivers and use our resources effectively.

Acknowledgements

We would like to recognize contributions from student research assistants at the Oklahoma Water Survey: Walter Chandler, Christina Gildea, Monica Ha, Angel Kroboth, Emily Rhodes, Helen Sanders, Jennifer Salvo, Ryan Totten, and Madeline Wade. Thank you to the City of Oklahoma City and Martin Nature Park for site access. This research was associated with a USGS 104(b) project – 2020 OK198B.

Author Bio and Contact Information

Grant Graves (corresponding author) is the Assistant Director and Research and Monitoring Coordinator for the Oklahoma Water Survey (OWS) at the University of Oklahoma and has 8+ years of professional experience in hydrology and water quality. He is responsible for overseeing multidisciplinary research and monitoring projects in water quality, wastewater sampling, watershed management, and hydrology at the OWS, including a statewide bacteria monitoring sampling effort for impaired streams and rivers and wastewater-based epidemiology sampling. He is pursuing his Ph.D. in Environmental Science at the University of Oklahoma. He may be contacted at grant.graves@ou.edu or 202 W. Boyd, Carson Engineering Center, Norman, OK 73019.

Jason R. Vogel, Ph.D., P.E., is a Professor in the School of Civil Engineering and Environmental Science at the University of Oklahoma, and currently serves as Director of the Oklahoma Water Survey. For more than 25 years, Vogel has worked to facilitate and develop solutions for water issues throughout the Great Plains of the United States and beyond. His research interests include hydrology, water quality, stormwater, and wastewater-based epidemiology. Prior to joining the University of Oklahoma, he held faculty and research positions at Oklahoma State University (OSU) in the Department of Biosystems and Agricultural Engineering and at the U.S. Geological Survey, respectively. He may be contacted at jason.vogel@ou.edu or 202 W. Boyd, Carson Engineering Center, Norman, OK 73019.

References

Baird, R. and L. Bridgewater. 2017. *Standard Methods for the Analysis of Water and Wastewater* (23rd Edition) Sections 9223B and 2540D. American Public Health Association, American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, D.C.

Bradshaw, J.K., B.J. Snyder, A. Oladeinde, D. Spidle, M.E. Berrang, R.J. Meinersmann, B. Oakley, R.C. Sidle, K. Sullivan, and M. Molina. 2016. Characterizing relationships among fecal indicator bacteria, microbial source tracking markers, and associated waterborne pathogen occurrence in stream water and sediments in a mixed land use watershed. *Water Research* 101: 498-509. Available at: https://doi.org/10.1016/j.watres.2016.05.014. Accessed February 10, 2023.

Brinkmeyer, R., R.M.W. Amon, J.R. Schwarz, T. Saxton, D. Roberts, S. Harrison, N. Ellis, J. Fox, K. DiGuardi, M. Hochman, S. Duan, R. Stein, and C. Elliott. 2015. Distribution and persistence of *Escherichia coli* and Enterococci in stream bed and bank sediments from two urban streams in Houston, TX. *Science of the Total Environment* 502: 650-658. Available at: https://doi.org/10.1016/j.scitotenv.2014.09.071. Accessed February 10, 2023.

Brock, F.V., K.C. Crawford, R.L. Elliott, G.W. Cuperus, S.J. Stadler, H.L. Johnson, and M.D. Eilts. 1995. The Oklahoma Mesonet: A technical overview. *Journal of Atmospheric and Oceanic Technology* 12: 5-19.

Christensen, V.G., P.P. Rasmussen, and A.C. Ziegler. 2002. Real-time water quality monitoring and regression analysis to estimate nutrient and bacteria concentrations in Kansas streams. *Water Science and Technology* 45(9): 205-219. Available at:

https://doi.org/10.2166/wst.2002.0240. Accessed February 10, 2023.

Crosby, S.C., N.C. Spiller, K.E. Tietz, J.R. Cooper, and P.J. Fraboni. 2019. Temporal and spatial variability of instream indicator bacteria (*Escherichia coli*) and implications for water quality monitoring. *Environmental Monitoring and Assessment* 191(12): 745. Available at: https://doi.org/10.1007/s10661-019-7930-1. Accessed February 10, 2023.

Desai, A.M. and H.S. Rifai. 2010. Variability of *Escherichia coli* concentrations in an urban watershed in Texas. *Journal of Environmental Engineering* 136(12): 1347-1359. Available at: https://doi.org/10.1061/(ASCE)EE.1943-7870.0000290. Accessed February 10, 2023.

Desai, A.M. and H.S. Rifai. 2013. *Escherichia coli* concentrations in urban watersheds exhibit diurnal sag: Implications for water-quality monitoring and assessment. *JAWRA Journal of the American Water Resources Association* 49(4): 766-779. Available at: https://doi.org/10.1111/jawr.12039. Accessed February 10, 2023.

Devane, M.L., E. Moriarty, L. Weaver, A. Cookson, and B. Gilpin. 2020. Fecal indicator bacteria from environmental sources; Strategies for identification to improve water quality monitoring. *Water Research* 185: 116204. Available at: https://doi.org/10.1016/j.watres.2020.116204. Accessed February 10, 2023.

Dwivedi, D., B.P. Mohanty, and B.J. Lesikar. 2013. Estimating *Escherichia coli* loads in streams based on various physical, chemical, and biological factors. *Water Resources Research* 49(5): 2896-2906. Available at: https://agupubs.onlinelibrary.wiley.com/doi/full/10.1002/wrcr.20265. Accessed February 10, 2023.

Gitter, A., K.D. Mena, K.L. Wagner, D.E. Boellstorff, K.E. Borel, L.F. Gregory, T.J. Gentry, and R. Karthikeyan. 2020. Human health risks associated with recreational waters: Preliminary approach of integrating quantitative microbial risk assessment with microbial source tracking. *Water* 12(2): 327. Available at: https://doi.org/10.3390/w12020327. Accessed February 10, 2023.

Gregory, L.F., A. Gitter, S. Muela, and K.L. Wagner. 2019. Should contact recreation water quality standards be consistent across hydrological extremes? *Journal of Contemporary Water Research and Education* 166: 12-23.

Haller, L., E. Amedegnato, J. Poté, and W. Wildi. 2009. Influence of freshwater sediment characteristics on persistence of fecal indicator bacteria. *Water, Air, and Soil Pollution* 203(1): 217-227. Available at: https://doi.org/10.1007/s11270-009-0005-0. Accessed February 10, 2023.

Hamilton, J.L. and I. Luffman. 2009. Precipitation, pathogens, and turbidity trends in the Little River, Tennessee. *Physical Geography* 30(3): 236-248. Available at: https://doi.org/10.2747/0272-3646.30.3.236. Accessed February 10, 2023.

Harmel, R.D., J.M. Hathaway, K.L. Wagner, J.E. Wolfe, R. Karthikeyan, W. Francesconi, and D.T. McCarthy. 2016. Uncertainty in monitoring *E. coli* concentrations in streams and stormwater runoff. *Journal of Hydrology* 534: 524-533. Available at: https://doi.org/10.1016/j.jhydrol.2016.01.040. Accessed February 10, 2023.

Jamieson, R.C., D.M. Joy, H. Lee, R. Kostaschuk, and R.J. Gordon. 2005. Resuspension of sediment-associated *Escherichia coli* in a natural stream. *Journal of Environmental Quality* 34(2): 581-589. Available at: https://doi.org/10.2134/jeq2005.0581. Accessed February 10, 2023.

McKergow, L.A. and R.J. Davies-Colley. 2010. Stormflow dynamics and loads of *Escherichia coli* in a large mixed land use catchment. *Hydrological Processes* 24(3): 276-289. Available at: https://doi.org/10.1002/hyp.7480. Accessed February 10, 2023.

McPherson, R.A., C.A. Fiebrich, K.C. Crawford, R.L. Elliott, J.R. Kilby, D.L. Grimsley, J.E. Martinez, J.B. Basara, B.G. Illston, D.A. Morris, K.A. Kloesel, S.J. Stadler, A.D. Melvin, A.J. Sutherland, and H. Shrivastava. 2007. Statewide monitoring of the mesoscale environment: A technical update on the Oklahoma Mesonet. *Journal of Atmospheric and Oceanic Technology* 24: 301-321.

Muirhead, R.W. and E.D. Meenken. 2018. Variability of *Escherichia coli* concentrations in rivers during base-flow conditions in New Zealand. *Journal of Environmental Quality* 47(5): 967-973. Available at: https://doi.org/10.2134/jeq2017.11.0458. Accessed February 10, 2023.

Oklahoma Water Resources Board (OWRB). 2017. Oklahoma Water Quality Standards. Oklahoma Administrative Code 785:45. Available at: https://www.owrb.ok.gov/rules/pdf/current/Ch45.pdf. Accessed February 21, 2023.

Petersen, F. and J.A. Hubbart. 2020. Physical factors impacting the survival and occurrence of *Escherichia coli* in secondary habitats. *Water* 12(6): 1796. Available at: https://doi.org/10.3390/w12061796. Accessed February 10, 2023.

Rochelle-Newall, E.J., O. Ribolzi, M. Viguier, C. Thammahacksa, N. Silvera, K. Latsachack, R.P.

Dinh, P. Naporn, H.T. Sy, B. Soulileuth, N. Hmaimum, P. Sisouvanh, H. Robain, J-L. Janeau, C. Valentin, L. Boithias, and A. Pierret. 2016. Effect of land use and hydrological processes on *Escherichia coli* concentrations in streams of tropical, humid headwater catchments. *Scientific Reports* 6(1): 32974. Available at: https://doi.org/10.1038/srep32974. Accessed February 10, 2023.

Sanders, E.C., Y. Yuan, and A. Pitchford. 2013. Fecal coliform and *E. coli* concentrations in effluent-dominated streams of the Upper Santa Cruz Watershed. *Water* 5(1): 243-261. Available at: https://doi.org/10.3390/w5010243. Accessed February 10, 2023.

Schultz-Fademrecht, C., M. Wichern, and H. Horn. 2008. The impact of sunlight on inactivation of indicator microorganisms both in river water and benthic biofilms. *Water Research* 42(19): 4771-4779. Available at: https://doi.org/10.1016/j.watres.2008.08.022. Accessed February 10, 2023.

Smith, S.J. and R.A. Esralew. 2010. *StreamStats in Oklahoma– Drainage-Basin Characteristics and Peak-Flow Frequency Statistics for Ungaged Streams*. U.S. Geological Survey Scientific Investigations Report 2009-5255. Available at: https://doi.org/10.3133/sir20095255. Accessed February 10, 2023.

Stocker, M.D., M. Penrose, and Y.A. Pachepsky. 2018. Spatial patterns of *Escherichia coli* concentrations in sediment before and after high-flow events in a first-order creek. *Journal of Environmental Quality* 47(5): 958-966. Available at: https://doi.org/10.2134/jeq2017.11.0451. Accessed February 10, 2023.

Stocker, M.D., J.G. Rodriguez-Valentín, Y.A. Pachepsky, and D.R. Shelton. 2016. Spatial and temporal variation of fecal indicator organisms in two creeks in Beltsville, Maryland. *Water Quality Research Journal* 51(2): 167-179. Available at: https://doi.org/10.2166/wqrjc.2016.044. Accessed February 10, 2023.

Stocker, M.D., J.E. Smith, C. Hernandez, D. Macarisin, and Y. Pachepsky. 2019. Seasonality of *E. coli* and enterococci concentrations in creek water, sediment, and periphyton. *Water, Air, and Soil Pollution* 230(9): 223. Available at: https://doi.org/10.1007/s11270-019-4263-1. Accessed February 10, 2023.

United States Department of Agriculture (USDA) Natural Resources Conservation Service (NRCS). Web Soil Survey. Available at: http://websoilsurvey.nrcs.usda.gov/. Accessed February 10, 2023.

United States Environmental Protection Agency (USEPA). 1986. Bacteriological Ambient Water Quality Criteria for Marine and Fresh Recreational Waters. EPA 440/5-84-002. Office of Water Regulations and Standards, Washington, D.C. Available at: https://www.epa.gov/sites/default/files/2019-03/documents/ambient-wqc-bacteria-1986.pdf. Accessed February 10, 2023.

United States Environmental Protection Agency (USEPA). 2010. Sampling and Consideration of Variability (Temporal and Spatial) for Monitoring of Recreational Waters. EPA-823-R-10-005. Office of Water, Washington, D.C. Available at: https://www.epa.gov/sites/default/files/2015-11/documents/sampling-consideration-recreational-waters.pdf. Accessed February 10, 2023.

United States Environmental Protection Agency (USEPA). 2012. Recreational Water Quality Criteria. Office of Water 820-F-12-058. Available at: https://www.epa.gov/sites/production/files/2015-10/documents/rwqc2012.pdf. Accessed February 10, 2023.

United States Geological Survey (USGS). 2014. National Field Manual for the Collection of Water-Quality Data. U.S. Geological Survey Techniques of Water-Resources Investigations, Book 9. Available at: http://pubs.water.usgs.gov/twri9A. Accessed February 10, 2023.

Verhougstraete, M.P., S.L. Martin, A.D. Kendall, D.W. Hyndman, and J.B. Rose. 2015. Linking fecal bacteria in rivers to landscape, geochemical, and hydrologic factors and sources at the basin scale. *Proceedings of the National Academy of Sciences* 112(33): 10419-10424. Available at: https://doi.org/10.1073/pnas.1415836112. Accessed February 10, 2023.

Vogel, J.R., J.D. Frankforter, D.L. Rus, C.M. Hobza, and M.T. Moser. 2009. *Water Quality of Combined Sewer Overflows, Stormwater, and Streams, Omaha, Nebraska, 2006-07*. U.S. Geological Survey Scientific Investigations Report 2009–5175. Available at: https://pubs.usgs.gov/sir/2009/5175/pdf/SIR2009-5175.pdf. Accessed February 10, 2023.

Whitman, R.L., M.B. Nevers, G.C. Korinek, and M.N. Byappanahalli. 2004. Solar and temporal effects on *Escherichia coli* concentration at a Lake Michigan swimming beach. *Applied and Environmental Microbiology* 70(7): 4276-4285. Available at: https://doi.org/10.1128/AEM.70.7.4276-4285.2004. Accessed February 10, 2023.

Universities Council on Water Resources
Journal of Contemporary Water Research & Education
Issue 177, Pages 103-112, April 2023

Research Note

Total Microcystin Concentration Variability in Water Samples and Recommended Minimum Volume (20 mL) for Freeze Thaw Cycles

*Brian E. Haggard[1,2] and Bradley J. Austin[1]

*[1]Arkansas Water Resources Center, University of Arkansas System Division of Agriculture, Don Tyson Center for Agricultural Sciences, Fayetteville, AR; [2]Biological and Agricultural Engineering Department, University of Arkansas, Fayetteville, AR; *Corresponding Author*

Abstract: Cyanobacterial harmful algal blooms (cyanoHABs) continue to be a monitoring and research focus, particularly on the occurrence of toxins like total microcystins. The objectives of this study were to evaluate sampling and analytical variability in measured total microcystin concentrations and then to evaluate the volume of raw water needed in the freeze thaw cycle to reduce sampling variability. Water samples were collected from a recreational lake with annual cyanoHABs, and then 2 mL was used in freeze thaw cycles before total microcystin analysis. Then, sample volumes used in the freeze thaw cycles varied from 2 to 300 mL for total microcystin analysis. With three separate experiments, we observed a great deal of sampling variability (when using 2 mL in the freeze thaw cycles) while analytical variability was much less. In fact, sampling variability could potentially account for temporal variability observed in the routine monitoring. However, when sample volume used in the freeze thaw cycles increased, total microcystin variability decreased. We recommend at least 20 mL to be used in the freeze thaw cycles when analyzing total microcystins in environmental samples.

Keywords: *total microcystins, ELISA analysis, freeze thaw volume, sampling variability*

Cyanobacterial harmful algal blooms (cyanoHABs) have been and continue to be a research focus across the USA and globe, where scientists are trying to understand the drivers in cyanobacterial toxin production. These blooms have been observed in freshwaters, mainly lakes, across the USA (Loftin et al. 2016b), but toxins can be measurable in streams (Loftin et al. 2016a; Graham et al. 2020; Austin and Haggard 2022). While toxins from cyanoHABs in lotic systems tend to be low, occurrences of elevated toxins, particularly total microcystins, have been reported in large rivers, e.g., Obryzyca River, Poland (Czyzewska et al. 2020) and Poteau River, Arkansas, USA (Haggard, B.E., unpublished data).

Microcystin in all its various forms (i.e., total microcystins) is one of the most studied cyanobacterial toxins in freshwaters, including lakes and lotic systems. Microcystins are usually present and often in high concentrations when other toxins like anatoxin, cylindrospermopsin, and saxitoxin are measured in water samples from lakes (Graham et al. 2010) and rivers (Loftin et al. 2016a; Czyzewska et al. 2020). Also, total microcystins (and nodularins) are easily measured by water labs using the enzyme linked immunoassay techniques (ELISA, Method 546; EPA 2016), but the ELISA Method is used by labs with skilled analysts. For these reasons, total microcystins are the focus of many studies on occurrence and drivers of cyanobacterial toxin production in freshwaters.

The ELISA technique for total microcystins has been shown to be quantitative, reliable, and quick (Nagata et al. 1997), although this technique is an indirect competitive assay or measure of this toxin. Generally, this technique can produce repeatable analytical results (Massey et al. 2020), and its method detection limit (MDL) is much less

Research Implications

- Variability in total microcystin concentrations was observed with repeated sampling and subsampling within an individual bottle.
- Some variability in total microcystin concentrations observed in lake studies might be due to sample volume used in freeze thaw cycles.
- Total microcystin variability decreased as sample volume used in three freeze thaw cycles increased.
- We suggest at least 20 mL for freeze thaw cycles, especially if water is not mechanically homogenized.

than recreational guidelines (e.g., 8 µg L^{-1} total microcystin; EPA 2019) and even slightly below drinking water limits (e.g., 0.3 µg L^{-1} for infants; EPA 2015). The method's freeze thaw cycles have been shown to produce strong recovery of intracellular total microcystins (Greenstein et al. 2021), so the use of raw water with this method provides a solid measure of extracellular (free in water) and intracellular total microcystin concentrations.

The volume from water samples from lakes and rivers used in the freeze thaw cycles varies (Table 1), but in general, most standard operating procedures, labs, and literature studies use 30 mL or less. Method 546 (EPA 2016) suggests 5 to 10 mL of well-mixed water be used in the freeze thaw cycles before ELISA analysis. We have a recreational lake (Lake Fayetteville, Northwest Arkansas) which experiences cyanoHABs each growing season, and on May 7, 2019 we measured 1.8 µg L^{-1} total microcystins at 13:00 and then over 11 µg L^{-1} at 16:00 (Figure 1). This variability between sampling events on the same day led to two questions: 1) could there be temporal variability in total microcystins during the cyanoHABs at this lake?, and 2) does the volume of raw water used in the freeze thaw cycles influence variability in measured concentrations of total microcystins? The purpose of our study was to answer these questions, but our efforts focused more on the second question to help guide future total microcystin analysis at the Arkansas Water Resources Center (AWRC) water quality lab.

Methods

Lake Fayetteville is a small recreational lake with a surface area of ~0.6 km^2 and catchment area of 24 km^2, which is managed by the City of Fayetteville, Arkansas. The first recorded study on this lake was in 1968 (Meyer 1971), which showed that the phytoplankton community was dominated by cyanobacteria at that time, and the annual pattern in dissolved nutrient supply was the same as today (Haggard et al. 2023a). Total microcystins were first measured on November 19, 2018 by a First-Year Engineering Honors Research Team under our mentorship and we were surprised to see total microcystins (0.442 µg L^{-1}) greater than MDL in late fall with colder water temperatures. We began routine cyanoHAB and total microcystins monitoring in March 2019, resulting in published studies on cyanoHABs, microcystin, and environmental drivers including Wagner et al. (2021) and Haggard et al. (2023a; 2023b). We have been collecting water samples from three access points along the north shore of the lake since 2019, and we sampled off the marina and kayak platforms near the dam in 2019 and 2020 to answer the question posed in this study (Figure 1). All water samples were collected approximately weekly, and each had total microcystins measured using the ELISA technique (Method 546; EPA 2016).

Our researchers collected additional water samples for total microcystins on select sampling dates beyond those collected for our routine monitoring. On June 11 and July 1, 2019, multiple water samples were collected 0.2 m below the water surface at the end of the kayak dock near the dam to evaluate potential sampling and analytical variability. On April 27, 2020, multiple water samples of the surface scum were collected to evaluate sampling and analytical variability; the surface scum was targeted intentionally, as it was likely to have greater toxin concentrations. Approximately 15 1-L samples were collected each date, and processed upon return to the lab. Each bottle had ~2 mL saved in 4 mL amber glass vials for the freeze thaw cycles, and one random bottle was subsampled ten times (~2 mL or less each time in 4 mL amber glass vials). Following three freeze thaw cycles, water with lysed contents

Table 1. Select references and labs providing volume used for freeze thaw cycles in analysis of total microcystins using enzyme linked immunosorbent assays techniques (based on Google Scholar search and eight pages viewed, as well as select personal communications).

ELISA Citation	Freeze Thaw Volume	Qualifier, If Any
Method 546; EPA 2016	5-10 mL	Well-mixed water
Thorpe and Brunet 2021	4 mL or less	Based on half of vial volume (8 mL)
Abdullahi et al. 2022	5 mL	Subsequent methanol extraction
Cullen 2009; Greenstien et al. 2021; Wilson 2022	~10 mL	Not applicable; Only small volume (µL) needed for Method 546 analysis
Wood et al. 2006	10 mL	100 mL water frozen initially; 10 mL two additional freeze thaw cycles
Ohio EPA 2018	20 mL	Additional preprocessing if chlorinated water sample
Klamath Blue Green Algae Working Group 2009	30 mL	Based on 25% of vial volume (120 mL or 4 oz vial in methods)
Nagata et al. 1997	30 mL	40 mL original volume
Olsen 2022	2-250 mL	If quick freeze thaw needed, the lesser volume in the range is used
Loftin et al. 2016a; 2016b	25-50 mL	Lysed sample filtered and stored; Frozen until analysis
Trout-Haney et al. 2016	750 mL or more	Entire volume three freeze thaw cycles and then centrifuged

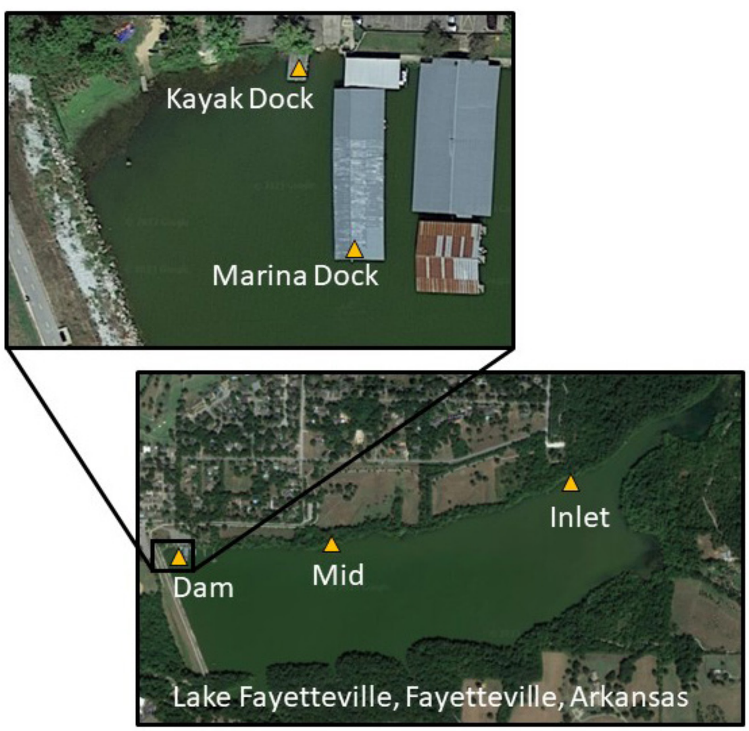


Figure 1. Map of sampling sites, marina dock, and kayak dock at Lake Fayetteville in Fayetteville, Arkansas.

was analyzed for total microcystins. One of the ten vials from subsampling was analyzed ten times; the vial was randomly selected. Variability across "between bottles," "within bottle," and "within vial" was compared by calculating the absolute residual for each individual concentration relative to the group mean. Mean absolute residuals were then compared across all bottles, within bottle, and within vial, using analysis of variance (ANOVA) with least significant difference (LSD) at an alpha of 0.05 ($p<0.05$). We used the software program, SigmaPlot Version 14.5 (Systat Software, Inc.) for all statistical comparisons.

On June 30 through July 1, 2019, we collected water samples to capture potential diurnal variability in total microcystin concentrations. The first water sample was collected at 17:00 from the end of the dock at the marina near the dam, and water samples were collected every hour until 23:00. Water samples were collected every 1.5 hr from 00:30 to 5:00 on July 1, and then sampling shifted back to hourly until 17:00 that day. A small volume (~2 mL) was put through freeze thaw cycles for total microcystin analysis from these samples.

On June 16th, 2020, we collected ~4 L of water from ~0.2 m below the surface at the end of the marina dock near the dam and near the shore, as well as sufficient volume of surface scum at both locations. The collected water was kept mixed at the lab by vigorously shaking each sample, while various subsample volumes were collected for the freeze thaw cycles. We used volumes of 2 mL (n=10), 5 mL (n=10), 10 mL (n=10), 20 mL (n=10), 60 mL (n=5), and 300 mL (n=3) for the below surface samples and the surface scum samples; a total of 48 subsamples for each, or 192 total for all four. All were put through three freeze thaw cycles and then analyzed for total microcystins. We assumed the true total microcystin concentration represented the mean of all subsample volumes, and then each individual concentration was converted to a Z-score to compare variability in sample volumes used in the processing and analysis. The Z-score allowed us to group each experiment, despite differences in measured total microcystin concentration before below surface and surface scum samples. Mean Z-scores of the various subsample volumes were compared using ANOVA, and means were separated using LSD ($p<0.05$).

Results and Discussion

Total microcystin concentrations were variable over time at Lake Fayetteville, showing a distinct bimodal pattern over 2019 and 2020 (Figure 2). For the most part, total microcystin concentrations are in relatively close agreement between the three sampling sites along the north shore of the lake across both study years. However, on occasion, total microcystin concentrations show increased variability between sites in 2019. As previously mentioned, lake samples from the same day (May 7, 2019) and site (dam), but just hours apart, had order of magnitude (1.78 versus 11.01 µg L^{-1}) differences in total microcystins. The second set of samples from that day were sent to the Wilson Lab at Auburn University (Table 1; https://www.wilsonlab.com/), where total microcystins at the dam site measured 4.23 µg L^{-1}. On May 27, 2019, total microcystin varied from 1.67 to 5.00 µg L^{-1} across the sites, and then the greatest measured total microcystin (15.38 µg L^{-1}) measured was observed on June 4, 2019, but other sites that day were less than 2 µg L^{-1}. This site variation persisted through late June, and then total microcystin differences between sites were less until October 29, 2019, when total microcystin varied from 2.00 µg L^{-1} at the dam to 0.31 µg L^{-1} or less at the other two sites. Considering the variability in total microcystin concentrations, we wanted to determine if these differences were real, or due to using subsample volumes on the low end of the range.

We did the first sampling variability experiment (Figure 3) at Lake Fayetteville on June 11, 2019, when total microcystin concentrations in the routine sampling varied from 0.34 µg L^{-1} at the mid-lake site to 2.96 µg L^{-1} near the dam. The sampling variability results showed significant differences and high variability, including:

- total microcystins varied from 0.39 to 2.98 µg L^{-1} across the 16 bottles collected, averaging 1.21 µg L^{-1} (± 0.60 standard deviation, SD) across all bottles that day from the kayak dock;
- total microcystins varied from 0.04 to 1.65 µg L^{-1} across the 9 vials used to subsample one bottle, averaging 0.51 µg L^{-1} (± 0.48 SD) across all vials; and
- total microcystins varied from 0.15 to 0.32

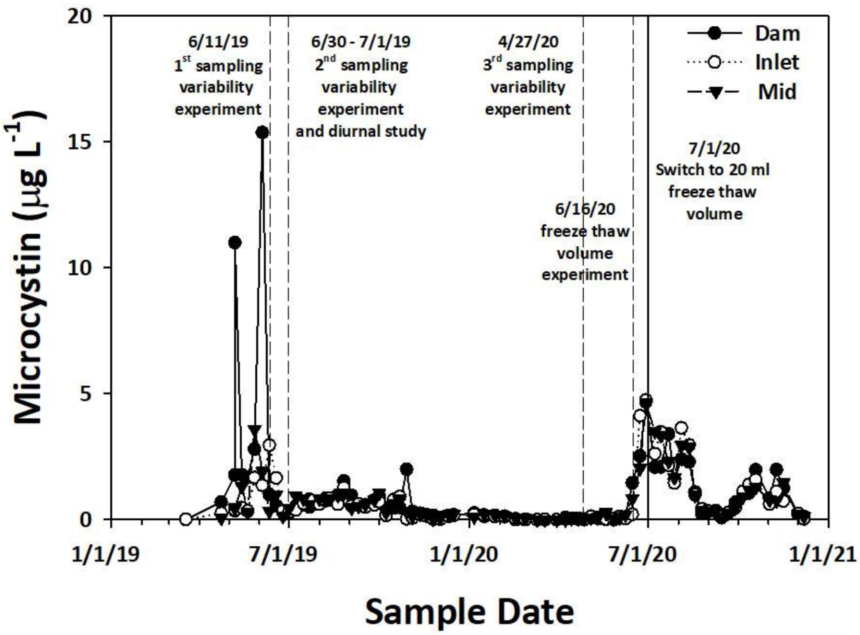


Figure 2. Microcystin concentrations measured during routine monitoring of three sites along the north shore of Lake Fayetteville from March 2019 through December 2020. Dashed vertical lines align with when each experiment was conducted over the two-year period. The solid vertical line shows when the lab switched freeze thaw sample volumes from 2 to 20 ml.

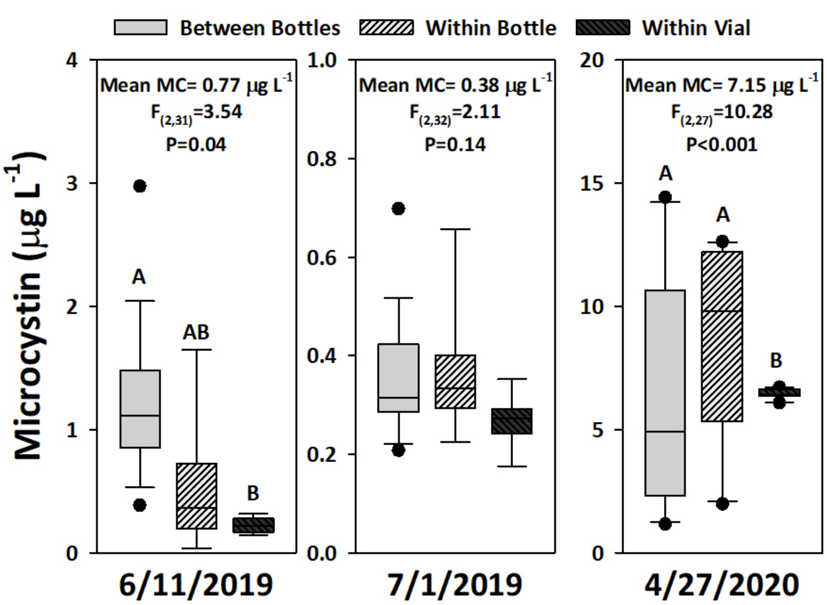


Figure 3. Box plots depicting variability in measured microcystin concentrations from sampling (Between Bottles), sample processing (Within Bottles), and analytical (Within Vial), when mean microcystin concentrations are moderate (6/11/2019), low (7/1/2019), and high (4/27/2020); letters above box plots show significant differences in the absolute value of the residuals (i.e., variability) for each experiment (ANOVA, LSD).

μg L^{-1} when the same random vial was analyzed several times.

The variability in total microcystin concentrations between bottles and within one bottle was much greater than that observed within one vial. These observations suggest that analytical variability of the ELISA method was low, as seen in other studies (Massey et al. 2020), particularly in relation to sampling variability. This leads to the questions regarding sampling variability both from the lake and sample processing within labs, i.e., subsample volume used in freeze thaw cycles.

On June 30, 2019, we looked at diurnal variability in total microcystin concentrations below the water surface from the marina dock near the Lake Fayetteville dam (Figure 4); water samples were collected every 1 to 1.5 hr for 24 hr. The concentrations of total microcystin ranged from ~0.1 μg L^{-1} at 16:00 July 1, 2019, to ~0.8 μg L^{-1} 12:00 the night before (June 30), but the variability in concentrations did not fit any diurnal patterns. For example, total microcystin concentrations were not greater just below the water surface at night when buoyancy might be increased in some cyanobacteria (Ibelings et al. 1991) nor were the concentrations greater during day when increased photosynthetically active radiation (PAR) has been positively correlated with microcystin production and content in a cyanobacteria (Wiedner et al. 2003). Even though potential diurnal patterns might be opposite, we observed variability in total microcystin concentrations, although mean total microcystin was less than 0.3 μg L^{-1} across the 24 hr period. We need to qualify that these represent water samples where only 2 mL or less was used in the freeze thaw process.

During this diurnal study, we repeated the sampling variability experiment at 15:00 on July 1, 2019. Again, sampling variability results showed no differences between subsampling and analysis but total microcystins varied two-fold to an order of magnitude difference (Figure 3), including:

- total microcystins varied from 0.21 to 0.70 μg L^{-1} across the 17 bottles collected, averaging 0.35 μg L^{-1} (± 0.12 SD) across all bottles that day from the kayak dock;
- total microcystins varied from 0.22 to 2.02 μg L^{-1} across the 10 vials used to subsample one bottle, averaging 0.53 μg L^{-1} (± 0.54 SD) across all vials; and
- total microcystins varied from 0.18 to 0.35

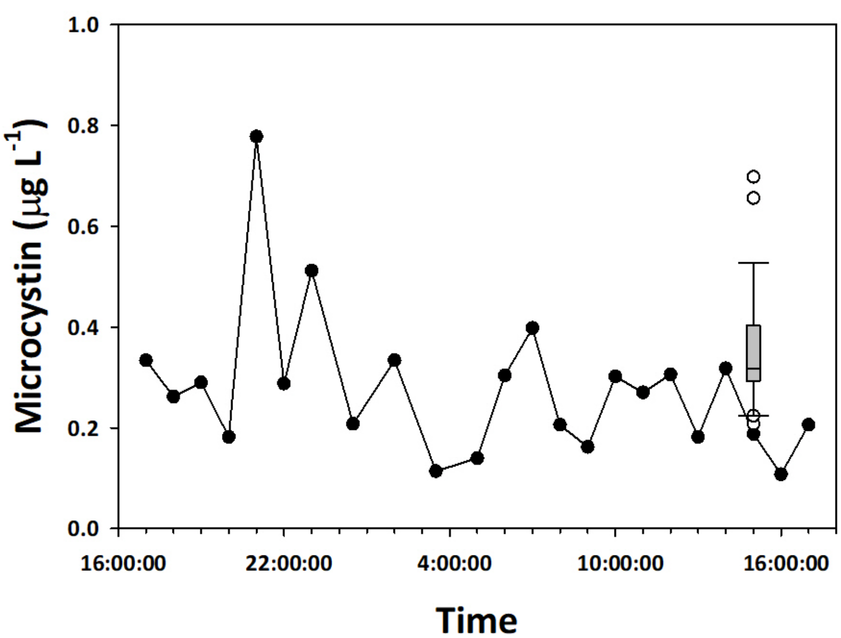


Figure 4. Diurnal variability in microcystin over a 24 hr period starting at 17:00 on 6/30/2019 to 17:00 on 7/1/2019; box plot at 15:00 on 7/1/2019 shows bottle and within bottle variability from the second sampling variability experiment, and open symbols represent the data outside the 90th percentile.

µg L^{-1} when the same random vial was analyzed several times.

These observations showed that the diurnal variability in total microcystins was within the sampling variability (0.21 to 0.70 µg L^{-1}), as well as the analytical variability (0.18 to 0.35 µg L^{-1}) when total microcystins were relatively low at this lake. Therefore, we really cannot make any conclusions about diurnal variability, when this experiment showed sampling variability may account for observed changes.

Next, we wanted to evaluate sampling variability like the previous two experiments, when total microcystin concentrations were extremely high and potentially exceeded recreational guidelines (i.e., 8 µg L^{-1}; EPA 2019). On April 27, 2021, we repeated the experiment showing:

- total microcystins varied from 1.17 to 14.43 µg L^{-1} across the 10 bottles collected, averaging 6.22 µg L^{-1} (± 4.68 SD) across all bottles that day from the kayak dock;
- total microcystins varied from 1.98 to 12.63 µg L^{-1} across the 10 vials used to subsample one bottle, averaging 8.77 µg L^{-1} (± 3.88 SD) across all vials; and
- total microcystins varied from 6.09 to 6.72 µg L^{-1} when the same random vial was analyzed several times (Figure 2).

These results showed that when total microcystin concentrations approached the recreational guidelines, measured concentrations were highly variable across the bottles and within one bottle. In fact, 10 out of 20 measured total microcystin concentrations in the bottles and within the one bottle exceeded 8 µg L^{-1}. However, analytical variability using this method was low, especially relative to sampling variability.

Now, we knew that sampling variability within the source water or within an individual bottle was much greater than analytical variability across a range from low to high concentration, when freeze thawing only used 2 mL. Our next question was – what is the minimum volume needed in the freeze thaw process to reduce sampling variability? The final experiment gave us the answer, or at least guidance, where total microcystins measured from 2 mL vials after three freeze thaw cycles had the greatest variance (i.e., mean Z-scores) from the mean across all analyzed vials (Figure 5). The Z-scores (relative to total microcystin analyses) significantly decreased as we increased sample volume used in the freeze thaw cycles, showing

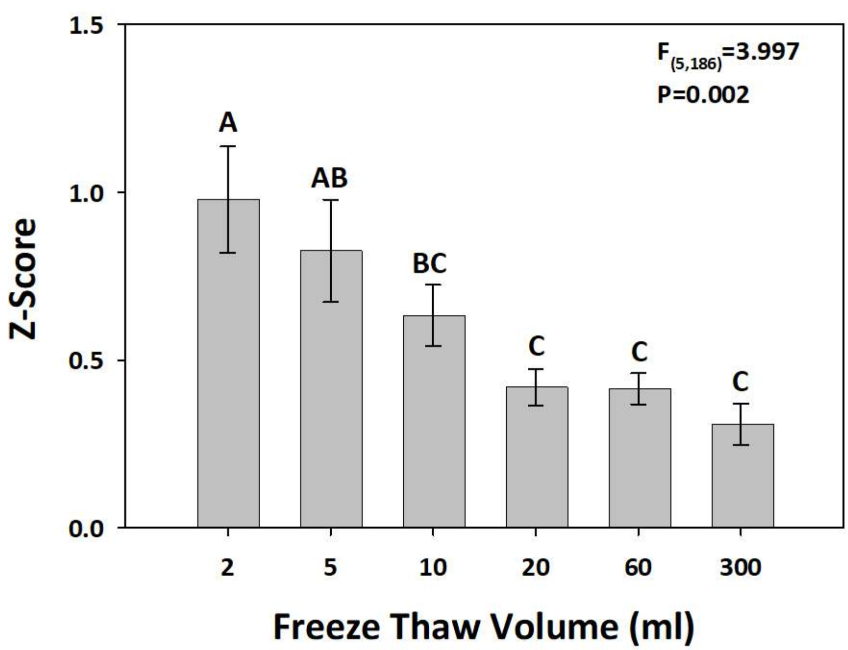


Figure 5. Mean Z-score (±1 standard error) based on microcystin freeze thaw volume (ml); Z-score's representing the amount of variability in measured microcystin concentrations relative to the overall mean for each freeze thaw volume; letters show differences in mean Z-scores across the volumes used in freeze thaw.

that at least 20 mL was needed for total microcystin analysis.

Our recommended minimum volume (i.e., 20 mL) needed in freeze thaw cycles fit with our review of research, state, and federal labs analyzing for total microcystins using the ELISA kits; these volumes ranged from 2 to 750 mL or more (Table 1). Method 546 (EPA 2016) suggests 5 to 10 mL of well-mixed water – we have mixed manually, inverting sample bottles several times. If mechanical mixing (e.g., frother) was used, we may have observed that smaller volumes for the freeze thaw cycles could work. The challenge with larger volumes is typically freeze space limitations; one possible option would be to freeze thaw larger volumes and then store back smaller volumes of (potentially filtered) water for total microcystin analysis.

In 2020, we switched from 2 mL for freeze thaw to 20 mL, and we did notice possible reduced sampling variability throughout that growing season (Figure 2). Total microcystin concentrations did vary between the three sites along the north shore at Lake Fayetteville, where maximum range was 2.037 to 4.115 µg L^{-1} on June 23, 2020, near the beginning of the cyanoHABs and 0.995 to 2.869 µg L^{-1} on August 18, 2020 near the end of the toxic bloom. If the minimum volume of 20 mL for freeze thaw does reduce sampling variability, then this might explain why such strong hierarchical structure existed between total microcystin concentrations and physiochemical properties at Lake Fayetteville in 2020 (Haggard et al. 2023a; 2023b).

Conclusions and Recommendations

Total microcystin concentrations in lake water samples can be influenced greatly by sampling variability, which might obscure data patterns or relationships (e.g., temporal variability). We showed that sampling variability may be high where natural variability in cyanobacterial blooms exists either below the water surface or in the surface scum. Therefore, we recommend at least 20 mL of sample volume be used in the three freeze thaw cycles for total microcystin analysis using ELISA kits; if the water is well-mixed mechanically, and not just by inversion, then smaller volumes might work. When we switched to 20 mL sample volume for freeze thaw, we saw reduced differences in total microcystin concentrations between sites (Figure 2) and strong relations between this toxin and physiochemical properties measured in the water.

Acknowledgements

Funding for this research was provided in part by the AWRC through the USGS WRRI 104b Base Funding Program, the USDA NIFA Hatch Program Project 2660, and the University of Arkansas System Division of Agriculture. The ability to use these funds, especially the USGS WRRI 104b Base Funding Program, to tackle state specific issues like nutrient and environmental drivers of cyanoHABs was key to the success of this manuscript, project, and continued monitoring (2019-present).

Author Bio and Contact Information

Dr. Brad Austin is a research scientist with the Arkansas Water Resources Center, where he provides and helps manage field and lab services for all research projects. Specifically, he managed the field work, lab analysis including microcystins, and statistical analysis for this research project at Lake Fayetteville. He has a Ph.D. in Biology from the University of Arkansas, a M.S. in Aquatic Biology and Limnology from Fort Hays State University, and a B.S. in Biological Sciences from Ottawa University. He may be contacted at bjaustin@uark.edu or 1371 West Altheimer Drive, Don Tyson Center for Agricultural Sciences, Fayetteville, AR 72704, USA.

Dr. Brian Haggard (corresponding author) has been focused on water quality issues since his graduate studies, and he has a keen interest in cyanoHABs. He is currently the director of the Arkansas Water Resources Center and a professor in the Biological and Agricultural Engineering Department with the University of Arkansas System Division of Agriculture. Prior to his current positions, he worked for the US Geological Survey and USDA Agricultural Research service in hydrology and water quality. He conceptualized the data collection and this sampling and analytical variability research project at Lake Fayetteville, as well as drafted the manuscript. He has a Ph.D. in Biosystems Engineering from Oklahoma State University, a M.S. in Agronomy from the University of Arkansas, and a B.S. in Life Sciences from the Missouri University of Science and Technology. He may be contacted at haggard@uark.edu or Engineering Hall 203, University of Arkansas, Fayetteville, AR 72701 USA.

References

Abdulla, H., Y. Tanimu, S.A. Akinyemi, M.C. Bittencourt-Oliveira, and M.A. Chia. 2022. Assessment of microcystins in surface water and irrigated vegetables in Kwaru stream, Hayin Danmani, Kaduna-Nigeria. *Environmental Science and Pollution Research* 29: 78303-78313.

Austin, B.J. and B.E. Haggard. 2022. Measurable microcystin in Ozark streams was rare during summer 2018 baseflow conditions. *Agriculture and Environmental Letters* 7(1): e20069. Available at: https://acsess.onlinelibrary.wiley.com/doi/full/10.1002/ael2.20069. Accessed February 8, 2023.

Cullen, B. 2009. Sampling and Analysis Plan for the Regional Examination of Harmful Algal Blooms (REHAB), King County Water and Land Division. Department of Natural Resources and Parks, Seattle, WA, USA.

Czyzewska, W., M. Piontek, and K. Luszczynska. 2020. The occurrence of potential harmful cyanobacteria and cyanotoxins in the Obrrzyca River (Poland), a source of drinking water. *Toxins* 12(5): 284. Available at: https://doi.org/10.3390/toxins12050284. Accessed February 8, 2023.

Environmental Protection Agency (EPA). 2015. Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins. EPA 820R15100.

Environmental Protection Agency (EPA). 2016. Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay. EPA 815-B-16-011.

Environmental Protection Agency (EPA). 2019. Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. EPA 822-R-19-001.

Graham, J.L., K.A. Loftin, M.T. Meyer, and A.C. Ziegler. 2010. Cyanotoxin mixtures and taste-and-odor compounds in cyanobacterial blooms from the Midwestern United States. *Environmental Science and Technology* 44: 7361-7368.

Graham, J.L., N.M. Dubrovsky, G.M. Foster, L.R. King, K.A. Loftin, B.H. Rosen, and E.A. Stelzer. 2020. Cyanotoxin occurrence in large rivers of the United States. *Inland Waters* 10(1): 109-117.

Greenstein, K.E., A. Zamyadi, and E.C. Wert. 2021. Comparative assessment of physical and chemical cell lysis methods for total microcystin-LR analysis. *Toxins* 13(9): 596.

Haggard, B.E., E. Grantz, B.J. Austin, A.L. Lasater, L. Haddock, A. Ferri, N.D. Wagner, and J.T. Scott. 2023a. Microcystin shows thresholds and hierarchical structure with physiochemical properties at Lake Fayetteville, Arkansas, May through September 2020. *Journal ASABE* 66(2): 307-317.

Haggard, B.E., E. Grantz, B.J. Austin, N.D. Wagner, and J.T. Scott. 2023b. Chlorophyll and phycocyanin raw fluorescence may inform recreational lake managers on cyanobacterial HABs and toxins: Lake Fayetteville case study. *Journal of Contemporary Water Research and Education* 177:63-71.

Ibelings, B.W., L.R. Murm and A.E. Walsby. 1991. Diurnal changes in buoyancy and vertical distribution in populations of Microcystis in two shallow lakes. *Journal of Plankton Research* 13: 419-436.

Klamath Blue Green Algae Working Group. 2009. Standard Operating Procedures, Environmental Sampling for Cyanobacteria for Cell Enumeration, Identification and Toxin Analysis. Developed for the 2009 AIP Measure 12 Water Quality Monitoring Activities.

Loftin, K.A., J.M. Clark, C.A. Journey, D.W. Kolpin, P.C. Van Metre, D. Carlisle, and P.M. Bradley. 2016a. Spatial and temporal variation in microcystin occurrence in wadeable streams in the southeastern United States. *Environmental Toxicology and Chemistry* 35: 2281-2287.

Loftin, K.A., J.L. Graham, E.D. Hilborn, S.C. Lehmann, M.T. Meyer, J.E. Dietze, and C.B. Griffith. 2016b. Cyanotoxins in inland lakes of the United States: Occurrence and potential recreational health risks in the EPA National Lakes Assessment 2007. *Harmful Algae* 56: 77-90.

Massey, I.Y., P. Wu, J. Wei, J. Luo, P. Ding, H. Wei, and F. Yang. 2020. A mini-review on detection methods of microsystins. *Toxins* 12(10): 641.

Meyer, R. 1971. *A study of phytoplankton dynamics in Lake Fayetteville as a means of assessing water quality*. Arkansas Water Resources Center Publication No. 10-A, 42 pp.

Nagata, S., T. Tsutsumi, A. Hasegawa, F. Yoshida, and Y. Ueno. 1997. Enzyme immunoassay for direct determination of microcystins in environmental water. *Journal of AOAC International* 80: 408-417.

Ohio EPA. 2018. Ohio EPA Total (Extracellular and Intracellular) Microcystins – ADDA by ELISA Analytical Methodology. Ohio EPA DES 701.0, Version 2.3.

Olsen, B. 2022. Division of Environmental Quality, Arkansas Department of Energy and Environment. Personal Communication July 8, 2022.

Thorpe, A. and C. Brunet. 2021. Standard Operating Procedure for: Microcystin (ELISA), University of Missouri Limnology Lab.

Trout-Haney, J.V., Z.T. Wood, and K.L. Cottingham. 2016. Presence of the cyanotoxin microcystin in Arctic lakes of Southwestern Greenland. *Toxins* 8(9): 256.

Wagner, N.D., E. Quach, S. Buscho, A. Ricciardelli, A. Kannan, S.W. Naung, G. Phillip, B. Sheppard, L. Ferguson, A. Allen, C. Sharon, J.R. Duke, R.B. Taylor, B.J. Austin, J.K. Stovall, B.E. Haggard, C.K. Chambliss, B.W. Brooks, and J.T. Scott. 2021. Nitrogen form, concentration, and micronutrient availability affect microcystin production in cyanobacterial blooms. *Harmful Algae* 103: 102002.

Wiedner, C., P.M., Visser, J. Fastner, J.S. Metcalf, G.A. Codd, and L.R. Mur. 2003. Effect of light on the microcystin content of Microcystis strain PCC 7806. *Applied and Environmental Microbiology* 69: 1475-1481.

Wilson, A. 2022. WilsonLab.com, Auburn University. Personal Communication July 6, 2022.

Wood, S.A., P.T. Holland, D.J. Stirling, L.R. Briggs, J. Sprosen, J.G. Ruck, and R.G. Wear. 2006. Survey of cyanotoxins in New Zealand water bodies between 2001 and 2004. *New Zealand Journal of Marine and Freshwater Research* 40: 585-597.

Universities Council on Water Resources
Journal of Contemporary Water Research & Education
Issue 177, Pages 113-139, April 2023

Review Article

Diversity, Equity, Inclusion, and Justice in Water Dialogues: A Review and Conceptualization

*Simone A. Williams[1,2], Susanna Eden[1], Sharon B. Megdal[1], and Valerisa Joe-Gaddy[1]

[1]*University of Arizona Water Resources Research Center, Tucson, Arizona*
[2]*Arid Lands Resource Sciences Graduate Interdisciplinary Program, University of Arizona, Tucson, Arizona*
**Corresponding Author*

Abstract: In the United States, the lack of diversity, equity, inclusion, and justice (DEIJ) in water governance and management has been identified as a serious problem that affects the validity of decisions. Because water governance and management institutions, processes, and practices at all scales involve dialogue, it is important to understand DEIJ in water dialogues. This paper reports on the results of a systematic literature survey that was undertaken to guide efforts by The University of Arizona Water Resources Research Center to improve diversity and inclusion in its engagement practices and outreach strategies. Three questions are explored: 1) How is DEIJ defined, conceptualized, and measured in water dialogues?, 2) How does a lack of DEIJ in water dialogues affect water-related outcomes and actors?, and 3) What are the approaches that can be used to increase DEIJ in water dialogues, especially with respect to underrepresented groups? The review synthesizes definitions of DEIJ and examines theories and methods from the literatures on discourse, diversity, social learning, and environmental justice. The lens of dialogue focused these disparate literatures on how people with diverse voices can be engaged and enabled to effectively participate in water dialogues. Despite the paucity of DEIJ literature relating to water resources in general, and to water dialogues more specifically, the review identified characteristics of DEIJ, factors that contribute to DEIJ issues, general lessons, and pathways that apply to increasing DEIJ in water dialogue participation. Further, this paper articulates a conceptual framework for understanding and addressing DEIJ failures in water dialogues. A concept of "just water dialogues" emerged that integrates insights from the literature reviewed with notions of environmental justice to help with identifying and resolving "water dialogue justice" (i.e., DEIJ failures). Review results suggest that DEIJ in water resources dialogues depends on the distribution of knowledge resources, and on broader issues that include cultural, political, and other often ignored contextual factors. Importantly, addressing DEIJ problems through the creation and maintenance of just water dialogues requires tackling power imbalances, enhancing individual and organizational capacity, and building bridges through effective engagement of diverse voices, especially those of underrepresented groups. Strategies that have demonstrated effectiveness in other contexts are highlighted, and future research needed to improve practices to enhance DEIJ in water dialogues is outlined.

Keywords: *water dialogues, discourse, diversity, equity, inclusion, environmental justice, engagement, water dialogue justice*

Effective governance and management are important for the long-term sustainability of water resources. Water resources governance is defined as the framework of water use laws, regulations, and customs, as well as the processes of engaging the public sector, the private sector, and civil society. It can include coordinating actions and decision-making between and among various jurisdictional levels and actors. On the other hand, water resources management consists of the actions to implement laws, policies, and regulations (Megdal et al. 2015; Petersen-Perlman et al. 2018).

Across the globe, one problem faced by the water sector is the lack of diversity, equity, inclusion, and justice (DEIJ) in water governance

Research Implications

- The literature survey supports the conclusion that this is the first paper that focuses on DEIJ in water dialogues.
- Examination of DEIJ in the context of water dialogues illustrates how inequities stem from unequal distribution of access to, benefits of, and protection from the harms associated with knowledge produced and transferred in water dialogues; exclusion from or inadequate participation in dialogue decision-making; and a failure to recognize the cultural identity and unique knowledge of underrepresented groups.
- Concepts from the literatures of discourse, diversity, social learning, and environmental justice provide a foundation for understanding the factors that influence equity in water dialogues.
- Just Water Dialogues, the conceptual framework that emerged from this review, applies a pluralistic approach and posits that failures along five interrelated dimensions of water dialogues can converge to undermine DEIJ, thereby affecting individuals, groups, and organizations engaged in, or potentially benefiting from, these dialogues.
- Findings from this study and recommendations for addressing DEIJ failures through effective engagement practice can be directly applied by individuals, groups, communities, and organizations engaged in water dialogues.

and management (World Bank 2019). Four characteristics of water provide insight into why DEIJ matters in water governance and management. These characteristics are: 1) water is essential for all life, 2) water goods and services provide multiple benefits to human well-being, 3) distribution of water resources is temporally and spatially uneven, and 4) power asymmetries affecting water governance result from this uneven resource distribution (Neal et al. 2014). These water attributes have resulted in the identification of various water governance and management issues that can emerge when DEIJ factors are considered or overlooked. If DEIJ is valued by society, then it is essential to pursue it in all aspects of water governance and management.

In the United States (U.S.), the lack of DEIJ in water governance and management has been identified as a serious problem that affects the validity of decisions (Wutich et al. 2013). Diversity in the water resources field remains low, despite recent efforts to attract new talent and expand dialogues. While population demographics in the U.S. have been trending toward greater diversity, these trends are not reflected in most water institutions, decision-making processes, or dialogues. Older males dominate water occupations more than in the general workforce. The median age of U.S. workers was 42 years in 2018, while the median age for water treatment operators was 46 years. In the same year, only 15% of water-related jobs were held by women, compared to an average of 47% of women in the national workforce (Kane and Tomer 2018). Additionally, women have lower recruitment rates in water occupations, have shorter average work tenure, and exit at higher rates than men (World Bank 2019). Racial minorities make up a lower share of the water sector workforce than the national labor force. Together, African American and Asian workers comprised 11.5% of water jobs compared to 18% of the national workforce (Kane and Tomer 2018). Similar patterns are seen within professional water associations and water education (King et al. 2018; Karsten 2019; Ali et al. 2021).

Although there is a growing consensus that the lack of diversity in geosciences presents an inequity requiring action (King et al. 2018), decades of research, policies, and projects have shown that diversifying water resource disciplines remains a challenge (Layne 2004; Neal et al. 2014; Zwarteveen and Boelens 2014; Kane and Tomer 2018; Hegde 2020). Previous DEIJ research has focused mainly on disparities in water resources access, impact of water hazards, and workforce composition (VanDerslice 2011; Balazs and Ray 2014; Liang 2016; Schaider et al. 2019; Statman-Weil et al. 2020). However, review of the literature did not yield any studies that examined DEIJ in relation to the dialogues associated with water resources, its governance, or its management, despite the significant role of water dialogues. Because water resources governance, institutions, processes, and practices at all scales involve dialogue, it is important to understand and explicitly account for DEIJ in water dialogues.

There are several barriers to increasing diversity and inclusion, but there are many benefits to overcoming them. In water resources, change has been slow (Hegde 2020) given that challenges may arise from feedback loops between low group diversity and exclusivity. When recruiting new members, groups exhibit a bias toward the familiar, which works against diversity (Razack et al. 2015). If a group's turnover rate is low, increasing diversity can take a long time, even in the absence of any bias (O'Brien et al. 2015). The relatively high percentages of males to females in water occupations persist (Kane and Tomer 2018; Hegde 2020), despite well-established evidence that gender diversity in the workplace can lead to positive outcomes (Hernandez et al. 2017). This is especially so where these outcomes are dependent on a variety of ideas and perspectives, such as information processing in teams (Chambers et al. 2017). Women have different types of knowledge, perceptions, experiences, and perspectives to apply to analyzing problems and tailoring solutions that may enrich water governance and management (World Bank 2019). This suggests there are benefits to increasing diversity and inclusion to enhance governance decision-making and outcomes.

Population growth and redistribution pressures, such as an aging workforce and increasing percentages of non-white demographic groups, are reshaping resource governance institutions in the U.S. These pressures, along with new technologies and methodologies, are driving changes; organizations are becoming more customer-focused (World Bank 2019). Considerations of DEIJ are important in times of change, especially when proposed changes to resource allocations, institutional rules, or physical systems will have societal impacts (Neal et al. 2014; Erkmen et al. 2021). As water governance personnel respond to pressures for change at all spatial scales, they will need to devise strategies, adaptations, and actions to address the varied requirements of an increasingly diverse population. So, understanding issues of DEIJ in the water sector takes on greater urgency.

Dialogues are present in institutions and processes including resource allocation; supply and infrastructure management; knowledge production and sharing; and individual, group, and organizational capacity development (King et al. 2018; Mercer-Mapstone et al. 2019; Erkmen et al. 2021; Lutz-Ley et al. 2021). The creation of inclusive dialogues within water organizations helps them embrace and effectively manage change, including changes prompted by diversifying participation (Razack et al. 2015; Day and Beard 2019). Creating and sustaining inclusive dialogues require an understanding of the differences among stakeholders, the system experiencing change, surrounding communities, and the organization's capacity to act. Dialogues also require an understanding of the interactions within specific contexts among different participants; their personal and organizational attributes, characteristics, and values; and how these attributes may hinder or support effective diversity actions.

There is no commonly accepted concept of what is meant by DEIJ in the literature. Inconsistent definition of key terms such as equity have emerged in policy documents, resulting in varying findings relating to DEIJ practices and initiatives (Tamtik and Guenter 2019). Many disciplines have unclear contextual variables and theoretical foundations in approaches to DEIJ. The water resources field is no exception. Even with the increasing prominence of discourse using political, technical, or economic rhetoric in relation to DEIJ, definition remains imprecise in the water resources governance literature. Research is needed to conceptualize DEIJ in water dialogues, develop methodologies to explore its properties, and devise theoretical approaches to explain its effects and impacts in and on organizations. Such actions can improve understanding, prediction, and management of DEIJ within dialogues in organizations or professional groups.

This paper draws from a broad DEIJ and social science literature to propose a conceptual foundation for understanding DEIJ in water resources dialogues. The paper provides a review of the literature applicable to defining and characterizing DEIJ and identifying best practices to address DEIJ issues in water resources, with the specific aim of diversifying water dialogues. Three questions are addressed: 1) How is DEIJ defined, conceptualized, and measured in water dialogues?, 2) How does a lack of DEIJ in water dialogues affect water-related outcomes and actors?, and 3) What approaches can be used to increase DEIJ in water dialogues, especially with respect to underrepresented groups? Theories and conclusions from social science research, particularly from the fields of discourse, diversity, social learning, and environmental justice (EJ), were analyzed and synthesized to articulate a conceptual framework for understanding and addressing DEIJ issues in water dialogues. The proposed conceptual framework integrates the analytical results and indicates pathways toward expanding DEIJ in dialogues relating to water governance and management.

Methods

Study Design

We performed a systematic review of literature relating to water resources, dialogues, and DEIJ. Figure 1 shows a flow chart of the study design. The varied nature of DEIJ in water resources necessitated an approach that considered the perspectives of multiple disciplines, theories, and information sources or types. Because most of the research reported in the literature is qualitative, the study's narrative synthesis is qualitative.

Search Strategy

Figure 2 shows the search strategy and data analysis methods applied in review and synthesis of the literature. Systematic searches were conducted of peer-reviewed publications in the Scopus and Google Scholar databases. Search terms relating to definitions, theories, characteristics, measurement, and engagement strategies within three categories, dialogues, water resources, and DEIJ, were incorporated into the search queries. The searches returned 263 papers and 84 were

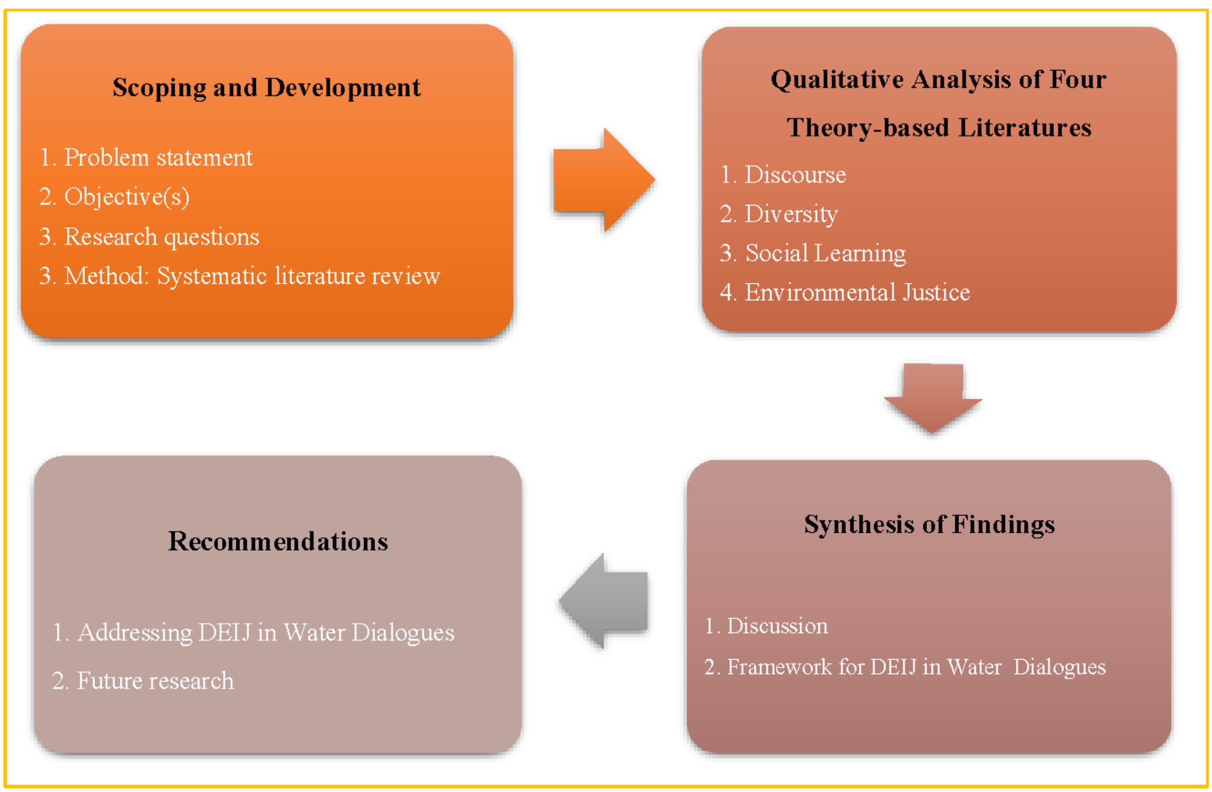


Figure 1. Flow chart of study methods and outputs.

selected for in-depth review based on defined exclusion criteria. An additional 23 citations from selected papers were pursued and reviewed. Additionally, grey literature, including reports and documents from conferences, workshops, and institutional websites, was scanned to identify current definitions, strategies, and practices that may not be captured in the published literature.

The study described herein employs an approach defined as theoretical pluralism: drawing upon multiple theoretical lenses to inform practice (Midgley 2011). The bulk of the studies identified for review came from four literatures: discourse, diversity, social learning, and EJ. Theoretical pluralism employs a systemic approach that requires examination of what each contributing

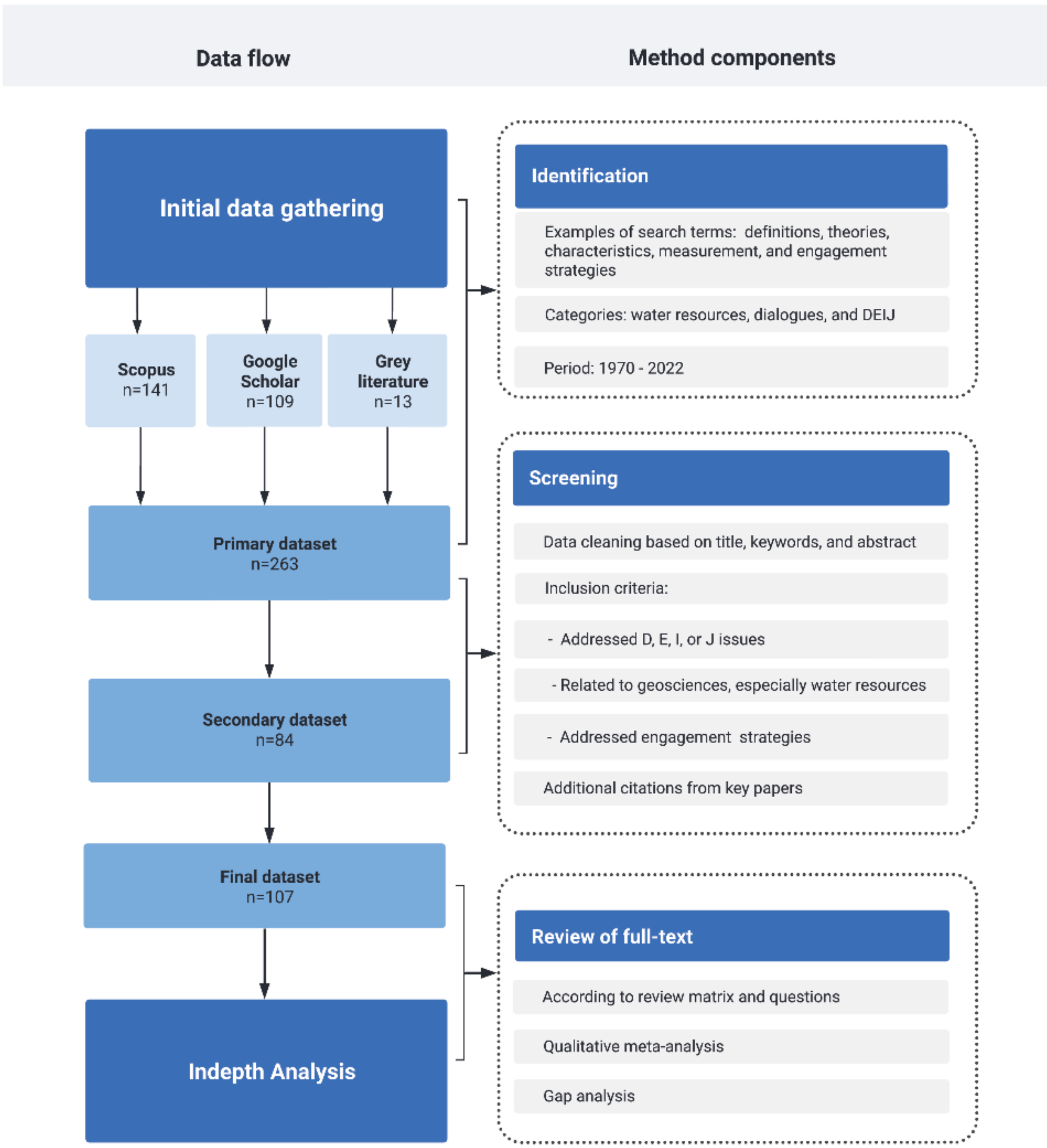


Figure 2. Data search and synthesis strategies.

perspective provides, how to decide among them, and how to reconcile conflicting perspectives into a coherent conceptual framework. This paper draws on predominant notions of dialogue to ground a single conceptual framework suggested by the literature of the four theoretical study areas listed above.

Definitions

Dialogue

Dialogue is a concept that has been characterized in many ways. It is the process of communicating, the forum of communication, and the output of a particular communication process. A dialogue can be open-ended learning, or a strategic undertaking aimed at achieving desired outcomes. It can be unstructured or designed with purpose and structure (Westoby and Dowling 2013; Mercer-Mapstone et al. 2017). Unlike ordinary discourse, dialogue is built on shared respect and the validity of all perspectives.

As the literature shows, dialogues are multi-dimensional spaces in which the characteristics of participants and their context interact. Bohm (1996) refers to "collective participation" to name an open-ended dialogue that convenes with no agenda, direction, topic, or theme, and that promotes fluid conversation while attempting to suspend personal opinions, beliefs, impulses, and judgments. Such dialogues focus on allowing all interests to voice concerns and individuals gain insight that is not achieved independent of the dialogue (Welp et al. 2006). The views of individual citizens are presented and defended, but participants do not negotiate positions, try to gain consensus, or win (Bohm 2004). On the other hand, Mercer-Mapstone et al. (2017) referred to dialogues in which a specific outcome is targeted, and dialogue processes are structured and consciously used to communicate and connect with the discourse of a certain field, discipline, agenda, or scenario. Water dialogues are structured communicative processes linking selected societal actors who are relevant for developing water governance and management, and professional and individual capacities. Relevant actors possess specialized knowledge, disparate life experiences, and insights that can vary from records on historical water allocation and management expertise. Typical settings for water dialogues include meetings, conferences, workshops, universities, and professional associations.

Diversity and Inclusion

Diversity refers to any dimension of differentiation and reflects unique experiences within social, historical, political, and other contextual settings (Roberson 2019). Diversity considers differences between individuals on any attribute that may lead to the perception that another person is different from self (Williams and O'Reilly 1998). In academic and organizational practice, the study of diversity is heavily dominated by a limited set of dimensions: age, race, color, ethnicity, gender, tenure, and functional background. However, in principle, diversity entails an almost limitless number of attributes, which may include nationality, religion, training or education, and skill set, as well as political opinions, general attitudes, and values.

Diversity attributes can be placed into three categories. Some attributes are categorized as demographic diversity (e.g., age, ethnicity, gender, religion, sexual orientation, nationality, and family structure), based on the assumption that shared characteristics may have similar effects on individual group members. Similarly, functional diversity refers to a group of attributes based on job-related requirements (e.g., educational background and veteran status). Some attributes are categorized based on deep level diversity, which relates to psychological variables (e.g., personality, attitudes, and values) that are not easily discernible (Van Knippenberg and Van Ginkel 2010). Additionally, the concept of diversity incorporates differences stemming from where people have lived, their thoughts, and life experiences.

Inclusion extends diversity a step further to incorporate a call to action. The concept of inclusion refers to the extent to which individuals feel valued for their unique attributes and have a sense of belonging as an important member of the group (Brimhall and Saastamoinen 2020). Inclusiveness means recognizing individual talents and encouraging the full participation and contribution of each person in both formal and informal group activities. As illustrated in Table 1, diversity and inclusion ask different questions and focus on different issues than efforts concerned with equity and justice (Stewart 2017). While an inclusive group is necessarily diverse, a diverse group is not always inclusive.

Equity, Justice, and Environmental Justice

Legal constructions of justice assert a uniform, formal framework for processes and outcomes based on the equality of all individuals before the law. In practice, however, frameworks that assert equality frequently ignore existing social differences, hierarchies, and implicit definitions of equality based on the characteristics, norms, standards, and interests of powerful groups (Boelens 2009). In contrast, equity indicates the consistent, systematic, fair, just, and impartial treatment of all persons, including those who belong to underserved communities that have been denied such treatment (US OPM 2021). Equity is defined by location, time, and group-based concepts of fairness. The ways society is ordered

Table 1. Comparison of Diversity, Equity, Inclusion, and Justice. Based on text from Stewart (2017), pg. 4, with quoted questions, copyright 2017 Inside Higher Ed.

Component	Focus	Types of Questions Asked	Measure of Success
Diversity	Valuing differences and increasing the numbers of underrepresented group members or perspectives.	1. "Who is in the room?" 2. "How many more of an underrepresented group do we have this year than last?"	▪ Representation from minoritized groups. ▪ Increases in numbers of minorities in group, forum, and institution. ▪ Incremental growth rates.
Equity	Reduction in harm via providing equal access based on need.	1. "Who is trying to get into the room but can't?" 2. "Whose presence in the room is under constant threat of erasure?" 3. "What conditions have we created that maintain certain groups as the perpetual majority here?"	▪ Increases in support for people's effective participation as reported by those who have been disadvantaged and targeted for inclusion. ▪ Types and degree of support provided relative to needs of minorities in the group.
Inclusion	Having a diverse candidate pool by fostering a sense of belonging, respect, and support.	1. "Has everyone's ideas been heard?" 2. "Is this environment safe for everyone to feel like they belong?"	▪ Records of balanced participation from all group members. ▪ Sources of all ideas considered show balanced impacts. ▪ Recognition for initiatives and credits for having a diverse candidate/membership pool.
Justice	Ensuring fair treatment, equitable access, effective practices, and accountability.	1. "Whose ideas won't be taken as seriously because they aren't in the majority?" 2. "Whose safety is being sacrificed and minimized to allow others to be comfortable maintaining dehumanizing views?"	▪ Getting rid of practices and policies that have disparate impacts on dominant versus underrepresented groups. ▪ Underrepresented group members' perception of fairness in participation.

are rooted in these specific contexts, which affect the distribution of resources, property, wealth, and authority (Zwarteveen 2006). This definition of equity, emphasizing its historical and place-based specificity, sets up tensions between different concepts of fairness. For instance, in educational science classes, efforts are growing to enhance representativeness to better reflect societal diversity (Layne 2004; Smith et al. 2009; Carr et al. 2015; Helitzer et al. 2016; Hoffman and Mitchell 2016; Irby-Butler 2017; West et al. 2018; Clark 2019; Tiwari et al. 2019). However, there is an inherent tension between these calls for representativeness in science classes and competitive student selection processes based on academic achievement. Political pushback from within academic excellence discourses has consistently prevailed over calls for greater demographic representativeness. Nonetheless, regardless of internal inconsistencies, examination of both formal justice founded on the principles of equality and socially perceived justice based on concepts of equity are necessary to gain a full understanding of EJ and justice in water matters (Boelens 2009).

Environmental justice is concerned less with equality and more with equity. It provides a lens through which equity and justice issues relating to water resources can be understood. The United States Environmental Protection Agency (US EPA 2020) defines EJ as the "fair treatment and meaningful involvement of all people regardless of race, color, national origin, or income with respect to the development, implementation, and enforcement of environmental laws, regulations, and policies." Likewise, Brulle and Pellow (2006) define EJ as "the principle that all people and communities are entitled to equal protection of environmental and public health laws and regulations." These definitions specify that there should be unbiased representation of all groups, classes, and races that may be impacted by specific risks (Nelson and Grubesic 2018).

Stakeholder Engagement

Stakeholder engagement is a method for involving in the deliberation process those who affect and are affected by a plan, policy, law, or other decision. Dialogue is a form of stakeholder engagement. The literature identifies four distinct stages of engagement: 1) problem framing and stakeholder identification, 2) dialogue forum preparation, 3) dialogue facilitation, and 4) participant capacity development (Day and Beard 2019). Addressing complex water problems that demand ongoing, inclusive, and adaptive problem-solving requires participation from multiple stakeholders, often with conflicting visions, and heightens the need for integrative and effective DEIJ engagement strategies. Engagement strategy is defined as the actions adopted to achieve the basic long-term goals and objectives of an entity and the allocation of resources necessary for conducting these actions (Guillaume et al. 2017). The strategies employed often determine the impact or outcome of engagement efforts.

Sustained multi-stakeholder dialogues can promote just outcomes from adaptive resource governance (Zwarteveen and Boelens 2014; Lutz-Ley et al. 2021). The roles of stakeholders in water dialogues vary as the purposes of specific dialogues change. Participants may limit their contributions to witnessing the governance process and commenting on policy outcomes, or they may have significant involvement in the generation of new solutions, knowledge, and meaning. The process provides the various stakeholders involved with an opportunity to examine assumptions, revise perspectives, and learn as individuals and as groups. Within dialogues, debate and other interactions may build consensus on empirical and value disputes, or at least identify areas of prevailing disagreement (Welp et al. 2006). This provides opportunities to adapt water governance and management toward just outcomes.

Theoretical Frames

Multiple theories inform understanding of DEIJ in Dialogues. The literature of four established theoretical study areas relevant to DEIJ were reviewed for explanations of what happens in dialogues. Figure 3 shows these four theoretical study areas: discourse, diversity, social learning, and EJ. These are discussed in this section in relation to water dialogues and provide insight on how DEIJ may affect water-related outcomes and actors.

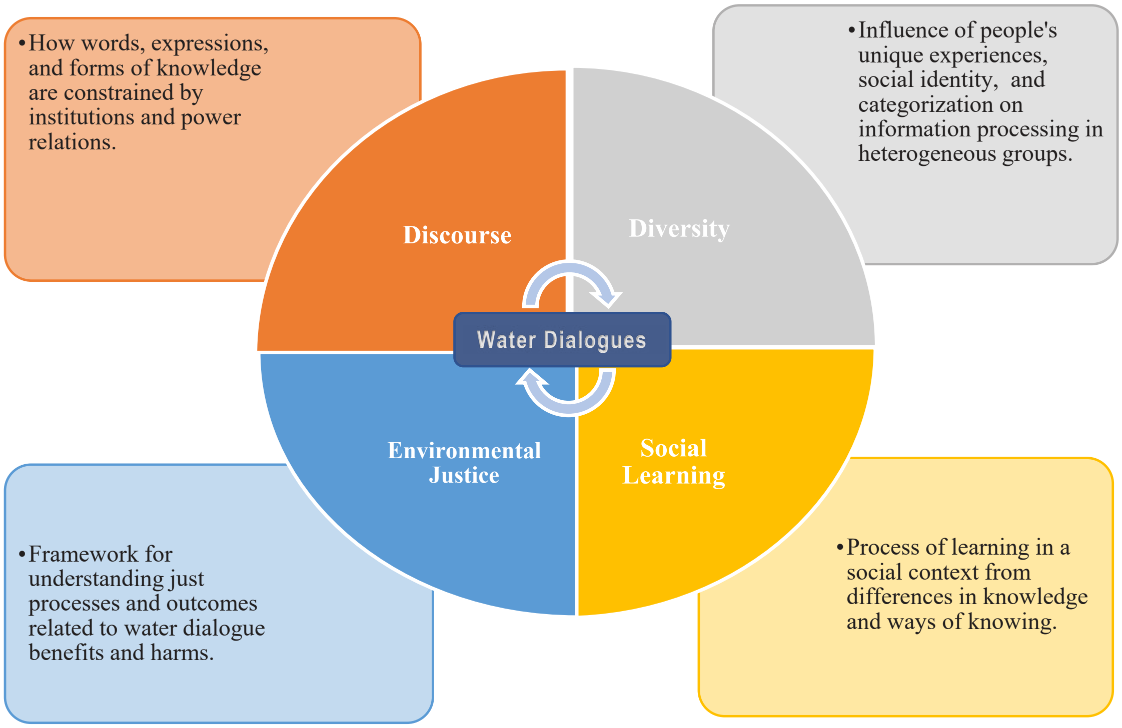


Figure 3. The four theoretical study areas contributing to a conceptual framework of DEIJ in water dialogues.

Discourse

Dialogues are the primary form of discourse. A discourse consists of statements, including those relating to truth, reality, morality, and behavior, which regulate how to talk about an issue. Within a discourse, it is difficult to think or act outside the boundaries imposed by these statements (Foucault 1975). This is because discursive practices are characterized by "delimitation of a field of objects, the definition of a legitimate perspective for the agent of knowledge, and the fixing of norms for the elaboration of concepts and theories" (Foucault 1977). Therefore, dialogues are theoretically grounded in the idea that discourse regulates social practices and the organization of social institutions (Razack et al. 2015).

Any institutional practice or technique in and through which social production of meaning takes place may be considered part of the discourse (Macdonell 1986). Meaning is expressed not only in speech and writing, but also in the consequence, order, and interchange of related verbal and non-verbal signs. Meaning is "embodied in technical processes, in institutions, in patterns for general behavior, in forms for transmission and diffusion and in pedagogical forms" (Foucault 1971; 1977). Discourse analysis requires examination of the forces that shape thinking and understanding at the individual, organizational, and other scalar levels. Key issues in discourse analysis are accounting for the positions from which people speak, and the institutions that store and distribute the views that are expressed (Foucault 1977). Macdowell (1986) illustrates how statements made, and the meanings of words used, depend on characteristics of the speaker and the context in which the statements are made (e.g., different social classes may use and interpret the same words differently).

Discourses reinforce power relations, which tend to advance certain interests and groups over others (Macdonell 1986). Power relations are created and maintained by influencing definitions of acceptable ways of believing, thinking, and acting, or delineations of social boundaries that define the voices and interests that are considered in any given context (Razack et al. 2015). Statements of "truth" can serve to generate practices or processes that organize, classify, and divide individuals. Such political positioning creates an obstacle to effective dialogue and positive change (Eden

2011), especially when norms and rules serve to rationalize and depoliticize dialogues. One popular strategy used to depoliticize water problems is to place contentious questions outside of the domain of public debate; for example, by defining a question as being uniquely scientific. However, showing deference to science by defining questions only in scientific terms may reproduce historical patterns of exclusion and undermine the purposes of the dialogue.

Diversity

Researchers have utilized various theoretical perspectives to understand diversity and its effects on organizations and actors. The study of diversity's influence on varied processes and outcomes has primarily been conducted within business organizations and has produced unique insights into people's professional diversity-related experiences (Kane and Tomer 2018; World Bank 2019). There has been a significant evolution in the understanding of the meaning, operation, and effects of diversity in organizations.

Scholarship on diversity has been historically grounded in social psychological theories of intergroup relations. Such theories articulate the formation and functionality of social stereotypes wherein differences are viewed as social distinctions that impede intergroup relations. Some researchers cite two main differences underlying diversity: differences in readily detectable attributes such as sex, age, and ethnicity (social category diversity); and differences in less visible underlying attributes that are more job-related, such as functional and educational background (informational/functional diversity) (Van Knippenberg et al. 2004). Regardless of categorical labels, diversity research and practice have focused on the impact of diversity on group process, performance, and diversity management. Two theoretical perspectives have dominated diversity research: the social categorization perspective and the informational resources perspective (Williams and O'Reilly 1998; Van Knippenberg and Van Ginkel 2010).

Three theories that frame the social categorization perspective studies include social identity theory, self-categorization theory, and similarity-attraction paradigm. Social identity theory proposes that people's definitions of self are shaped by their group memberships, so they are motivated to enhance their self-concept by seeking a positively valued distinctiveness from those groups (Tajfel 1978). Self-categorization theory posits that people tend to categorize themselves and others based on the social environments in which they are located. As social categories become salient, people tend to view themselves more as representatives of social categories than as unique individuals. Such differentiation manifests as biases favoring members of ingroups over those they view as belonging to other social categories (Hogg and Turner 1987; Hogg and Terry 2000). The similarity-attraction paradigm (Byrne 1971) expands theories of social identity or categorization. It hypothesizes that people are attracted to those they perceive as similar and are inclined to seek interactions with similar persons. This is based on salient factors such as demographic characteristics and expressions of (or assumptions about) values and attitudes. In self-defined meritocracies, privileged individuals seeking admission can use explicit strategies to "fit in" and demonstrate their recognition of established power dynamics (Razack et al. 2015) - a strategy not available to most individuals from disadvantaged groups. Similarity-attraction is likely to produce distinctions between in-groups and out-groups and shapes social interactions between groups (Roberson 2019).

Within the informational resource perspective on diversity, the value-in-diversity hypothesis (Cox and Stacy 1991) points to evidence that categorical dissimilarity creates variances in skills, knowledge, and experiences in groups. It assumes that heterogeneous groups have access to larger and more varied informational resources and therefore are more likely to generate better quality solutions to problems. Empirical research provides evidence of this performance advantage (Cox and Stacy 1991; Roberson 2019).

Some scholars have tried to integrate and reconcile theories of the influences of social identity and categorization with those of information processing in heterogeneous groups. In particular, the categorization-elaboration model (Van Knippenberg et al. 2004) posits that intergroup biases emanating from social categorization processes may interrupt information exchange crucial to realizing the value in diversity. Guillaume et al. (2017) identify various contingency factors that determine the degree to which diversity leads to positive or negative outcomes. Facilitation may be needed to prevent intergroup biases from blocking the performative benefits of diversity and to foster the exchange of knowledge and perspectives derived from diversity. Individual and group information processing, information expansion, and the exchange and integration of knowledge-based resources within the group may also require facilitation (Roberson 2019).

Social Learning

Social learning, as the process and outcome of working together on a shared problem or question, bears directly on water dialogues. In dialogues, social learning provides a mechanism to connect diverse ways of knowing, producing, and sharing knowledge (Owen et al. 2019). Social learning theory suggests that differences drive learning in social contexts. Social learning theory emphasizes processes of observing, modeling, and emulating the behaviors, attitudes, and emotional reactions of others. Both environmental and cognitive factors interact to influence human learning and behavior (Balazs and Ray 2014). New knowledge emerges from working together in the social learning context and interactions change the understanding and beliefs of participants relative to the problem (Faysse et al. 2014; Akpo et al. 2015). The value of social learning as a process for fostering dialogue and as a product of dialogue has been demonstrated in the literature. Learning occurs when dialogues incorporate multiple viewpoints and create space for individual and organizational transformation (Owen et al. 2019).

Dialogue, as a group communication and interaction process, plays a key role in social learning. Welp et al. (2006) described three primary outcomes of water dialogues: production of new knowledge, increased odds of this knowledge being used in governance and decision-making, and improved capacity to develop and utilize water knowledge. The specific knowledge held by different actors can vary from scientific or technical expertise, through management or administrative experience, to the observations of community members and citizens. In contrast to discourse, social learning dialogues accept different kinds of knowledge on an equal footing. Meaning flows freely between participants, and individuals gain insight that is not achieved independent of the dialogue (Bohm 2004). Scientists need access to the knowledge of stakeholders to better understand, represent, and analyze water problems, define models, and identify solutions (Welp et al. 2006). As a social learning process, water dialogues help to build an expert belief system through communication and interaction with stakeholders that provides a more realistic and complete picture of water issues.

Environmental Justice

As described above, multiple notions of what constitutes justice exist simultaneously in EJ. Contributions have come from various disciplines; each adds valuable insight by applying different perspectives and approaches to EJ research (Nelson and Grubesic 2018). EJ theories have expanded significantly in several ways since their inception in the 1970s. Early EJ theories focused primarily on distributive equity; profoundly uneven social and geographical access to environmental amenities and exposure to environmental harms were viewed as demonstrating injustice (Wutich et al. 2013). Initial discussions and actions focused on prevention or mitigation of pollution and the allocation of pollution impacts and costs. Later, EJ examined demands for a focus on environmental outcomes (Zeitoun et al. 2014) and restorative actions based on historical responsibility. The per capita equity theories of Jamieson (2001) and Singer (2004) applied existing notions of distributive justice to the climate debate, while Caney (2006) took a rights-based approach to climate justice. In addition, the scope of EJ discourse and research has expanded to include a broader range of topics, geographic areas, new methods (such as spatial analysis) (Sze and London 2008), and demographic categories (e.g., ethnic groups, women, and youth), rather than only place-specific communities/individuals. The use of the term has diffused vertically to issues such as food security or Indigenous rights, and horizontally to alternative ideas, meanings, and framings from outside the U.S. (Walker 2009a; 2009b).

Recently the EJ discourse has moved toward a framework wherein both the natural and non-human environment interact to create the conditions for justice (Schlosberg 2013). Early notions of environment as wilderness were combined with a broad recognition of environment as including places where humans live, work, and play (Novotny 2000; Agyeman 2005), thus acknowledging the value of natural systems to both human and non-human well-being. EJ moved beyond description and documentation of inequity to the cultural and institutional structures that contribute to it. Nelson and Grubesic (2018) showed that EJ research came to focus on the distribution of environmental amenities. This conceptual shift considers that a working environment is required for justice and involves creating material flows and human practices that do not weaken environmental processes and systems. Drawing from EJ work that examines the reallocation of incomes, resources, and power because of changes to the environment, Schlosberg (2004) conceptualized EJ as a trivalent construct that includes dimensions of resource distribution, cultural recognition, and participation in decision-making. In this construct, justice requires not only an appreciation of unjust distribution of environmental benefits or harm and lack of recognition of the cultural identities of marginalized groups by dominant institutions, but also the interaction between the two in political and social processes and decisions that affect their environment (Zwarteveen and Boelens 2014). Schlosberg (2007) expanded the trivalent construct to include a capabilities dimension, which entails the rights of individuals to the things that allow or assist us to translate basic goods and services into conditions necessary to live a good life. In this case, capabilities move beyond only being concerned with the amount of goods an individual gets, to consider what those goods do for the individual's well-being.

Fricker (2007) contributed the concept of epistemic justice, which is people's right to be respected in their capacities and identities as knowers. This demands that the experiential and observational knowledge of local environmental conditions be given the same weight in decision-making as the knowledge of credentialed experts (Ottinger et al. 2017). Zwarteveen and Boelens (2014) articulated the concept of water justice by adding a socio-ecological integrity component that considers the relational coexistence of human and non-human ecologies as a matter of justice. Injustice issues may arise due to the interplay of power and politics with natural resources allocation and ways of thinking and talking about resources via complex, contested processes. The evolution of EJ conceptual models suggests the convergence of theoretical approaches on the value of DEIJ and, by extension, the importance of diversifying water dialogues. The paucity of studies applying EJ theories to water dialogues, however, leaves unillumined important factors, including how relevant information and knowledge is shared, who participates, and how dialogue processes may affect the creation and perpetuation of injustices in the water sector (Tamtik and Guenter 2019).

Discussion

Theoretical constructs contribute to a framework for understanding DEIJ in water resource dialogues. In some ways, the water discourse landscape may seem far removed from the urgency of local struggles over water access experienced by underserved people and communities. Some dialogues reveal issues of EJ due to low DEI that never generate disputes but manifest instead as silent hardships. Water dialogues frequently relate to active conflicts over whose interests will be prioritized in allocating and regulating water use. The initiation of dialogue on water reallocations or other forms of change, such as constructing dams that displace communities, can ignite conflict (Vos et al. 2006; Ahlers 2010; Zwarteveen and Boelens 2014). However, some actions trigger exclusion from access to and benefits of dialogue knowledge resources, especially when change advocates challenge the culture of existing dialogues and forms of knowledge. Although some of these situations attract significant attention, many involve subtle and extended struggles by underrepresented groups.

Insights from the four literatures reviewed herein (Figure 2) can inform understanding of DEIJ issues present in water dialogues. These four theoretic literatures contain several concepts that are important to identifying, understanding, analyzing, and addressing the lack of DEI in water dialogues, which may lead to justice issues. The concepts open opportunities to deepen understanding of specific and interconnected political, socioeconomic, technical, biophysical, and cultural drivers that promote or inhibit DEIJ in water dialogues.

Just Water Dialogues: A Conceptual Framework of DEIJ in Water Dialogues

In this section, a conceptual framework is provided to show how the lack of, or low levels of, DEI in water dialogues can lead to injustices, especially for persons in underrepresented groups. We term these DEIJ failures in water dialogues as "water dialogue justice." Building on the exposition of the four components of justice by Schlosberg (2013), Zwarteveen and Boelens (2014), and others (i.e., distribution, recognition, capabilities, and participation), it is posited that the domain of DEIJ in water dialogues contains five interrelated dimensions. These interrelated dimensions: 1) knowledge distribution, 2) participation, 3) social boundaries, 4) capabilities, and 5) scale and measurement, can converge to create instances of water dialogue justice and affect individuals, groups, organizations, and networks engaged in water dialogues. Figure 4 illustrates the proposed conceptual framework for just water dialogues and the interactions among and between the five dimensions.

Knowledge Distribution. The knowledge distributional dimension of water dialogues relates to questions of: Who has access to water dialogues?, How is information produced in these dialogues?, and How is access to information allocated? The ultimate distribution of benefits and harms depends on access to information. A lack of equitable access to information provided via dialogues can create injustices. Kibler et al. (2014) show how uneven access to hydrometeorological data and information in a river basin differentially affected the capacity

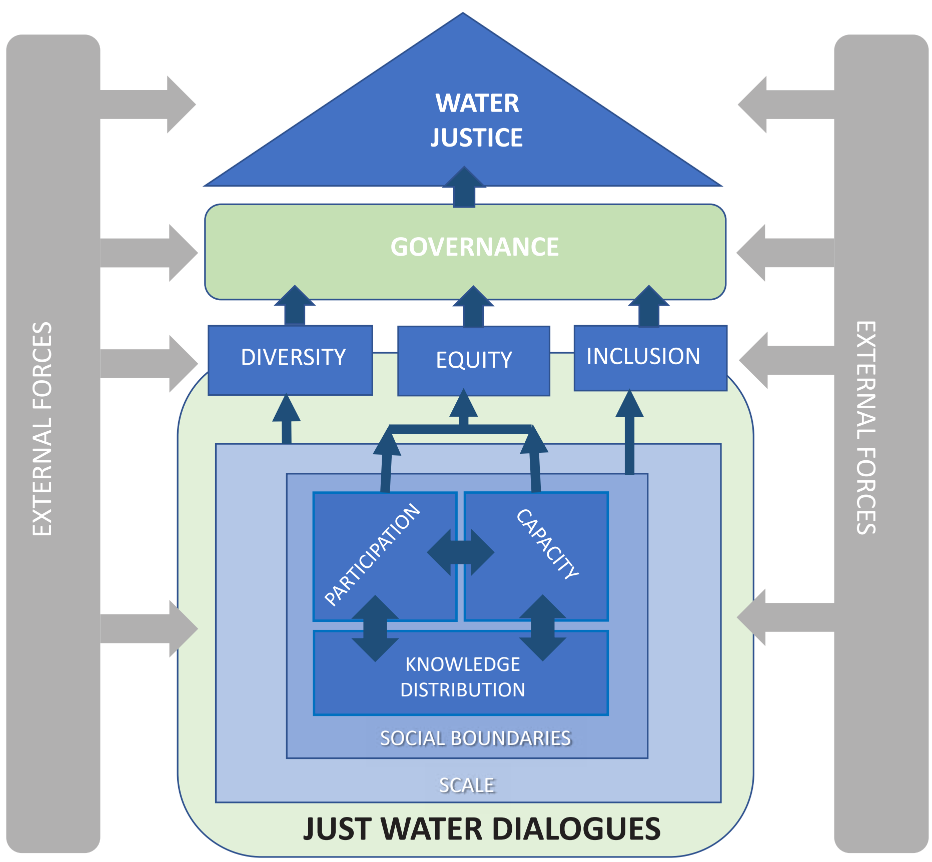


Figure 4. Conceptual framework of Just Water Dialogues.

of groups or individuals to increase their resilience to extreme hydrometeorological events. Despite codifying principles of equal access to data in formal agreements on basin-wide water allocation, at sub-basin levels, hydrometeorological data access principles were often ignored, and data were seldom shared across organizations operating in different sub-basin or geopolitical units. In the most downstream nation in the basin, precipitation and discharge stations fell significantly below minimum recommended densities, resulting in vastly ungauged areas and extremely limited data. The disparity in access to, and distribution of data and information impeded the application of basin-wide, data-driven, empirical models to forecast extreme events. Additionally, it may lead to injustice in terms of knowledge and capacity in some communities for increasing resilience to extreme events.

Science and technology can aid or inhibit knowledge production and distribution in ways that may lead to unjust outcomes. Two aspects of science and technology have important implications for knowledge-generating practices in water dialogues and decision-making. First, choice of technology can have unequal effects, benefiting some people to the exclusion of others (Ottinger et al. 2017). It can also advantage some values or knowledge systems. For example, dialogues that take place virtually, requiring broadband access, or at spatially concentrated, in-person meetings, have historically favored densely populated, urban communities over sparse, rural communities. Similarly, the use of scientific and technical language, and the communication method may affect participation by some underrepresented groups in the production and distribution of knowledge via water dialogues. Second, the professional practices, identities, and ethical codes of scientists can shape knowledge resource availability in marginalized communities (Ottinger et al. 2017). Despite the evident contribution of engineers toward DEIJ in industrial and development water projects, the enduring engineering culture that prioritizes technical over social aspects of solutions (Felt et al. 2016) tends to impede progress toward more equitable resource access outcomes and knowledge production. Some groups may deem other non-engineered solutions valuable. Therefore, such imbalance in access to and coproduction of water knowledge may lead to suboptimal outcomes for underrepresented groups, especially when the knowledge is used in water governance and management.

Participation. The participation dimension concerns not only who participates, but also whose participation, priorities, and interests are privileged in water dialogues. In water resource professional dialogues, Shames and Wise (2017) found tensions relating to gender and methodology, wherein scientific methods are privileged. Research shows the dominance of specific methods and contribution types in the dialogue at geoscience conferences, along with considerable gender differences in the methods used, intellectual contributions made, and practical justifications articulated by presenters (King et al. 2018). Often, such dominance discourages the participation of women and racial minorities in many governance public processes and dialogues. The result is that the voices of disadvantaged groups may be subordinated by more privileged participants due to contextual and procedural factors. These factors include the fact that men have historically dominated water resources and other geoscience fields, and continue to dominate subfields of certain methods; that men disproportionately enter and remain in certain subfields and the larger water workforce; and that men are more likely to enter graduate programs with more mathematical and science training. Also, women may seek female mentors (Kane and Tomer 2018; King et al. 2018), but with fewer female mentors available, women are less likely to advance to leadership. It is important to also recognize that when access costs are high, economic hardship makes equitable participation in water dialogues difficult for multiple reasons (Zwarteveen and Boelens 2014).

Scientific knowledge may be used to limit the ability of individuals and groups to participate effectively in the design of and discussion within water dialogue processes. Increasing DEIJ in water dialogues is hampered by the practice of seeking scientific solutions for contentious political questions, thus avoiding public debate. Evidence from the literature shows that even when the search for solutions remains in the public realm, expert knowledge is privileged over other ways of knowing in public processes and dialogues in water governance and management. Public comments on issues are also routinely reinterpreted and reframed using scientific constructs (Mercer-Mapstone et al. 2019), which discourages participation by individuals with non-scientific knowledge.

Ottinger et al. (2017) explain that when not framed as expert knowledge, individual, group, or community input is marginalized, misunderstood, or overlooked. However, local and experiential knowledge often serve as the basis for community-initiated investigations of water pollution and key water resource solutions. These authors show that knowledge generated through local ways of knowing often has driven action to address water pollution hazards, like the contamination of drinking water supplies that occurred in Flint, Michigan in 2014. Community groups may use different standards of proof, ask different questions, seek ways to sustain resource access and values, and seek to address threats to the resource, rather than only assess regulatory compliance (Ottinger and Cohen 2011; Ottingeret al. 2017). Although there is merit in non-scientific, local knowledge and perspectives, they may be excluded from water dialogues.

Social Boundaries. Shared norms, practices, resources, tools, routines, language, interests, and histories create boundaries within and around each water dialogue. These boundaries are negotiable and may be fluid (Wenger 2010a; 2010b), but they determine whose norms or rules are accommodated or determine the distribution of and access to knowledge produced in the dialogue. Learning occurs through social interactions within and across these boundaries, such as when people are challenged to recognize new points of view, new approaches, and new problems (Owen et al. 2019). Organizations like The University of Arizona Water Resources Research Center can function as bridging organizations that reach across boundaries to communicate, convene, consult, collaborate, and build capacity through water dialogues (Mott Lacroix and Megdal 2016; Mott Lacroix et al. 2016; Owen et al. 2019).

Boundaries may be used to distinguish in-groups from others, sometimes based on class, ethnicity, gender, or age. The norms shared in bounded groups may become entrenched and serve to rationalize failures in DEIJ (Zwarteveen and Boelens 2014). A boundary separates scientists and water resource professionals from other dialogue participants. Scientists and professionals integrate their cultural identities, interpretations, and experiences into their work, and these personal characteristics often shape dialogue norms and rules. The procedural and interpretive choices involved in research and related dialogues necessitate value judgments (Zwarteveen and Boelens 2014; Roberson 2019), such as which questions to study and methods to use. Scientists often make these judgments based on group norms without understanding how their values might differ from those of other groups or communities. People without scientific or professional credentials integrate their cultural identities, assessments of scientific claims, and local, vocational, and experiential knowledge into their understanding of water resources, and that may be quite different from that of scientists and professionals (Wynne 1996). The result of a deference to scientific or expert knowledge has been a longstanding marginalization of local knowledge and non-scientific ‘ways of knowing’ based on socially constructed boundaries.

The marginalization of local and non-scientific knowledge and underrepresented groups in water dialogues, due to socially constructed boundaries, is then justified. The low participation of racial minority group members is often deemed a lack of interest, or the natural result of few minority group members being in the field, rather than as a problem of social boundaries, power relations, or information distribution. Frequently, common information dissemination methods such as the internet, organizational websites, and email listservs, are used without examining the effect on participation DEIJ. Public dissemination channels are often used to distribute information to depoliticize participation (or lack of participation) in the dialogue, despite these channels being ineffective in reaching some marginalized groups (Zwarteveen and Boelens 2014). An example is that using only virtual distribution channels when isolated rural and/or poor communities have low internet connectivity limits their participation. Various theories suggest that any choice mediated by humans, influenced by their power relationships, and subjected to individual or group membership biases and norms, can construct participation scarcity beyond natural occurrence (Hogg and Terry 2000; Roberson 2019), and may drive injustice. The assumptions made distract attention from the choices made by in-groups, based on membership biases and norms that act as selective barriers, and can explain low participation rates by some groups. Additionally, exclusion of underrepresented groups may occur because conveners of dialogues do not know how to effectively engage them and, at the same time, marginalized groups do not have information about the opportunities to participate.

Capabilities. Within theoretic literatures, knowledge is a contestable, contingent set of socially produced claims that are intertwined with relations of power rather than existing separately from the political sphere (Fricker 2007). Water dialogues, meanings, and the production of facts are intrinsic to inequitable water policies. Concepts of truth and meaning emerge through social processes in which agreement, persuasion, belief, culture, and viewpoint play a role (Zwarteveen and Boelens 2014). The capacities of individuals and organizations to influence water governance and management are enhanced via three primary benefits that result from interaction in water dialogues: new practice-based knowledge, increased odds that this knowledge will be used in decision-making, and enhanced capacity to develop and utilize practical water knowledge (Welp et al. 2006). Pierre Bourdieu's theory of capitals proposes the concept of capital as the resource from which capabilities are derived. Within water dialogues, several types of social, economic, cultural, and symbolic capital compete, interact, and mediate individual standing and opportunities to influence the process and outcomes (Razack et al. 2015). Individuals may occupy various positions of power depending on their specific capital endowments relative to others operating and competing within dialogue processes.

The relative power of individuals may determine "Whose truth prevails?" in dialogues. The knowledge that is formed through dialogues may reflect power relations in ways that are consequential for water dialogue DEIJ (Zwarteveen and Boelens 2014; Razack et al. 2015). People and organizations endowed with high economic and political capital hold advantaged positions in water dialogues. Money and status flow to actors, programs, and organizations exploring topics of concern to government and corporate interests at the expense of topics important to less powerful groups. Because resources that enable research and dialogue flow overwhelmingly to areas of interest of political and economic elites, underrepresented communities living with water hazards tend to face systematically incomplete or unrepresentative knowledge (Hess 2007; Frickel et al. 2010). Therefore, dialogues dominated by elites have the potential to aggravate DEIJ failures in water resources, especially when people rely on these dialogues to gain knowledge and develop skills to effectively participate in decision-making (Ottinger et al. 2017). This often translates to a capability effect for individuals and groups who have limited access to knowledge transferred through dialogues that is diverted to others with less need.

Advantaged institutions, organizations, and individuals also frequently control problem framing. Water resource problems tend to be complex and subject to both factual uncertainty and conflicts over values. They are difficult to frame in ways that produce consensus on acceptable solutions. Empirical research indicates that how an issue is framed strongly influences the answers people give to related questions (Wynne 2005). Dialogues can be framed using specific discursive strategies to legitimize organizational perspectives. Strategically framed corporate communication has been used to gain prominence and public support in a social media context. Providing evidence-based facts and external experts as reliable and neutral sources and echoing words and actions of supporters are strategies used for advancing the organization's perspective. Also, when challenged, organizations manage dialogue by delegitimizing arguments that run counter to their view (Ravazzani and Maier 2017).

Framing is central to water dialogues because it can enhance or depress DEIJ. In water dialogues, framing shapes meaning by stipulating what is included, excluded, emphasized, and contextualized (Ravazzani and Maier 2017). Thus, framing has real consequences for water governance and management outcomes. For example, public misunderstanding, mistrust, or skepticism of the scientific discourse on risk, may relate to how risk issues are defined and how the risk discourse is constructed (Welp et al. 2006). Political priorities and public behaviors at odds with natural resources and public health recommendations are the likely result.

Scale and Measurement. Measurement and the scale of measurement can serve to perpetuate DEIJ issues in organizations and dialogue programs. For instance, dominant water scarcity narratives start from the perceived imbalance between water supply and demand, which implies that solutions involve strategies to increase supplies or reduce demand. Scholars have criticized the narratives and frames of absolute water scarcity in policy debates for prioritizing quantitative metrics and ignoring issues of poverty, uneven water access and distribution, and the appropriation of water by powerful interests (Jairath 2010). If water scarcity is socially constructed, implicit in the question "is there sufficient water?" is the related question "sufficient for what, for whom, and where?" (Jaffee and Case 2018). In their study of conflict over groundwater extraction, Jaffee and Case (2018) showed how various actors deploy contextual ambiguities in water scarcity narratives or discourses to advance or defend their positions. The research illustrates the use of power relations, language, and framing to define water scarcity at convenient geographic and temporal scales, volumes, and economic impacts. They concluded that the issue of hydrologic scarcity masks deeper issues of economic and social justice at the heart of the water resources conflict. Therefore, measurement considerations are crucial to understanding water dialogue justice.

Water sector DEIJ issues are scale dependent. Assessment of fairness may change with the temporal and spatial units of analysis. Spatial and temporal scales used to evaluate DEIJ are contested social constructs that change with choice, definition, and decisions about scales (Zwarteveen and Boelens 2014). Evaluating the DEIJ of a water dialogue depends on how the boundaries of the system are defined. For instance, the diversity of participants in national professional associations may look entirely different from local counterparts. While membership and leadership in a national association may reflect low DEIJ, in any local association DEIJ may be high due to local factors. Equally, a local chapter may reflect low DEIJ relative to local population demographics. Shifting scales may be used as a strategy to change perceptions of inequity. DEIJ within an online seminar offered by a local entity may rise over time by expanding the area from which participants are recruited, but this expansion may be detrimental to local participants. Local participants may unfairly lose access to effective participation, leadership positions offered by the local association, and the knowledge transferred in the seminar. However, the seminar may be deemed inclusive because it includes participation from a larger and potentially more diverse national audience. Therefore, to address DEIJ deficits in water dialogues, explicit consideration needs to be given to understanding the contextual functioning of scale, including how it affects DEIJ measurement.

From Theory to Practice

The four groups of theories applied herein to conceptualize DEIJ issues in water dialogues come from a variety of sources, represent a range of fields, and reflect multiple aspects of water resource policy and practice. In combination, these theories point toward best practices for enhancing DEIJ outcomes in water dialogues.

Marginalized groups face unique challenges to engage in and benefit from dialogues. Dialogues that explicitly consider issues of DEIJ may be better positioned to reach these groups. Omitting consideration of DEIJ variables from evaluation of organization and program performances may limit the ability to serve marginalized communities, or even worse, may create new disparities (Ramos et al. 2021). Policies and initiatives to promote DEIJ can be undermined if the indicators used to define and measure DEIJ attainment reinforce existing disparities and hierarchies (Chambers et al. 2017). Karakhan et al. (2021) identify ten indicators that influence the achievement of DEIJ. The literature suggests that metrics and frameworks that perform exceptionally well are those that combined multiple standardized and validated measures with scales of measurement. However, while the literature provides various broad frameworks for assessing DEIJ, there are few studies that provide specifics on how to identify applicable indicators and determine their level of influence.

The just water dialogues framework can be deployed to evaluate DEIJ issues by illuminating the influences and interactions of access to knowledge, participation, social boundaries, capabilities, scale, and measurement. The framework anticipates methods for addressing DEIJ in water dialogues that differ and may conflict. Despite the interconnectedness of cultural, representational, distributional, and capabilities elements that leads to DEIJ failures in water dialogues, there is value in distinguishing them. For instance, in professional organization dialogues, recognition of racial minorities and women often means calling attention to people as members of a category and then affirming their value to organizations and dialogues. Alternatively, redistribution of access to leadership roles may require eliminating economic or political categorizations that underpin group norms. These solutions promote the right to equity as individuals instead of focusing on the right to be different as an identifiable group. Acknowledging such tension between satisfying individual or group claims is crucial to advancing DEIJ goals. Therefore, it is important to examine how bridges can be established across differences to address inequities in water dialogues, including through effective DEIJ engagement across contexts, locations, scales, and identities (Schlosberg 2004; Zwarteveen and Boelens 2014).

Research on admission to educational institutions indicates that policymakers addressing diversity and equity issues should explicitly recognize the power dynamics at play. This can enable greater inclusion by promoting multiple kinds of excellence, thereby challenging traditional notions of the meritocracy (Razack et al. 2015; King et al. 2018; Tamtik and Guenter 2019). It also helps to avoid unwanted exclusion based on one authoritative definition of excellence that consistently prevails over greater demographic representativeness. Recognizing inherent power relations also aids in identifying entrenched privilege in group selection processes that challenge the claims of meritocracy.

Methods proposed to improve participation of marginalized groups in water dialogues tend to apply "one size fits all" engagement systems. Two types of approaches are evident in the literature: broad engagement frameworks and general strategies (Akhmouch and Clavreul 2016). General strategies identified in the literature to enhance engagement, recruitment, and retention from underrepresented groups include professional advocacy, mentorship, improving the participation environment, maintaining the flexibility of methods and modalities, and enhancing educational opportunities for new participants (Mallett et al. 2021). There is limited empirical evidence on application of these strategies and frameworks to guide practitioners seeking to improve engagement of diverse participants in water dialogues. The Center for Diversity and Global Initiatives reported several effective strategies for facilitating outreach and dialogues: case studies; simulation; coaching; role-modeling; and integrative dialogic practices that link various knowledge bases to intellectual, ethical, and technical decision-making (NLN 2017). The suitability and efficacy of each strategy may depend on the dialogue context, including the element of DEIJ characteristics and the targeted audience.

The current focus on engagement as a mechanism for addressing water dialogue justice is hampered by a lack of studies looking at the experiences of marginalized communities and the barriers that prevent their full participation in dialogues. Critically questioning established discourse norms, power relations, and contextual factors in water dialogues can result in recontextualizing and reorganizing the power relations in dialogues. It can also expose the specifics of place, time, and position of the knower(s) associated with dialogue outcomes (Zwarteveen and Boelens 2014). Making research useful in engagement practice requires sensitivity to the effects of certain discursive representations and frames on experiences, problems, and solutions and on knowledge generation and transfer in water dialogues. Deriving participant engagement best practices would involve visualizing power mechanisms operating within established discourse and illustrating how to address factors that can disguise distributional and representational issues.

A Convergence of Recommendations Suggests Effective Pathways to Increasing DEIJ in Water Dialogues

The literature described herein provides an initial set of parameters with which to identify and address water dialogue justice via diverse, equitable, inclusive, and just engagement in water dialogues. The proposed five-dimensional framework of Just Water Dialogues links the literature to the water resources field and water dialogues specifically. The following recommendations are made to inform practices to enhance DEIJ in water dialogues.

- Knowledge production and distribution are key to enlarging engagement of diverse voices in water dialogues. Increasing DEIJ requires breaching of social boundaries that inhibit knowledge distribution. Sufficiently broad knowledge distribution will depend on insights into norms and rules that perpetuate exclusivity based on power relations, normative practices, and interactions with contextual factors and structures at various scales.
- There is a basic need for awareness and overt consideration of the ways discourse frames realities, problems, and solutions in knowledge generation and transfer, and how certain discursive representations and power relations affect individuals and groups.
- The water sector faces the challenge of developing processes for increasing DEIJ that account for the limits of scientific knowledge and the need to incorporate experiential knowledge into dialogue and decision-making. More work is needed to understand why science and expert knowledge are deemed authoritative in some cultural and socio-political contexts but not in others. This work should consider whether and how assertions or assumptions of scientists and other experts are at odds with community values and views.
- Because of the strong context dependence of water dialogues, standard, one-size-fits-all engagement methods proposed to improve the participation of underrepresented groups may be insufficient or may not lead to diversity improvement. Context-sensitive engagement design can improve DEIJ in water dialogues (Ottinger et al. 2017; Brimhall and Saastamoinen 2020).
- Promoting diversity and inclusion in organization mission, leadership, staff, outreach, dialogue programs, and processes is important. Emphasizing efforts to recruit, retain, and engage a diverse group of water dialogue participants is especially crucial, as is planning and executing an inclusive water dialogue program, and establishing partnerships that support increased diversity, equity, and inclusion.
- Several practices are recommended in relation to the four stages of engagement. First, early in the problem-framing and stakeholder identification and selection, water dialogue facilitators should engage varied marginalized communities and share control over framing the engagement program scope. Practitioners argue for more nuanced, emergent means for stakeholder identification to promote inclusivity, since existing 'top-down' frameworks for engagement have tended to exclude some stakeholders, particularly from minority groups (Mercer-Mapstone et al. 2019). Second, while preparing for dialogue forums, the facilitators should work closely with marginalized stakeholders to identify and address barriers that prevent their inclusion. Third, throughout dialogue facilitation, activities should be used that allow members of underrepresented groups to provide knowledge and input in ways that are most comfortable to them. Fourth, dialogue facilitators should work to enhance the capabilities and capacity of participants to understand information, communicate effectively, and deal with conflict (Day and Beard 2019).
- Dialogue facilitators should explicitly monitor DEIJ attainment using a combination of variables based on specific dialogue and DEIJ context. Reliance on standardized indicators to assess the distribution of water-related benefits/harms may produce or perpetuate inadequate responses to problems, missed opportunities for effective policies, and perceptions of inequitable management (Dawson et al. 2018). Defining and evaluating equity in the distribution of water-related benefits/harms implies engagement and involvement with those whose experiences and environments are the foci of dialogues.

Future Research

DEIJ research has moved away from simple, main effect approaches and toward examining variables that influence the effects of diversity. While there is no shortage of primary studies linking diversity with positive or negative outcomes, it remains unclear which contingent factors make diversity work, including the factors that make demographic differences salient, produce or prevent intergroup bias, and enhance or weaken information elaboration (Guillaume et al. 2017). Research that gives greater clarity to the influence of context is essential to understanding where, when, and how diversity dynamics evolve in organizations (Joshi and Roh 2009; Roberson 2019). Enhancing the theoretical rigor and practical relevance of diversity research, therefore, requires considerations of structural, normative, and relational features of context. Essential research on elements of DEIJ and on how context affects perceptions of and reactions to it would benefit from studies that account for broad social and cultural influences (Roberson 2019). Diversity management practices can be examined in different settings, such as professional associations, universities, and utilities. As DEIJ performance indicators tend to be industry-specific, examining the diversity-performance relationship in non-business settings may provide new insights.

Conclusions

This paper draws from the broad DEIJ literature to propose a conceptual framework for understanding DEIJ in water dialogues and identifying best practices to create just water dialogues by addressing DEIJ failures. Theories from the literatures of discourse, diversity, social learning, and EJ provide the basis for understanding the factors that influence DEIJ outcomes in water dialogues. The just water dialogues framework applies a pluralistic approach to posit that five interrelated dimensions of DEIJ (i.e., knowledge distribution; participation; social boundaries; capabilities; and scale and measurement) can converge to create instances of DEIJ success or failures, affecting individuals, groups, organizations, and networks engaged in water dialogues. Water dialogue inequities stem from but are not limited to: distribution of access, benefits, and harms associated with knowledge produced and transferred in dialogues; participation in dialogue decision-making; and recognition of the cultural identities and unique knowledge of underrepresented groups.

Discourse theory suggests that DEIJ in water dialogues starts with recognizing the limits on discourse, from monopolization of water knowledge to subtle normalization of dominant perspectives within a dialogue. Diversity theories focus on individual biases and the cultural contexts in which these biases are nurtured and propose pathways to DEIJ based on structural adjustments in organizations and engagement programs. Social learning theory provides an approach for designing and evaluating engagement strategies aimed at more diverse participation in water dialogues. In practice, social learning uses the co-creation of knowledge from reciprocal exchange among diverse stakeholders participating in water dialogues. EJ theories suggest that participation in dialogue design and management is required for just water dialogue outcomes. Within EJ theory, different ways of organizing around and discussing water, and of addressing recognition issues, will counteract socially or traditionally embedded rules and practices of water discourse that silence diverse voices (Zwarteveen 2010).

A focus on the context of water dialogues can contribute to understanding the deeper epistemic dimensions of DEIJ in these dialogues. This understanding will inform practices with potential to improve DEIJ in water dialogues, governance, and management. Solutions start with recognizing the many manifestations of injustice within water dialogues specifically, and water resources generally. Ultimately, change in water sector DEIJ will only occur with the meaningful and impactful involvement of previously unrepresented or underrepresented groups. Just water dialogues provide pathways for effective involvement of underrepresented groups. Design of engagement strategies should consider contextual factors and recognition of issues that discourage effective participation from underrepresented groups, and should actively involve these groups.

Acknowledgements

This research was supported in part by a federal Water Resources Research Act Section 104b Grant Number

G21AS00517 from the U.S. Geological Survey through The University of Arizona Water Resources Research Center, and by the Technology Research Initiative Fund administered by The University of Arizona Office for Research, Innovation, and Impact, funded under Proposition 301, the Arizona Sales Tax for Education Act in 2000.

Author Bio and Contact Information

Simone A. Williams (corresponding author) is a Ph.D. Candidate in Arid Lands Resource Sciences at The University of Arizona and a Graduate Research Associate at the Water Resources Research Center (WRRC). Her research focuses on enhancing groundwater vulnerability and risk assessment to improve policy and management outcomes. She has a Master's of Earth and Environmental Resources Management degree from University of South Carolina, and over 24 years of professional experience spanning government, business, and civil society organizations. She worked extensively in international, multi-stakeholder contexts on sustainable development and building the resilience of water resources, vulnerable communities, and marginalized groups in areas susceptible to hydrometeorological disasters. She can be contacted at sawillms@arizona.edu or by mail at The University of Arizona Water Resources Research Center, 350 N. Campbell Ave., Tucson, AZ 85719.

Dr. Susanna Eden retired from the position of Assistant Director for The University of Arizona WRRC in January 2021 and currently serves the WRRC as part-time Research/Outreach Programs Officer. She holds a Ph.D. in Water Resources Administration from UA and has been engaged with water resources research and outreach for more than 25 years. Her research centers on policy and decision-making in water resources, stakeholder engagement, and the use of scientific information. She also is responsible for development of the WRRC's annual Arroyo publication. She can be contacted at seden@arizona.edu.

Dr. Sharon B. Megdal is Director of The University of Arizona WRRC, Professor of Environmental Science, C.W. and Modene Neely Endowed Professor, and Distinguished Outreach Professor. She aims to bridge the academic, practitioner, and civil society communities through water policy and management research, education, and engagement programs. The geographic scope of Dr. Megdal's work ranges from local to international. Applied research projects include analysis of water management, policy, and governance in water-scarce regions, groundwater recharge, and transboundary aquifer assessment. Professor Megdal holds a Ph.D. degree in Economics from Princeton University. She can be contacted at smegdal@arizona.edu.

Dr. Valerisa Joe-Gaddy is an alumna of The University of Arizona receiving her Ph.D. in Environmental Science with an emphasis in microbiology. Dr. Joe-Gaddy's research and extension interests include developing and validating methods to assess microbial water quality and communicating modern water quality and produce safety methods for growers. In addition, she is passionate about environmental science literacy and serving the Diné people through outreach and engagement. Currently, her work is addressing diversity, equity and inclusion within water resource research. She can be contacted at vmjoe@arizona.edu.

References

Agyeman, J. 2005. *Sustainable Communities and the Challenge of Environmental Justice*. New York University Press, New York, NY.

Ahlers, R. 2010. Fixing and nixing: The politics of water privatization. *Review of Radical Political Economics* 42(2): 213-230. Available at: https://doi.org/10.1177/0486613410368497. Accessed February 16, 2023.

Akhmouch, A. and D. Clavreul. 2016. Stakeholder engagement for inclusive water governance: "Practicing what we preach" with the OECD Water Governance Initiative. *Water* 8(5): 204. Available at: https://doi.org/10.3390/w8050204. Accessed February 16, 2023.

Akpo, E., T.A. Crane, P.V. Vissoh, and R.C. Tossou. 2015. Co-production of knowledge in multi-stakeholder processes: Analyzing joint experimentation as social learning. *The Journal of Agricultural Education and Extension* 21(4): 369-388. Available at: https://doi.org/10.1080/1389224x.2014.939201. Accessed February 16, 2023.

Ali, H.N., S.L. Sheffield, J.E. Bauer, R.P. Caballero-Gill, N.M. Gasparini, J. Libarkin, K.K. Gonzales, J. Willenbring, E. Amir-Lin, J. Cisneros, D. Desai, M. Erwin, E. Gallant, K.J. Gomez, B.A. Keisling, R. Mahon, E. Marín-Spiotta, L. Welcome, and B. Schneider. 2021. An actionable anti-racism plan for geoscience organizations. *Nature Communications* 12(3794). Available at: https://doi.org/10.1038/s41467-021-23936-w. Accessed February 16, 2023.

Balazs, C.L. and I. Ray. 2014. The drinking water disparities framework: On the origins and persistence of inequities in exposure. *American Journal of Public Health* 104(4): 603-611. Available at: https://doi.org/10.2105/AJPH.2013.301664. Accessed February 16, 2023.

Boelens, R. 2009. The politics of disciplining water rights. *Development and Change* 40(2): 307-331. Available at: https://doi.org/10.1111/j.1467-7660.2009.01516.x. Accessed February 16, 2023.

Bohm, D. 1996. *On Dialogue*. Routledge, London and New York.

Bohm, D. 2004. *On Dialogue.* 2nd edition. L. Nichol (Ed.). Taylor & Francis e-Library.

Brimhall, K.C. and M. Saastamoinen. 2020. Striving for social good through organizational inclusion: A latent profile approach. *Research on Social Work Practice* 30(2): 163-173. Available at: https://doi.org/10.1177/1049731519832103. Accessed February 16, 2023.

Brulle, R.J. and D.N. Pellow. 2006. Environmental justice: Human health and environmental inequalities. *Annual Review of Public Health* 27: 103-124. Available at: https://doi.org/10.1146/annurev.publhealth.27.021405.102124. Accessed February 16, 2023.

Byrne, D.E. 1971. *The Attraction Paradigm*. Academic Press, New York.

Caney, S. 2006. Cosmopolitan justice, rights and global climate change. *Canadian Journal of Law & Jurisprudence* 19(2): 255-278. Available at: https://doi.org/10.1017/S0841820900004100. Accessed February 16, 2023.

Carr, P.L., C.M. Gunn, S.A. Kaplan, A. Raj, and K.M. Freund. 2015. Inadequate progress for women in academic medicine: Findings from the national faculty study. *Journal of Women's Health* 24(3): 190-199. Available at: https://doi.org/10.1089/jwh.2014.4848. Accessed February 16, 2023.

Chambers, D., L. Preston, A. Topakas, S. de Saille, S. Salway, A. Booth, J. Dawson, and J. Wilsdon. 2017. Review of Diversity and Inclusion Literature and an Evaluation of Methodologies and Metrics Relating to Health Research. *Working Paper* - May 2017, Issue. Available at: https://wellcome.org/sites/default/files/review-of-diversity-and-inclusion-literature.pdf. Accessed February 16, 2023.

Clark, D.K. 2019. Improving utility succession and workforce development planning. *Journal American Water Works Association* 111(8): 12-22. Available at: https://doi.org/10.1002/awwa.1338. Accessed February 16, 2023.

Cox, T.H. and B. Stacy. 1991. Managing cultural diversity: Implications for organizational competitiveness. *Academy of Management Perspectives* 5(3): 45-56. Available at: https://doi.org/10.5465/AME.1991.4274465. Accessed February 16, 2023.

Dawson, N., A. Martin, and F. Danielsen. 2018. Assessing equity in protected area governance: Approaches to promote just and effective conservation. *Conservation Letters* 11(2): e12388. Available at: https://doi.org/10.1111/conl.12388. Accessed February 16, 2023.

Day, L. and K.V. Beard. 2019. Meaningful inclusion of diverse voices: The case for culturally responsive teaching in nursing education. *Journal of Professional Nursing* 35(4): 277-281. Available at: https://doi.org/10.1016/j.profnurs.2019.01.002. Accessed February 16, 2023.

Eden, S. 2011. Lessons on the generation of usable science from an assessment of decision support practices. *Environmental Science & Policy* 14(1): 11-19. Available at: http://dx.doi.org/10.1016/j.envsci.2010.09.011. Accessed February 16, 2023.

Erkmen, C.P., K.A. Ortmeyer, G.J. Pelletier, O. Preventza, and D.T. Cooke. 2021. An approach to diversity and inclusion in cardiothoracic surgery. *The Annals of Thoracic Surgery* 111(3): 747-752. Available at: https://doi.org/10.1016/j.athoracsur.2020.10.056. Accessed February 16, 2023.

Faysse, N., M. Errahj, A. Imache, H. Kemmoun, and T. Labbaci. 2014. Paving the way for social learning when governance is weak: Supporting dialogue between stakeholders to face a groundwater crisis in Morocco. *Society and Natural Resources* 27(3): 249-264. Available at: https://doi.org/10.1080/08941920.2013.847998. Accessed February 16, 2023.

Felt, U., R. Fouché, C.A. Miller, and L. Smith-Doerr (Eds.). 2016. *The Handbook of Science and Technology Studies*. 4th edition. The MIT Press, Cambridge, MA. Available at: https://ebookcentral.proquest.com/lib/uaz/detail.action?docID=5052910&pq-origsite=primo#. Accessed February 16, 2023.

Foucault, M. 1971. *The Archaeology of Knowledge and the Discourse on Language*. Pantheon Books, New York.

Foucault, M. 1975. *Discipline and Punish: The Birth of the Prison*. Pantheon Books, New York.

Foucault, M. 1977. *Language, Counter-Memory, Practice: Selected Essays and Interviews*. Cornell University Press, Ithaca, N.Y.

Frickel, S., S. Gibbon, J. Howard, J. Kempner, G. Ottinger, and D.J. Hess. 2010. Undone science: Charting social movement and civil society challenges to research agenda setting. *Science, Technology, & Human Values* 35(4): 444-473.

Fricker, M. 2007. *Epistemic Injustice: Power and the Ethics of Knowing*. Oxford University Press.

Guillaume, Y.R.F., J.F. Dawson, L. Otaye-Ebede, S.A. Woods, and M.A. West. 2017. Harnessing demographic differences in organizations: What moderates the effects of workplace diversity? *Journal of Organizational Behavior* 38(2): 276-303. Available at: https://doi.org/10.1002/job.2040. Accessed February 16, 2023.

Hegde, S. 2020. Barriers and bias: Women in water utilities are breaking them all. *wH2O: The Journal of Gender and Water* 7(2). Available at: https://repository.upenn.edu/wh2ojournal/vol7/iss1/2. Accessed February 16, 2023.

Helitzer, D.L., S.L. Newbill, G. Cardinali, P.S. Morahan, S. Chang, and D. Magrane. 2016. Narratives of participants in national career development programs for women in academic medicine: Identifying the opportunities for strategic investment. *Journal of Women's Health* 25(4): 360-370. Available at: https://doi.org/10.1089/jwh.2015.5354. Accessed February 16, 2023.

Hernandez, P. R., B. Bloodhart, R.T. Barnes, A.S. Adams, S.M. Clinton, I. Pollack, E. Godfrey, M. Burt, and E.V. Fischer. 2017. Promoting professional identity, motivation, and persistence: Benefits of an informal mentoring program for female undergraduate students. *PLoS One* 12(11): e0187531. Available at: https://doi.org/10.1371/journal.pone.0187531. Accessed February 16, 2023.

Hess, D.J. 2007. *Alternative Pathways in Science and Industry: Activism, Innovation, and the Environment in an Era of Globalizaztion*. The MIT Press, Cambridge, MA.

Hoffman, G.D. and T.D. Mitchell. 2016. Making diversity 'everyone's business': A discourse analysis of institutional responses to student activism for equity and inclusion. *Journal of Diversity in Higher Education* 9(3): 277-289. Available at: https://doi.org/10.1037/dhe0000037. Accessed February 16, 2023.

Hogg, M.A. and D.J. Terry. 2000. Social identity and self-categorization processes in organizational contexts. *The Academy of Management Review* 25(1): 121-140. Available at: https://doi.org/10.2307/259266. Accessed February 16, 2023.

Hogg, M.A. and J.C. Turner. 1987. Intergroup behaviour, self-stereotyping, and the salience of social categories. *British Journal of Social Psychology* 26(4): 325-340. Available at: https://doi.org/10.1111/j.2044-8309.1987.tb00795.x. Accessed February 16, 2023.

Irby-Butler, T. 2017. The kaleidoscope view of workforce diversity and inclusion. *Journal American Water Works Association* 109(8): 66-69. Available at: https://doi.org/10.5942/jawwa.2017.109.0108. Accessed February 16, 2023.

Jaffee, D. and R.A. Case. 2018. Draining us dry: Scarcity discourses in contention over bottled water extraction. *Local Environment* 23(4): 485-501. Available at: https://doi.org/10.1080/13549839.2018.1431616. Accessed February 16, 2023.

Jairath, J. 2010. Advocacy of water scarcity: Leakages in the argument. In: *The Limits to Scarcity: Contesting the Politics of Allocation*, L. Mehta (Ed.). Routledge, New York, NY, pp. 215-232.

Jamieson, D. 2001. Climate change and global environmental justice. In: *Changing the Atmosphere: Expert Knowledge and Environmental Governance*, C. Miller and P.N. Edwards (Eds.). The MIT Press, Cambridge, MA, pp. 287-307.

Joshi, A. and H. Roh. 2009. The role of context in work team diversity research: A meta-analytic review. *Academy of Management* 52(3): 599-627. Available at: https://doi.org/10.5465/amj.2009.41331491. Accessed February 16, 2023.

Kane, J. and A. Tomer. 2018. *Renewing the Water Workforce: Improving Water Infrastructure and Creating a Pipeline to Opportunity*. Metropolitan Policy Program at Brookings Institution, Washington, D.C. Available at: http://hdl.voced.edu.au/10707/495012. Accessed February 16, 2023.

Karakhan, A.A., J.A. Gambatese, D.R. Simmons, and A.J. Al-Bayati. 2021. Identifying pertinent indicators for assessing and fostering diversity, equity, and inclusion of the construction workforce. *Journal of Management in Engineering* 37(2): 04020114. Available at: https://doi.org/10.1061/(ASCE)ME.1943-5479.0000885. Accessed February 16, 2023.

Karsten, J.L. 2019. Insights from the OEDG program on broadening participation in the geosciences. *Journal of Geoscience Education* 67(4): 287-299. Available at: https://doi.org/10.1080/10899995.2019.1565982. Accessed February 16, 2023.

Kibler, K.M., R.K. Biswas, and A.M.J. Lucas. 2014. Hydrologic data as a human right? Equitable access to information as a resource for disaster risk reduction in transboundary river basins. *Water Policy* 16(S2): 36-58. Available at: https://doi.org/10.2166/wp.2014.307. Accessed February 16, 2023.

King, L., L. MacKenzie, M. Tadaki, S. Cannon, K. McFarlane, D. Reid, and M. Koppes. 2018. Diversity in geoscience: Participation, behaviour, and the division of scientific labour at a Canadian geoscience conference. *Facets* 3(1): 415-440. Available at: https://doi.org/10.1139/facets-2017-0111. Accessed February 16, 2023.

Layne, P. 2004. Examining diversity in the drinking water industry. *Journal American Water Works Association* 96(8): 48-53. Available at: https://doi.org/10.1002/j.1551-8833.2004.tb10671.x. Accessed February 16, 2023.

Liang, J. 2016. Defining environmental justice communities for regulatory enforcement: Implications from a block-group-level analysis of New York State. *Review of Policy Research* 33(6): 666-685. Available at: https://doi.org/10.1111/ropr.12202. Accessed February 16, 2023.

Lutz-Ley, A.N., C.A. Scott, M. Wilder, R.G. Varady, A. Ocampo-Melgar, F. Lara-Valencia, A.A. Zuniga-Teran, S. Buechler, R. Díaz-Caravantes, A. Ribeiro Neto, N. Pineda-Pablos, and F. Martín. 2021. Dialogic science-policy networks for water security governance in the arid Americas. *Environmental Development* 38: 100568. Available at: https://doi.org/10.1016/j.envdev.2020.100568. Accessed February 16, 2023.

Macdonell, D. 1986. *Theories of Discourse: An Introduction*. B. Blackwell, New York.

Mallett, P., A. Thompson, and T. Bourke. 2021. Addressing recruitment and retention in paediatrics: A pipeline to a brighter future. *Archives of Disease in Childhood: Education and Practice Edition* 107: 57-63. Available at: https://doi.org/10.1136/archdischild-2020-319696. Accessed February 16, 2023.

Megdal, S.B., A.K. Gerlak, R.G. Varady, and L.-Y. Huang. 2015. Groundwater governance in the United States: Common priorities and challenges. *Groundwater* 53(5): 677-684. Available at: https://doi.org/10.1111/gwat.12294. Accessed February 16, 2023.

Mercer-Mapstone, L., W. Rifkin, W. Louis, and K. Moffat. 2019. Power, participation, and exclusion through dialogue in the extractive industries: Who gets a seat at the table? *Resources Policy* 61: 190-199. Available at: https://doi.org/10.1016/j.resourpol.2018.11.023. Accessed February 16, 2023.

Mercer-Mapstone, L., W. Rifkin, K. Moffat, and W. Louis. 2017. Conceptualising the role of dialogue in social licence to operate. *Resources Policy* 54: 137-146. Available at: https://doi.org/10.1016/j.resourpol.2017.09.007. Accessed February 16, 2023.

Midgley, G. 2011. Theoretical pluralism in systemic action research. *Systemic Practice and Action Research* 24(1): 1-15. Available at: https://doi.org/10.1007/s11213-010-9176-2. Accessed February 16, 2023.

Mott Lacroix, K.E. and S.B. Megdal. 2016. Explore, synthesize, and repeat: Unraveling complex water management issues through the stakeholder engagement wheel. *Water* 8(4): 118. Available at: https://www.mdpi.com/2073-4441/8/4/118. Accessed February 16, 2023.

Mott Lacroix, K.E., B.C. Xiu, and S.B. Megdal. 2016. Building common ground for environmental flows using traditional techniques and novel engagement approaches. *Environmental Management* 57(4): 912-928. Available at: https://doi.org/10.1007/s00267-016-0656-8. Accessed February 16, 2023.

National League for Nursing (NLN). 2017. *Diversity and Inclusion Toolkit*. Center for Diversity and Global Initiatives. Available at: https://www.nln.org/docs/default-source/uploadedfiles/professional-development-programs/diversity-toolkit.pdf. Accessed February 16, 2023.

Neal, M.J., A. Lukasiewicz, and G.J. Syme. 2014. Why justice matters in water governance: Some ideas for a 'water justice framework'. *Water Policy* 16(S2): 1-18. Available at: https://doi.org/10.2166/wp.2014.109. Accessed February 16, 2023.

Nelson, J.R. and T.H. Grubesic. 2018. Environmental justice: A panoptic overview using scientometrics. *Sustainability* 10(4): 1022. Available at: https://www.mdpi.com/2071-1050/10/4/1022. Accessed February 16, 2023.

Novotny, P. 2000. *Where We Live, Work, and Play: The Environmental Justice Movement and the Struggle for a New Environmentalism*. Praeger, Westport, CT.

O'Brien, K.R., M. Scheffer, E.H. van Nes, and R. van der Lee. 2015. How to break the cycle of low workforce diversity: A model for change. *PLoS One* 10(7). Available at: https://doi.org/http://dx.doi.org/10.1371/journal.pone.0133208. Accessed February 16, 2023.

Ottinger, G., J. Barandiaran, and A.H. Kimura. 2017. Environmental justice: Knowledge, technology, and expertise. In: *The Handbook of Science and Technology Studies*, U. Felt, R. Fouché, C.A. Miller, and L. Smith-Doerr (Eds.). The MIT Press, Cambridge, MA, pp. 1029-1057.

Ottinger, G. and B.R. Cohen (Eds.). 2011. *Technoscience and Environmental Justice: Expert Cultures in a Grassroots Movement*. The MIT Press, Cambridge, MA.

Owen, G., D.B. Ferguson, and B. McMahan. 2019. Contextualizing climate science: Applying social learning systems theory to knowledge production, climate services, and use-inspired research. *Climatic Change* 157(1): 151-170. Available at: https://doi.org/10.1007/s10584-019-02466-x. Accessed February 16, 2023.

Petersen-Perlman, J.D., S.B. Megdal, A.K. Gerlak, M. Wireman, A.A. Zuniga-Teran, and R.G. Varady. 2018. Critical issues affecting groundwater quality governance and management in the United States. *Water* 10(6): 735. Available at: https://doi.org/10.3390/w10060735. Accessed February 16, 2023.

Ramos, G., C. Ponting, J.P. Labao, and K. Sobowale. 2021. Considerations of diversity, equity, and inclusion in mental health apps: A scoping review of evaluation frameworks. *Behaviour Research and Therapy* 147: 103990. Available at: https://doi.org/https://doi.org/10.1016/j.brat.2021.103990. Accessed February 16, 2023.

Ravazzani, S. and C.D. Maier. 2017. Strategic organizational discourse and framing in hypermodal spaces. *Corporate Communications: An International Journal* 22(4): 507-522. Available at: https://doi.org/10.1108/CCIJ-06-2017-0063. Accessed February 16, 2023.

Razack, S., B. Hodges, Y. Steinert, and M. Maguire. 2015. Seeking inclusion in an exclusive process: Discourses of medical school student selection. *Medical Education* 49(1): 36-47. Available at: https://doi.org/10.1111/medu.12547. Accessed February 16, 2023.

Roberson, Q.M. 2019. Diversity in the workplace: A review, synthesis, and future research agenda. *Annual Review of Organizational Psychology and Organizational Behavior* 6: 69-88. Available at: https://doi.org/10.1146/annurev-orgpsych-012218-015243. Accessed February 16, 2023.

Schaider, L.A., L. Swetschinski, C. Campbell, and R.A. Rudel. 2019. Environmental justice and drinking water quality: Are there socioeconomic disparities in nitrate levels in U.S. drinking water? *Environmental Health* 18(3). Available at: https://doi.org/10.1186/s12940-018-0442-6. Accessed February 16, 2023.

Schlosberg, D. 2004. Reconceiving environmental justice: Global movements and political theories. *Environmental Politics* 13(3): 517-540. Available at: https://doi.org/10.1080/0964401042000229025. Accessed February 16, 2023.

Schlosberg, D. 2007. *Defining Environmental Justice Theories, Movements, and Nature*. Oxford University Press, Oxford.

Schlosberg, D. 2013. Theorising environmental justice: The expanding sphere of a discourse. *Environmental Politics* 22(1): 37-55. Available at: https://doi.org/10.1080/09644016.2013.755387. Accessed February 16, 2023.

Shames, S.L. and T. Wise. 2017. Gender, Diversity, and Methods in Political Science: A Theory of Selection and Survival Biases. *PS: Political Science & Politics* 50(03): 811-823. Available at: https://doi.org/10.1017/s104909651700066x. Accessed February 16, 2023.

Singer, P. 2004. *One World: Ethics of Globalisation.* 2nd edition. Yale University Press, New Haven, CT.

Smith, S.G., P.A. Nsiah-Kumi, P.R. Jones, and R.J. Pamies. 2009. Pipeline programs in the health professions, part 1: Preserving diversity and reducing health disparities. *Journal of the National Medical Association* 101(9): 836-840+845-851. Available at: https://doi.org/10.1016/S0027-9684(15)31030-0. Accessed February 16, 2023.

Statman-Weil, Z., L. Nanus, and N. Wilkinson. 2020. Disparities in community water system compliance with the Safe Drinking Water Act. *Applied Geography* 121: 102264. Available at: https://doi.org/10.1016/j.apgeog.2020.102264. Accessed February 16, 2023.

Stewart, D. 2017. Language of appeasement. *Inside Higher Ed* 30. Available at: https://www.insidehighered.com/views/2017/03/30/colleges-need-language-shift-not-one-you-think-essay. Accessed February 16, 2023.

Sze, J. and J.K. London. 2008. Environmental justice at the crossroads. *Sociology Compass* 2(4): 1331-1354. Available at: https://doi.org/10.1111/j.1751-9020.2008.00131.x. Accessed February 16, 2023.

Tajfel, H. (Ed.). 1978. *Differentiation between Social Groups: Studies in the Social Psychology of Intergroup Relations*. Academic Press, New York.

Tamtik, M. and M. Guenter. 2019. Policy analysis of equity, diversity and inclusion strategies in Canadian universities–How far have we come? *Canadian Journal of Higher Education* 49(3): 41-56. Available at: https://www.erudit.org/en/journals/cjhe/1900-v1-n1-cjhe05066/1066634ar.pdf. Accessed February 16, 2023.

Tiwari, T., C.L. Randall, L. Cohen, J. Holtzmann, J. Webster-Cyriaque, S. Ajiboye, L. Schou, M. Wandera, K. Ikeda, M. Fidela de Lima Navarro,

M. Feres, H. Abdellatif, E. Al-Madi, S. Tubert-Jeannin, C.H. Fox, E. Ioannidou, and R.N. D'Souza. 2019. Gender inequalities in the dental workforce: Global perspectives. *Advances in Dental Research* 30(3): 60-68. Available at: https://doi.org/10.1177/0022034519877398. Accessed February 16, 2023.

U.S. Environmental Protection Agency (US EPA). 2020. *EPA Annual Environmental Justice Progress Report FY 2020*. EPA Publication Number 230R20002. Available at: https://www.epa.gov/sites/production/files/2021-01/documents/2020_ej_report-final-web-v4.pdf. Accessed February 16, 2023.

U.S. Office of Personnel Management (US OPM). 2021. *Government-wide Strategic Plan to Advance Diversity, Equity, Inclusion, and Accessibility in the Federal Workforce*. Available at: https://www.whitehouse.gov/wp-content/uploads/2021/11/Strategic-Plan-to-Advance-Diversity-Equity-Inclusion-and-Accessibility-in-the-Federal-Workforce-11.23.21.pdf. Accessed February 16, 2023.

Van Knippenberg, D., C.K.W. De Dreu, and A.C. Homan. 2004. Work group diversity and group performance: An integrative model and research agenda. *Journal of Applied Psychology* 89(6): 1008-1022. Available at: https://psycnet.apa.org/doi/10.1037/0021-9010.89.6.1008. Accessed February 16, 2023.

Van Knippenberg, D. and W.P. Van Ginkel. 2010. The categorization-elaboration model of work group diversity: Wielding the double-edged sword. In: *The Psychology of Social and Cultural Diversity*, R.J. Crisp (Ed.). Wiley-Blackwell, Malden, MA, pp. 257-280.

VanDerslice, J. 2011. Drinking water infrastructure and environmental disparities: Evidence and methodological considerations. *American Journal of Public Health* 101: S109-S114. Available at: https://doi.org/10.2105/AJPH.2011.300189. Accessed February 16, 2023.

Vos, H.D., R. Boelens, and R. Bustamante. 2006. Formal law and local water control in the Andean Region: A fiercely contested field. *International Journal of Water Resources Development* 22(1): 37-48. Available at: https://doi.org/10.1080/07900620500405049. Accessed February 16, 2023.

Walker, G. 2009a. Beyond distribution and proximity: Exploring the multiple spatialities of environmental justice. *Antipode* 41(4): 614-636. Available at: https://doi.org/10.1111/j.1467-8330.2009.00691.x. Accessed February 16, 2023.

Walker, G. 2009b. Globalizing environmental justice: The geography and politics of frame contextualization and evolution. *Global Social Policy* 9(3): 355-382. Available at: https://doi.org/10.1177/1468018109343640. Accessed February 16, 2023.

Welp, M., A. De La Vega-Leinert, S. Stoll-Kleemann, and C.C. Jaeger. 2006. Science-based stakeholder dialogues: Theories and tools. *Global Environmental Change* 16(2): 170-181. Available at: https://doi.org/10.1016/j.gloenvcha.2005.12.002. Accessed February 16, 2023.

Wenger, E. 2010a. Communities of practice and social learning systems: The career of a concept. In: *Social Learning Systems and Communities of Practice,* C. Blackmore (Ed.). Springer, London, pp. 179-198.

Wenger, E. 2010b. Conceptual tools for CoPs as social learning systems: Boundaries, identity, trajectories and participation. In: *Social Learning Systems and Communities of Practice,* C. Blackmore (Ed.). Springer, London, pp. 125-143. Available at: https://doi.org/10.1007/978-1-84996-133-2_8. Accessed February 16, 2023.

West, M.A., S. Hwang, R.V. Maier, N. Ahuja, P. Angelos, B.L. Bass, K.J. Brasel, et al. 2018. Ensuring equity, diversity, and inclusion in academic surgery: An American Surgical Association white paper. *Annals of Surgery* 268(3): 403-407. Available at: https://doi.org/10.1097/SLA.0000000000002937. Accessed February 16, 2023.

Westoby, P. and G. Dowling. 2013. *Theory and Practice of Dialogical Community Development: International Perspectives*. Routledge, New York, NY. Available at: https://doi.org/10.4324/9780203109946. Accessed February 16, 2023.

Williams, K.Y. and C.A. O'Reilly. 1998. Demography and diversity in organisations: A review of 40 years of research. In: *Research in Organisational Behaviour,* B.M. Staw and L.L. Cummings (Eds.). JAI Press, Greenwich, CT.

World Bank. 2019. Women in Water Utilities: Breaking Barriers. Washington, D.C. Available at: https://openknowledge.worldbank.org/bitstream/handle/10986/32319/140993.pdf?sequence=9&isAllowed=y. Accessed February 16, 2023.

Wutich, A., A. Brewis, A.M. York, and R. Stotts. 2013. Rules, norms, and injustice: A cross-cultural study of perceptions of justice in water institutions. *Society and Natural Resources* 26(7): 795-809. Available at: https://doi.org/10.1080/08941920.2012.723302. Accessed February 16, 2023.

Wynne, B. 1996. May the sheep safely graze? A reflexive view of the expert-lay knowledge divide. In: *Risk, Environment, and Modernity: Towards a New Ecology*, S. Lash, B. Szerszynski, and B. Wynne (Eds.). SAGE Publications, pp. 44-83.

Wynne, B. 2005. Risk as globalizing "democratic" discourse? Framing subjects and citizens. In: *Science and Citizens: Globalization and the Challenge of Engagement,* M. Leach, I. Scoones, and B. Wynne (Eds.). Zed Books, New York, NY, pp. 66-82.

Zeitoun, M., J. Warner, N. Mirumachi, N. Matthews, K. McLaughlin, M. Woodhouse, A. Cascão, and T.J.A. Allan. 2014. Transboundary water justice: A combined reading of literature on critical transboundary water interaction and 'justice', for analysis and diplomacy. *Water Policy* 16(S2): 174-193. Available at: https://doi.org/10.2166/wp.2014.111. Accessed February 16, 2023.

Zwarteveen, M. 2010. A masculine water world: The politics of gender and identity in irrigation expert thinking. In: *Out of the Mainstream: Water Rights, Politics and Identity,* R. Boelens, D. Getches, and A. Guevara-Gil (Eds.). Earthscan from Routledge, New York, NY, pp. 75-98.

Zwarteveen, M.Z. 2006. *Wedlock or Deadlock? Feminists' Attempts to Engage Irrigation Engineers*. Wageningen Universiteit, The Netherlands. Available at: https://edepot.wur.nl/121803. Accessed February 16, 2023.

Zwarteveen, M.Z. and R. Boelens. 2014. Defining, researching and struggling for water justice: Some conceptual building blocks for research and action. *Water International* 39(2): 143-158. Available at: https://doi.org/10.1080/02508060.2014.891168. Accessed February 16, 2023.

Contents

UCOWR
UNIVERSITIES COUNCIL ON WATER RESOURCES

Universities Council on Water Resources
Mail Code 4526
Southern Illinois University Carbondale
1231 Lincoln Drive
Carbondale, IL 62901
www.ucowr.org