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Spatial patterns of enzymatic activity in large water bodies: Ship-borne measurements of beta-D-glucuronidase activity as a rapid indicator of microbial water quality

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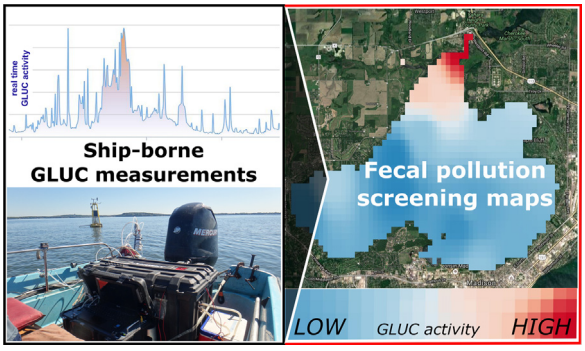
HIGHLIGHTS

- Ship-borne GLUC measurements were used for fecal pollution screening.
- GLUC screening maps indicated contaminant in-put on large water bodies.
- Surface water GLUC activity was primarily related to hydrologic inputs.
- Human-dominated water sources are key drivers of GLUC activity in surface waters.

GRAPHICAL ABSTRACT



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ABSTRACT

This study used automated enzymatic activity measurements conducted from a mobile research vessel to detect the spatial variability of beta D glucuronidase (GLUC) activity in large freshwater bodies. The ship-borne observations provided the first high-resolution spatial data of GLUC activity in large water bodies as rapid indication of fecal pollution and were used to identify associations with hydrological conditions and land use. The utility of this novel approach for water quality screening was evaluated by surveys of the Columbia River, the Mississippi River and the Yahara Lakes, covering up to a 500 km river course and 50 km^2 lake area. The ship-borne measurements of GLUC activity correlated with standard *E. coli* analyses ($R^2 = 0.71$) and revealed the effects of (1) precipitation events and urban run-off on GLUC activity in surface waters, (2) localized point inlets of potential fecal pollution and (3) increasing GLUC signals along gradients of urbanization. We propose that this ship-borne

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Ship-borne
Land use
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water quality screening to be integrated into future water inventory programs as an initial or complementary tool (besides established fecal indicator parameters), due to its ability to provide near real-time spatial information on potential fecal contamination of large surface water resources and therefore being helpful to greatly reduce potential human health risks.

1. Introduction

Our current understanding of the microbiology of large water bodies, especially concerning the fate, transport and pathways of microbial pollutants, is predominantly based on assays that require extensive sampling and laboratory analysis efforts, resulting in limited spatial and temporal resolution (Cabral, 2010). Rivers and lakes receive discharge from numerous and diverse sources, including urban, industrial and agricultural areas that often contain pathogenic bacteria (Bradford et al., 2013; Ferguson et al., 2003; Pachepsky et al., 2006). Waterborne pathogens are a major issue of global concern and are the cause of large disease outbreaks that affect human health and impair freshwater systems (WHO, n.d.). Managing water resources to mitigate human health risk is challenging, as microbial pollutants are spatially and temporally heterogeneous due to variability among sources and complex transport processes. Thus, health-related water quality research, as well as the management, allocation and use of surface water resources, would greatly benefit from an enhanced spatial and temporal resolution of microbial parameters.

In recent years, various methods have been developed to detect or indicate the presence of microbes or microbiological parameters online and near real-time. These include on-site flow-cytometry (Besmer et al., 2016, 2014), optical detection of suspended particles including the differentiation between bacteria and particles (Højris et al., 2016), indirect indicators of bacterial activity such as ATP (Vang et al., 2014) or sensors directly sensing bacteria by contact with the sensor (Ji et al., 2004; Park et al., 2014). Today, instruments using these technologies are already available on the market (e.g. 'Bactosense' by bNovate Technologies ("Automated Flow Cytometry||bNovate Technologies," n.d.), 'Bacmon' by Grundfos ("BACMON automated bacteria monitoring solution," n.d.)). However, at the current state of the art, the specificity of such on-site biosensor-based instruments is not sufficient for a real-time monitoring of specific bacterial targets, such as bacteria indicating fecal pollution (Deshmukh et al., 2016).

The detection of enzymatic activities has been proposed as a rapid surrogate for specific microbiological water pollution monitoring (Cabral, 2010; Farnleitner et al., 2001, 2002). Measurements of beta D glucuronidase (GLUC) activity are significantly correlated to the abundance of fecal indicator bacteria (FIB) *E. coli* in rivers (Farnleitner et al., 2001, 2002), ponds (George et al., 2000) and coastal waters (Fiksdal et al., 1994). The correlation is especially strong for waters impacted by municipal sewage (Farnleitner et al., 2001, 2002) and manure (Stadler et al., 2016). Therefore, the dominant sources of GLUC activity in waters influenced by urban areas are assumed to be wastewater treatment plant effluents (Hendricks and Pool, 2012), the input of surface-associated fecal matter due to urban run-off (McCarthy et al., 2012; McLellan et al., 2007) and feces of small mammals inhabiting drain pipes in some locations (such as raccoons (Bondo et al., 2016)). Leaking sewer lines may be a significant diffuse source of untreated wastewater, even reaching storm drains in municipalities with separate storm and sanitary sewer systems (Sercu et al., 2011, 2009). In agricultural areas, the dominant source of GLUC activity in waters is assumed to be inputs of livestock feces or slurry manure application on crop fields (Bradford et al., 2013; Farnleitner et al., 2011; Pachepsky et al., 2006). A relevant source of FIB, and consequently GLUC, at lake beaches can be water birds, such as geese (McLellan and Salmore, 2003; Meerburg et al., 2011; Whitman and Nevers, 2004). While GLUC activity is predominantly correlated with the abundance of FIB *E. coli* in water, cross-sensitivities, as well as interferences of enzymatic activity by non-fecal compounds, such as algae or organic matter, have been studied previously (Biswal et al., 2003; Fiksdal and Tryland, 2008). Although these mechanisms of interference may limit the usefulness of GLUC as a surrogate to quantify *E. coli*, they were shown to be less important in terms of the applicability of GLUC as a qualitative indicator for fecal pollution of water resources (Ender et al., 2017; Koschelnik et al., 2015; Ryzinska-Paier et al., 2014; Stadler et al., 2016).

Automated on-site measurements of enzymatic activity are now technically feasible and have been used for near real-time indication of microbiological contamination in a variety of aquatic monitoring stations, ranging from pristine groundwater (Ryzinska-Paier et al., 2014) to sediment-laden surface waters (Ender et al., 2017; Stadler et al., 2016). While these prior efforts have been extremely useful in assessing temporal enzymatic dynamics in single locations (Ender et al., 2017; Stadler et al., 2016), the utility of these automated tools would increase significantly if they could also be applied across large areas to address the pronounced spatial heterogeneity in microbial pollution within and among individual water bodies. Thus, the goal of our study was to assess the spatial variability of enzymatic activity in surface waters for the first time by means of rapid and automated GLUC activity measurements from a mobile research vessel. Specifically, we ask: (a) can automated measurements of GLUC activity serve as an indicator for fecal pollution of large water bodies?; and (b) what are the spatial patterns of GLUC activity within an individual lake or river and are they related to land use and hydrological dynamics? The surveys presented here exemplify a novel approach for water quality screening of inland waters and are focused on gaining a better understanding of the spatial patterns in water quality, as well as the fate of fecal indicators in surface waters. Suggestions for further applications in environmental science, water management and early warning systems are provided.

2. Material and methods

2.1. Rapid determination of GLUC activity

The rapid GLUC on-site assay is based is on specific bacterial hydrolysis of the substrate 4 methylumbelliferyl β D glucuronide (MUG) and fully automated fluorescence detection (excitation: 365 nm, emission: 455 nm) of the enzymatic reaction product 4 methylumbelliferone (MU) ("*Enzymatic Assay of β-Glucuronidase (EC 3.2.1.31) From E. coli [WWW Document]*," n.d.; Fishman and Bergmeyer, 1974). The automated measurements were performed in batches using 6.5 ml of sample per measurement, and a flow-through photometric measurement-chamber enabled a high-resolution fluorescence analysis of the enzymatic reaction product MU. The measurement step takes 15 min and the assay has been calibrated to Modified Fishman Units (MFU/100 ml), based on the enzyme unit definition for beta D glucuronidase activity (Fishman and Bergmeyer, 1974). The prototype used for automated and mobile GLUC measurements in this study was housed in a weatherproof case suitable for on-site and outdoor operation. The construction and function of the same prototype design have been described in detail by Koschelnik et al., 2015 and Stadler et al., 2016.

To assess the applicability of rapid GLUC measurements as an indicator for *E. coli*, a sample campaign was initiated during July 2016. Water samples (n = 18) from different beaches of the Madison Lakes (described below) were analyzed with both a defined substrate ISO 9308-2:2012 assay (IDEXX Colilert18®) and the prototype that was deployed for the ship-borne GLUC measurements. The samples were taken during the weekly water quality monitoring program of public beaches in Dane County, conducted during swimming season by Public Health Madison and Dane County ("*Madison, and Dane County Beaches - Water Quality - Public Health - Madison, and Dane County - City of Madison, Wisconsin [WWW Document]*," n.d.).

2.2. Instrumentation

The essential technical base for this study was the Fast Limnology Automated Measurement (FLAMe) platform described by Crawford et al., 2015. The core feature of the FLAMe is a flow-through system that allows for ship-borne sampling (temporal resolution of up to 1 Hz) of inland waters at both low and high speeds. A high output diaphragm pump delivers surface water (from approx. 20 cm below water level) to an array of sensors mounted inside the boat, including a YSI EXO2 multiparameter sonde (temperature [°C], pH, specific conductivity (SPC; [μS/cm]), turbidity [FNU], fluorescent dissolved organic matter (fDOM; [RFU]), and chlorophyll *a* [μg/l]) and a Satlantic SUNA V2 optical nitrate analyzer (nitrate (NO_3-N [mg/l])). The FLAMe can integrate additional sensors with simple modifications. In this study, we used a peristaltic pump to deliver water immediately and unaltered (upstream of the sensors and diaphragm pump) from the lake or river to a prototype instrument capable of rapidly measuring GLUC activity of waters. All measurements were georeferenced with an onboard GPS (WAAS enabled), time-corrected based on internal flow rates and sensor response times, and merged using time stamps (R Core Team, 2016). All sensors and equipment for ship-borne measurements were powered by 12 V DC on-board power and batteries.

2.3. Data interpretation

The aim of the presented research is to provide a methodology for an initial, rapid and complementary assessment of the spatial patterns of potential fecal pollution across large water bodies. The described approach does not intend to replace any established microbial fecal indicators, but shall enable a rapid overview to support a more purposeful and resourceful study design on large or understudied water bodies (e.g. localization of strategic sample points).

To visualize GLUC spatial patterns, we generated maps across the water surfaces using an inverse distance weighting (IDW) algorithm (QGis Development Team, 2009). GLUC activity was measured every 15 min, and measurements were assumed to be independent (calculated Moran's I for all sites < 0.08, Anselin et al., 2006) such that all data were used for interpolation. We note that the presented GLUC activity maps are estimated values across each water body and are intended for qualitative screening proposes, rather than for a quantitative determination of GLUC values over the sampled water body.

To assess drivers of GLUC activity, a comparison of the point measurements of enzymatic activity with simultaneously quantified limnological variables was used for an enhanced data interpretation with respect to contaminant pathways and transport processes. Correlations between GLUC activity and the isochronal measured limnological variables were examined using linear regression analyses.

2.4. Test sites and survey details

To evaluate the utility of rapid GLUC mapping across a range of environmental conditions, we used three distinct study systems: the Yahara lakes, a midwestern U.S. chain of lakes embedded in an agricultural and urban landscape; the Lower Columbia River in the northwestern U.S., which drains a catchment with pronounced climatological and land use variation and includes both impounded (reservoir) and free-flowing reaches; and a large, physically variable navigational pool within the Upper Mississippi River. Within the Yahara lakes, mapping was done within the first lake (Lake Mendota) as well as between lakes to further evaluate patterns of enzymatic activity and relationships between GLUC and other water chemistry parameters at different spatial scales.

2.4.1. Lake Mendota

Lake Mendota is a medium sized eutrophic lake located in Wisconsin, USA (Table 2). The lake has been the subject of many studies in aquatic ecology and limnology over several decades (Brock, 2012; Bryson and Suomi, 1952; Carpenter et al., 2007; Jones et al., 2012) and is regularly monitored by the North Temperate Lakes Long Term Ecological Research (NTL-LTER) program. With a surface area of 39.9 km^2, it is the largest and northernmost lake in a chain of four lakes on the Yahara River (Brock, 2012). Tributaries flowing into the lake's northern bay drain predominately agricultural watersheds. Urban areas (including the City of Madison) are located in the immediate surroundings of the lake and route stormwater run-off directly into the lake. The lake is a popular recreation site and several public beaches are monitored weekly for FIB and cyanobacteria toxins during swimming season by Public Health Madison and Dane County ("*Madison, and Dane County Beaches - Water Quality - Public Health - Madison, and Dane County - City of Madison, Wisconsin [WWW Document]*," n.d.). The dominant pathways and sources of GLUC activity for Lake Mendota are hypothesized to be: (a) tributaries draining urban and agricultural catchments (in the northern part of the lake), (b) leaks from sanitary sewers, (c) geese inhabiting recreational beaches and (d) fecal contamination from diffuse urban sources that reaches the lake via storm drains during storm run-off conditions (in the southern part of the lake).

To assess the impact of hydrological events on the spatial patterns of GLUC in lake water (Table 1), we conducted three surveys on the lake. Each tour lasted 3 h when motor-boating at ~50 km/h. Our goal was to achieve a representative distribution of GLUC measurements over the lake area but also to capture specific locations of interest, such as the confluences of the Yahara River (north shore), Pheasant Branch Creek (WNW shore), and a discharge point of a stormwater run-off channel (south shore). We sampled the lake on three dates that varied with respect to time since precipitation, from 7 h to 6 days after an event. Yahara River discharge and precipitation data for these periods were derived from the USGS stream gauge #05427850 (Yahara River at State Highway 113).

To determine the effects of urban runoff on the GLUC activity of lake water, one spatial survey, focused in detail on the highly urbanized south shore of Lake Mendota (Table 1).

2.4.2. Yahara Lakes

Continuing downstream from Lake Mendota, the Yahara River flows first into Lake Monona and then Lake Waubesa (Brock, 2012). These lakes are smaller than Lake Mendota, with surface areas of 13 and 8 km^2, respectively. While the northern part of Lake Mendota primarily receives water from agricultural watersheds, the proportion of urban areas relative to the catchment size increases moving down the lake chain (ratio lake size to neighboring urban area: 1:2 for Lake Mendota, 1:4.6 for Lake Monona and 1:5 for Lake Waubesa). The dominant pathways and sources of GLUC activity for the chain of lakes are hypothesized to be similar to those described for Lake Mendota, but with an increasing influence of urban sources downstream. We note that wastewater from the greater Madison metropolitan area is routed downstream of the Yahara lake chain.

A one-day measurement-tour followed the Yahara River downstream from its confluence at the north end of Lake Mendota, through Lake Monona and further into Lake Waubesa (Table 1).

Table 1
List of surveyed water bodies. Including key facts such as focus of the survey, water body type, location, dates, weather, measured area and length, number of conducted measurements and distance between measurement points.

Focus of survey	Water body	Type	Location	Date of surveys	Number of surveys	Weather during surveys	Measured stretch (rivers), area (lakes) per survey	Total number of GLUC measurements	Distance between GLUC measurements
Hydrological events	Lake Mendota	Lake	Wisconsin, USA	June 21st, June 29th, July 6th, 2016	3	Dry - rain	40 km^2	38	1.5 km
Urban run-off	Lake Mendota	Lake	Wisconsin, USA	June 21st, 2016	1	Dry	4 km^2	17	0.5 km
Land use	Yahara Lakes	Lake	Wisconsin, USA	June 29th, 2016	1	Dry	50 km^2	23	1.7 km
Land use	Lower Columbia River (LCR)	River	Oregon/Washington, USA	July 12th–18th, 2016	1	Dry	500 km	80	6.0 km
Land use	Upper Mississippi River (UMR)	River	Wisconsin/Minnesota, USA	August 3rd, 2016	1	Dry	50 km	23	3.5 km

2.4.3. Columbia River

The Columbia River is the fourth largest river (by flow) in North America. For the last ~200 km, the Lower Columbia River (LCR) forms the border of Washington and Oregon, ultimately discharging into the Pacific Ocean. Much of the river has been heavily modified by dam constructions, and the river serves as a navigation route and is a significant source of hydroelectrical power and irrigation. The eastern part of the LCR basin is characterized by an arid high-desert environment and is divided by the Cascade Mountain Range from the wet and intensively forested coastal part of the basin. In the eastern LCR watershed, irrigation supports agriculture; e.g., in the Umatilla and Yakima River basins. The western LCR includes large population centers (such as Portland, OR) in addition to a mixture of agriculture and forest land uses. Microbiological studies in the LCR basin have primarily focused on tributaries and the estuary (Crump et al., 1999; Cuffney et al., 2000). The LCR was chosen as a test site because of its variability in climate and land use along the river course. The dominant sources and pathways of GLUC activity into the LCR are assumed to be both from agricultural and urban areas. Primary agricultural sources of GLUC are hypothesized to be areas with livestock, particularly for the Yakima and the Umatilla basins (Cuffney et al., 2000; Leland, 1995). Urban sources of GLUC are assumed to be dominated by the input of wastewater treatment plant effluents into the LCR and its tributaries from population centers, such as Richland, Pasco, Hermiston, The Dalles and Portland (Wünsch et al., 2016).

A survey was conducted on the primary channel of the LCR (Table 1) The research vessel was navigated into select tributaries, including the Snake, Yakima, Umatilla, John Day and Willamette Rivers. A short segment of the LCR (between the Dalles and Bonneville Dams) was not surveyed because it was not safely navigable due to strong winds and high waves. More information describing this survey can be found in Crawford et al., 2017.

2.4.4. Mississippi River

The Mississippi River is the largest river in North America, draining parts of 37 U.S. states before discharging into the Gulf of Mexico. The Upper Mississippi River (UMR) drains one of the most intensively used agricultural regions in the world (NRCS, 2012 "Corn Belt"), known as the U.S. Corn Belt. Heavily impacted by agricultural runoff, the UMR has been the subject of studies focusing on nutrient controls and dynamics as well as greenhouse gas emissions (Crawford et al., 2016; Pellerin et al., 2014; Turner et al., 2016). A system of low-head locks and dams facilitate navigation through the UMR. The dams divide the river into "Pools", which encompass a variety of aquatic habitats, including primary channels, side channels, impounded areas, and backwaters. We surveyed Pool 8, which is an ~50 km section of the UMR near the city of La Crosse, Wisconsin (Table 1). Limnological variables of this pool offer a strong contrast to the LCR (Table 2). The sources and pathways of GLUC activity into Pool 8 of the UMR are hypothesized to be dominated by inputs from agricultural areas from upstream sources of the primary stem and tributaries discharging directly into Pool 8, such as the La Crosse and Root Rivers. Relevant urban sources of GLUC are assumed to be the effluents of wastewater treatment facilities of population centers upstream of Pool 8 (such as Minneapolis) as well as the city of La Crosse adjacent to Pool 8.

3. Results

3.1. Correlation of GLUC activity and conventional E. coli analyses

Grab samples of lake water from different beaches of the Madison Lakes were analyzed in the laboratory with both a defined substrate ISO 9308-2:2012 assay (IDEXX Colilert18®) and the same prototype that was deployed for the ship-borne GLUC measurements. These analyses showed a positive correlation ($R^2 = 0.71$, $p < 0.001$, $n = 18$)

Table 2
Limnological variables and GLUC activities for Lake Mendota, Lower Columbia River and Upper Mississippi River measured during the presented surveys (including confluences, n = number of measurements).

	Lake Mendota			Lower Columbia River			Upper Mississippi River		
	June 21, June 29, & July 6			*July 12–July 18*			*Aug 3*		
	Median	(Min–max)	n	Median	(Min–max)	n	Median	(Min–max)	n
Temperature [°C]	24.6	(22.4–27.3)	*31,153*	20.1	(17.7–28.5)	*90,596*	27.3	(25.1–30.39)	*22,720*
Turbidity [FNU]	2.4	(0.3–19.9)	*31,148*	1.5	(0.0–9.7)	*90,596*	6.1	(1.1–25.95)	*22,718*
SPC [μS/cm]	521	(345–796)	*31,153*	127	(87–419)	*90,596*	371	(210–549)	*22,720*
pH	8.4	(7.5–8.7)	*31,151*	8.0	(7.4–9.7)	*90,596*	7.5	(6.9–8.3)	*22,720*
NO_3-N [mg/l]	0.26	(0.10–2.05)	*1718*	0.11	(0.02–4.14)	*8126*	2.49	(0.0–5.44)	*1898*
Chlorophyll *a* [μg/l]	2.0	(0.4–56.7)	*31,153*	1.0	(<0.1–348.3)	*90,596*	13.0	(3.8–92.1)	*22,720*
fDOM [RFU]	7.8	(6.0–29.6)	*31,153*	0.0	(<0.1–24.5)	*90,596*	28.8	(0.79–30.65)	*22,720*
GLUC [mMFU/100 ml]	6.2	(<0.8–32.9)	*38*	1.8	(<0.8–20.3)	*80*	7.5	(5.0–15.0)	*23*

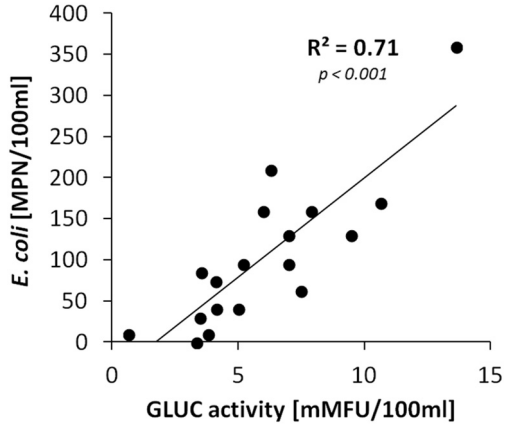

Fig. 1. The correlation of GLUC activity with conventional *E. coli* analyses. Samples were collected from lake water near beaches on Lake Mendota, Lake Monona and Lake Waubesa. The observed R^2 of 0.71 demonstrates the applicability of rapid GLUC measurements to indicate *E. coli* contamination ($n = 18$, p-value < 0.001).

between rapid GLUC activity measurements and results from defined substrate-based *E. coli* analyses (Fig. 1). During the summer season 2016 *E. coli* levels ranged from <10 to 1900 MPN/100 ml at Lake Mendota, from <10 to >24,000 MPN/100 ml at Lake Monona and from <10 to 11,000 MPN/100 ml at Lake Waubesa beaches, respectively (data not shown). The beach closure limit is 1000 MPN/100 ml. Grab samples are collected from shallow water to evaluate the risk for kids. In general *E. coli* results decline with depth.

3.2. Influence of hydrological events on the GLUC activity of lake water

Depending on the time since the last precipitation event and its intensity, the GLUC activity of lake water showed distinct spatial variability (compare Fig. 2A, B, C and D, E, F). While the deep central basin of Lake Mendota had GLUC values close to the limit of detection (0.8 mMFU/100 ml), higher values were observed along the shore, especially at confluences (Yahara River up to 32.9 mMFU/100 ml). Major variation of the GLUC activity was observed in the river-lake transition zone of the Yahara River. When we sampled promptly after a rain event (Fig. 2B, C and E, F), the Yahara River GLUC plume extended further into the lake compared to sample dates after longer periods of no rain (Fig. 2A and D). Elevated GLUC values were observed for all water sources (including Pheasant Branch Creek and the storm drainage on the south shore) just hours after an intense local storm event (Fig. 2C). In Lake Mendota, GLUC activity had the strongest correlation with turbidity ($R^2 = 0.60$, $p < 0.001$) and fDOM ($R^2 = 0.58$, $p < 0.001$) (Table 3).

3.3. GLUC activity in urban lake water

The south edge of Lake Mendota, which was adjacent to a highly urbanized area, had maximum GLUC values immediately adjacent to a storm drain and near the lake's outlet (during dry weather conditions up to 5.0 mMFU/100 ml) (Fig. 3) Within this urban transect, SPC and GLUC activity were negatively correlated (Fig. 3C, $R^2 = 0.73$, $p < 0.001$).

At the larger between-lake scale, we observed an increase in GLUC activity along the gradient of increasing urbanization across the Yahara Lakes chain (Fig. 4). During dry weather conditions, GLUC activity was elevated in the Yahara River confluence zone at 28.7 mMFU/100 ml but settled to an average baseline level within Lake Mendota of 4.1 mMFU/100 ml (STDEV = 1.2). Continuing down the lake chain, the mean GLUC activity increased to 7.3 (STDEV = 2.8) and 9.7 (STDEV = 2.3) mMFU/100 ml for Lake Monona and Lake Waubesa, respectively. Step increases in GLUC activity (up to 13.3 mMFU/100 ml) corresponded to sections of the lakes that received inputs from distinct urban sources, such as storm drains or small urban tributaries that underwent limited mixing with the rest of the lake system (Fig. 4). Across the three lakes, GLUC activity showed strongest correlations with chlorophyll ($R^2 = 0.86$, $p < 0.001$) and NO_3-N ($R^2 = 0.80$, $p < 0.001$) (Table 3).

3.4. Allochthonous sources affecting GLUC activity in rivers

GLUC values along the LCR and selected tributaries (Fig. 5) ranged between <0.8 and 20.3 mMFU/100 ml, with a mean of 2.8 mMFU/100 ml and a median of 1.8 mMFU/100 ml (Table 1). Over long stretches, especially upstream of the John Day River confluence, GLUC values within the LCR primary channel were predominately close to the limit of quantification (0.8 mMFU/100 ml). High GLUC values were observed at the confluences of the Yakima River (20.3 mMFU/100 ml) and the Umatilla River (15.5 mMFU/100 ml), both of which drain agricultural areas (Fig. 5). We observed GLUC values up to 7.3 mMFU/100 ml in the Willamette River as it passed through the highly urbanized area of Portland, OR (Fig. 5). Three major sections of distinct GLUC activity could be recognized along the entire LCR transect. The upper stretch (median: 0.9 mMFU/100 ml) between Richland and Biggs Junction was characterized by low GLUC values in the main channel that were interrupted by brief but high peaks due to tributaries from agricultural areas. A middle section (median: 2.0 mMFU/100 ml) between the John Day River confluence and Willamette River confluence had an increased GLUC activity in the main channel compared to the upper stretch, although local increases associated with tributaries were less pronounced. Finally, the lower section (median: 3.5 mMFU/100 ml), associated with the City of Portland, was characterized by further GLUC increases that corresponded to increasing amounts of waters from urban and agricultural areas accumulating downstream. GLUC measurements decreased in the reach of the Dalles Dam from 3.7 to 1.7 mMFU/100 ml along the length of the impoundment (Fig. 5).

GLUC activity in the LCR was most strongly correlated with chlorophyll ($R^2 = 0.55$, $p < 0.001$) and fDOM ($R^2 = 0.38$, $p < 0.001$) (Table 3). In the fairly homogenous main channel of the LCR, GLUC activity was associated with tributary inlets, which tend to have higher SPC than the main LCR channel (SPC vs GLUC: $R^2 = 0.32$, $p < 0.001$).

Within the UMR, GLUC values in Pool 8 and select confluences ranged between 5.0 and 15.0 mMFU/100 ml, with a mean of 8.0 mMFU/100 ml and a median of 7.5 mMFU/100 ml (Fig. 6, Table 1). Maximum GLUC signals were recorded at the confluences of the La Crosse River (15.0 mMFU/100 ml) and the Root River (13.7 mMFU/100 ml), as both of these tributaries drain agricultural catchments. GLUC values were consistently higher in the UMR compared to the LCR (Figs. 5 and 6), corresponding to a greater proportion of agricultural and urban land use in its watershed. Spatial variability of GLUC activity was greater within the 50-km survey section of the UMR (range of GLUC values excluding tributaries: 5.6 mMFU/100 ml), compared with the almost 500 km section surveyed at the LCR (range of GLUC values excluding tributaries: 4.1 mMFU/100 ml). The correlation with GLUC activity was negative for fDOM ($R^2 = 0.65$, $p < 0.001$) and positive for turbidity ($R^2 = 0.48$, $p < 0.001$). Tributaries had lower fDOM and higher GLUC activity compared to Pool 8.

4. Discussion

4.1. Indicator applicability of GLUC activity

The significant correlation between GLUC and FIB observed for lake water is concordant to previous sampling campaigns conducted at surface water monitoring stations (Ender et al., 2017; Stadler et al., 2016). The results of this sampling campaign point out the potential of rapid GLUC measurements to indicate microbial fecal pollution of water resources. It has been reported that *E. coli* can survive in the environment and therefore be sourced from nonfecal and environmental sources

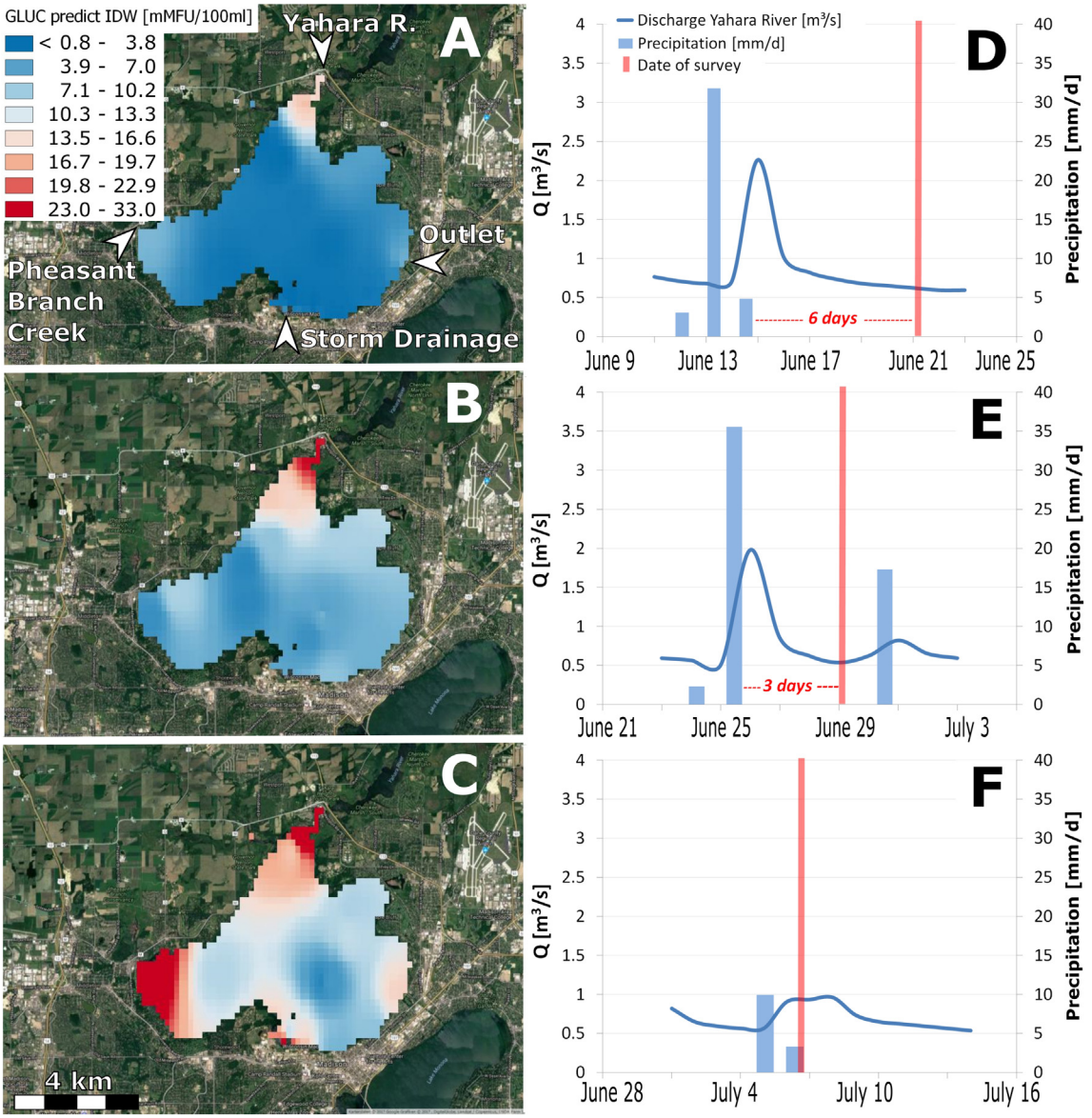

Fig. 2. GLUC activity screening maps of Lake Mendota on the left (A, B, and C) show the diverse spatial patterns of GLUC activity on the lake depending on time since last precipitation event. On the right (D, E, and F) the corresponding discharge of the Yahara River (blue line), precipitation amount (blue bars) and date of survey (red bar) are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Byappanahalli et al., 2003; Garzio-Hadzick et al., 2010; LaLiberte and Grimes, 1982; Lau and Ingham, 2001) which impacts its fecal indicator applicability significantly. We cannot exclude that the results shown here are not affected by an environmental growth of *E. coli*.

Regarding interference effects between algae and GLUC, we emphasize that the presented surveys aimed for a qualitative water quality screening. The mentioned cross sensitivities between GLUC and organic matter may limit the quantitative validity of the absolute GLUC values presented. However, several recent studies conducted in various aquatic habitats all showed that a relative increase of GLUC activity is capable to indicate a potential microbial contamination in waters (Ender et al., 2017; Stadler et al., 2017, 2016).

Table 3
Correlation (linear regression) R^2 between GLUC activity and limnological variables for the surveyed water bodies. Star code indicated the significance level (***: p-value < 0.001, **: p-value < 0.005, *: p-value < 0.05, n = number of measurements). A negative correlation is indicated by (−).

	Lake Mendota			Yahara Lakes			Lower Columbia River			Upper Mississippi River		
GLUC vs.	n	R^2	p	n	R^2	p	n	R^2	p	n	R^2	p
Turbidity [FNU]	*38*	0.60	***	*23*	0.41	***	80	0.25	***	*23*	0.48	***
SPC [µS/cm]	*38*	0.04		*23*	0.00		80	0.32	***	*23*	0.20	*
fDOM [RFU]	*38*	0.58	***	*23*	0.68	***	80	0.38	***	*23*	(−) 0.65	***
Chlorophyll *a* [µg/l]	*38*	0.35	***	*23*	0.86	***	80	0.55	***	*23*	0.17	*
NO_3-N [mg/l]	*24*	0.16	*	*23*	0.80	***	80	0.26	***	*23*	0.00	

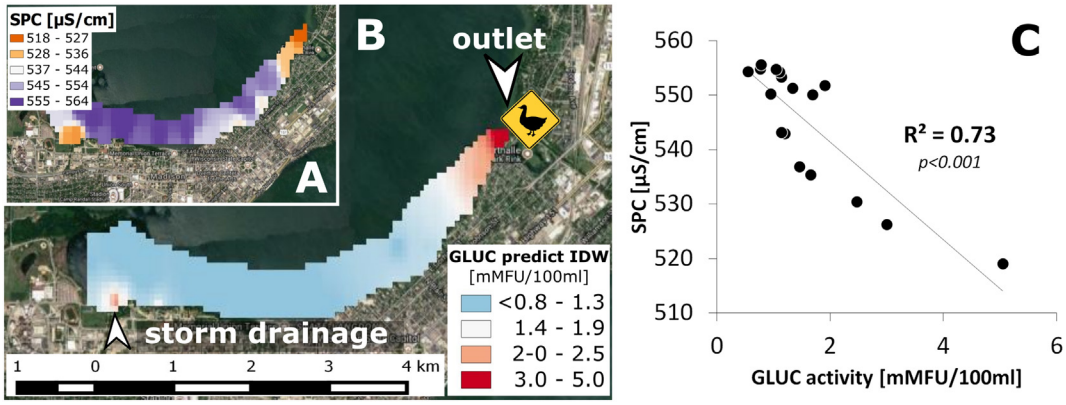

Fig. 3. SPC (A) and GLUC activity (B) screening maps along an urban shoreline of Lake Mendota showing SPC and GLUC activity at a storm drain and near a park at the lake's outlet (goose symbol). GLUC activity in lake water neighboring the City of Madison was negatively correlated with specific conductivity (C, $R^2 = 0.73$, n = 17, p-value < 0.001).

4.2. GLUC activity in lake water

Hydrological processes were determined to be the primary driver of spatial GLUC heterogeneity in Lake Mendota. GLUC signals were determined predominantly by the influence of tributaries and confluences on the receiving water body, rather than in-lake processes (such as thermal- or wind induced mixing, browning or local aggregations of algae). As turbidity and fDOM are derived from tributaries, the association of these parameters with GLUC activity is interpreted as the dominant influence of rivers, such as the Yahara River, on the GLUC activity of Lake Mendota. The rivers that flow into the lake's northern bay drain agricultural catchments that support row crop and dairy farms, and thus carry large suspended sediment and nutrient loads and presumably contain fecal pollution from livestock waste, especially during high flow (Kitchell, 2012; Lathrop et al., 1998). In terms of indicator applicability of GLUC for a potential fecal pollution the series of three surveys conducted during different times after rain events, can be interpreted as positive and negative control: During high flow, shortly after a storm we assumed increased FIB levels in stream water (such as the Pheasant Branch Creek). The survey conducted on July 6th enabled a positive control, as high GLUC values above 20 mMFU/100 ml were recorded in the western part of the lake (Fig. 2C). During low flow we assume a minimal fecal contamination of the Pheasant Branch Creek. The survey conducted on June 21st, during dry weather enabled a negative control when GLUC values below 10 mMFU/100 ml were measured in the same area.

4.3. Influence of urban run-off on GLUC activity

The negative correlation of SPC and GLUC within the urban bay of Lake Mendota is interpreted as an indicator for urban run-off. Following the last rain event, the low-SPC overland flow had yet to completely mix with lake water, leading to localized areas of low SPC water near the shore (Fig. 3A). These areas with low SPC were associated with higher GLUC activity, supporting the assumption that the presence of FIB in the lake is predominantly caused by inputs of allochthonous water, likely urban runoff following heavy precipitation. The increased GLUC values at the confluence of the storm-drainage (Fig. 3B) were likely due to urban drainage water. The second area of high GLUC activity is adjacent to a public park where geese frequently congregate (Heftey, 2011) (Fig. 3B, goose symbol), thus bird feces were the likely source of fecal contamination.

Strong correlations between GLUC activity, chlorophyll and NO_3-N were determined along a gradient of urbanization along the chain of

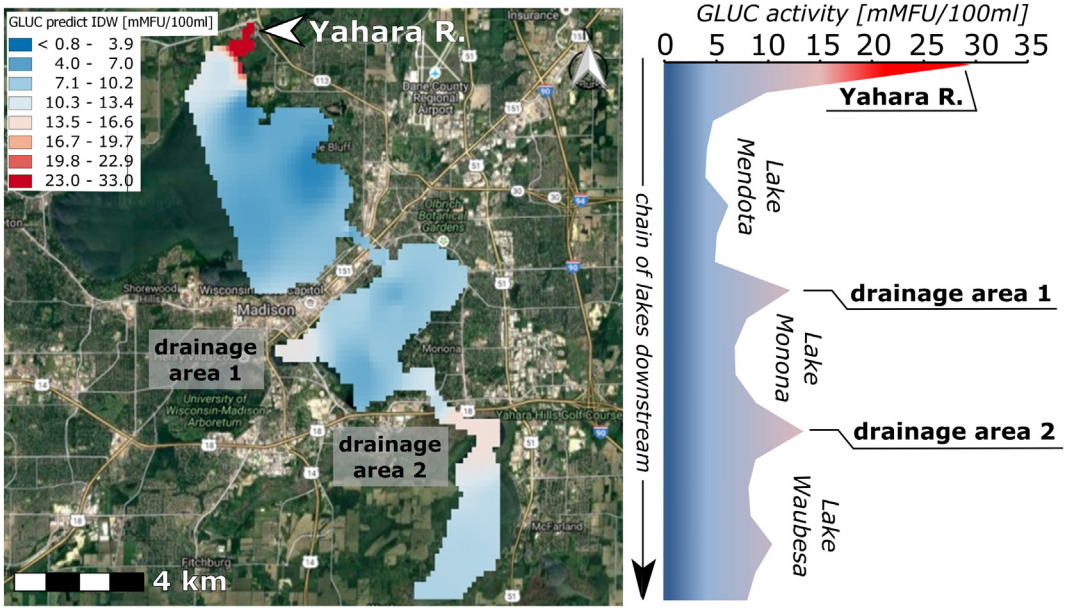

Fig. 4. On the left, GLUC activity screening map along a gradient of urbanization following the chain of the Madison Lakes downstream through Lake Mendota, Lake Monona and Lake Waubesa. Areas that are assumed to contribute significant amounts of urban run-off are marked as drainage area 1 and drainage area 2.

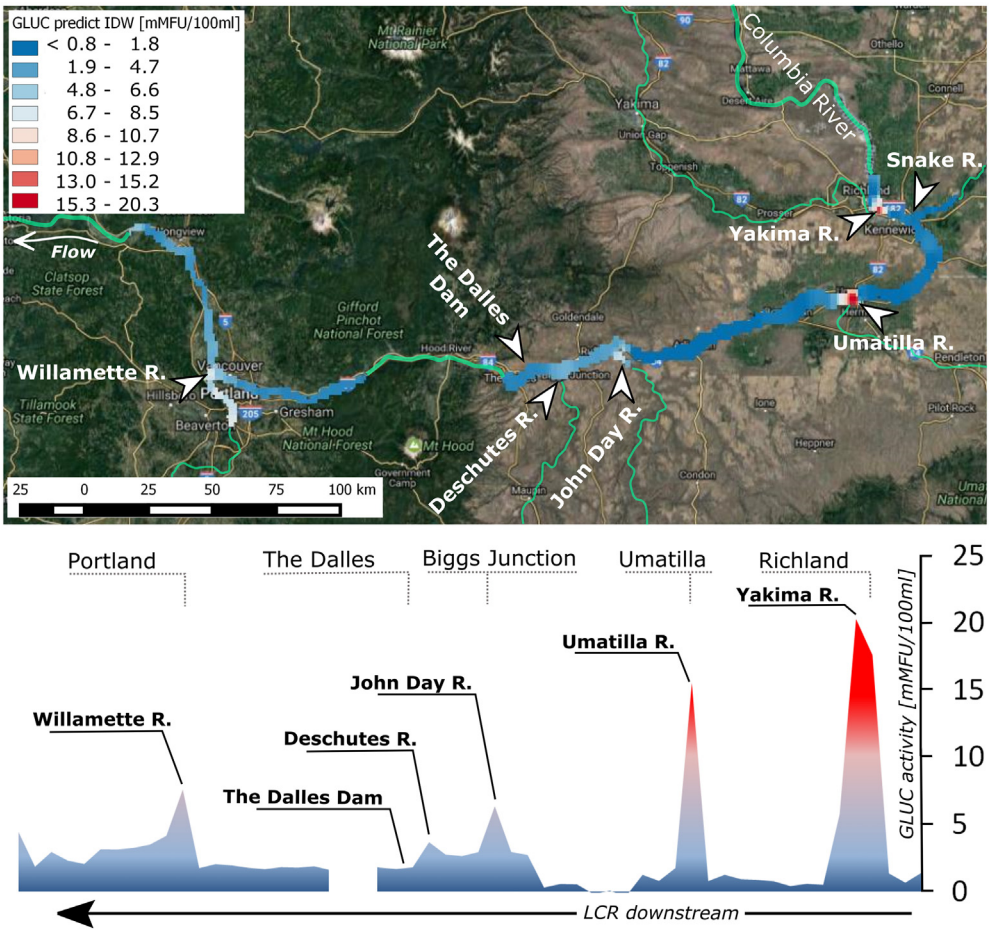

Fig. 5. GLUC activity screening map (top), shows GLUC activity in the LCR and its confluences. The graph on the bottom shows the corresponding course of GLUC activity, showing peaks of GLUC activity due to tributaries and population centers, a decrease of GLUC values within the reach of The Dalles Dam and an increase of GLUC activity in the LCR heading downstream.

lakes. We believe this correlation was a consequence of hydrologic transport leading to parallel signals. Nutrients promoting algae growth reach the lower lakes via storm drainages and surface runoff. Fecal pollution from pet waste or urban wildlife and suburban drainage areas, as well as leaking sewer lines, migrate along the same pathway, leading to similar spatial patterns.

4.4. GLUC activity in rivers

Tributaries draining agricultural and urban areas were the predominant driver of the spatial patterns of GLUC activity in both the LCR and the UMR. Anthropogenic influences were indicated by the association between GLUC activity and nutrients, fDOM and consequently chlorophyll. The association of GLUC with turbidity is interpreted as a result of the influence of tributaries draining agricultural catchments susceptible to soil erosion that are likely to deliver fecal contamination from livestock waste.

The higher variability of GLUC activity in the UMR, compared to the LCR, is assumed to be a consequence of the braided fluvial network of backwaters and side channels and has been reported for a variety of other variables, such as nutrients, carbon gases and aquatic macrophytes (Crawford et al., 2016, 2015; De Jager and Houser, 2012; Houser and Richardson, 2010).

A potential influence of river impoundments on the fate of GLUC active organisms has been recorded in the LCR within the impoundment of the Dalles dam. The retention of particle-associated microorganisms due to increased sedimentation within impoundments has been reported (Gannon et al., 1983) and is a plausible explanation for the observed decrease of GLUC activity within the impoundment of the Dalles dam.

4.5. Capability of ship-borne GLUC measurements

Preventing human exposure to water-borne pathogens and providing better insights into the fate and transport of fecal indicators are challenging tasks to which ship-borne GLUC measurements can contribute. We have been able to demonstrate that ship-borne GLUC measurements are a promising screening tool that can provide near real-time maps that indicate fecal *E. coli* contamination of water resources. The ship-borne strategy can give an initial overview on the microbial variability at understudied sites and allow a more purposeful selection of sampling locations. This tool can improve health-related surveillance of waters and potentially lead to faster decision-making and management actions (e.g., beach closures). High spatial resolution GLUC measurements are also valuable for contaminant transport models. The GLUC values presented in this study were consistent with previously published studies, ranging between nearly unpolluted groundwater (Ryzinska-Paier et al., 2014) and surface waters impacted by municipal sewage (Farnleitner et al., 2002; Garcia-Armisen et al., 2005; George et al., 2000) and manure (Stadler et al., 2016). However, as a tool to replace conventional microbiological assays, we urge caution; a reasonable correlation between GLUC and *E. coli* was observed for lake water, but this is below the 0.95 threshold required for proxy parameters (Stadler et al., 2010). We encourage following up these enzymatic activity screening surveys with additional culture-based methods.

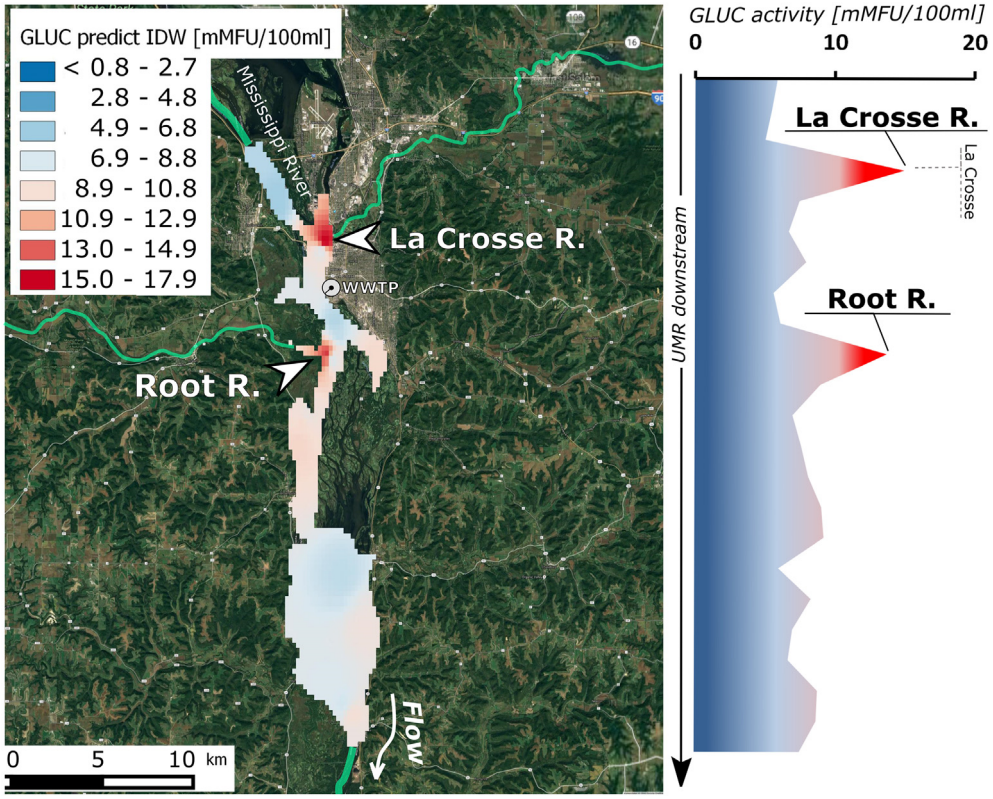

Fig. 6. On the left, GLUC activity screening map of Pool 8 of the Upper Mississippi River (UMR), showing the influence of the La Crosse and the Root Rivers, both draining agricultural catchments. The graph on the right shows the corresponding course of GLUC activity, showing peaks of GLUC activity due to the tributaries.

4.6. Expanded developments and future perspectives

To ask ecological questions that go beyond fecal microbial contamination, the chemical enhancement of available substrates (in terms of e.g. temperature stability of MUG) and the preparation and application of new substrates will enable a more diverse assessment of enzymatic activities in waters. Developments of buffers and reagents are underway to allow automated enzymatic assays in saline environments.

5. Conclusions

Surface water GLUC activity varied among the study sites and was primarily related to hydrologic inputs. More specifically, our surveys point to human-dominated water sources as key drivers of GLUC activity in surface waters. The comparison between the LCR and the Yahara Lakes highlighted increasing downstream GLUC signals that corresponded to greater anthropogenic influences in both systems. In general, each system had different correlations between GLUC and limnological variables, but the results from all surveys point to externally derived GLUC, indicated by abrupt changes in water chemistry associated with tributaries and other water sources. For future studies, we do not recommend using these correlations to predict GLUC activity, but rather to use them to infer distinct water masses that may differ in their microbial quality.

The ship-borne approach of combining rapid microbial methods with limnological observations described in this study is not constrained to enzymatic assays: In general, we see the integration of emerging rapid microbiological on-line methods into platforms for ship-borne measurements of physicochemical variables as a powerful methodology to improve data interpretation and process understanding within the fields of aquatic ecology, health-related water quality and water resource management.

Notes

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