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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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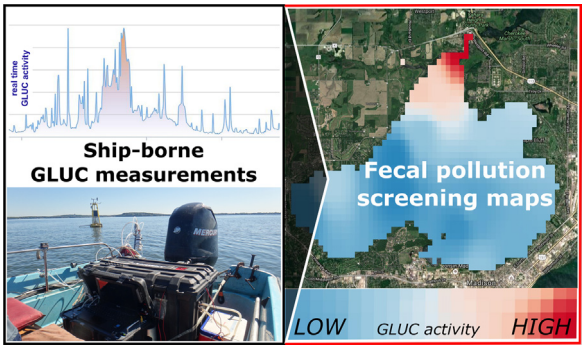
HIGHLIGHTS

- 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.