Doctoral Thesis

Rapid on-site determination of enzymatic activity in water resources: From the technical assessment towards new perspectives in water quality monitoring.

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Abstract

Health-related water quality research, but also the management, allocation and utilization of water resources could highly benefit from an enhancement of the temporal and spatial resolution of microbial parameters. Recent technological developments have brought automated on-site measurements of enzymatic activity within the reach of real time monitoring and the detection of enzymatic activities has been proposed as a rapid surrogate for the microbiological pollution monitoring of water resources. Such automated enzymatic assays, operated on-site and online have the potential to indicate microbial contamination of waters in near-real time, are therefore of great interest to be implemented into early warning systems and could significantly enhance our understanding of contaminant transport processes and pathways.

By proceeding from technically and method oriented science questions towards the applied utilization of a novel on-site monitoring technique, the goals of this thesis are: To test the technical realization of prototypes for the rapid and automated determination of beta-D-glucuronidase (GLUC) activity in sediment sediment laden streams, to disclose the indicator applicability of such assays for culture-based fecal indicator bacteria *E. coli*. and to use this novel technique within an interdisciplinary framework to improve modern-day water quality research.

A long-term and continuous field deployment of measurement equipment is the key to generate time-series data, suitable to capture both event- and seasonal dynamics of crucial parameters, such as GLUC activity. In Chapter 2 an auto-sampler is presented, that was designed to be used as sample pretreatment in order to allow on-site measuring apparatuses to be operated in sediment laden water resources. The device was called SAMP-FIL and delivered automatically a filtered ambient water sample to the connected measurement equipment. Focus has been set on minimal sample alteration as well as on an effective self-cleaning procedure. It was controlled by a RaspberryPi microcomputer. The SAMP-FIL sample pretreatment was tested for over one year for rapid and on-site GLUC determination in sediment-laden stream water. The installation of the SAMP-FIL increased the error-free running time and measurement accuracy of the connected devices and enabled the continuous operation of the measuring devices in a technically challenging environment.

Using the development from the previous chapter and having the technical challenges for long-term on-site operation of GLUC measurements disclosed, the next step was composed of testing the method´s robustness and assessing the plausibility of gathered data. GLUC activity values yielded with two different prototype construction designs have been compared and the temporal dynamics of these signals, their variation due to hydrological events and seasons as well as their correlation with microbial standard analyses have been assessed in Chapter 3. Four prototypes for rapid GLUC measurements have been operated at the stream monitoring station at the experimental HOAL catchment for over two years. Field tests, reference sample campaigns and event monitoring were used to

highlight the capability and limits of rapid GLUC measurements in surface waters. The experiments suggest very consistent results of the instrument pairs with the same construction design, and somewhat lower consistency for different designs. GLUC activity is less well associated with suspended sediment concentrations than *E. coli* which is interpreted in terms of indicator applicability. This chapter shows that this rapid assay can yield consistent results over a long period of on-site operation in technically challenging habitats.

Different water resources, such as ground- or surface water, constitute fundamentally differing operation environments for rapid enzymatic assays in regards to the range and temporal dynamics of signals as well as the concentration of organic and inorganic matter causing potential interference effects. Chapter 4 evaluates the operation of rapid GLUC measurements in different water resources. It is shown that the evaluated apparatuses for automated enzymatic activity measurements were technically robust for long-term on-site monitoring at diverse sites, ranging from pristine groundwater to sediment-laden stream water. Near real-time automated enzymatic activity measurement can thus be considered to be realized successfully from a technical point of view. The generated near-real-time data on GLUC activity point to habitat-specific differences with regard to their proxy capacities for culture-based fecal pollution detection.

While Chapter 3 and Chapter 4 are focused on the temporal dynamics of GLUC activity at definite monitoring stations in different water resources, Chapter 5 assesses the capability of automated enzymatic activity measurements conducted from a mobile research vessel to detect the spatial variability of beta-D-glucuronidase (GLUC) activity on large fresh water bodies. Surveys have been performed on the Columbia River, the Mississippi River and on Lake Mendota covering up to 500 km river course or 50 km^2 lake area, respectively. The observations provide for the first time high resolution spatial data of GLUC activity on large water bodies and document its association to hydrological conditions and land use. The ship-borne measurements disclosed effects of precipitation events and urban run-off on the GLUC activity of inland waters, localized point in-lets of potential fecal pollution and showed an increasing GLUC signal along a gradient of urbanization.

The results of this thesis point out, that automated on-site methods based on specific enzymatic activity monitoring have great potential to be integrated in early warning systems, use oriented protection of water resources and process control. By determining the potential and constrains of rapid on-site enzymatic methods, the thesis paves the ground for a purposeful integration of such prototypes as a novel complementary parameter into well-established monitoring systems to improve data interpretation and process understanding within the fields of health related water quality and water resource management. Innovative applications of automated enzymatic assays, such as ship-borne measurements of GLUC activity, are described in this thesis for the first time to highlight new perspectives in water quality monitoring.

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Chapter 1

Introduction

1.1. Background and research questions

Although the ***transport of microbial contaminants***, like pathogenic microorganisms, is mainly driven by the hydrology, in many cases ***hydrologic observations*** have not been coupled with measurement of concentrations of microorganisms (Pachepsky et al., 2006). To date the behavior of pollutants in soils and their interactions with hydrologic processes ***at catchment or regional scales*** has still been only partially understood (Botter et al., 2009). Compared to nutrient parameters, microbial flux analysis received much less attention from research and policy communities (Kay et al., 2007). Relating to water safety planning or management approaches there is a need for better understanding the dynamics of microbial transport (Tyrrel and Quinton, 2003). The "*Rotorua Declaration*" (adopted at a joint meeting of the IWA ***Health Related Water Microbiology*** Symposium and the ***Diffuse Pollution*** Conference held in Rotorua, NZ in Sept. 2011) urges the need for measurements in high temporal resolution to reflect the highly episodic nature of microbial flux. Furthermore, the importance of understanding the complex indicator-pathogen relationships through specific research is advised. ***Rivers and lakes*** are as receiving water bodies widely impacted by discharge from urban, industrial or agricultural areas often containing pathogenic bacteria (Bradford et al., 2013; Ferguson et al., 2003; Mawdsley et al., 1995; Pachepsky et al., 2006; Tyrrel and Quinton, 2003). Health-related water quality research, but also the ***management, allocation and utilization*** of such water resources could highly benefit from an enhancement of the spatial resolution of microbial parameters. Yet, the understanding of large water bodies´ microbiology, especially concerning fate, transport processes and pathways of microbial pollutants, is predominantly based on assays requiring elaborate sampling and laboratory efforts resulting in limited spatial and temporal resolution (João P. S. Cabral, 2010; Savio et al., 2015).

A parameter that indicates the microbiological water quality and can be measured on-site in high temporal resolution would be a valuable tool within an interdisciplinary framework to address these needs.

Cultivation based standard analyses of fecal pollution typically take one to several days and are therefore not suitable for rapid water quality assessment (J. P. S. Cabral, 2010). Instead, methods involving ***enzymatic activity*** have been tested in various aquatic habitats and suggested as a surrogate for culture-based microbiological pollution monitoring (Farnleitner et al., 2001, 2002; Fiksdal and Tryland, 2008; Garcia-Armisen et al., 2005). The determination of fecal indicator bacterial members by means of specific enzymatic activity of e.g ***beta-D-***

Glucuronidase (GLUC), galactosidases and esterases are nowadays commonly used and there are various chromogenic and fluorogenic substrates for the specific detection of these enzymatic activities (Fiksdal et al., 1994a; Morikawa et al., 2006; Noble and Weisberg, 2005; Rompré et al., 2002; Wildeboer et al., 2010). Over the last two decades, several studies have suggested the use of direct enzymatic activity determination to monitor microbiological contamination in various water sources (Farnleitner et al., 2002; Fiksdal et al., 1994b; Fiksdal and Tryland, 2008; George et al., 2001). A high correlation of GLUC activity with *E. coli* has been reported for waters impacted by distinct sources of fecal contamination, such a municipal sewage (Farnleitner et al., 2001, 2002). Nevertheless, these common enzymatic activity measurements for fecal indicator bacteria require laboratory facilities and elaborate sampling methods (Lebaron et al., 2005; Rompré et al., 2002); Rompré et al. stated in 2002 that such methods, compared to culture-based standard assays, are more expensive and incubation time remains too long for same-day results. Valuable ***technological progress*** occurred in the last decade and modern prototypes allow nowadays a ***fully automated and rapid on-site determination*** of enzymatic activity, suitable for a near-real time monitoring of waters (Koschelnik et al., 2015; Ryzinska-Paier et al., 2014; Zibuschka et al., 2010). Measurements were reported to be possible in less-than-hourly intervals, reaching a temporal resolution of up to 15 minutes (Koschelnik et al., 2015; Stadler et al., 2016). These on-line enzymatic methods could be highly beneficially in a ***health related water quality surveillance*** and can be a valuable complementary tool for a high temporal resolution monitoring (Cabral, 2010). The potential of near-real time monitoring of enzymatic activity seems to be specifically high for the implementation into early warning systems and ***industrial process control***. Moreover, this method will help to improve the ***understanding of catchment behavior*** as well as contaminant transport processes and pathways in different habitats.

In order to utilize such novel monitoring techniques, ***there is a need for studies*** testing prototypes for automated and on-site enzymatic activity determination to ***evaluate the consistency of measurement*** results, ***technical robustness*** during long-term on-site operation, and ***proxy capability*** for culture-based microbiological analyses in the observed habitat. An innovative utilization of automated and on-site enzymatic activity determination is needed to determine the method´s potential and constrains. The ***coupling of on-line hydrological observations*** with near-real time data from automated enzymatic activity measurements will enhance our knowledge of microbial transport dynamics, ranging from ***event- to seasonal scale***. Using transportable prototypes to generate GLUC activity data from mobile research vessels can help to disclose previously unknown ***spatial patterns of enzymatic activity*** on large water bodies, reflecting the potential fecal associated contamination of water resources. Such endeavors can yield new tools for a ***rapid water quality screening*** and can help to localize point-inlets of potential fecal pollution in near-real time. Furthermore, it can enable a ***more purposeful arrangement and selection of sampling points*** for elaborate microbiological methods, such as DNA extraction and quantification of bacterial DNA.

By ***proceeding from technically and method oriented science questions towards the applied utilization*** of a novel on-site monitoring technique, the goals of this thesis are: To ***test the technical realization*** of prototypes for the rapid and automated determination of beta-D-glucuronidase (GLUC) activity in water resources, to ***disclose the indicator applicability*** of such assays for culture-based fecal indicator bacteria *E. coli*. and to use this novel technique within an interdisciplinary framework to ***improve modern-day water quality research***. In order to achieve these goals following research questions are addressed within this thesis:

- What are the technical requirements to conduct automated enzymatic activity measurements long-term and on-site in sediment laden stream water? (Chapter 2)
- Can low-cost microcomputers be used by researches to control automated on-site devices?(Chapter 2)
- Are measurements of enzymatic activity conducted by different prototype constructions comparable? (Chapter 3)
- What dynamics of beta-D-Glucuronidase can be captured in stream water draining an agriculturally used headwater catchment? (Chapter 3)
- How are automatically measured beta-D-Glucuronidase activity and culture-based *E. coli* analyses correlated? (Chapter 3)
- Is the association of automatically measured beta-D-Glucuronidase activity and culture-based *E. coli* analyses catchment/habitat specific? (Chapter 4)
- Can rapid enzymatic activity measurements be used in an interdisciplinary framework to reveal spatial patterns of beta-D-Glucuronidase activity on large surface water resources? (Chapter 5)
- Can ship-borne measurements of beta-D-Glucuronidase activity be used as a rapid screening-tool to localize potential sources and pathways of fecal contamination? (Chapter 5)

1.2. Structure of the thesis

This thesis is organized by chapters. ***Chapter 1*** is a consolidating introduction. The chapters 2 to 5 are each based on a manuscript that has been published by or will be submitted to a scientific peer-reviewed journal or scientific peer-reviewed book. ***Chapter 2*** describes the design and field test of an automated and programmable auto sampler that has been deployed at the HOAL catchment for rapid enzymatic activity measurements. Chapter 2 has been published in Talanta - International Journal of Pure and Applied Analytical Chemistry (Stadler et al., 2017a). ***Chapter 3*** compares prototypes for rapid enzymatic activity determination in sediment laden stream water. Chapter 3 has been published in the journal Water

Research (Stadler et al., 2016). ***Chapter 4*** assesses the applicability of rapid enzymatic activity determination in various water resources. Chapter 4 has been published in the Handbook of Online and Near-real-time Methods in Microbiology (Stadler et al., 2017b). ***Chapter 5*** uses ship-borne and rapid enzymatic activity measurements to disclose the spatial variability of enzymatic activity on large surface water resources. Chapter 5 is the final draft stage of a manuscript to be submitted to the journal Environmental Science & Technology. ***Chapter 6*** concludes the previous chapters in a comprehensive manner.

Chapter 2

Development and evaluation of a self-cleaning custom-built auto sampler controlled by a low-cost RaspberryPi microcomputer for online enzymatic activity measurements

2.1. Abstract

A fully automated on-site device (SAMP-FIL) that enables water sampling with simultaneous filtration and effective cleaning procedures of the device's components was developed and field-tested. The SAMP-FIL was custom-built using commercially available components and was controlled by a RaspberryPi single-board computer operating open-source software. SAMP-FIL was designed for sample pre-treatment with minimal sample alteration to meet the requirements of on-site measurement devices that cannot handle coarse suspended solids within the measurement procedure or cycle. A highly effective cleaning procedure provides a fresh and minimally altered sample for the connected measurement device. The construction and programmed software facilitates the use of SAMP-FIL for different connected measurement devices. The SAMP-FIL sample pretreatment was tested for over one year for rapid and on-site enzymatic activity (beta-D-glucuronidase, GLUC) determination (BACTcontrol) in sediment-laden stream water. The formerly used proprietary sampling set-up was assumed to lead to significant damping of the measurement signal due to its susceptibility to clogging, debris accumulation and bio-film accumulation. The implementation of SAMP-FIL considerably increased the error-free running time and measurement accuracy of BACTcontrol devices. This chapter describes how low-cost microcomputers, such as the RaspberryPi, can be used by operators to substantially improve established measuring systems via effective sampling devices. Furthermore, the results of this study highlight the importance of adequate sample pretreatment for the quality of on-site measurements.

2.2. Introduction

On-site monitoring of chemo-physical and bio-chemical parameters in surface waters are currently standard procedures in various fields, such as hydrology, limnology and civil engineering. Although technological progress and scientific

questions have advanced on-site monitoring of water resources to higher temporal and spatial resolutions, measurement systems are still technically challenged by common environmental factors, such as suspended solid concentrations. Emerging parameters to be monitored on-site (e.g., enzymatic activity) require measurement devices with complex construction design, including valves and hoses with diminutive apertures (Stadler et al. 2008; Zibuschka et al. 2010; Ryzinska-Paier et al. 2014; Koschelnik et al. 2015; Stadler et al. 2016). The effect of suspended organic and inorganic matter on the accuracy of measurement results and the device running time of such methods are particularly high in stream draining catchments susceptible to soil erosion (e.g., agricultural catchments). In such cases, sample pretreatment becomes an unavoidable component of the procedure (Stadler et al. 2016) to meet the technical requirements of the measuring devices and prevent valves or tubing from clogging. The required sample pretreatment procedure includes filtering of the natural water sample and filling a vessel from which the connected instruments draw the sample for the intended measurement. As manufacturers may have limited insight and comprehension into the determining factors of specific monitoring locations, proprietary solutions occasionally do not meet the specific demands necessary to enable the optimal operation of the respective measurement instruments. Pre-assembled and commercially available modules for filtration, which allow back flushing and filter cleaning in combination with adequate pumping, can be used for on-site sample pretreatment. These modules are often limited in terms of sampling volume and filter size or have a fixed filter mesh width and thus have strong constraints regarding the conversion of the set-up for other purposes (e.g., connection to another measurement instrument). Furthermore, such modules may provide considerable inside surfaces and cavities for debris and bio-film accumulation, leading to potential adulteration of the measurement signal.

Several types of low-cost microcomputers (e.g., Arduino, RaspberryPi) were released during the last decade and are currently often used within a wide user area of the open-source community (Richardson & Wallace 2012; Upton & Halfacree 2012; Ali et al. 2013; Hut et al. 2013; Monk 2013). The possibility to connect and control peripheral devices (e.g., sensors, relays) due to embedded ports (e.g., USB) or general-purpose input/output (GPIO) and the ability to operate self-programmed scripts (e.g., Python) have great potential within the field of environmental science, particularly regarding measurement engineering and monitoring. Recent studies have described the application of low-cost single-board computers for implementation in monitoring systems, e.g., as a strategic interface or data-logger (Hut et al. 2013; van de Giesen et al. 2014, 2014; Salam et al. 2014; Vujovic & Maksimovic 2014; Sukaridhoto et al. 2015). However, there is a lack of scientific literature describing the selection and assembly of hardware and software in a comprehensive manner to enable the reader to reproduce the same or similar set-ups.

The authors of this chapter describe how a robust and effective RaspberryPi controlled autosampler for sample pretreatment, necessary for the on-site enzymatic activity determination in surface water, was developed in a step-by-step manner. Instructions from open-source platforms (e.g.,

www.raspberrypi.org/forums) have been adapted to this special demand, and the construction was designed following the authors operating experience of automated on-site enzymatic activity determination in surface water.
The main objective was to construct a programmable sample pretreatment set-up that has an insignificant impact on the natural water sample and is resistant against in-device debris and bio-film accumulation. The hardware components and programmed software are selected and constructed in a manner that easily enables the connection of the SAMP-FIL to another on-site instrument by modification of the sample volume, filter size and operation cycles. A 16-month field campaign was conducted to test the constructed device (SAMP-FIL) in terms of its robustness for continuous long-term automated operation, its impact on measurement accuracy, and the running time of the connected instruments (BACTcontrol).

2.3. Material and Methods

On-site detection of enzymatic activities has been suggested as a rapid surrogate for the monitoring of microbiological pollution in water resources (Cabral 2010; Fiksdal et al. 1994; George et al. 2000; Farnleitner et al. 2002). Due to the potential short measuring intervals, this method has high potential as a near-real-time water quality monitoring tool and can contribute important information for identifying faecal contamination. To understand the dynamics and transport processes of faecal-associated contamination in stream water, two devices (BACTcontrol, MicroLAN, Netherlands) for the automated and rapid determination of enzymatic activity (beta-D-glucuronidase) were operated for stream water monitoring at the catchment outlet since 2012. During the measurement process, the sample mixed with specific assay reagents generated an increasing fluorescence signal that reflected the level of enzymatic activity, which was monitored over time. The construction design and sampling and measurement procedures of the BACTcontrol devices have been described in detail by Zibuschka et al. (2010) and Ryzinska-Paier et al. (2014). Although the measurement principle yielded consistent long-term data from automated on-site operation in ground water monitoring (Ryzinska-Paier et al. 2014), the use of these instruments for surface water monitoring is a technical challenge due to the high suspended solid concentrations during event run off conditions. Outages of BACTcontrol devices due to technical failure or service are substantially higher when operated in surface water monitoring compared to ground water monitoring (Ryzinska-Paier et al. 2014; Stadler et al. 2016). To establish long-term on-site operation, in this case, sample pretreatment (filtration through 100 µm) was an unavoidable step (Stadler et al. 2016). The BACTcontrol instruments conducted measurements every three hours and were housed in an air-conditioned measurement station situated at the brookside, one meter above the water level.
The proprietary sample pretreatment set-up (Fig. 1 A) was based on a submersible pump that was placed in the stream and constantly pumping water with a flow rate of 1.5 l/min through a flow-through housing with a 100 µm filter into a 10 l sample container. The sample container was equipped with an overflow, enabling

a constant flow-through and complete exchange of the pre-treated water within less than 7 minutes. The dimensions and flow-through rates were designed to enable sedimentation of fine material (<100 µm) within the container. The BACTcontrol devices obtained the required sample volume (100 ml) for the GLUC measurements from this sample container every 180 minutes (Fig. 1 A). The filter cartridge and hoses had to be manually cleaned on a biweekly basis. The components of this set-up were designed for long-term usage and proved technically robust, with failure-free running times up to 12 months. Nevertheless, evaluation of the continuously measured GLUC signal showed that a majority of GLUC measurements were delayed up to several hours from the hydrological parameters monitored in parallel, such as the stream discharge and turbidity. In particular, during event runoff conditions, the GLUC activity is assumed to be strongly correlated with discharge and turbidity, as these parameters indicate the potential input and transport of surface-associated faecal pollution in the stream. Furthermore, the monitored peaks of GLUC activity appeared to be significantly damped in many cases. The described phenomena occurred despite regular cleaning of the filter cartridge and hoses, indicating that substantial parts of the inner surfaces of the components (e.g., pump, fittings, tubing) are not accessible for the on-site and manual cleaning procedure and that the constant water flow led to bio-film growth, deposition of debris and sintering, causing the retention and delayed release of beta-D-glucuronidase-producing organisms (e.g., E. coli) into the measuring device.

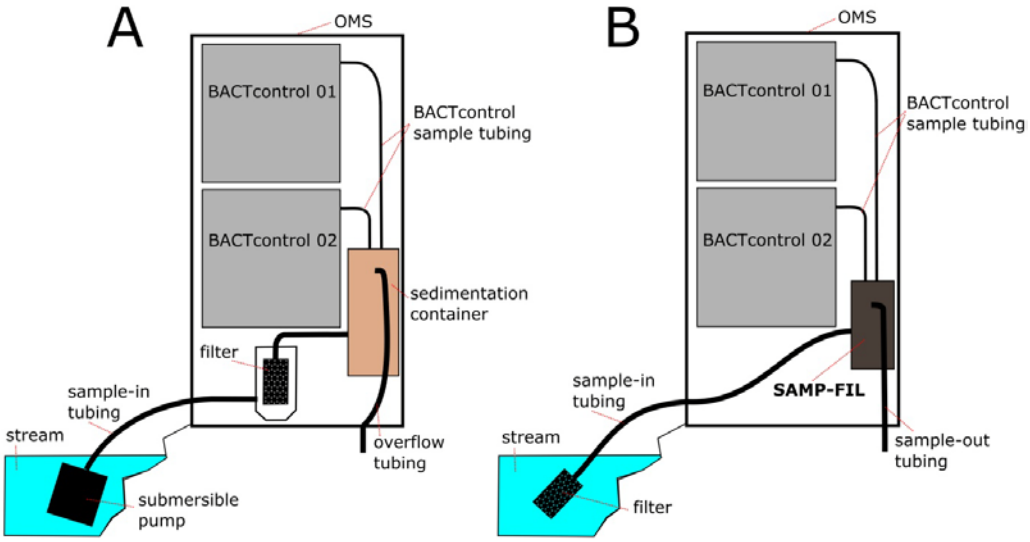

Fig. 1: Schematic of the basic sample pretreatment set-ups and fundamental components of the outdoor monitoring station (OMS). A: The proprietary set-up with the constantly operated submersible pump located in the stream, filter (cartridge enclosed in housing) and sedimentation container. B: Set-up with SAMP-FIL, where the filter cartridge is located in the stream and sample water is provided to the BACTcontrol devices by SAMP-FIL in coordination with their measurement intervals.

2.3.1. Test site

The Hydrological Open Air Laboratory (HOAL, (Blöschl et al., 2015, 2011)) in Petzenkirchen (Lower Austria) is operated and maintained by the Institute for Land and Water Management Research (*Federal Agency for Water Management, Austria*) and the Vienna Doctoral Programme of Water Resource Systems (*Centre for Water Resource Systems, Vienna University of Technology, Austria*). The catchment is 0.66 km² in area and is drained by a 620 m stream. Twelve point discharges contribute to the discharge of the stream. These include tile drains, springs and surface tributaries (Exner-Kittridge et al. 2013). The mean annual precipitation is 823 mm/yr (1990-2014). The land use of the catchment area is dominated by agriculture, consisting of 83% arable land, 7% grassland, 7% forested area, and 3% paved surface. The hydrogeology is characterized by porous and fissured aquifers consisting of clay, marl and sand. Soils show medium to limited infiltration capacities. The annual sediment erosion is approximately 1 t/ha (Eder et al. 2010). The monitored stream shows high discharge dynamics (minimum discharge 2014: 0.5 l/s, maximum discharge 2014: 73 l/s) with rapid reactions during rain events. The turbidity in the monitored stream is highly event-linked, as rain events promptly cause an increase of suspended solids (TSS) in the stream water. Maximum TSS concentrations of over 3 g/l were recorded during the test period in July 2014 and January 2015. Depending on the hydrologic condition of the catchment, the stream water turbidity is a diverging combination of eroded sediment flushed into the stream by surface runoff and re-suspended riverbed sediment (Eder et al. 2014). The main source of faecal contamination of ground and surface water is swine manure applied periodically to the fields.

2.3.2. Design and Construction

To overcome the problem of suboptimal sample pretreatment, a compact and robust auto sampler (SAMP-FIL) that samples and provides a filtered water sample scheduled to the measurement intervals of the connected BACTcontrol devices was constructed (Fig. 1B). The objective was to conduct the sampling and filtering procedure as quickly as possible to minimize the retention time and adulteration of the sampled water. The flow-through rates were kept high to achieve turbulent pipe-flow to minimize debris and bio-film accumulation within the device. Flushing out the complete equipment with pressurized air enabled filter cleaning and allowed the equipment to dry while idle. To schedule the time sequence of the array of required components, a RasperryPi single-board computer was chosen because the guidance support by the on-line community is high, it allows on-device programming and it has been reported to be robust for numerous long-term operations (Horan 2013). All components of the SAMP-FIL are mounted within a casing of manageable size, where the sampling tubing with the filter, outlet and BACTcontrol devices are connected (Fig. 2).

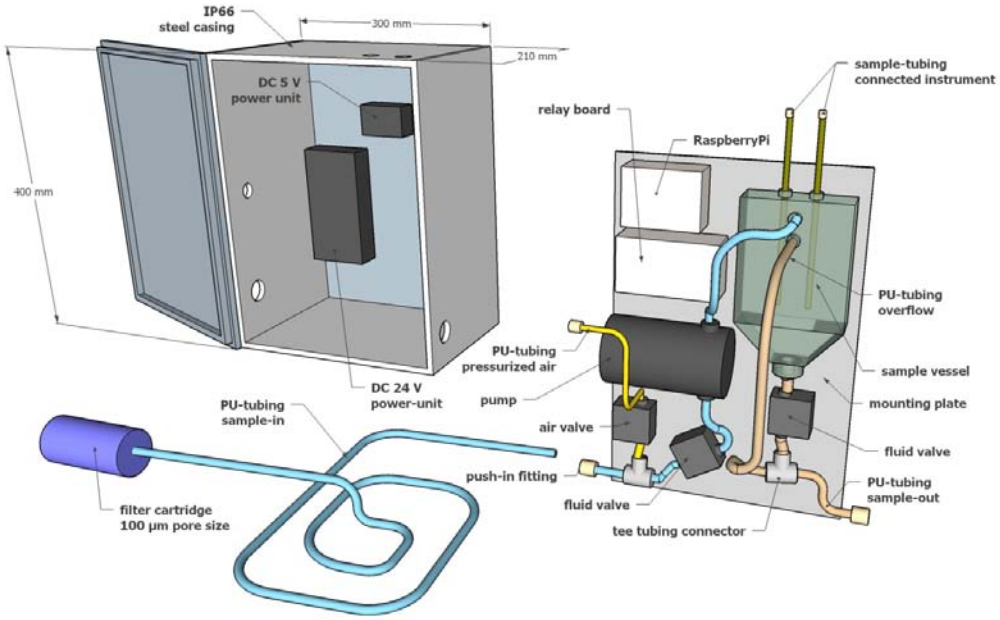

Fig. 2: Construction plan of the SAMP-FIL hardware. Core components are installed on a detachable mounting plate (right). The IP66 casing houses this plate and the power units. Connections for the sample-in tubing, sample-out tubing, pressurized air tubing and main power are made on the casing. Sample tubing from the connected devices (BACTcontrol) is similarly linked via push-in fittings through the casing.

2.3.3. Hardware

The SAMP-FIL (Fig. 2) was housed in a commercial compact steel enclosure (Rittal, AE 1034.5, width: 300 mm, height: 400 mm, depth: 210 mm) with protection category IP 66. The sampling was conducted using a rotary diaphragm pump (Charles Austen, RD5 DC, suction pump, flow rate: 5 l/min, empty lift: 8 m, DC: 24 V). Sample inlets and outlets as well as pressurized air inlets were controlled by an array (Fig. 3) of two fluid control valves (SMC Pneumatics, VX 232, 2-port solenoid valve, normally closed, nominal diameter: 8 mm, DC: 24 V) and one air control valve (SMC Pneumatics, VX 220, 2-port solenoid valve, normally closed, nominal diameter: 6 mm, DC: 24 V).
Both fluid- and air control values operate on DC 24 V. Pressurized air (2 bar) was delivered from a compressor (Jun Air, 6-15) and used to clean the auto-sampler and achieve dry conditions during idle. Pressurized air was also used for the automated cleaning of in situ probes (s::can spectrolyser and Nadler ion-sensitive probe) at the same monitoring station. The sample tubing extended from the SAMP-FIL into the stream. A commercial filter cartridge (Acqua SAN, cartridge size: 1 inch, stainless steel mesh) with a pore size of 100 µm was mounted at the end of this tubing (Fig. 2, Fig. 3). All fluid and air tubings are polyurethane (PU) hoses (fluid: SMC Pneumatis, TU 1065, outer diameter: 10 mm, inner diameter: 6.5 mm, air: SMC Pneumatis, TU 0604, outer diameter: 6 mm, inner diameter: 4 mm). The tubing was connected with the filter, valves, pump and sample vessel via one-touch (push-in) fittings (SMC Pneumatics, KQ 2).

For each operation cycle, a filtered sample was delivered into the sample vessel (volume: 500 ml, material: HDPE, format: square). The required sample volume (100 ml) was taken from this sample vessel for on-site GLUC measurement by the BACTcontrol devices.

Operation of the aforementioned components (all sourced DC 24 V) was controlled by a relay board (SainSmart, 8-channel DC 5 V relay module, high current relay: AC 250 V, 10 A or DC 30 V, 10 A, driver current: 15 – 20 mA, indication of relay output status: LED), which was triggered by the RaspberryPi (Raspberry Pi 1 model B, DC 5 V) via GPIO (general purpose input/output) pins. The RaspberryPi has no default real-time clock (RTC) and retrieves the system time from a network time protocol (NTP) server (time server) whenever it is connected to the Internet. When operated off-line, in cases of power outages (e.g., due to thunderstorms), the RTC is required such that the RaspberryPi reboots with the correct system time after it is reconnected to power. Therefore, a peripheral RTC (DS 1307 RTC) was connected to the RaspberryPi via GPIO to access an accurate and current system time during off-line operation (as described in: "Raspberry Pi • View topic - The Correct way to add a RTC," n.d.).

The RaspberryPi and relay board (both sourced by DC 5 V) are connected (Fig. 3) to a 5 V commercial power supply unit (2 A). All other components operate on DC 24 V (Fig. 3) and are connected to an adequate commercial power supply unit (6 A). Both 5 V and 24 V power supply units are affiliated with AC 230 V main power connections (Fig. 3).

The BACTcontrol devices and SAMP-FIL were mounted in an air-conditioned, weatherproof outdoor monitoring station (OMS, Fig. 1). The option to mount the SAMP-FIL within the OMS was chosen as the least elaborate from a technical perspective because the length of the tubing was kept minimal and frost-proof housing was assured.

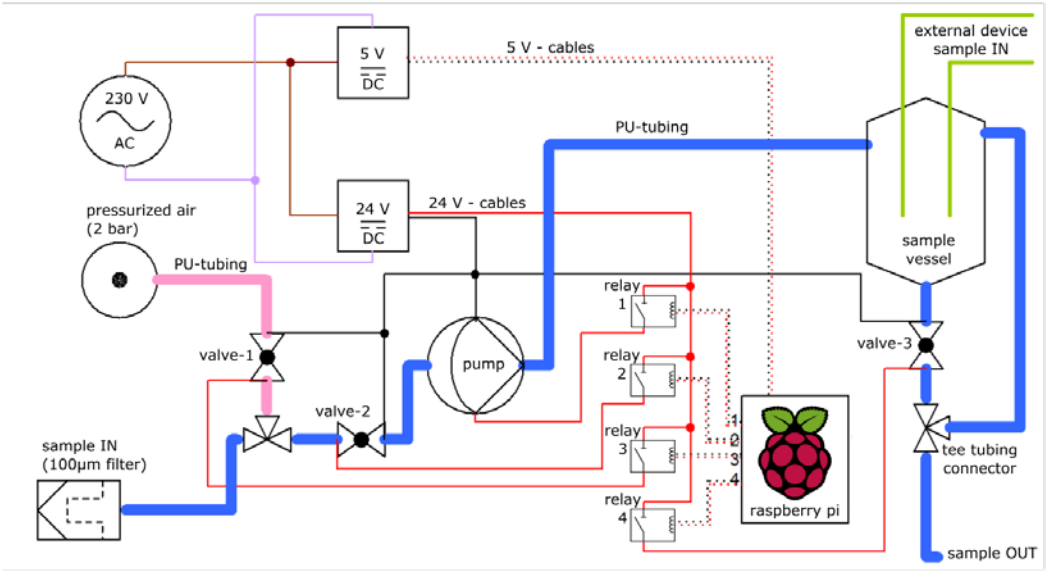

Fig. 3: Schematic diagram of the current lines, tubing and core components of the SAMP-FIL. Fluid tubing is marked in blue, pressurized air tubing is marked in pink and the sample-in tubing of the connected BACtcontrol devices is marked in green. Dashed red and black lines indicate 5 V cables, and

solid red and black lines indicate 24 V cables. Brown and purple lines show 230 V main power connections.

In addition to the robustness of the components, the main focus was set on a design enabling highly turbulent sample-water flow within the tubing and valves to reduce debris, bio-film and sinter deposition on the inner surface of the equipment. The flow rate of the high-performance pump (5 l/min) and the chosen inner diameter of the fluid tubing (6.5 mm) theoretically enabled at straight intercepts of the tubing a flow velocity (v) of 2.5 m/s, with a Reynolds number (Re) of over 12,500. A flow velocity of 1.7 m/s and a Reynolds number over 10,500 were calculated for the inside of the fluid valves (cylindrical aperture with a nominal diameter of 8 mm). Although this is an estimation and friction due to specific material roughness, bent tubing sections and intersections between components is neglected, highly turbulent flow behaviour within the tubing and valves can still be assumed (Marriott 2009). Calculations were made for a water temperature of 10°C, which is close to the annual mean stream water temperature of 10.3°C, using the following equations (Marriott 2009):

$$v = \frac{4Q}{\pi D^2} \qquad (1)$$

$$Re = \frac{DV\rho}{\mu} \qquad (2)$$

where Q is the volumetric flow rate, D is the pipe diameter, ρ is the density of the fluid and μ is the dynamic viscosity.

2.3.4. Software

The operating system (OS) of choice was the Linux-based Raspbian, which was installed using the "New Out Of The Box Software" package ("Installing Raspbian with NOOBS | Raspberry Pi Learning Resources," n.d.) on the RaspberryPi's secure digital memory (SD) card. The Raspbian image includes an integrated developer environment (IDE) used for Python programming (IDLE). Four GPIO pins of the RaspberryPi board are used as output to trigger the corresponding relay on the relay-board and are controlled by an executable Python script. The core features of the script are a file-type access to the GPIOs (no additional libraries and packages are required) and the Python *time.sleep* command, which suspends execution for a given number of seconds. This allows for coding a simple time-sequenced state machine that iteratively activates the peripheral devices for a certain time, as specified in the script, with the *time.sleep* command. Following this, the script schedules all necessary operations for one complete sampling cycle. *Cron* was used to schedule the sampling cycle to the measurements of the connected BACTcontrol devices (fixed measurement interval of 180 minutes). *Cron* is a software-tool to configure scheduled tasks using the system time on Unix systems, e.g., to schedule executable scripts to run at a fixed interval. The programmed Python script was set to be executed by *Cron* shortly before each BACTcontrol measurement starts. To keep track of the working steps conducted by SAMP-FIL, the Python logging module is used within the script. Thus, each programmed operation was logged together with a

timestamp in a text file, allowing potential failures to be traced and aligned to erroneous BACTcontrol measurements.

2.3.5. Function

Each sampling cycle of the SAMP-FIL includes seven steps (Table 1). During the "CLEANING" step, the pump, "sample-in" fluid valve, "sample-out" fluid valve and pressurized air valve are activated. All equipment, including the filter cartridge, tubing, fluid valves, pump and sample vessel, are flushed through by pressurized air (Table 1). During the "FLUSHING" step, the valve for pressurized air is closed and all of the equipment is flushed through with recent sample water (Table 1). The "SAMPLING" step enables the filling of the sample vessel by closing the "sample-out" valve while keeping the pump and "sample-in" fluid valve activated (Table 1). During the "READY" step, the pump is deactivated, all valves stay closed and the sampled water remains in the sample vessel from where it is retrieved by the BACTcontrol devices (Table 1). When the abstraction of the water sample by the BACTcontrol devices is completed, the "sample-out" valve is opened to empty the sample vessel (Table 1). After draining the sample vessel, the "sample-out" valve stays open, and the "sample-in" valve, pump and pressurized air valve are activated (Table 1); this "CLEANING" step is identical to step 1 (Table 1), i.e., the residuals of the sample water are flushed out from the tubing, pump, valves and sample vessel, and the filter mesh is cleaned. After these six steps, the SAMP-FIL goes into "IDLE" mode, where no peripherals are activated, and the equipment remains dry (Table 1). This sequence of steps assures the residence of sample water within the device only when it is required and enhances long-term and continuous operation before and after each sampling cycle as the SAMP-FIL is flushed completely by pressurized air to prevent the filter cartridge from clogging, and any residual water is discarded from the previous cycle.

Table 1: Scheduled sequences for one complete operation cycle of SAMP-FIL.

sequence No.	task	function	status pump *(relay 1)*	status valve-1 *(relay 3)*	status valve-2 *(relay 2)*	status valve-3 *(relay 4)*
1	Cleaning	*Pressurized airflow through the complete system*	active	active	active	active
2	Flushing	*Flushing the system with recent sample water*	active	inactive	active	active
3	Sampling	*Filling of sample vessel*	active	inactive	active	inactive
4	Ready	*Connected devices abstract sample*	inactive	inactive	inactive	inactive
5	Emptying	*Emptying of sample-vessel*	inactive	inactive	inactive	active
6	Cleaning	*Pressurized airflow through the complete system*	active	active	active	active
7	Idle	*All components remain dry until next cycle*	inactive	inactive	inactive	inactive

2.3.6. Field test

A field test with 3 phases was conducted to test the influence of proprietary sample-pretreatment and SAMP-FIL on the on-site measured GLUC activity. During "Phase 1" (23 March 2014 to 23 April 2014), both devices for on-site

GLUC measurements (BACTcontrol 01 and BACTcontrol 02) were connected to the proprietary sample pretreatment. During "Phase 2" (27 July 2014 to 19 August 2014), the BACTcontrol 01 was connected to the SAMP-FIL, whereas BACTcontrol 02 remained connected to the proprietary sample-pretreatment. In "Phase 3" (13 September 2014 to 13 October 2014), both BACTcontrol devices were connected to the SAMP-FIL.
To test the technical capability of the SAMP-FIL for long-term on-site operation, it was continuously operated since the installation in July 2014 for 16 months until November 2015.

2.3.7. Reference analyses

Several studies described the attachment of faecal indicator bacteria to suspended particulate matter in aquatic habitats (Brettar & Höfle 1992; Crump et al. 1999; Edberg et al. 2000; Garcia-Armisen & Servais 2009; Savio et al. 2015). Enzymatic activity has been reported to be linked to fractions of suspended particulates in stream water (Farnleitner et al. 2002, 2001). As sample pre-filtration is an unavoidable step for automated GLUC measurements in sediment-laden waters, the authors tested the impact of a 100 µm filter on GLUC measurements and performed culture-based *E. coli* analyses. Grab samples were taken from the stream during different catchment conditions, i.e., regarding the hydrologic state and microbiological impact. One portion of each sample was filtered through a 100 µm filter (as mounted in the SAMP-FIL), whereas the other portion remained unaltered. Both portions were analyzed for GLUC activity and *E. coli*. GLUC activity measurements were performed with a ColiMinder laboratory measurement device. Although the units of the on-site and laboratory GLUC measurements are different, both ColiMinder (laboratory) and BACTcontrol (on-site) provide the same target-parameter, namely, the determination of beta-D-glucuronidase activity in waters (Stadler et al. 2016). Both constructions for GLUC determination yield results with an average one-to-one ratio between mMFU/100 ml (ColiMinder) and pmol/min/100 ml (BACTcontrol) (Stadler et al. 2016). The *E. coli* analyses were conducted using the Colilert18 method (ISO 9308-2:2012, MPN/100 ml).

2.4. Results

2.4.1. Field test: Phase 1

Measurements recorded by BACTcontrol devices connected to the proprietary pretreatment set-up showed significant damping and delay of the GLUC signals (Fig. 4). These effects were particularly pronounced during runoff events, when stream parameters, such as discharge and suspended sediment, showed a rapid response to changes in hydrologic conditions, whereas the response of GLUC signals was either delayed (for several hours) from that of parallel monitored stream parameters or appeared considerably damped (Fig. 4). Linear regression

analysis of measurement data showed consistency between both BACTcontrol devices, with an R^2 of 0.72 and a slope of 1.24. Measurements with negative GLUC values (Fig. 4) indicate malfunction of the BACTcontrol 02 device due to clogged reagent dosing or a contaminated fluorescence measurement window, presumably conditioned by insufficient sample pretreatment.

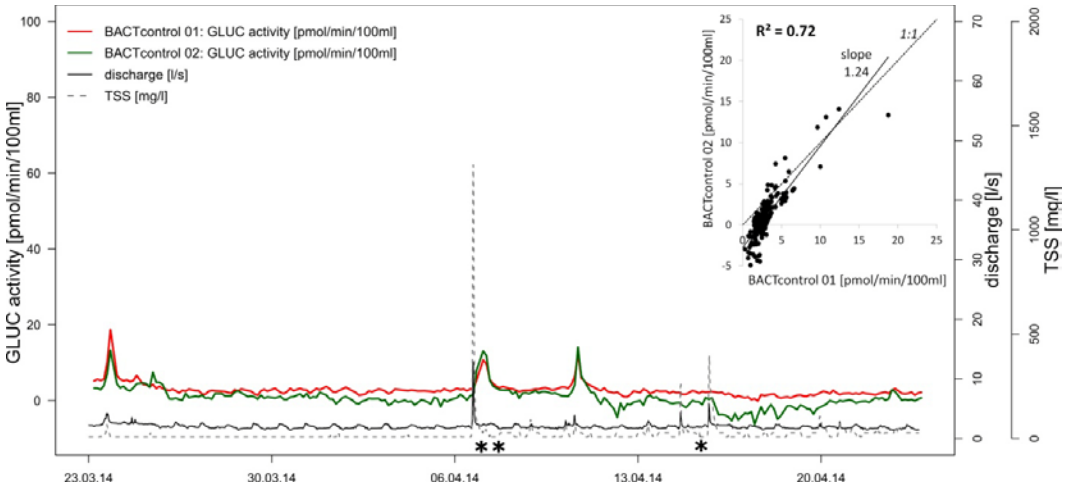

Fig. 4: GLUC signals of BACTcontrol 01 (red) and BACTcontrol 02 (green) (both connected to proprietary sample pretreatment), discharge (black line) and TSS (dashed line) at the stream monitoring location during test phase 1. The linear regression analysis of BACTcontrol data for the same time period is shown on the right. The occurrences of GLUC signal damping (*) and delay (**) are highlighted with asterisks.

2.4.2. Field test: Phase 2

Damping and delay of GLUC signals due to the proprietary pretreatment set-up indicated during "Phase 1" were disclosed in "Phase 2", in which BACTcontrol 01 was connected to the SAMP-FIL and BACTcontrol 02 retrieved sample water from the proprietary set-up (Fig. 5). Several precipitation events occurred during the test period, causing a potential input of faecal-associated contamination into the stream. BACTcontrol 01 recorded a significant peak of GLUC activity for each of these events, whereas BACTcontrol 02 showed a damped delayed response or even no response (Fig. 5). A linear regression coefficient R^2 of 0.15 was found between the measurement results of BACTcontrol 01 and BACTcontrol 02. The regression slope of 0.16 (Fig. 5) demonstrates the higher sensitivity of the device connected to SAMP-FIL (BACTcontrol 01).

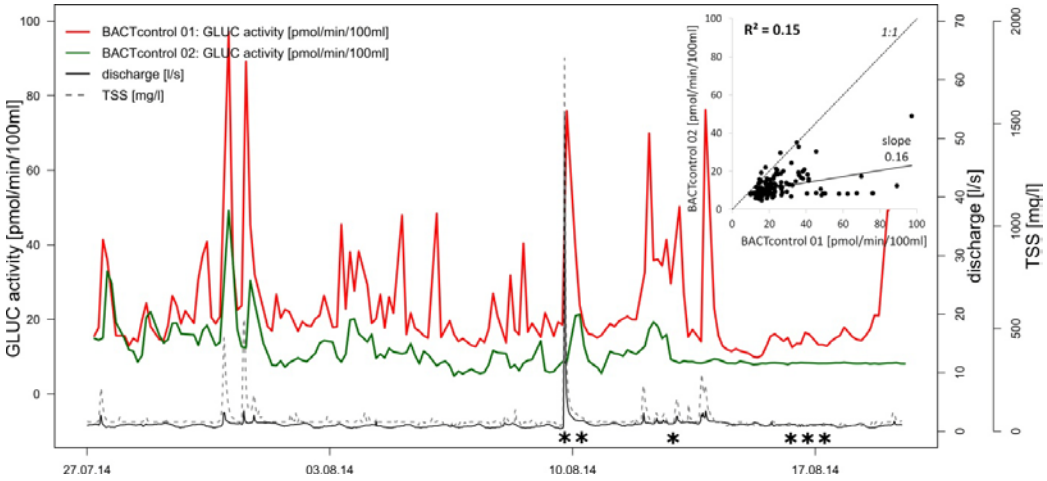

Fig. 5: GLUC signals of BACTcontrol 01 (red, connected to the SAMP-FIL) and BACTcontrol 02 (green, connected to the proprietary sample pretreatment), discharge (black line) and TSS (dashed line) at the stream monitoring location during test phase 2. Linear regression analysis of BACTcontrol data for the same time period is shown on the right and indicates the higher sensitivity of BACTcontrol 01 connected to SAMP-FIL. The occurrences of GLUC signal (BACTcontrol 02) damping (*), delay (**) and no response (***) are highlighted with asterisks.

2.4.3. Field test: Phase 3

Connection of both the BACTcontrol apparatuses to SAMP-FIL resulted in highly consistent measurements between BACTcontrol 01 and BACTcontrol 02 and prompted responses of GLUC signals to changes in hydrologic conditions (Fig. 6). A linear correlation coefficient R^2 of 0.94 was found between GLUC values gathered with BACTcontrol 01 and BACTcontrol 02. Both sets of GLUC signals are highly comparable regarding timing and range (Fig. 6). A regression slope of 0.74 indicates an offset between both devices (on average, BACTcontrol 01 yielded higher results than BACTcontrol 02 by 2.2 pmol/min/100 ml). For the first time since the installation of BACTcontrol devices at the monitoring location in 2012, diurnal fluctuations of GLUC activity (Fig. 6) in stream water during dry weather periods were captured by the BACTcontrol devices (Stadler et al. 2016).

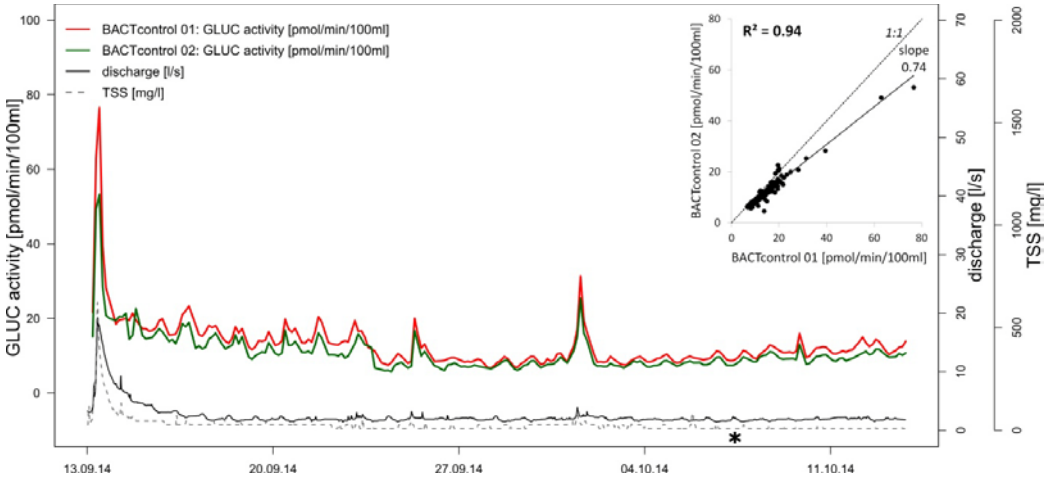

Fig. 6: GLUC signals of BACTcontrol 01 (red) and BACTcontrol 02 (green) (both connected to the SAMP-FIL), discharge (black line) and TSS (dashed line) at the stream monitoring location during test phase 3. The linear regression analysis of BACTcontrol data for the same time period is shown on the right. A high correlation (R^2=0.94, p-value<0.001) of GLUC measurements was achieved during this test period. The diurnal fluctuations in the GLUC activity in stream water were captured (marked with an asterisk).

2.4.4. Continuous long-term operation

The SAMP-FIL was continuously operated from July 2014 to November 2015 (16 months). No technical failures of mechanical and electronic components occurred during this period. The coded script was autonomously continued after sporadic power outages (e.g., due to thunder storms). The error of the installed RTC of ±1 min per month was maintained and corrected approximately every 3 months on-site by accessing the RaspberryPi via remote desktop. The filter cartridge remained unclogged but was preventively changed after 6 months of operation. No crucial sediment or bio-film accumulation within the tubing and sample vessel could be detected by visual inspection after the test period. The SAMP-FIL was preventatively flushed with decalcifying and disinfecting cleaning solution after the test period before on-going operation continued.
After the installation of SAMP-FIL, the GLUC measurements of the connected BACTcontrol devices achieved their highest quality in terms of consistency and sensitivity. The capability for long-term operation of BACTcontrol apparatuses in this technically challenging habitat increased as error-free running time and service intervals of up to 6 months were achieved.

2.4.5. Impact of sample pretreatment on GLUC activity and *E. coli* concentrations

Laboratory measurements of GLUC activity and culture-based *E. coli* analyses did not indicate a systematic influence of the 100 µm filter on the measured signals (Fig. 7). Consequently, it is assumed that the chosen pore size does not adulterate on-site GLUC measurements.

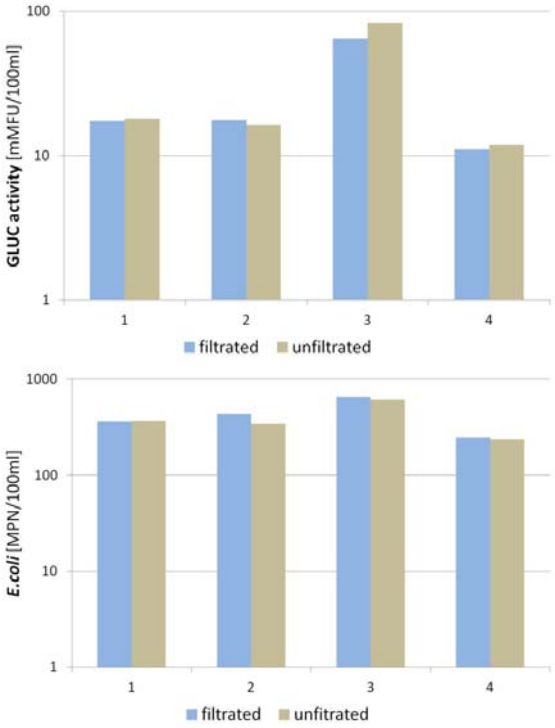

Fig. 7: Graphs of results from GLUC (left) and E. coli (right) analyses of four stream water samples, each portioned into a filtrated (through 100 µm) and unfiltered part. No systematic impact of the tested filter on GLUC activity or E. coli concentration was found.

2.6. Discussion and Perspective

Low-cost equipment that allows the assembly of affordable equipment to be used as data loggers, sensors or accessory units will likely become of increased importance within various fields of today's environmental research, such as hydro-meteorological monitoring. Available inexpensive equipment has the potential to promote research and science into new fields and scales: To overcome the dearth of meteorological monitoring stations in the sub-Saharan Africa, Van de Giesen et al. (2014) described the extensive installation of multitudinous monitoring stations based on new cost-effective technologies. The use of open-source technologies for the construction of low-cost laboratory equipment was delineated and discussed by Pearce (2013).

The authors are aware that the capability of the used single-board computer was not fully exploited with the task described in this study. Nevertheless, the RaspberryPi proved to be the ideal choice for the intended purpose due to its robustness, usability, and low price (RaspberryPi 2 B: < 35€). This allowed resources to be used for the purchase of professional high-performance components (e.g., pumps, valves, casing, and tubing), resulting in failure-free on-site operation in a technically challenging habitat for 16 months (and on-going).

This work demonstrated that researchers can develop an effective device, controlled by a low-cost single-board computer, with a basic working knowledge in electronics but no background in electrical engineering or information

technology (IT). The planning and construction of SAMP-FIL was exclusively conducted within the Vienna Doctoral Programme on Water Resource Systems (TU Wien).

The experimental results obtained during the various test phases demonstrated the relevance of best possible sample pretreatment with regard to minimal sample adulteration and the prevention of device fouling. This study quantitatively demonstrates how inadequate pretreatment procedures result in falsified and biased on-site measurements.

The SAMP-FIL version described in this chapter is a prototype. Further endeavours will focus on the on-line connection of the SAMP-FIL, in particular, linking the SAMP-FIL to BACTcontrol to trigger SAMP-FIL activities by the connected measurement device.

Chapter 3

Real-time monitoring of beta-D-glucuronidase activity in sediment laden streams: A comparison of prototypes

3.1. Abstract

Detection of enzymatic activities has been suggested as a rapid surrogate for culture-based microbiological pollution monitoring of water resources. This chapter tests four fully automated prototype instruments for on-site monitoring of beta-D-glucuronidase (GLUC) activity. The tests are performed on sediment laden stream water in the Hydrological Open Air Laboratory (HOAL) during March 2014 to March 2015. The dominant source of fecal pollution in the stream is swine manure applied to the fields within the catchment. The experiments suggest very consistent results of the instrument pairs with the same construction design (R^2=0.96 and R^2=0.94), and somewhat lower consistency for different designs (R^2=0.71). Correlations of on-site measured GLUC activity and culture-based *E.coli* analyses over the entire study period give R^2=0.52 and R^2=0.47 for the two designs. At the event scale, the correlations tend to be higher. GLUC activity is less well associated with suspended sediment concentrations than *E.coli* which is interpreted in terms of indicator applicability and the role of time since manure application. The study shows that this rapid assay can yield consistent results over a long period of on-site operation in technically challenging habitats. For the observed habitat, the use as a proxy for culture-based assays could not be proven, though the study suggests that this biochemical indicator has high potential for the implementation into early warning systems.

3.2. Introduction

Agricultural activities may cause fecal pollution of surface and ground water (Blann et al., 2009; Bradford et al., 2013; Buck et al., 2004; Farnleitner et al., 2011). Streams receiving agricultural runoff often contain pathogenic bacteria from manure (Hutchison et al., 2004; Jones, 1999; Mawdsley et al., 1995; Tyrrel and Quinton, 2003). Real time detection of fecal pollution of surface waters therefore has a high potential for use-orientated protection of water resources.
Cultivation based standard analyses of fecal pollution typically take one to several days and are, therefore, not suitable for rapid water quality assessment (J. P. S.

Cabral, 2010). Instead, methods involving enzymatic activity have been tested in various aquatic habitats and suggested as a surrogate for culture-based microbiological pollution monitoring (Farnleitner et al., 2001, 2002; Fiksdal and Tryland, 2008; Garcia-Armisen et al., 2005). There are various chromogenic and fluorogenic substrates for the specific detection of these enzymatic activities, e.g. beta-D -glucuronidases (GLUC), galactosidases and esterases (Fiksdal et al., 1994a; Morikawa et al., 2006; Noble and Weisberg, 2005; Rompré et al., 2002; Wildeboer et al., 2010). Whereas these common enzymatic activity measurements for fecal indicators require laboratory facilities and elaborate sampling methods (Lebaron et al., 2005; Rompré et al., 2002), research within the last two decades has focused on developing rapid enzymatic assays(Fiksdal et al., 1994a; George et al., 2000). Yet these assays still require manual sampling and laboratory analytics.
Recent technological development have brought automated on-site measurements of enzymatic activity within the reach of real time monitoring (Koschelnik et al., 2015; Ryzinska-Paier et al., 2014; Zibuschka et al., 2010). These studies have been mainly conducted for groundwater. For surface waters the measurements are more challenging because of larger temperature variations and potentially high sediment concentrations. The purpose of this chapter is to perform a field test of instruments for automated on-site enzymatic activity detection for stream water with high suspended sediment loads resulting from runoff events in order to understand the strengths and limitations of the instruments and optimise the measurement setup.

3.3. Material and Methods

3.3.1. Site description

The methodological basis of the field test conducted in this study is a comparison of automated rapid on-site GLUC measurements with culture-based microbiological measurements as well as with hydrological data in the Hydrological Open Air Laboratory (HOAL, (Blöschl et al., 2015, 2011). The HOAL in Petzenkirchen (Lower Austria) is operated and maintained by the Institute for Land and Water Management Research (*Federal Agency for Water Management, Austria*) and the Vienna Doctoral Programme of Water Resource Systems (*Centre for Water Resource Systems, TU Wien, Austria*).
The HOAL catchment is 0.66 km² in size and drained by a stream 620 m in length. Twelve point discharges contribute to the stream, including tile drains, springs and surface tributaries (Michael Exner-Kittridge et al., 2013). The mean annual precipitation is 823 mm/yr (years 1990 – 2014). Land use of the catchment is dominated by agriculture, consisting of 87 % arable land, 5% grass land, 6 % forested area and 2 % paved land. The hydrogeology is characterized by porous and fissured aquifers made up of clay, marl and sand. Soils show medium to limited infiltration capacities. Yearly sediment erosion is approximately 10 t/km² (Eder et al., 2010). Main source of fecal contamination of ground- and surface water is swine manure applied to the fields. Application in 2014 took place during

March, April, August and October with a typical rate of 20m³/0.1km².
The stream has high discharge dynamics (Table 2) with rapid response to rain events, causing significant peaks of suspended sediment concentration in the stream water. Typically, early in the event, sediments re-suspended from the riverbed control the sediment concentrations, while later in the event sediments from the hillslopes dominate (Eder et al., 2014). A considerable proportion of sediments stem from tile drainages. Relatively short, intense events can cause a great increase in sediment concentrations. Consequently, the site is ideal for testing measurement methods under demanding conditions with strong variations of the weather conditions, hydrology, land use management and microbiological impact.
The instrumentation of the HOAL includes on-line measurements of water level for discharge determination, electrical conductivity (EC), turbidity and water temperature (Table 2) at the stream monitoring station MW (Fig. 8), which is located at the catchment outlet (see Blöschl et al., 2015 for details). The turbidity measurements are calibrated with grab samples and referenced to total suspended solids concentration (TSS mg/l).
Winter and spring 2014 were characterized by fairly low discharges resulting in a yearly average of 2.4 l/s for 2014. Rain events in late spring, summer and autumn caused several high discharge peaks with a maximum (hourly average) in May 2014 at station MW of 73.4 l/s (Table 2). The minimum 2014 discharge of 0.5 l/s was recorded in August. Stream water temperature was continuously monitored because of the importance of temperature regarding enzymatic activity in aquatic habitats (Chróst, 1989). Stream water temperature generally tracked the yearly trend of air temperature. Water temperature reached a minimum of 0.2 °C in January 2014 and a maximum of 20 °C in July 2014 (Table 2). The average water temperature in 2014 was 10.3 °C. Diurnal fluctuations of water temperature (up to ± 7°C in April 2014) show maximum values in the afternoon and a minimum in the early morning. Turbidity in the monitored stream is highly event linked, as rain events promptly cause an increase of suspended solids in the stream water. Maximum suspended sediment concentrations of over 3 g/l TSS (Table 2**)** were recorded in July 2014 and January 2015.

Table 2: Range of key-parameters in the HOAL stream during the test period (March 2014 – March 2015) (n = number of samples). The figures for GLUC activity (ColiMinder and BACTcontrol) as a biochemical indicator are printed in bold.

			Min	Max	Median	Mean
Discharge	[l/s]	n = 8760	0.5	73.4	2.3	2.6
Suspended solids	[TSS mg/l]	n = 8760	0.0	3210	8.0	18.7
Electrical conductivity	[µS/cm]	n = 8760	260	856	769	765
Water temperature	[°C]	n = 8760	0.2	20.0	10.7	10.3
Air temperature	[°C]	n = 7099	−8.7	34.9	12.2	11.6
E. coli	[MPN/100 ml]	n = 54	<1	3450	172	632
GLUC activity (ColiMinder)	[mMFU/100 ml]	n = 3360	**0.8**	**170**	**10.9**	**15.8**
GLUC activity (BACTcontrol)	[pmol/min/100 ml]	n = 846	**1.1**	**108**	**9.7**	**11.5**

3.3.2 Automated on-site GLUC measurements

At location MW (Fig. 8) two ColiMinder devices (VWM GmbH, Austria) for rapid on-site GLUC monitoring have been operating in parallel since March 2014. At the same location two BACTcontrol devices for rapid on-site GLUC

monitoring (MicroLan, Netherlands) have also been operating in parallel since 2012 (in this study only measurements after the installation of an improved sampling set-up in July 2014 are used). Both devices detect beta-D-glucuronidase enzymatic activity and record and transmit the data on a continuous basis.

The ColiMinder is based on a flow-through photometric measurement-chamber (patent: PCT/AT2011/000497) which enables a high resolution fluorescence analysis. The shape of the measuring-chamber and the fluidic system are optimized for automated water sampling, reagent dispensing and effective cleaning process. In order to get accurate fluorescence readings independent of turbidity, a data correction algorithm (patent: PCT/AT2014/050036) was used. The GLUC activity measurements were performed in batches using 6.5 ml of sample per measurement. The measurement step takes about 15 minutes and the full measurement cycle, including cleaning and sample conditioning, lasts 30 to 40 minutes. ColiMinder is calibrated to Modified Fishman Units (MFU/100ml), based on the enzyme unit definition for beta-glucuronidase activity (Fishman and Bergmeyer, 1974); (Bergmeyer, 2012). The measurement interval has been chosen as 60 minutes.

The BACTcontrol devices (formerly Coliguard) have a different design. Construction design as well as sampling- and measurement procedure of the BACTcontrol devices are described in detail by Zibuschka et al. (2010) and Ryzinska-Paier et al. (2014). The devices provide units pmol/min/100 ml. The measurement interval was 3 hours.

Both ColiMinder and BACTcontrol devices were connected to 230 V AC power and accommodated in temperature-controlled, weatherproof casings, where also reagents and cleaning solutions were stored (Fig. 8). Sample intakes of both constructions are located 20 cm below water level on opposite stream sides. During the measurement process, the sample mixed with specific assay reagents (proprietary information) generates an increasing fluorescence signal reflecting the level of enzymatic activity, which is monitored over time. Internal control parameters, such as fluorescence signal, linearity of fluorescence slope, temperature of measurement-chamber, device´s environmental temperature, measurement duration and blank value measurements are available for each data point and were used for quality checking the measurement results. All devices were connected to an on-site wireless data transfer GPRS-modem via Ethernet interface, enabling on-line access to the measurement data.

The fundamental differences between the two constructions (Fig. 8) are: The fluorescence measurement-chamber (ColiMinder: Measurement-chamber not accessible, BACTcontrol: Measurement-chamber accessible), the pore size of the filter mounted at the sample intake (ColiMinder: 1 mm, BACTcontrol: 0.1 mm), the pump used to deliver water samples to a sample container shared by devices of each construction (ColiMinder: Peristaltic pump, BACTcontrol: Rotary diaphragm pump) and the arrangement of reagent and cleaning solutions (ColiMinder: Shared reagent- and cleaning solution containers for both devices, BACTcontrol: Separate reagent- and cleaning solution containers per devices) and, most importantly, different reagent and cleaning solutions and different photometric measurements.

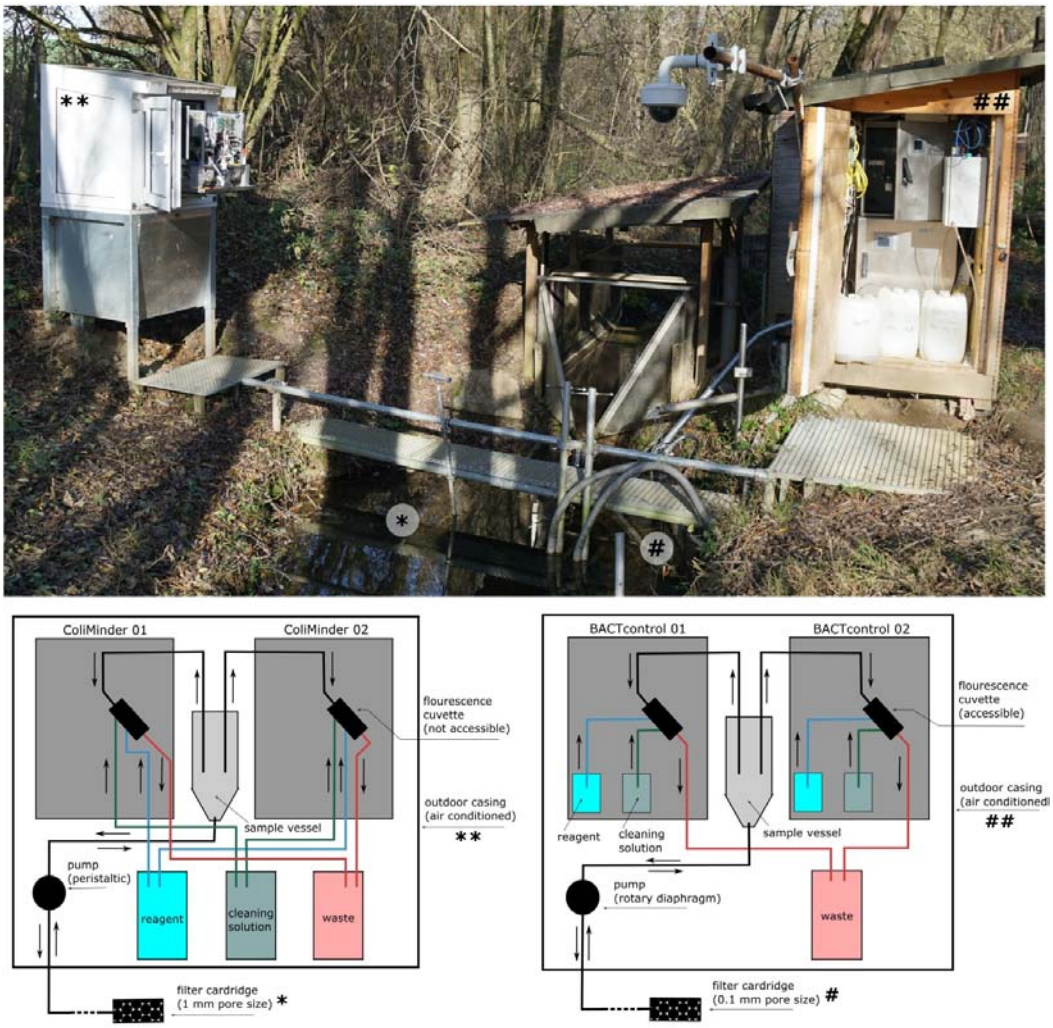

Fig. 8: Top: Photography of the measurement station "MW" showing the monitored stream, outdoor casings of ColiMinder (left, marked by asterisks) and BACTcontrol (right, marked by hash tags) as well as the discharge flume. Sample intake of ColiMinder is located on the right stream side (marked by asterisk), that of BACTcontrol is located on the left stream side (marked by hash tag). Bottom: Schematic of the basic construction of ColiMinder (left) and BACTcontrol (right).

3.3.3. Inter-comparison of on-site GLUC measurements

To test the consistency of the on-site GLUC measurements, data from two prototypes of the same design were compared with each other. In addition, data from the instruments with different designs were compared. For the latter comparison, measurement data were aligned to the following full hour for consistency, as ColiMinder devices did not yet allow arbitrary time stamps.
Although the units of the measurements are different, all designs provide the same target-parameter, namely the determination of beta-D-glucuronidase activity in waters. The unit pmol/min/100ml of BACTcontrol measures enzymatic activity (pmol of fluorophore per minute and per 100 ml). The unit mMFU/100ml of ColiMinder is similar, however, it references the enzymatic activity to known conditions (i.e. those published by Fishman). While, for any direct comparison of

the measurements it would be ideal to determine the hydrolysis rate of each device using series of standard solutions with known enzymatic activity, pH and reaction temperature, this information is proprietary to the companies, so could not be used in this chapter. Therefore, direct comparisons of the measurement results with different units are shown.

3.3.4. Quality screening of on-site GLUC measurements

In addition to internal control parameters, comparisons of measurements from devices with the same design were used to assess their validity. The normalised absolute difference of the readings, Δ S, in percent was calculated as follows:

$$\Delta S = \mathrm{abs}\frac{(S01-S02)}{S01} * 100 \quad (1)$$

Where S_{01} and S_{02} are the readings of the two devices.
For quality screening the Δ S (%) values were compared to the GLUC measurements for each construction. To determine potential effects of environmental parameters on the consistency of measurements, Δ S (%) values were compared with turbidity, water temperature, air temperature, suspended sediment concentrations and discharge.

3.3.5. Reference sampling survey of culture-based *E. coli*

To test the capability of on-site GLUC measurements as a quantitative proxy for fecal derived *E. coli*, GLUC measurements were compared to microbiological standard assays. 54 grab samples were taken manually during the test period for reference and analyzed with the Colilert18 (ISO 9308-2:2012) method. This total number comprises samples from a continuous reference sampling campaign conducted on an approximately monthly basis (n=10), samples from three runoff events (n=31) and intermittent grab samples during base flow conditions (n=13). Microbiological analyses (ISO 9308-2:2012 and ISO 16649-1) data from monthly grab samples are available for a period of three years (2012-2015) characterizing the range of fecal indicator bacteria (FIB) during base flow in the monitored stream. Because of the strong correlation between *E. coli* concentrations determined with the ISO 9308-2:2012 (MPN/100ml) and ISO 16649-1 (CFU/100ml) methods (R^2=0.94, n=25, p-value<0.001, monthly grab samples 2012-2015), and because of the higher number of reference samples analyzed during the test period with ISO 9308-2:2012, MPN values were used in this study as a proxy for standard culture based assays.

3.3.6. Event monitoring of culture-based *E. coli*

To investigate the utility of the instruments for capturing abrupt changes of GLUC activity during rainfalls, several runoff events were sampled in more detail. Automated sampling devices (ISCO sampler 6712), triggered by water level-thresholds were used for event sampling. ISCO samplers were linked to a pressure

transducer (GE Sensing, PTX 1830 or Ott PSI) that measures the water level. Two auto-samplers capable of sampling up to 30 hours with a total of 48 bottles were installed at station MW. The first device sampled at 15 minute intervals when the programmed water-level threshold was exceeded. After the first 24 bottles were filled, autosampling automatically switched to a second device, which sampled every hour. For microbiological event monitoring the auto samplers were equipped with sterile bottles. One bottle in each auto sampler was employed as a blank for quality control purposes in order to detect any inadvertent coliform contamination that may result from the procedure. The "blank bottle" followed an identical procedure as the sample bottles and stayed in the auto sampler from installation to removal without being filled. Instead, it was rinsed after the sampling campaign with 100 ml of sterile water that was subsequently analyzed with IDEXX Colilert18 for *E. coli* and coliform bacteria. The MPN analyses of "blank bottles" indicated an absence of contaminant *E. coli* and coliforms, confirming the validity of the procedure. Event-samples were retrieved within 5 hours, refrigerated, and analyzed within 8 hours after sampling.
Precipitation and air temperature data from a weather station located in the center of the catchment were also used in the analyses.

3.4. Results

3.4.1. Consistency of measurements

GLUC measurements of the devices with the same construction design were highly consistent throughout the measurement period (Fig. 9A, Fig. 9B). Linear correlation coefficients R^2 of 0.94 were found between the two ColiMinder devices (Fig. 9A) and R^2 of 0.96 for the two BACTcontrol devices (Fig. 9B) (all p-values <0.001). The regression slopes are 0.88 and 0.89, respectively, and the offsets are small. The correlations between devices with different designs (Fig. 9C, Fig. 9D) shows very reasonable consistency (R^2=0.71) with a slope of 0.85 and 0.98. ColiMinder and BACTcontrol were drawing samples from opposite sides of the stream (Fig. 8) and the sampling times may differ by up to one hour which likely contributes to the lower correlations. Nevertheless, measurement results of the two designs show a highly symmetrical range of signals and an average one-to-one ratio between mMFU/100ml (ColiMinder) and pmol/min/100ml (BACTcontrol) (Fig. 9**,** Fig. 11**C and** Fig. 11**D**). The same construction design have an offset relative to each other that is slightly less than half the mean base-GLUC activity monitored during a large part of the test period (i.e. ColiMinder 01 gave consistently higher results than ColiMinder 02 by +2 mMFU/100ml, and BACTcontrol 01 gave consistently higher results than BACTcontrol 02 by +1.5 pmol/min/100ml). The offset of ColiMinder exceeded their lower limit of detection (0.8 mMFU/100ml) while the offset of BACTcontrol was within the lower limit of detection (1.5 pmol/min/100ml).

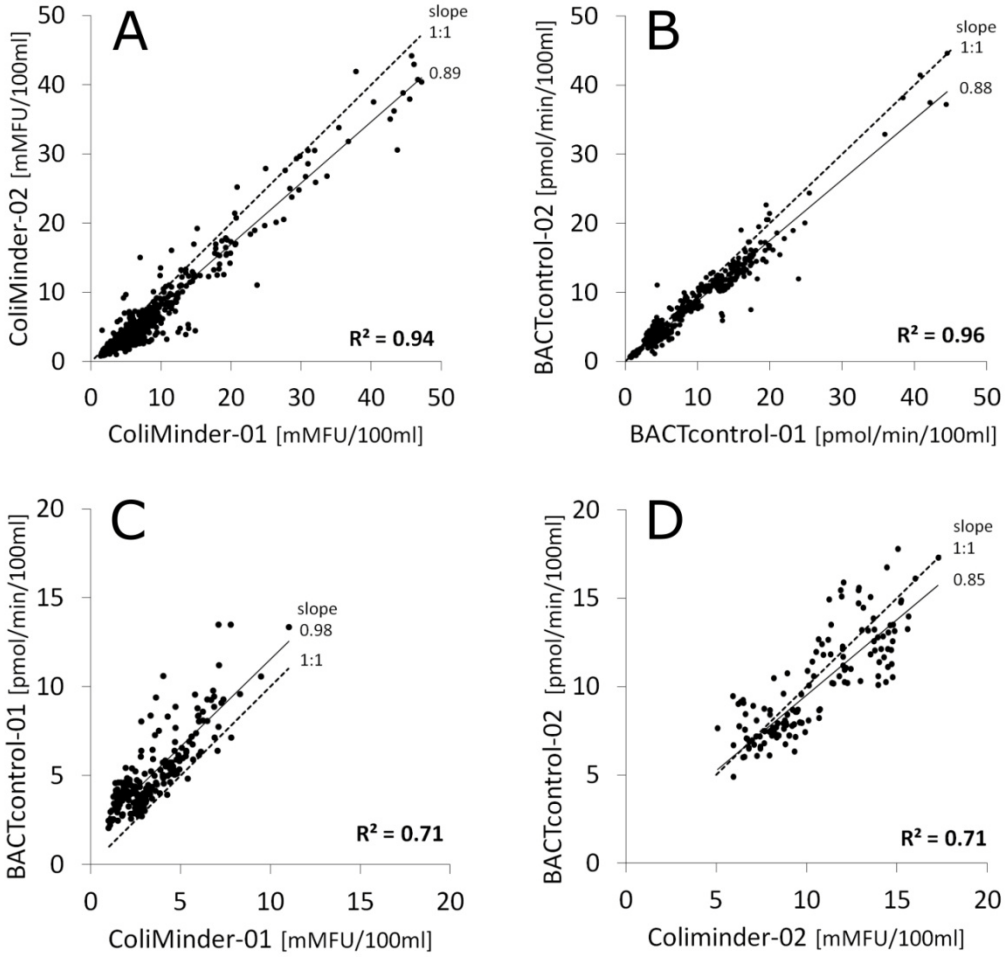

Fig. 9: Comparison of GLUC measurements from ColiMinder and BACTcontrol prototypes operated in parallel. A and B: Data from devices with same design give very consistent results (R^2>0.90, p-value<0.001). C and D: Data from devices with different designs give comparable results (R^2=0.71, p-values<0.001). Range of signals varies between panels C and D due to different test periods.

3.4.2. Influence of environmental parameters on GLUC measurement consistency

As a first step, the normalised absolute differences of the readings, Δ S, of similar devices were compared with the GLUC readings themselves (Fig. 10A). The BACTcontrol devices generally give higher differences of readings than ColiMinder. The average Δ S does not exceed 40% for either of the designs. A comparison of the Δ S values with sediment concentrations (TSS) (Fig. 10C) does not indicate a systematic increase of Δ S with higher TSS values. Clearly, the concentration of suspended solids in stream water (up to 3200mg/l TSS) did not directly affect the consistency of the measurement results. This applies likewise to the comparison of Δ S values with discharge (Fig. 10D). There was, similarly, no evidence for negative effects of water temperature (0.2°C-20°C) on the consistency of the measurements (not shown here). In contrast, the comparison of Δ S values with air temperature (Fig. 10B) indicated for the case of ColiMinder

significant higher measurement deviations for air temperatures exceeding 25°C (Fig. 10B, grey shade). As a consequence of these deviations, the substrate tempering within the instrument was improved by installing a thermoelectric cooling module (Peltier cooler) which eliminated this negative effect.

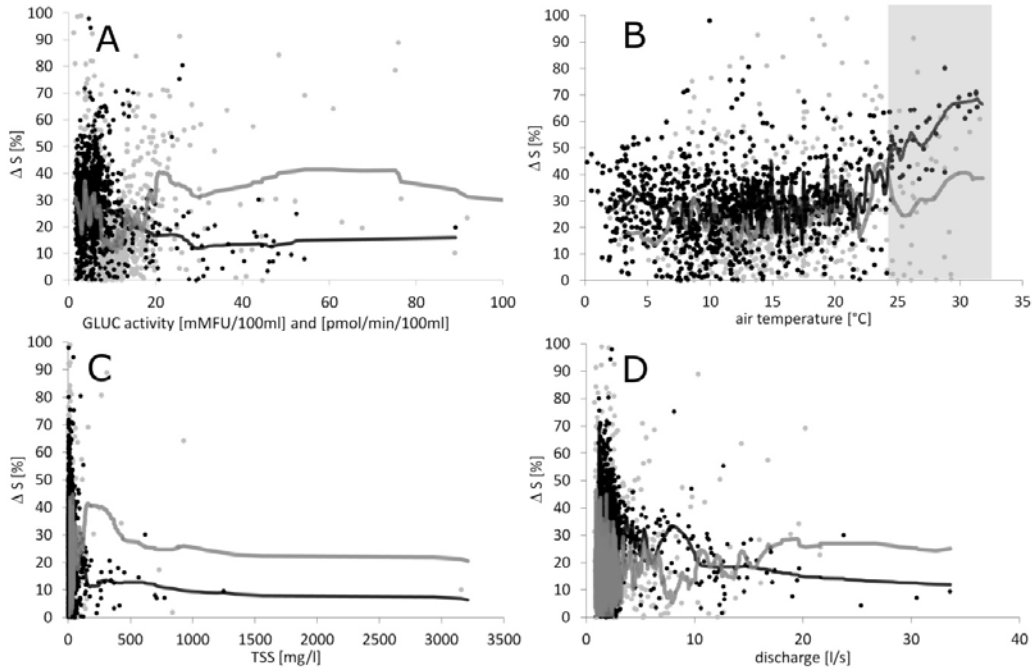

Fig. 10: Influence of environmental parameters on the consistency of GLUC measurements (ColiMinder: black dots, moving average: black line. BACTcontrol: grey dots, moving average: grey line). GLUC activity (A), air temperature (B), total suspended solids (TSS) (C) and discharge (D). Notes: A: ColiMinder generally gave smaller readings than BACTcontrol. B: Air temperatures above 25°C caused increased signal deviation for ColiMinder (grey shaded area). C and D: High TSS concentrations and discharges do not deteriorate consistency of GLUC readings.

3.4.3. Comparison with culture-based analyses

On-site GLUC measurements and grab sample analyses with culture-based methods of *E. Coli* measurements yielded good consistency (Table 3). For the entire data set (Table 3) ColiMinder vs. MPN gave correlations of R^2=0.52, and BACTcontrol vs. MPN gave R^2=47 (n=50, p-values<0.001). If individual runoff events were examined, the correlation tended to increase. For example, the event in February 2014 (shown in Fig. 11D below) gave R^2=0.8 (p-value<0.001, n=13).
In the test period (March 2014 till March 2015) *E. coli* concentrations in monthly base flow had a maximum in July 2014 (*E. coli*: 780 CFU/100 ml and 770 MPN/100 ml) and a minimum in March 2014 (*E. coli*: 2.6 CFU/100ml and < 1 MPN/100 ml). The GLUC data from all four instruments exhibit a similar pattern. The monthly mean of the GLUC signals increases tenfold from March 2014 to August 2014 (Fig. 11**B**) which agrees well with the microbiological standard assays.

Table 3: Correlation (linear regression) R^2 between GLUC activity (ColiMinder and BACTcontrol), *E.coli* (MPN) and hydrological parameters. Star code indicates significance level (*: p-value≤0.001, **: p-value≤0.05, for R^2≤0.1), n=number of measurements, NRMSE=normalized root mean squared error). Both constructions for GLUC measurements show very reasonable correlations to *E.coli*. *E.coli* concentrations are more strongly related to the hydrological parameters, especially those indicated runoff events such as EC, than GLUC measurements are.**

	GLUC ColiMinder [mMFU/100 ml]	GLUC BACTcontrol [pmol/min/100 ml]	*E. coli* [MPN/100 ml]	Discharge [l/s]	Electrical conductivity (EC) [µS/cm]	Sediment concentration (TSS) [mg/l]	Water temperature [°C]
GLUC ColiMinder [mMFU/100 ml]							
GLUC BACTcontrol [pmol/mon/100 ml]	0.71*** *n = 378* *NMRSE = 0.35*						
E. coli [MPN/100 ml]	0.52*** *n = 54* *NMRSE = 0.74*	0.47*** *n = 51* *NMRSE = 0.94*					
Discharge [l/s]	0.22*** *n = 3792* *NMRSE = 0.85*	0.18*** *n = 836* *NMRSE = 0.89*	0.63*** *n = 54* *NMRSE = 0.62*				
Electrical conductivity (EC) [µS/cm]	0.08 *n = 3792* *NMRSE = 0.06*	0.12*** *n = 844* *NMRSE = 0.06*	0.68*** *n = 54* *NMRSE = 0.06*	0.05 *n = 6917* *NMRSE = 0.07*			
Sediment concentration (TSS) [mg/l]	0.24*** *n = 3558* *NMRSE = 2.50*	0.22*** *n = 836* *NMRSE = 1.53*	0.51*** *n = 53* *NMRSE = 1.11*	0.47*** *n = 6571* *NMRSE = 2.85*	0.21*** *n = 6571* *NMRSE = 3.47*		
Water temperature [°C]	0.11*** *n = 3792* *NMRSE = 0.26*	0.10*** *n = 845* *NMRSE = 0.26*	0.14*** *n = 54* *NMRSE = 0.18*	0.01 *n = 6917* *NMRSE = 0.28*	0.17*** *n = 6917* *NMRSE = 0.26*	0.00 *n = 6571* *NMRSE = 0.28*	
Air temperature [°C]	0.01 *n = 2353* *NMRSE = 0.42*	0.00 *n = 523* *NMRSE = 0.36*	0.18** *n = 30* *NMRSE = 0.30*	0.04 *n = 5272* *NMRSE = 0.39*	0.06 *n = 5272* *NMRSE = 0.38*	0.00 *n = 4936* *NMRSE = 0.37*	0.74*** *n = 5272* *NMRSE = 0.10*

3.4.4. Event dynamics

All devices were able to detect rapid fluctuations of enzymatic activity in stream water caused by changes in the hydrological conditions in the catchment (Fig. 11B, Fig. 11D). All monitored runoff events caused an increase of GLUC activity in stream water. GLUC measurements from the two construction designs showed the same trends and dynamics regarding timing and amplitude (Fig. 11D). Due to the different measuring intervals of the tested prototypes (ColiMinder: 1h, BACTcontrol: 3h) the ColiMinder measurements reflect quick changes in GLUC activity in more detail (Fig. 11D). While the sediment concentration in the stream (Fig. 11A) is strongly correlated with the intensity of rain events or changing run-off conditions (with higher rain intensity usually resulting in higher TSS concentration), events with peak values of GLUC activity are not necessarily associated with high intensity precipitation or high TSS concentrations. This behaviour is illustrated in Fig. 11A and Fig. 11B where the left grey bar shows a period of consistency between TSS- and GLUC peaks (Fig. 11AB). The right grey bar shows a period of inconsistency. Table 3 also shows that *E.coli* concentrations in the monitored stream are more strongly correlated with hydrological parameters, such as discharge, TSS and EC, than with GLUC activity. Especially the electrical conductivity EC of stream water is related strongly to *E.coli* concentrations (R^2=0.68), but not to GLUC activity (R^2=0.08 and R^2=0.12). Furthermore, comparisons with culture-based FIB analyses showed that the GLUC and *E.coli* responses during events are quite similar regarding the timing of the rising limb, although there were differences regarding the amplitude and the recession (Fig. 11D).

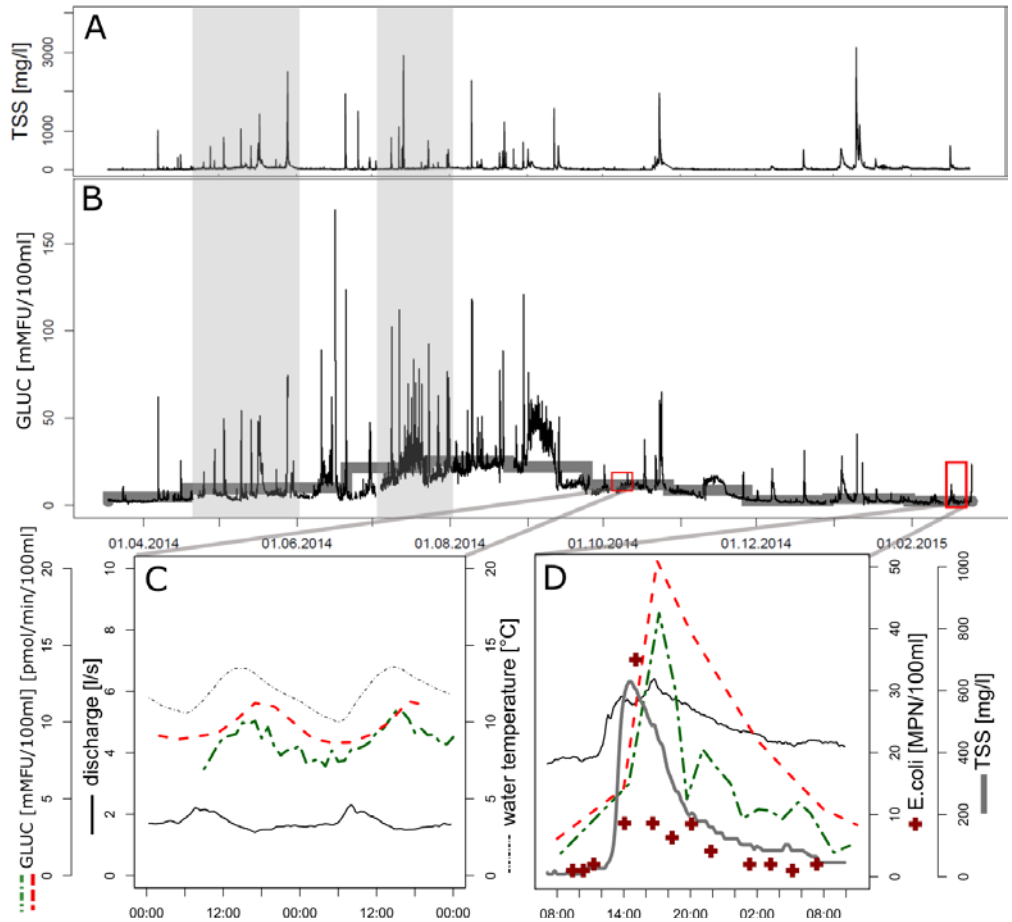

Fig. 11: A: Sediment concentrations TSS in stream water during the test period (March 2014-March 2015). Peaks of TSS indicate runoff events. B: GLUC activity in stream water during the test period (black) and monthly mean (grey). Left grey bar in A and B highlights a period of consistency between sediment concentrations and GLUC, right bar a period of inconsistency. C: Diurnal dynamics of GLUC activity (green: ColiMinder, red: BACTcontrol), water temperature (dashed line) and discharge (black). D: Event dynamics of GLUC activity (green: ColiMinder, red: BACTcontrol), TSS (grey), discharge (black) and *E.coli* (crosses). Both the diurnal and event dynamics of GLUC are consistent between devices. At the event scale (D) ColiMinder (green) has a better time resolution but both devices exhibit a similar dynamic as *E.coli*.

3.4.5. Diurnal fluctuations

Diurnal fluctuations of enzymatic activity (Fig. 11C) in stream water were recorded with the ColiMinder devices installed in March 2014. Daily variation ranged up to 4 mMFU/100ml. After improving the sampling procedure of the BACTcontrol devices in July 2014, diurnal fluctuation were also captured with these devices within the range of 4 pmol/min/100 ml. During dry periods all four devices recorded a maximum GLUC activity in the late afternoon with decreasing activity during night hours leading to minimum values in the early morning. This pattern has a different phase as the daily discharge fluctuations driven by riverine transpiration, and is more closely related to diurnal water temperature (Fig. 11**C**).

3.4. Discussion

3.4.1. General operation of the devices

All tested devices proved to be reliable under the diverse set of field conditions they were subjected to. The tests showed that consistent and continuous on-site measurement data can be gathered for of up to 6 months without technical failure. Of particular concern was the role of high suspended sediment loads. The devices turned out to provide valid measurement data even in the presence of TSS concentrations of up to 3g/l. However, biweekly intervals for manual cleaning the instruments were necessary. This is a considerably shorter interval than the monthly maintenance reported for applications involving ground water (Ryzinska-Paier et al., 2014). Damping of the signal because of fine filters was not detected. A rinsing water (de-ionized water) consumption of 85 ml (ColiMinder) and 100 ml (BACTcontrol) per measurement and the chosen temporal resolution of measurements required a weekly refill. Clogging of hoses and valves due to debris deposition sometimes led to erroneous measurements and made it necessary to alternately disconnect the devices for servicing after 3 (ColiMinder) to 6 (BACTcontrol) months of continuous operation. The longer running time until dismounting of the BACTcontrol devices suggests that a 0.1 mm filter should be preferred over a 1 mm filter. Improvements regarding sample pre-filtration, cleaning fluids compounds and temperature control within the devices' out-door casing were conducted following the results of this study. Efforts are still underway regarding the most appropriate cleaning and rinsing solutions and overall optimization for the specific operating environment, particularly with respect to the prevention of biofilm formation and accumulation of particulates within the device. Such upcoming amendments might significantly enhance runtimes between required maintenance. Nevertheless, more research on the effects of pre-filtration upon GLUC activity measurements is needed.
All prototypes of both constructions were able to conduct comparable measurements reflecting the dynamics of GLUC activity in stream water on various time scales (seasonal, event, diurnal). Because of the lower signal deviation between the ColiMinder devices, compared to BACTcontrol and especially due to the shorter measurement intervals, ColiMinder devices might be preferable if one is interested in high temporal resolution. The measurement-chamber that is accessible for manual cleaning by the operator is a significant benefit of the BACTcontrol construction.

3.4.2. Range of values

The observed GLUC activity varied during the test period from 0.8 mMFU/100ml to 170 mMFU/100ml (ColiMinder) and 1.1 pmol/min/100ml to 108 pmol/min/100ml (BACTcontrol), ranging from the lower limit of detection to a value of signifying fecal contamination. These results are consistent with an agricultural catchment subject to periodic manure application on the crop fields. The GLUC magnitudes are also consistent with previous published studies,

ranging between GLUC levels of almost unpolluted ground water (Ryzinska-Paier et al., 2014) and stream water influenced by municipal sewage (Farnleitner et al., 2002; Garcia-Armisen et al., 2005; George et al., 2000; Ouattara et al., 2011). The event monitoring showed that GLUC peaks tend to be aligned with the first flush of event stream runoff. It seems that the discharge increase in the early phase of the event may occasionally produce a "wash-out" effect (data not shown). This phenomenon has also been reported in studies on event scale transport of fecal derived coliforms, where analyses were based on culture-based assays (Krometis et al., 2007). Regarding the diurnal fluctuations of GLUC activity method and construction based temperature compensation problems can be eliminated from a technical point of view, as the diurnal fluctuations of enzymatic activity were recorded with BACTcontrol devices for the first time after the sampling procedure was improved but the measurement principle did not change. The reported temperature dependence of bacterial activity is suggested to cause these dynamics in the stream.

3.4.3. Indicator applicability

Methods of enzymatic activity measurements are ideally capable of detecting the enzymatic activity from all metabolically active target bacteria, including the so-called VBNC (viable but non-cultivable) subpopulation, whereas culture based methods are not (J. P. S. Cabral, 2010). The association between *E.coli* and GLUC, described in this study, lies within the range of correlations reported in previous studies. Tight associations between fecal indicator bacteria and GLUC were reported in at least three studies (Farnleitner et al., 2001, 2002; Fiksdal et al., 1994a; George et al., 2001) that focused on catchments with influences from municipal sewage (human origin). However, the correlation between *E. coli* and GLUC for catchments under the influence of ruminant fecal sources tends to be poor ((Ryzinska-Paier et al., 2014). Although data from the aforementioned studies have not been compared statistically in this chapter, the contrasting behaviour suggests that the differences in the association between *E.coli* and GLUC is strongly dependant on the habitat, runoff patterns in the catchment and fecal contamination source types and ages. A dominant source of fecal contamination of stream water in the HOAL is the application of swine manure. The coliform loads, but also the GLUC activity, in the stream likely vary seasonally with changing land management practices and runoff, resulting in an alternating influence of fecal contamination with varying proportions of the VBNC subpopulation associated to different compartments in the catchment (e.g. soil water, hyporheic zone or overland flow). One would assume that the highest correlations between *E. coli* and GLUC can be found in catchments under the influence of non-ruminant fecal pollution originating predominantly from one of these compartments. Such conditions have likely occurred in the HOAL through contaminated runoff during hydrological events, reflected by a R^2 of 0.80 (p-value<0.001) between *E. coli* and GLUC for single events such as that in February 2015, where no precipitation occurred and air temperatures rose slight

above zero which melted frozen soil water in the catchment and produced a quite significant discharge into the stream (Blöschl et al., 2015).
Comparison of GLUC measurements with hydrologic parameters showed that, although GLUC activity in stream water is fairly poorly correlated with hydrological parameters, it is most closely aligned with TSS. *E.coli* concentrations determined by cultivation based methods, have a stronger correlation with hydrological parameters, especially the electrical conductivity of stream water, which indicates predominantly the influence of event water in the stream. Although the number of observations from grab samples and those from on-site measurements differ, this suggests that the dynamics of *E.coli* in the HOAL catchment are mainly event driven, whereas the variations of the GLUC signal in stream water are only to some extent linked to runoff events and particle transport. Rather catchment conditions, such as the hydrologic state or land management practices as well as the aforementioned source and age of fecal contamination play a significant role too.
Cross-sensitivity and interference of GLUC activity with non-fecal compounds, such as algae or organic matter have also been reported (Biswal et al., 2003; Fiksdal and Tryland, 2008; Molina-Munoz et al., 2007) and may play an additional role in the correlation of enzymatic methods with microbiological standard assays. Furthermore (Togo et al., 2010) reported amplifying as well as inhibitory effects on GLUC activity, due to the presence of different ions in water samples. (Chang et al., 1989) described the abundance of fecal derived *E.coli* not active in respect to beta-D-glucuronidase. Further research is needed to assert the applicability of on-site enzymatic methods as a specific indicator for fecal associated bacteria in different habitats, the role of non fecal associated components and their actual influence on the on-site measured GLUC signal.

3.4.4. Outlook

Current investigations focus on the influence of hydrologic conditions (baseflow or event runoff) on the relation between *E.coli* and GLUC activity. Further research is also needed to quantify the GLUC activity of different compartments (soil water, hyporheic zone and overland flow) contributing to the stream flow (M. Exner-Kittridge et al., 2013). Research in the HOAL catchment investigates the influence of land management procedures (e.g. manure application, plowing) on the dynamics of enzymatic activity in stream water and uses the GLUC time series in combination with hydrological methods to identify the pathways and transport processes of potential fecal pollution. Detailed monitoring and field experiments focusing on the diurnal fluctuations of GLUC activity in stream water are presently undertaken.

3.5. Conclusions

The results of this study suggest that the potential of real time monitoring of beta-D-glucuronidase (GLUC) activity is enormous. The implementation of on-site GLUC measurements as a quantifying proxy parameter for culture-based *E.coli*

analyses could not be proven in the observed habitat, nevertheless, this biochemical indicator that may be available on-site and with high temporal resolution is of great value for understanding catchment behaviour as well as contaminant transport processes in different habitats. The assessment of the instruments paves the ground for a wider application of on-site and online measurements of physicochemical parameters. Automated on-site methods based on specific enzymatic activity monitoring will likely become a cornerstone of early warning systems, use oriented protection of water resources and process control.

Chapter 4

Automated near-real-time monitoring of enzymatic activities in water resources

4.1. Abstract

In recent decades, studies have described the laboratory detection of enzymatic activities in water samples from various aquatic habitats and have suggested such assays as a surrogate for culture-based microbiological pollution monitoring. Recent technological developments have brought automated measurements of enzymatic activity within the reach of on-site and near-real-time monitoring. Both laboratory and automated on-site enzymatic activity analyses are based on fluorogenic substrates for the specific detection of enzymatic activities, for example, those involving beta-D-glucuronidase (GLUC), beta-D-galactosidase or esterases.

This chapter reviews findings from two independently conducted studies testing prototypes for automated and on-site enzymatic activity determination in ground water resources (Ryzinska-Paier et al., 2014) and surface water (Stadler et al., 2016) to address the following research questions: (i) Whether technical devices for automated measurements of enzymatic activity are technically robust for long-term on-site operation at groundwater and surface water-monitoring locations, (ii) Whether automated GLUC determination can be used as a proxy for culture-based *E. coli* analyses in various habitats, and (iii) Whether enzymatic GLUC activity measurements can be used as a general indicator for the fecal contamination of water resources.

The authors show that the evaluated apparatuses for automated enzymatic activity measurements were technically robust for long-term on-site monitoring at sites ranging from pristine groundwater to sediment-laden stream water. Near-real-time automated enzymatic activity measurement can thus be considered to be realized successfully. However, in contrast to previous studies based on laboratory analyses, the successful use of on-site-measured GLUC activity as a proxy for culture-based fecal pollution analysis could not be demonstrated. The generated near-real-time data on GLUC activity point to habitat-specific differences with regard to their proxy capacities for culture-based fecal pollution detection. In addition, further investigation is needed to evaluate the capacity of GLUC to serve as a conservative, cultivation-independent biochemical indicator of fecal pollution for water resources. Future research should also focus on the application/development of alternative substrates for enzymatic activity

measurement, as this particular biochemical sensor technology holds great promise to complement automated process monitoring to support water resource safety management in the future.

4.2. Introduction

Sensitive and rapid detection of microbiological contaminants is essential for the sustainable and proactive management of water resources. Because cultivation-based standard analyses of fecal pollution typically require more than one working day, these methods are not suitable for rapid water quality assessment (J. P. S. Cabral, 2010). Alternative methods involving enzymatic activity measurements have been tested in various aquatic habitats (Farnleitner et al., 2001, 2002; Fiksdal and Tryland, 2008; Garcia-Armisen et al., 2005). Over the last two decades, several studies have suggested the use of direct enzymatic activity determination to monitor microbiological contamination in various water sources (Farnleitner et al., 2002; Fiksdal et al., 1994b; Fiksdal and Tryland, 2008; George et al., 2001). These common enzymatic activity measurements for fecal indicators require laboratory facilities and elaborate sampling methods (Lebaron et al., 2005; Rompré et al., 2002), and studies have reported a tight association between measurements of enzymatic activity and culture-based analyses (Farnleitner et al., 2002; Garcia-Armisen et al., 2005; George et al., 2000; Ouattara et al., 2011), but these measurements remain too time-consuming for rapid application.

Up-to-date technological developments have enabled fully automated measurements of enzymatic activity (e.g., using glucuronidases or galactosidases) that are suitable for on-site and real-time monitoring (Koschelnik et al., 2015; Ryzinska-Paier et al., 2014; Zibuschka et al., 2010). Measurements were reported to be possible in less-than-hourly intervals (Koschelnik et al., 2015; Stadler et al., 2016). The potential for real-time monitoring of enzymatic activity seems to be high, specifically for implementation into early warning systems and process control. Moreover, this method will help to improve the understanding of catchment behavior as well as contaminant transport processes in different habitats. In this respect, there is a need for studies testing prototypes for automated and on-site enzymatic activity determination to evaluate the consistency of measurement results, technical robustness during long-term on-site operation, and proxy capability for culture-based microbiological analyses in the observed habitat.

These specific questions were already addressed in recent research by Ryzinska-Paier et al., 2014 and Stadler et al., 2016, focusing on prototype operations either in groundwater (Ryzinska-Paier et al., 2014) or in surface water (Stadler et al., 2016). The test sites of these studies differ in hydrogeology, microbiological impact and hydrological catchment dynamics. However, up until now, no scientific overview exists that compares the application of automated measurements in these contrasting environments. The aim of this chapter is: i) to evaluate whether technical devices for automated measurements of enzymatic activity are technically robust for long-term on-site operation at groundwater and

surface water monitoring-locations, (ii) whether automated GLUC determination can be used as a proxy for culture-based *E. coli* analyses in various habitats, and (iii) whether enzymatic GLUC activity measurements can be used as a general indicator for the fecal contamination of water resources. In addition, further research needs regarding this innovative technology are discussed.
The experimental results from the aforementioned studies were used in this chapter to describe the application of on-site enzymatic methods. This chapter´s objective is to assess the question of whether, from an engineering point of view, automated measurements of enzymatic activity are possible in differing and technically challenging aquatic habitats. Furthermore, aspects of the proxy capability of on-site measured GLUC activity for culture-based analyses and the indicator potential of GLUC activity for the fecal contamination of water are addressed.

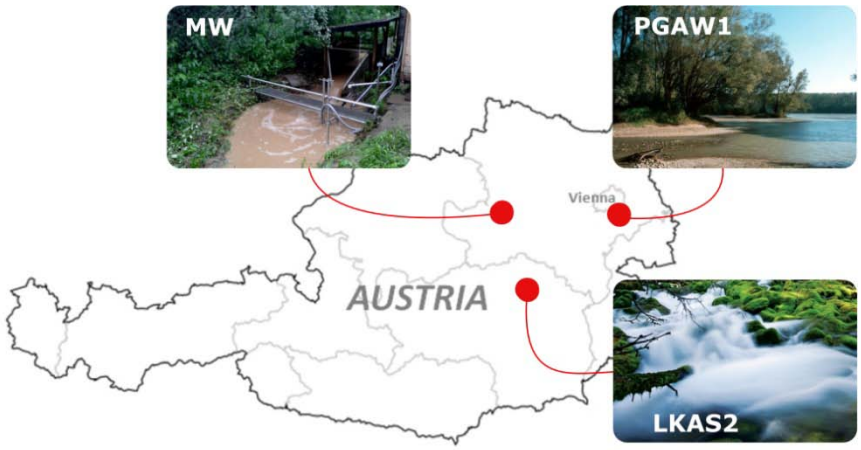

Fig. 12: Map of Austria. Test sites LKAS2, PGAW1 and MW are marked with red dots.

4.3. Materials and Methods

4.3.1. Data

The basis for this work emerges from research published by Ryzinska-Paier et al. (2014) and Stadler et al. (2016) describing the realization of automated on-site measurements of beta-D-glucuronidase in ground water (Ryzinska-Paier et al., 2014) and surface water (Stadler et al., 2016). The authors chose these studies as excellent sources of data to comparatively review the application of these measurements in contrasting water resources, including surface and groundwater. The following key characteristics of the studies support their comparison: i) both studies were conducted independently on test sites but provide a detailed background description of the aquifer characteristics, microbiological impact range and dynamics; and ii) the tested prototypes used the same substrate for the specific determination of beta-D-glucuronidase (GLUC) activity. Furthermore, data from laboratory analyses of GLUC activity are available in the literature (Farnleitner et al., 2002; Garcia-Armisen et al., 2005; George et al., 2000;

Ouattara et al., 2011), and therefore, a comparison between on-site and laboratory assays is appropriate.

4.3.2. Test sites

4.3.2.1. Karstic limestone aquifer

The karst spring LKAS2 is located in the Northern Calcareous Alps in Austria (Fig. 12). The hydrogeological catchment reaches altitudes of up to approx. 2200 m, with a total area of approx. 60 km². The hydrological regime is highly dynamic (Table 4) and is characterized by a prompt hydraulic response to intense precipitation events (Stadler et al., 2008). During the test period, a discharge maximum of almost 30 $m^3 s^{-1}$ and a minimum of 1.1 $m^3 s^{-1}$ have been recorded. The water temperature is fairly constant over the year, and the median of 5.4 °C represents an alpine catchment in this geographic region. Turbidity values reached a maximum of 4.5 FNU (Formazine Nephelometric Units) during intense runoff events. LKAS2 is highly vulnerable, and the dominant source of fecal pollution (ruminant) is surface-associated input during the grazing period in the summer due to hydrologic events (Reischer et al., 2008).

4.3.2.2. Porous groundwater aquifer

The well PGAW1 (Fig. 12) is located in an alluvial backwater area downstream of Vienna (Vierheilig et al., 2013). The water quality is stable throughout the year, and the impact of fecal contamination is widely not present. Contamination only tends to occur during flood events of the Danube River (Kirschner et al., 2014). The physico-chemical parameters (Table 4) coincide with a well assigned to a porous groundwater aquifer.

4.3.2.3. Surface water

MW is a monitoring location at a stream discharging a 0.66 km² catchment with 87% agricultural land use. The HOAL (Hydrological Open Air Laboratory, Blöschl et al., 2015) catchment is an experimental catchment in western Lower Austria (Fig. 12); it is characterized by elevated discharge dynamics (Table 4) with a rapid response to precipitation events (M. Exner-Kittridge et al., 2013). During the test phase, a discharge maximum of 73 l/s and a minimum of 0.5 l/s were recorded. During event runoff conditions, the stream water is sediment-laden, as indicated by turbidity values of up to 3210 FNU. The stream water temperature ranged between 0.2 °C and 20 °C following the trend of the yearly air temperature. The main source of fecal contamination is manure applied periodically on crop fields (Stadler et al., 2016).

Table 4: Comparative overview of the ranges of the measured basic hydrological parameters and fecal pollution by the cultivation-based standard fecal indicator *E. coli* at the three test sites (n=number of measurements, FNU=Formazine Nephelometric Units, MPN=Most Probable Number)

	LKAS2 (2010–2011)				PGWA1 (2010–2011)				MW (2014–2015)			
	n	median	min	max	*n*	median	min	max	*n*	median	min	max
Discharge (LKAS2, MW) Abstraction rate (PGWA1) [l/s]	*99423*	4107	1112	29795	*7589*	65	0	262	*8760*	2.3	0.5	73.4
Turbidity [FNU]	*110368*	0.3	0.0	4.5	*101370*	0.2	0.1	0.9	*8760*	8	0	3210
Conductivity [µS/cm]	*105013*	195	159	222	*11904*	563	493	650	*8760*	769	260	856
Temperature [°C]	*104933*	5.4	4.9	5.9	*13449*	11.4	9.8	13.1	*8760*	10.7	0.2	20
E. coli [MPN/100 ml]	*206*	0	0	435	*214*	0	0	0	*54*	172	0	3450

4.3.3. On-site GLUC measurements

For automated enzymatic activity determination, prototypes of two different devices were tested (BACTcontrol, formerly Coliguard: MicroLan, Netherlands; ColiMinder: VWM, Austria). Both designs detect beta-D-glucuronidase enzymatic activity and record and transmit the data in near real-time. The measurement principle is based on a photometric measurement chamber that enables high-resolution fluorescence analysis. During the measurement process, the sample mixed with specific assay reagents (proprietary information) generates an increasing fluorescence signal reflecting the level of enzymatic activity, which is monitored over time. Internal control parameters, such as the fluorescence signal, linearity of the fluorescence slope, temperature of the measurement chamber, the device´s environmental temperature, the measurement duration and blank value measurements are available for each data point and were used to initially quality check the measurement results. All devices were connected to a GPRS modem for data transfer and online access to the measurement device. GLUC activity measurements from both devices were performed in batches. ColiMinder devices used 6.5 ml of sample per measurement. The full ColiMinder measurement cycle, including cleaning and sample conditioning, lasts 30 to 40 minutes. BACTcontrol devices (Ryzinska-Paier et al., 2014; Zibuschka et al., 2010) enable adjustable sampling volumes (100 ml up to 5000 ml). For ground and spring water monitoring, 1000 ml was used; for surface water, 100 ml sample volume was used. The full BACTcontrol measurement cycle, including cleaning and sample conditioning, lasts 180 minutes. ColiMinder is calibrated to Modified Fishman Units (MFU/100 ml), based on the enzyme unit definition for beta-glucuronidase activity (Fishman and Bergmeyer, 1974); (Bergmeyer, 2012). BACTcontrol provides units of pmol/min/100 ml. Stadler et al., 2016 suggests an average one-to-one ratio between mMFU/100 ml and pmol/min/100 ml. Further technical details and information about the comparison of BACTcontrol and ColiMinder can be found in Stadler et al., 2016.

At test sites PGAW1 and LKAS2, BACTcontrol devices (MicroLan, Netherlands) for automated beta-D-glucuronidase (GLUC) activity were operated over a two-year period (2010 to 2011). At location MW, both BACTcontrol and ColiMinder were operated in parallel for over a year (2014 to 2015).

On-site measured GLUC data from all three test sites were compared to physico-chemical parameters monitored in parallel. The GLUC signals gathered at location MW with devices having two different constructions were compared with each other by performing linear regression analysis.

4.3.4. Hydrological and microbiological parameters

E. coli was used as a microbiological standard parameter of fecal pollution (Farnleitner et al., 2010). Water samples were analyzed for the cultivation-based bacterial standard fecal indicator *E. coli* using Colilert18 (ISO 9308-2:2012, MPN/100 ml). The sampling intervals ranged from biweekly (PGAW1, LKAS2) to monthly (MW).
At all test sites, online sensors were used to gather hydrological as well as physico-chemical parameters. On-site measurements of discharge (Q), electrical conductivity (EC), water temperature and turbidity in high temporal resolution enabled the ascertainment of the hydrological conditions in the observed catchment (Ryzinska-Paier et al., 2014; Stadler et al., 2016). The captured hydrologic dynamics, characteristic for each test-site, were used to assess the plausibility of automated GLUC measurements (e.g., event runoff conditions determined by decreasing the electrical conductivity and increasing the turbidity as an indicator for the potential influence of contaminated event water). The data from environmental background parameters were compared to GLUC measurements using Spearman´s rank correlation.

4.3.5. Comparison with standard laboratory enzymatic assays

Ryzinska et al. (2014) performed laboratory experiments to assess the comparability of automated enzymatic activity measurements (BACTcontrol) and standard enzymatic assays. A Sigma Aldrich assay was applied and methylumbelliferyl-□-D-glucuronide (MUG) was used as a substrate. Two comparative analyses were conducted, ranging from a short incubation time up to 75 minutes of incubation. The release of methylumbelliferyl (MUF) was evaluated using high performance liquid chromatography (HPLC).

4.4. Results

4.4.1. Technical realization

4.4.1.1. Technical application and long-term on-site operation

The tested prototypes proved to be reliable for continuous long-term operation for enzymatic measurements at the various test sites (Table 2). Measuring and cleaning procedures were conducted automatically and the measurement results were transmitted using the GPRS network. However, considerably shorter intervals for manual maintenance were needed for surface water operation

compared to those reported for ground water (Ryzinska-Paier et al., 2014; Stadler et al., 2016). On-site measurement data could be gathered for up to 6 months without technical failure at all locations, even in the presence of a total suspended solid concentration of up to 3 g/l (MW).
At pristine groundwater resources (Table 1, Table 2), such as site PGWA1, the level of GLUC was below the detection threshold of the assay. At sediment-laden stream water (MW), the main technical challenge was the high suspended solid (TSS) load in the monitored water during event runoff conditions. In this case, on-site sample pre-filtration was necessary to prevent the tubing and valves from clogging (Stadler et al., 2016). Reference analytics of unfiltrated and filtrated water samples showed no significant effect of filtration through 100 µm pore size on *E. coli* concentrations and enzymatic activity (Stadler et al., 2016).

Table 5: Comparative data for the tested devices at the contrasting surface and groundwater locations (modified from Stadler et al., 2016 and Ryzinska-Paier et al., 2014)

	ColiMinder	BACTcontrol
Test-site	***MW***	***PGAW1, LKAS2, MW***
Company	Vienna Water Monitoring (Austria)	MicroLan (Netherlands)
Tested substrate	beta-d-glucuronidase (GLUC)	beta-d-glucuronidase (GLUC)
Parameter	mMFU/100 ml	pmol/min/100 ml
Limit of quantification	0.8 mMFU/100 ml	1.5 pmol/min/100 ml
Time resolution (measurement incl. cleaning cycle)	60 min	180 min
Data transfer	GPRS modem	GPRS modem
Internal control parameters (metadata)	Fluorescence signal, slope of signal, temperature (measuring chamber, device), measurement duration, blank value measurement	Fluorescence signal, slope of signal, temperature (measuring chamber, device, LED), measurement duration, pump rating, blank value measurement
Blank value measurement (programmable)	every 12 hours	every 24 hours
Total test time	***MW*:** 12 months	***MW*:** 6 months* ***PGAW1, LKAS2*:** 12 months
2 devices operated in parallel	***MW*:** ColiMinder-01, ColiMinder-02	***MW*:** BACTcontrol-01, BACT control-02
Regular service interval (e.g., reagent refill, cleaning of tubing and filter)	***MW*:** biweekly	***MW*:** biweekly ***PGAW1, LKAS2*:** monthly
Technical service (e.g., re-calibration)	3–6 months	6–12 months

** BACTcontrol devices were operated at MW since 2012, but in this study, only measurements after the installation of an improved sampling setup in July 2014 are used.*

4.4.1.2. Comparability of automated GLUC measurements between devices

At location MW, four prototypes having two different constructions (2x BACTcontrol, 2x ColiMinder) were operated in parallel (Stadler et al., 2016). Linear regression analyses (all p-values<0.001) showed highly consistent results for devices with the same construction. A linear correlation coefficient, R^2, of 0.94

was found between the two ColiMinder apparatuses, and an R^2 value of 0.96 was obtained for the two BACTcontrol devices (Stadler et al., 2016). The correlations between devices with different designs showed reasonable consistency, with an R^2 value of 0.71 (Fig. 13).
BACTcontrol and ColiMinder drew samples from opposite sides of the stream, and sampling times may have differed by up to one hour, which likely contributed to the lower correlation. Overall, measurements from the two designs had a highly symmetrical range and an average one-to-one ratio of signals (Stadler et al., 2016).

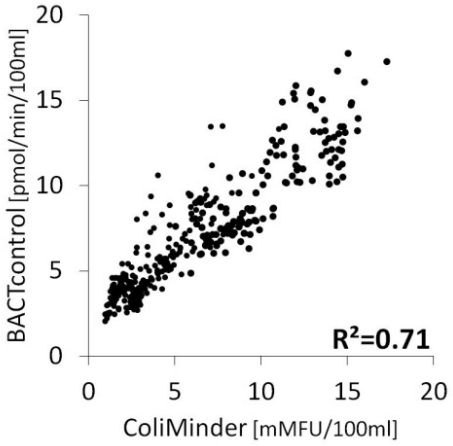

Fig. 13: Scatter plot showing the correlation of GLUC measurements conducted with devices having different construction (modified from Stadler et al., 2016)

4.4.1.3. Comparability to laboratory standard enzymatic assays

For both tested incubation times, the automated enzymatic measurements yielded results that were highly comparable to a standard Sigma assay (Ryzinska-Paier et al., 2014).

4.4.2. Range and dynamics of GLUC signals

GLUC signals monitored on-site in high temporal resolution had fundamentally different ranges and dynamics of enzymatic activity at the various test sites. GLUC data ranged from the limit of detection (PGAW1) to considerable levels of enzymatic activity (MW). The dynamics of GLUC activity were in general related to the changing hydrological conditions in the respective catchment, as indicated through the physico-chemical and hydrological parameters measured in parallel.
At the porous alluvial ground water resource (PGAW1), GLUC activity showed almost no variation (Fig. 14), ranging throughout the year within the limit of detection (Ryzinska-Paier et al., 2014).
At LKAS2, the GLUC signal reflected the very dynamic nature of a limestone-karstic aquifer (Fig. 14). In particular, events of intense summer precipitation caused peaks of enzymatic activity, reaching maxima of up to 6.0 pmol/min/100 ml. In the winter, one flooding event caused an increase of GLUC values up to 2.5 pmol/min/100 ml (Ryzinska-Paier et al., 2014).

The GLUC activity at the stream monitoring location MW was highly dynamic (Fig. 14). Characteristic for this test site was the increased background of GLUC activity during the summer months. The base signal of GLUC activity reached its maximum in August/September (Fig. 14), decreased during autumn and had a minimum in late winter (Fig. 14). Measurements taken with BACTcontrol ranged from 1.1 pmol/min/100 ml up to 108 pmol/min/100 ml (not shown here). ColiMinder recorded a minimum of 0.8 mMFU/100 ml and a maximum of 120 mMFU/100 ml. Precipitation events caused significant peaks of enzymatic activity in the stream water (Fig. 14). While all of the recorded hydrological events caused a prompt increase in GLUC activity in stream water, the amplitude of GLUC peaks was not exclusively determined by the intensity of the event, stream water turbidity or discharge (Stadler et al., 2016).

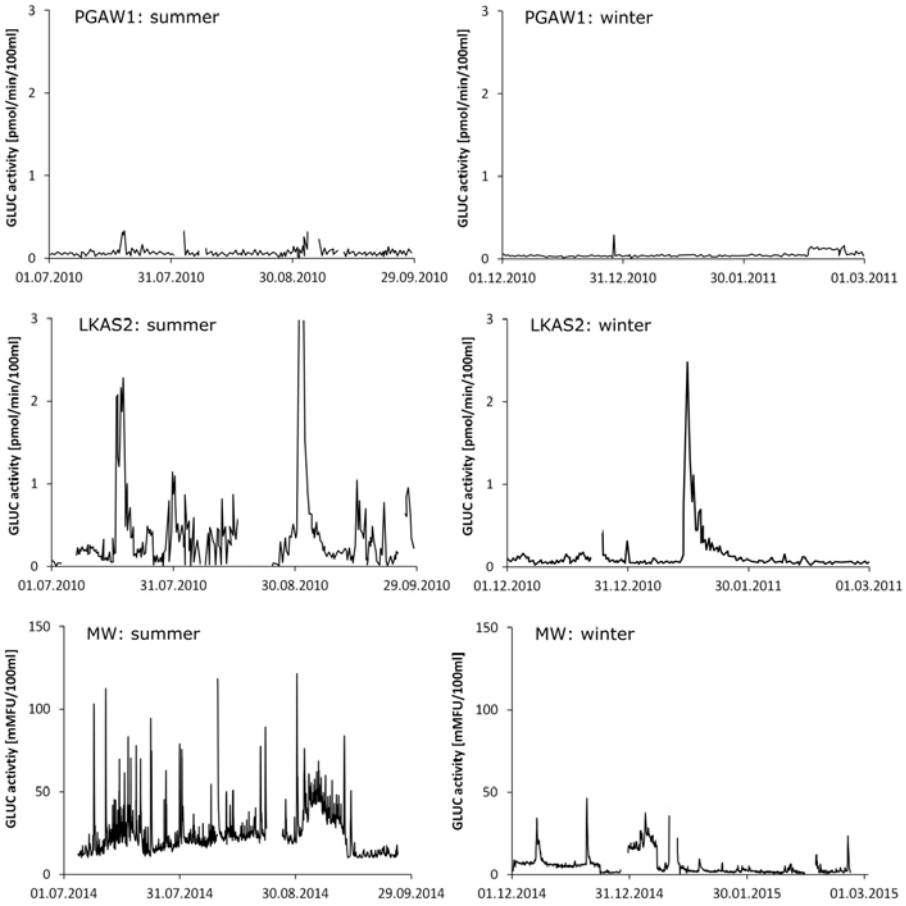

Fig. 14: Graphic showing on-site measured GLUC activity at the test sites. Left plot shows summer months; right plot shows measurements during winter (plot for PGAW1 and LKAS2 reproduced from Ryzinska-Paier et al. (2014) with permission from the copyright holders, IWA Publishing, plot for MW modified from Stadler et al. (2016)).

4.4.3. Comparison of GLUC data with hydrological and microbiological parameters

Cultivation-based *E. coli* were not detected in 100 ml samples, and the automated GLUC values did not exceed the limit of quantification at site PGAW1 throughout the test period.

At location LKAS2, the cultivation-based *E. coli* concentrations ranged from undetectable to 435 MPN/100 ml. Analyses of cultivation-based *E. coli* from the stream water samples collected at location MW revealed a range from undetectable to 3450 MPN/100 ml. Spearman´s rank correlation (Table 6) of cultivation-based *E. coli* with isochronal on-site GLUC measurements showed a ρ of 0.53 at site LKAS2 (Ryzinska-Paier et al., 2014). At location MW, the correlation between *E. coli* and GLUC values was higher. Here, ρ of 0.71 for BACTcontrol measurements and ρ of 0.83 for ColiMinder measurements were found (Table 6).

At LKAS1, the GLUC measurements were more strongly correlated with the physico-chemical parameters, such as discharge (ρ =0.77), than with cultivation-based *E. coli*. At location MW, the GLUC signals gathered with both constructions were more tightly correlated with cultivation-based *E. coli*. Still, the GLUC measurements conducted with ColiMinder were more tightly correlated with the physico-chemical parameters than those conducted with BACTcontrol. This may be interpreted as an effect of the higher temporal resolution of ColiMinder measurements, allowing a clearer tracking of promptly changing hydrological conditions (e.g., precipitation events). The correlations of GLUC values with the water temperature at MW revealed comparable levels for both BACTcontrol (ρ =0.43) and ColiMinder (ρ =0.35).

Table 6: Comparison of GLUC measurements with environmental hydrological parameters and cultivation-based *E. coli* enumeration (ρ =Spearman´s rank correlation coefficient, n=number of samples, P=p-value).

	LKAS2 BACTcontrol			**MW** BACTcontrol			**MW** ColiMinder		
	n	**ρ**	*P*	*n*	**ρ**	*P*	*n*	**ρ**	*P*
GLUC vs. discharge	*1804*	**0.77**	*<0.001*	*846*	**0.08**	*<0.05*	*1564*	**0.17**	*<0.001*
GLUC vs. turbidity	*1804*	**0.69**	*<0.001*	*846*	**0.35**	*<0.001*	*1564*	**0.72**	*<0.001*
GLUC vs. water temperature				*846*	**0.43**	*<0.001*	*1564*	**0.35**	*<0.001*
GLUC vs. *E. coli*	*113*	**0.53**	*<0.001*	*52*	**0.71**	*<0.001*	*52*	**0.83**	*<0.001*

4.5. Discussion

4.5.1. Technical application

The tested devices for automated enzymatic activity determination proved to be reliable and robust under the diverse sets of field conditions they were subjected to. The time and staff required for the regular maintenance of the apparatuses was manageable and reasonable for on-site devices that are continuously operated,

even for the monitoring of sediment-laden stream water. These biweekly to monthly service intervals appear realistic for potential monitoring applications, such as the surveillance of process systems, wastewater treatment plants or bathing waters.
Reference analyses with a standard enzymatic assay showed a high level of agreement between the results from automated devices and laboratory analyses. The comparison of different constructions of devices for the automated measurement of enzymatic activity showed comparable results.
The GLUC signals gathered at the various test sites appeared to be plausible with respect to the fact that they generally reflected the hydrological dynamics and catchment characteristics (Fig. 14). Furthermore, the ranges of GLUC activity determined for the examined test sites appeared reasonable when they were compared to each other and to previously published results (for further details, see 4.2.).

4.5.2. Range and dynamics of GLUC signals

Previous studies reported GLUC values of up to 10^6 pmol/min/100 ml in water heavily influenced by municipal sewage (Farnleitner et al., 2002; Garcia-Armisen et al., 2005; George et al., 2000; Ouattara et al., 2011). The GLUC signals at station MW were consistent with an agricultural catchment subject to periodic manure application on the crop fields, ranging from the lower limit of detection to a value of signifying fecal contamination with maximum values of 108 pmol/min/100 ml and 120 mMFU/100 ml. The values for GLUC activity in fairly unpolluted karst water at site LKAS2 reached maximum values of 6 pmol/min/100 ml. At a protected groundwater resource, such as PGWA1, the values were below the limit of detection. On-site GLUC measurements at MW reached values almost twenty times higher than those at LKAS2. While the GLUC dynamics at the karst water resource LKAS2 were event-driven, the GLUC signal captured at MW was not exclusively determined by the hydrologic conditions. An increase in the baseline enzymatic activity signal during the summer was interpreted as the influence of ambient and water temperatures, as well as land management practices, on enzymatic activity.

4.5.3. GLUC as a proxy indicator for cultivation-based *E. coli* enumeration?

The evaluation of the data gathered at LKAS2 and MW showed significant differences in the association between GLUC data and *E. coli* analyses by means of culture-based enumeration. Therefore, the eligibility of automated measured GLUC activity as a proxy for standard microbiological methods was not verified. These results are in contrast to studies testing the comparability of laboratory enzymatic assays and culture-based analyses, where high associations between fecal indicator bacteria and GLUC were reported (Farnleitner et al., 2001, 2002; Fiksdal et al., 1994a; George et al., 2001). The discrepancy between results from GLUC enzymatic measurements and cultivation-based *E. coli* analyses, and the

dependence of this association on the observed habitat, was demonstrated for the first time by the automated and on-site gathered GLUC measurements in the different investigated catchments.
Studies reporting tight correlations between GLUC and *E. coli* have focused on catchments with a recent point-source influence from municipal sewage effluents (Farnleitner et al., 2001, 2002; Fiksdal et al., 1994a; George et al., 2001). In contrast, the dominant sources of fecal contamination in the catchments studied herein are considered recent and aged swine manure application (MW) and ruminant feces (LKAS2) applied as non-point sources to the surface. The correlation between cultivation-based *E. coli* and GLUC values at site MW lies within those reported for sites under the impact of municipal sewage and the low association reported for karstic groundwater (Ryzinska-Paier et al., 2014). The authors assume that the runoff patterns and discharge dynamics in the studied catchment as well as the fecal contamination source type and age play a significant role. It is hypothesized that the association between the GLUC activity and culture-based *E. coli* analyses strongly depends on the observed habitat. GLUC measurement methods are capable of detecting activities from all enzymatic active target bacteria, including the so-called VBNC (viable but non-cultivable) sub-populations, whereas culture-based methods are not (J. P. S. Cabral, 2010). Furthermore, the highest correlations between the cultivation-based *E. coli* and GLUC values are expected in habitats under the influence of fecal pollution originating predominantly from a recent point source of contamination, such as wastewater treatment plant effluents. Additionally, the complexity of runoff patterns, as well as various sources and ages of fecal contamination, lead to varying proportions of the VBNC sub-population, which are associated with different compartments in the catchment. This will consequently weaken the associations between cultivation-based *E. coli* enumeration and enzymatic activity measurements.

4.5.4. GLUC as a conservative biochemical indicator of fecal pollution?

A further question focuses on the capacity of automated GLUC measurements as a conservative biochemical marker of microbial fecal pollution, targeting all types of fecal-associated cells, whether live, dead or dormant. However, there exist no data to date which enable the specific evaluation of this research question for contaminated water resources. Detailed studies need to be performed in the future.
In this respect, cross-sensitivities as well as interferences of enzymatic activity by non-fecal compounds, such as algae or organic matter, have been reported previously (Biswal et al., 2003; Fiksdal and Tryland, 2008; Molina-Munoz et al., 2007). These mechanisms of interference may limit the capacity of GLUC as a conservative biochemical marker for microbial fecal pollution. Togo et al., 2010 also reported amplifying the inhibitory effects on GLUC activity, due to the abundance of different ions in the water samples. Furthermore, Chang et al., 1989 described the presence of fecal-derived *E. coli* that are not active with respect to beta-D-glucuronidase activity.

4.6. Conclusions and perspectives

As it is currently state-of-the-art for gathering chemo-physical parameters, the automation of microbial monitoring will likely become increasingly relevant in the near future. In this respect, the automated determination of enzymatic activities has great potential to make a significant contribution to complement online water quality monitoring. It has been clearly demonstrated that the automated monitoring of enzymatic activities is now technically feasible. However, the type of substrates that can be used and their ability to serve as meaningful indicators still require further scientific evaluation.

Notably, the application of enzymatic activity monitoring is not limited to raw water resources, but is likely of high interest wherever enzymatic activities can be applied as a useful indicator of microbial activities. This is likely to be the case for process monitoring, such as screening for bacterial re-growth potential in engineered systems.

Further development of available substrates and the application of different substrates may also enable a more specific and diverse assessment of enzymatic activities. For example, xylanase or invertase can be evaluated as enzymes involved in C transformation, urease or amidase can be evaluated as enzymes involved in N transformation, arylsulfatase can be used as an enzyme involved in organic S transformation, and alkaline phosphatase and acid phosphatase can be evaluated as enzymes involved in organic P transformation ((Burns and Dick, 2002; Chrost, 2012; Hoppe, 1991, 1983).

Furthermore, technological advances in the automated monitoring of enzymatic activity will permit a transition from static on-site operation at monitoring stations to mobile applications using portable devices.

Chapter 5

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

5.1. Abstract

The automated and rapid determination of enzymatic activity of water resources is an emerging biochemical parameter that can be measured on-site and in near-real time. Recent applications of this novel technology suggested that such measurements can serve as a rapid indicator for microbial pollution monitoring. Up to now automated on-site measurements of enzymatic activity have been conducted following a static approach focusing on the temporal variability at definite monitoring locations. This study assessed the capability of automated enzymatic activity measurements conducted from a mobile research vessel to detect the spatial variability of beta-D-glucuronidase (GLUC) activity on large fresh water bodies. Surveys have been performed on the Columbia River, the Mississippi River and on Lake Mendota covering up to 500 km river course or 50 km^2 lake area, respectively. The observations provide for the first time high resolution spatial data of GLUC activity on large water bodies and document its association to hydrological conditions and land use. The ship-borne measurements disclosed effects of precipitation events and urban run-off on the GLUC activity of inland waters, localized point in-lets of potential fecal pollution and showed an increasing GLUC signal along a gradient of urbanization. The habitat specific relation of the enzymatic assay with standard *E. coli* analyses for an aestival lake environment showed a reasonable correlation (R^2=0.71).

5.2. Introduction

Science questions have accompanied the technological progress to achieve observations in higher temporal and spatial resolution focusing on various fields of today´s environmental research (Cuffney et al., 2000; van de Giesen et al., 2014; “WHO | Water quality assessments,” n.d.). More affordable equipment of manageable size allows the on-site measurement of a broad range of physico-chemical and bio-chemical parameters in waters and lead to new findings

concerning hydrological, biological and ecological processes(Dent and Grimm, 1999; Gish et al., 2005; Grayson and Blöschl, 2001; Guan et al., 2011). The increasing abundance of monitoring stations and sample locations at water resources allows us a better insight into the spatial distribution of relevant parameters and improved modeling as well as the theoretical understanding of natural systems(Beven, 1989; Brettar and Höfle, 1992; Collins and Rutherford, 2004; Dent and Grimm, 1999; Farnleitner et al., 2002; Fiksdal et al., 1994a; Fleckenstein et al., 2010; Savio et al., 2015; Stadler et al., 2010, 2008). Still the deployment of static monitoring stations at definite locations accommodates constrains regarding their spatial distribution. Therefore endeavors have been undertaken to bridge the spatial gap by means of measurements from mobile platforms (Crawford et al., 2015; "HydroSphere Drifter Brings Lagrangian Sampling To Freshwater," 2016). The achievement of such mobile and vastly autonomous platforms shed new light into lagrangian monitoring and the spatial heterogeneity of crucial quality parameters, such as nutrients, in water resources (Crawford et al., 2016, 2015; Pellerin et al., 2014).

Yet, the understanding of large water bodies´ microbiology, especially concerning fate, transport processes and pathways of microbial pollutants, is predominantly based on assays requiring elaborate sampling and laboratory efforts resulting in limited spatial and temporal resolution (João P. S. Cabral, 2010; Savio et al., 2015). Rivers and lakes are as receiving water bodies widely impacted by discharge from urban, industrial or agricultural areas often containing pathogenic bacteria (Bradford et al., 2013; Ferguson et al., 2003; Mawdsley et al., 1995; Pachepsky et al., 2006; Tyrrel and Quinton, 2003). Health-related water quality research, but also the management, allocation and utilization of such water resources could highly benefit from an enhancement of the spatial resolution of microbial parameters. The detection of enzymatic activities has been proposed as a rapid surrogate for the microbiological pollution monitoring of water resources (João P. S. Cabral, 2010; Farnleitner et al., 2001, 2002). Automated on-site measurements of enzymatic activity are nowadays feasible from a technical point of view, have been conducted at stationary monitoring stations on different water resources and can be used as an indicator for microbiological contamination of water on a catchment specific basis (Ender et al., 2017; Ryzinska-Paier et al., 2014; Stadler et al., 2017, 2016).

In this work, spatial variability of enzymatic activity on surface water resources is highlighted for the first time by means of rapid and automated beta-D-glucuronidase (GLUC) activity measurements from a mobile research vessel (Fig. 15). The aim of the conducted research was to answer the science questions, (a) if ship-borne GLUC measurements on water bodies varying fundamentally in respect do their catchments´ land use and water quality (Table 7) are technically applicable, (b) if previously unknown spatial patterns of GLUC activity on large freshwater bodies and its variation due to differing land use and hydrological conditions can be disclosed using the presented approach and (c) if ship-borne geo-referenced enzymatic activity data can be used for the generation of GLUC activity screening maps for the respective water body. The intend of these maps is to provide a biochemical snapshot of surface waters applicable to rapidly localize

point in-lets of potential fecal pollution, such as tributaries or storm drainages in near-real time. The four presented surveys exemplary visualize a novel approach for water quality screening of inland waters and are focused on the better understanding of microbial transport 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.

5.3. Material and Methods

5.3.1. Instrumentation

The essential technical base for the conducted research was the FLAMe platform (Fig. 15) for fast automated and ship-borne limnological measurements, described by Crawford et al. (Crawford et al., 2015). The core feature of the FLAMe is a flow through system that allows the ship-borne sampling (temporal resolution of up to 1 Hz) of inland waters at both low and high speeds. A high output diaphragm pump delivered ambient water to an array of probes mounted inside the boat, such as an YSI EXO2 multiparameter probe (parameters: 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 (parameter: nitrate (NO_3-N [mg/l]). Additionally a sprayer type equilibration system was used to equilibrate dissolved gases, which were measured for carbon dioxide (CO_2; [ppm]) and methane (CH_4; [ppm]) using a Los Gatos Research ultraportable greenhouse gas analyzer. The FLAMe can integrate additional sensors with simple modifications. Here, we used a peristaltic pump to deliver water immediately and unaltered (upstream of the sensors and diaphragm pump) from the FLAMe system to an instrument prototype capable of rapid measurements of enzymatic activity (described below). All measurements were georeferenced with an onboard GPS (WAAS enabled), time-corrected based on internal flow rates and processing times, and merged using time stamps. All measuring devices had a time lag less than one minute after sample abstraction.

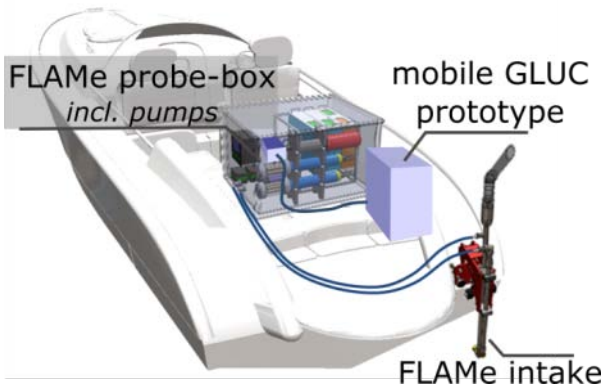

Fig. 15: Schematic showing the FLAMe monitoring platform used for shipborne enzymatic activity (GLUC) measurements (modified after Crawford et al., 2015). The FLAMe intake enables sample abstraction at various speeds from approx. 30 cm water depth. The FLAMe probe-box contains pumps

and sensors (such as an YSI multiparamter sonde). The prototype for mobile GLUC activity measurements received sample water from the FLAMe box via a peristaltic pump connected upstream of the main pump.

In this chapter, we used a mobile prototype capable of rapidly measuring GLUC activity of waters. GLUC activity has been found to be significantly associated to the abundance of *E. coli* in waters and was therefore suggested as an indicator parameter for the rapid assessment of the microbiological water quality (João P. S. Cabral, 2010; Farnleitner et al., 2001; Fiksdal et al., 1994a; George et al., 2000; Morikawa et al., 2006). The construction and function of the prototype used for automated and rapid enzymatic measurements has been described by Koschelnik et al. (Koschelnik et al., 2015) and Stadler et al. (Stadler et al., 2016). Recent applications of a portable prototype for measuring enzymatic activity of waters in remote locations have been conducted by Ender et al. (Ender et al., 2017). The automated measurements of enzymatic activity are based on a flow-through photometric measurement-chamber which enables a high resolution fluorescence analysis. The shape of the measuring-chamber and the fluidic system are optimized for automated water sampling, reagent dispensing and effective cleaning process. In order to get accurate fluorescence readings independent of turbidity, a data correction algorithm was used (Koschelnik et al., 2015). The measurements were performed in batches using 6.5 ml of sample per measurement. The measurement step takes 15 minutes and the assay has been calibrated to Modified Fishman Units (MFU/100ml), based on the enzyme unit definition for beta-D-Glucuronidase (GLUC) activity (Fishman and Bergmeyer, 1974); (Bergmeyer, 2012). Previous studies showed degeneration effects on the glucoronidase substrate during exposure (>24 hours) at ambient temperatures above 25 °C (Stadler et al., 2016).Therefore, for on-board operation the glucuronidase substrate has been apportioned in vials, each holding a small substrate amount adequate for only 50 measurements. One substrate vial was deployed in the prototype, while stock was kept constantly refrigerated in an ice chest and supplied when necessary. The prototype for automated and mobile GLUC measurements has been housed in a weatherproof case of manageable size (Pelican: type 1440) suitable for on-site and outdoors operation. All probes and equipment for ship-borne measurements were supplied by 12 V DC on-board power and batteries.

5.3.2. Potential sources of GLUC activity

A high correlation of GLUC activity with *E. coli* has been reported for waters impacted by municipal sewage(Farnleitner et al., 2001, 2002) as well as manure(Stadler et al., 2016). The dominant sources of GLUC activity in waters impacted by urban areas are assumed to be waste water treatment plant effluents(Hendricks and Pool, 2012), the in-put of surface associated fecal matter due to urban run-off(McCarthy et al., 2012) as well as in some places feces of small mammals inhabiting drain pipes (such as raccoons(Bondo et al., 2016)). Leaking sewer lines causing untreated wastewater to reach storm drains are a diffuse source of concern, also in places facilitated with separated sewer

systems(Sercu et al., 2011, 2009). In agricultural areas the dominant source of GLUC activity in waters is assumed to be the in-put of manure, due to life-stock or slurry application on crop-fields, respectively (Bradford et al., 2013; Farnleitner et al., 2011; Pachepsky et al., 2006). A relevant source of fecal indicator bacteria (FIB) and consequently GLUC at lake beaches can be water birds, such as geese(Meerburg et al., 2011; Whitman and Nevers, 2004). Furthermore, the growth and survival of *E. coli* in lake environments has been reported to be affected by algae (Byappanahalli et al., 2003; Englebert et al., 2008; Kaplan and Bott, 1989; Whitman et al., 2003).
Cross-sensitivities as well as interferences of enzymatic activity by non-fecal compounds, such as algae or organic matter, have been discussed in literature previously (Biswal et al., 2003; Fiksdal and Tryland, 2008; Molina-Munoz et al., 2007). These mechanisms of interference may limit the capacity of GLUC as a quantifying surrogate for *E. coli* but showed to be secondary in terms of the applicability of GLUC as a qualitative indicator for a potential fecal pollution of water resources(Ender et al., 2017; Koschelnik et al., 2015; Ryzinska-Paier et al., 2014; Stadler et al., 2016).

5.3.3. Data interpretation

To visualize spatial patterns, we generated maps of each variable across the water body surfaces using an inverse distance weighting algorithm. For the limnological data, we randomly selected 10% of each dataset to improve processing time and account for autocorrelation. Because GLUC activity was measured less frequently (i.e., every 15 minutes) measurements were assumed to be independent and all data were used for interpolation. We note that presented GLUC activity maps are estimated values across each water body and intended for qualitative screening proposes, rather than for a quantitative determination of GLUC values over the probed water body.
To assess drivers of GLUC activity, parallel monitored limnological parameters were used for an enhanced data interpretation in respect to contaminant pathways and transport processes. Associations of GLUC activity with the isochronal measured physico-chemical parameters were examined using linear regression analyses (Table 8).

5.4. Test sites

5.4.1. Lake Mendota:

Lake Mendota is a medium sized eutrophic lake located in Wisconsin, USA. The lake has been object of many studies in aquatic ecology and limnology during the last decades(Brock, 2012; Bryson and Suomi, 1952; Carpenter et al., 2007) and it is regularly monitored by the North Temperate Lakes Long Term Ecological Research (NTL-LTER) program. Covering a surface are of 39.9 km² it is the largest and northernmost lake of a chain of four lakes on the Yahara River.(Brock,

2012) Lake Mendota´s catchment area is ~560 km² with approximately 70% of the lake's water budget coming from the Yahara River and Sixmile Creek (Brock, 2012). These rivers flow into the lake's northern bay and drain predominately agricultural watersheds. Several urban areas (including the City of Madison) are located in the immediate surroundings of the lake, many of which route storm water run-off directly into the lake. The lake is a popular recreation site with several public beaches that are monitored weekly for fecal indicator bacteria and blue green algae during swimming season by the Public Health Madison and Dane County ("Madison & Dane County Beaches - Water Quality - Public Health - Madison & Dane County - City of Madison, Wisconsin," n.d.). The dominant pathways and sources of GLUC activity for Lake Mendota are assumed to be (a) tributaries on the north shore such as the Pheasant Branch Creek and the Yahara River, draining agriculturally used (with live stock) catchments and receiving effluents of waste water treatment plants in places (b) the population of geese inhabiting recreational beaches, (c) fecal contamination of various urban sources that reaches the lake via storm drains during storm run-off conditions.

5.4.2. Three lakes

Continuing downstream from Lake Mendota, the Yahara River flows into Lake Monona and Lake Waubesa (Brock, 2012). Lake Monona and Lake Waubesa are smaller than Lake Mendota with surface areas of 13 and 8 km², respectively. Similar to Lake Mendota, both lakes have a highly agricultural and urbanized watershed. While the northern part of Lake Mendota is mainly influenced rivers draining predominately agricultural watersheds, the proportion of urban areas relative to the catchment size increases moving downstream. A survey of Lake Mendota, Monona, and Waubesa was conducted along this urbanization gradient. The dominant pathways and sources of GLUC activity for the chain of lakes are assumed to be similar to those described for Lake Mendota, but with an increasing influence of urban storm-drains downstream.

5.4.3. Columbia River

The Columbia River is the fourth largest river (by flow) in the United States. Its watershed includes parts of British Columbia and Alberta, Canada and the U.S. states of Montana, Idaho, Wyoming, Utah, Nevada, Oregon, and Washington. 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, is a relevant route for navigation as well as a significant source of hydro electrical 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 many parts of the eastern LCR watershed (e.g., the Umatilla and Yakima Rivers), irrigation is intensively used to support agriculture. The western LCR includes large population centers (such as Portland, OR) in addition to a mixture of predominately agriculture and forest

land uses. Microbiological studies in the LCR basin focused mainly on tributaries and the estuary (Crump et al., 1999; Cuffney et al., 2000). The LCR was chosen as a riverine test site because of its significant variability of climate and land use along the course. The dominant sources and pathways of GLUC activity into the LCR are both from agricultural and urban areas. Primary agricultural sources of GLUC are assumed to be areas with live stock, such as the Yakima and the Umatilla basin. Urban sources of GLUC are assumed to be dominated by the input of waste water treatment plant effluents into the LCR and its tributaries from population centers, such as Richland, Pasco, Hermiston, The Dalles and Portland.

5.4.4. Mississippi River

The Mississippi River is the largest river in North America, draining parts of 37 U.S. states and ultimately discharging into the Gulf of Mexico. The Upper Mississippi River (UMR) drains one of the most intensively used agricultural regions in the world (“Corn Belt | 2012 <acronym title="National Resources Inventory">NRI</acronym> | NRCS,” n.d.), known as the U.S. Corn Belt. Heavily impacted by agricultural runoff, the UMR has been subject for 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). Low-head dam and lock constructions facilitate navigation through the UMR. The dams divide the river into “Pools”, which encompass a variety of aquatic areas including: main channels, side channels, impounded areas, and backwaters. Our survey included Pool 8, which is a 30 km section of the UMR near the city of La Crosse, Wisconsin. The surface area of Pool 8 is ~100 km², but varies seasonally based on discharge and dam operations(Turner et al., 2016). Pool 8 was chosen as a fluvial system with contrary characteristics, compared to the LCR, regarding land use patterns and water quality. The sources and pathways of GLUC activity into Pool 8 of the UMR are assumed to be dominated by in-puts from agricultural areas with live stock from both up-stream sources, as well as tributaries discharging directly into Pool 8, such as the La Crosse and the Root River. Relevant urban sources of GLUC are assumed to be the effluents of waste water treatment facilities of both population centers upstream of Pool 8 (such as Minneapolis) as well as the city of La Crosse neighboring Pool 8.

Table 7: Table showing minimum, median and maximum values of limnological parameters as well as GLUC activity for Lake Mendota, Lower Columbia River and Upper Mississippi River determined during the presented surveys (including confluences). Comparison of parameters highlights the significant differences of the water bodies in regards to physico-chemical characteristics as well as nutrient impact and GLUC activity.

	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)	*31153*	20.1	(17.7 - 28.5)	*90596*	27.3	(25.1 - 30.39)	*22720*
Turbidity [FNU]	2.4	(0.3 - 19.9)	*31148*	1.5	(0.0 - 9.7)	*90596*	6.1	(1.1 - 25.95)	*22718*
SPC [µS/cm]	521	(345 - 796)	*31153*	127	(87 - 419)	*90596*	371	(210 - 549)	*22720*
pH	8.4	(7.5 - 8.7)	*31151*	8.0	(7.4 - 9.7)	*90596*	7.5	(6.9 - 8.3)	*22720*
NO3-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)	*31153*	1.0	(-0.3 - 348.3)	*90596*	13.0	(3.8 - 92.1)	*22720*
fDOM [RFU]	7.8	(6.0 - 29.6)	*31153*	0.0	(-0.4 - 24.5)	*90596*	28.8	(0.79 - 30.65)	*22720*
GLUC [mMFU/100ml]	6.2	(0.8 - 32.9)	*38*	1.8	(0.8 - 20.3)	*80*	7.5	(5.0 - 15.0)	*23*

5.5. Surveys

5.5.1. Survey 1: GLUC activity in a lake - spatial heterogeneity and indicator applicability, Lake Mendota

The survey on Lake Mendota consisted of different work phases with the aim (i) to test the technical applicability of prototypes for the ship-borne determination of enzymatic GLUC activity, (ii) to disclose previously unknown spatial and temporal patterns of enzymatic GLUC activity in lake environments, as well as the impact of precipitation events and urban run-off on such patterns and (iii) to gain information about the habitat specific correlation of rapid GLUC measurements with standard culture-based assays for fecal indicator bacteria.

Survey 1a: We conducted three surveys of the spatial pattern of GLUC in Lake Mendota in June and July 2016. The survey dates varied with respect to time since precipitation events. On each date, we followed a grid-like track that covered the entire lake surface, generating 12 to 13 GLUC measurements. Each tour lasted 3 hours when motor-boating at ~50 km/h. Focus was set on a representative distribution of GLUC measurements over the lake area but also to capture relevant points, such as the confluences of the Yahara River (north shore) and Pheasant Branch Creek (WNW shore) and a discharge point of a storm water run-off channel (south shore). Tours were preformed with a time-lag ranging from 6 days to several hours after heavy summer-storm precipitation events. Yahara River discharge and precipitation data for these periods was derived from the USGS stream site 05427850 (Yahara River at State Highway 113).

Survey 1b: To disclose the effects of urban run-off on the GLUC activity of lake water, one measurement-tour in June 2016 focused only on the south shore of Lake Mendota and yielded high resolution spatial GLUC data (n=17) between a channel for urban storm water run-off and the lake outlet. Survey 1b has been conducted during dry weather, with 6 days since the last precipitation event.

Survey 1c: To assess the indicator applicability of rapid GLUC measurements for culture based *E. coli*, a reference sample campaign was initiated during July 2016. Water samples (n=18) from different beaches of the Madison Lakes were analyzed with both the culture-based ISO 9308-2:2012 assay (Colilert18) and the prototype that was deployed for the ship-borne GLUC measurements. The samples were abstracted within the weekly water quality monitoring program of public beaches in Dane county, conducted during swimming season by the Public Health Madison and Dane County ("Madison & Dane County Beaches - Water Quality - Public Health - Madison & Dane County - City of Madison, Wisconsin," n.d.).

5.5.2. Survey 2: GLUC activity along a gradient of urbanization, Three Lakes

In July 2016 a one-day measurement-tour followed the Yahara River downstream from its confluence at the north of Lake Mendota, through Lake Monona and further into Lake Waubesa. The whole course was navigable and GLUC measurements (n=23) were taken in all three lakes, as well as the stream intercepts and wetlands of urban storm drainage connecting these lakes. Survey 2 has been conducted during dry weather, with no immediate influence of precipitation events on the lakes.

5.5.3. Survey 3: Influence of land use on the GLUC activity of river waters, Lower Columbia River

From July 12th to 18th 2016 a survey has been conducted during prevailing dry weather conditions on the main channel of the LCR, covering approx. 500 km of river course and yielding 80 GLUC measurements. The route started downstream of the Priest Rapids Dam (46.644, -119.909), and ended near Westport, Washington (46.143, -123.381) ~55 km from the river terminus at the Pacific Ocean. 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.

5.5.4. Survey 4: Influence of land use on the GLUC activity of river waters, Upper Mississippi River

The Navigation Pool 8 of the UMR has been surveyed during dry weather conducting a one day measurement-tour in August 2016. Ship-borne GLUC measurements (n= 23) have been conducted along the main navigation channel, within impounded backwater areas as well as at the confluences of the La Crosse River and the Root River, tributaries of the UMR draining agriculturally used watersheds in Wisconsin and Minnesota.

5.6. Results and Discussion

5.6.1. Survey 1a: Impact of hydrological events on the GLUC activity of lake water

The influence of storm water run-off, both from agricultural and urban areas, on the GLUC activity of lake water has been disclosed during survey 1a. Depending on the time since the last precipitation event and its intensity, the GLUC activity of lake water shows distinct spatial variability (Fig. 16). While the center of the lake, with its deepest water column showed GLUC values constantly close to the limit of detection (0.8 mMFU/100ml), higher values have been observed along the shore – especially at confluences (Yahara River up to 32.9 mMFU/100ml). Major variation of the GLUC activity signals were found in the plume of the Yahara River confluence, especially regarding its changing spatial extent (Fig. 16A, B and C). The smaller the time-lag between the measurement tour and the last precipitation event was, the further the Yahra River Plume reached into the lake (Fig. 16A, B and C). Significantly increased GLUC values were found at all confluences and inlets (including Pheasant Branch Creek and the storm drainage on the south shore) in the immediate aftermath of a local and intense storm event (Fig. 16C). The hydrograph of the Yahara River did not show a significant peak in this case, as precipitation likely occurred very locally and not in the headwater. It is assumed that this short and intense storm resulted predominantly in overland flow around the lake area. Strong westerly winds, resulting in an eastbound swell and an input of surface associated debris may account for the high GLUC signals on the west shore of the lake, at the confluence of the Pheasant Branch Creek. Results show also an increase of GLUC activity (up to 26.4 mMFU/100ml) at the S and SSE shore of the lake due to run-off from the urban areas of Madison.

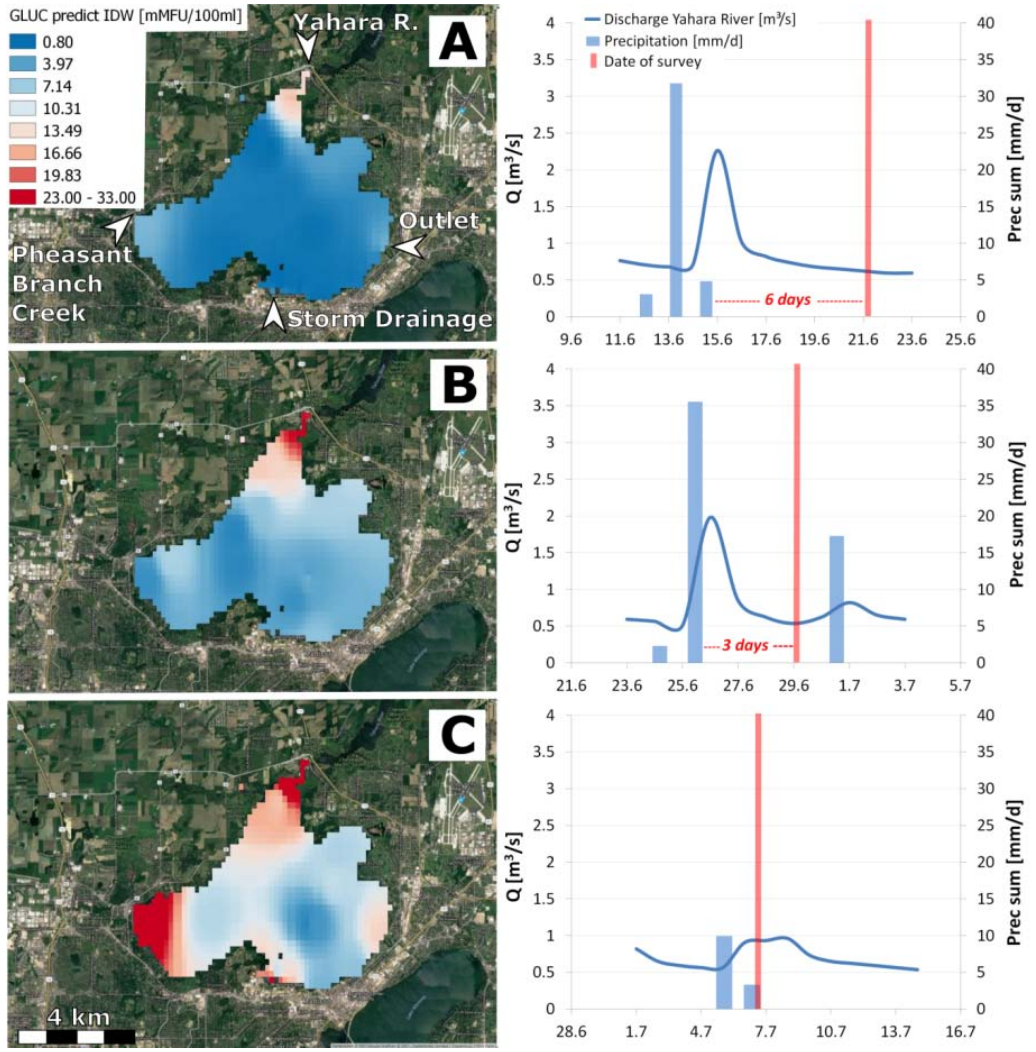

Fig. 16: Results of the survey 1a focusing on the impact of hydrological events on the GLUC activity of Lake Mendota. GLUC activity screening maps on the left (A, B, C) were generated using inverse distance weighting and show the diverse spatial patterns of GLUC activity on the lake depending on time since last precipitation event. On the right the corresponding hydrograph of the Yahara River (blue graph), precipitation amount (light blue bars) and date of survey (red bar) are shown.

5.6.2. Survey 1b: GLUC activity in urban, bankside lake water

High resolution ship-borne measurements disclosed the spatial variability of GLUC values in lake water directly adjacent to an urban area at dry weather conditions (Fig. 17). During survey 1b, significant lower GLUC values (up to 5.0 mMFU/100ml) have been observed at the south shore of Lake Mendota compared to the north shore (survey 1a, up to 32.9 mMFU/100ml). The south shore, neighboring an urban area, showed the maximum GLUC values at the inlet of a storm-drainage and at the lake´s outlet. This was indicated already during survey 1a (Fig. 16C), but with coarser resolution.

Linear regression analyses between electrical conductivity and high resolution GLUC activity measurements of lake water (Fig. 17) supported the assumption that the input of fecal indicator bacteria into the lake is, in this part, predominantly

caused by the input of surface associated matter due to urban run-off as a result of heavy precipitation events: The input of precipitation water and consequently urban run-off causes a decrease of the electrical conductivity of the bankside lake water (during seasons where no road salt is applied). For survey 1a, with the dominant influence of the Yahara River, GLUC activity shows no statistical association with electrical conductivity (R^2=0.08, p>0.05). The results from the survey 1b focusing only on the urban lake shore show that GLUC activity values are negatively correlated (R^2= 0.73, p<0.001) with the electrical conductivity. The increased GLUC values at the confluence of the storm-drainage (Fig. 17) can clearly be aligned to urban drainage water. Highest GLUC values and lowest SPC were observed in lake water during survey 1b at Tenney Beach, next to the lakes outlet (Fig. 17).

Geese lingering at Tenney Beach and the nearby park were a potential source for fecal contamination and precipitation water may have flushed feces into the lake. The shallow bank and a wave trap could be factors hindering a mixing and dilution with unpolluted lake water.

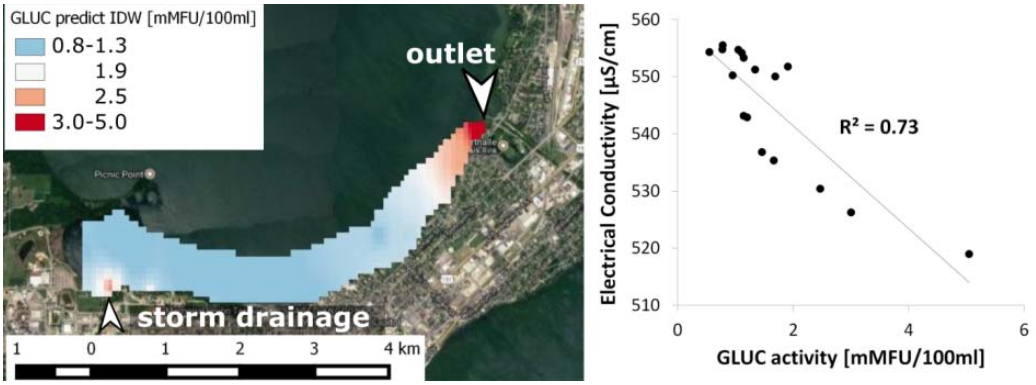

Fig. 17: Results of survey 1b focusing on the effect of urban run-off on the GLUC activity of bankside lake water. The GLUC activity screening map on the left was generated using inverse distance weighting and shows higher GLUC activity at the confluence of a storm drainage and at the lake´s outlet. The plot on the right shows a negative correlation (R^2=0.73, n=17, p-value<0.001) of GLUC activity with electrical conductivity for lake water neighboring an urban area, indicating the input of GLUC active organisms from non-lake water sources.

5.6.3. Survey 1c: Correlation of GLUC activity and culture based *E. coli* analyses

Results from the reference sample campaign found a positive correlation (R^2=0.71, p<0.001) between rapid GLUC activity measurements and culture-based *E. coli* analyses (Fig. 18).

Previous studies showed that this association is habitat specific (Ender et al., 2017; Ryzinska-Paier et al., 2014; Stadler et al., 2016) and is likely to vary seasonally. Still, the results of the reference sample campaign confirm the indicator applicability of rapid GLUC measurements for microbiological pollution monitoring of summery lake water.

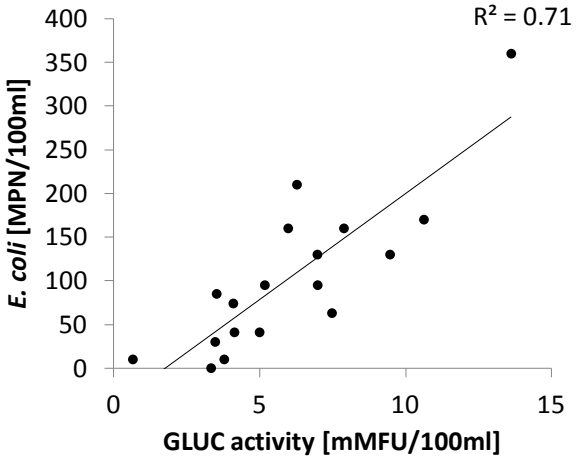

Fig. 18: Plot showing the correlation of GLUC activity with culture based *E. coli* analyses. Samples were abstracted from beaches on Lake Mendota, Labe Monona and Lake Waubesa. A R^2 of 0.71 proves the indicator applicability of rapid GLUC measurements for culture based *E. coli* analyses (n=18, p-value<0.001).

5.6.4. Survey 2: GLUC activity in lake water along a gradient of urbanization

The results of survey 2 showed an increase of GLUC activity in lake water along a gradient of urbanization following the Yahara River downstream through Lake Mendota, Lake Monona and Lake Waubesa (Fig. 19). During dry weather conditions the mean GLUC activity in Lake Mendota is 4.1 mMFU/100ml, with the highest values at the confluence of the Yahara River (28.7 mMFU/100ml). For Lake Monona a mean GLUC activity of 7.3 mMFU/100ml and for Lake Waubesa a mean GLUC activity of 9.7 mMFU/100ml has been determined. Moreover locations with increased GLUC activity (up to 13.3 mMFU/100ml) were localized in the two lower lakes.

These sections of the lakes receive inputs from distinct urban sources, such as storm drains and are due to their position protected from mixing to the rest of the lake system (Fig. 19).

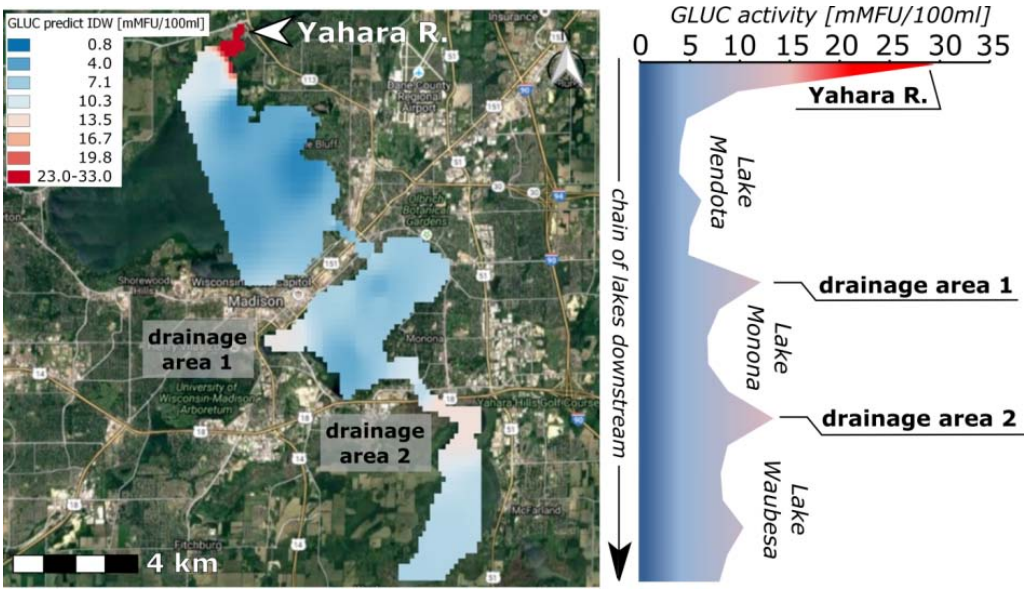

Fig. 19: On the left, GLUC activity screening map along a gradient of urbanization following the chain of the Madison Lakes downstream through Lake Mendota, Lake Monona and Lake Waubesa. Areas that are assumed to originate significant amounts of urban drainage and run-off are marked as drainage area 1 and drainage area 2. Graph on the right shows the course of GLUC activity along the chain of lakes downstream, highlighting hotspots of GLUC activity at the Yahara River confluence and at the waters adjacent the drainage areas 1 and 2. An increasing GLUC activity in the lakes could be observed following the gradient of urbanization.

5.6.5. Survey 3: Effects of land-use on the GLUC activity in river water, Lower Columbia River

GLUC measurements along the LCR and selected tributaries (Fig. 20) showed values between 0.8 and 20.3 mMFU/100ml, with a mean of 2.8 mMFU/100ml and a median of 1.8 mMFU/100ml (Table 7). Over long stretches, especially upstream of the John Day River confluence GLUC values of the LCR main channel were predominately close to the limit of quantification (0.8 mMFU/100ml). Significant higher GLUC singles were measured at the confluences of the Yakima River (20.3 mMFU/100ml) and the Umatilla River (15.5 mMFU/100ml), both draining agriculturally used areas (Fig. 20).Yet, no distinguished increase of the GLUC activity within the LCR main channel has been determined downstream of these two confluences. At the confluences of John Day River (average discharge 59 m^3/s) and Deschutes River (average discharge 200 m^3/s) GLUC values of 6.3 mMFU/100ml and 3.7 mMFU/100ml, respectively were measured. Those GLUC values are decisively lower compared to Yakima River (average discharge 99 m^3/s) and Umatilla River (average discharge 14 m^3/s). Nevertheless, downstream of the confluences of John Day River and Deschutes River the main LCR channel shows relatively increased GLUC values of up to 3.7 mMFU/100ml (Fig. 20).

This is interpreted as an effect of tributaries volume contribution relative to the LCR main channel and a dilution effect due to waters with very low GLUC activities of the upper stretch of the LCR and the Snake River (average discharge 1550 m^2/s). Because of the low GLUC activity in the LCR main channel no

significant impact of dams in general on the fate of GLUC active organisms could be asserted. Still, the measurements in the beginning of the backwater and downstream of the The Dalles Dam indicate a decrease of GLUC activity from 3.7 to 1.7 mMFU/100ml in the reach of the dam (Fig. 20). The retention of particle associated microorganisms due to constrained sedimentation by the impoundment of river waters has been previously reported (Gannon et al., 1983, 2005). Alongside the urban area of Portland GLUC values up to 7.3 mMFU/100ml were measured in the Willamette River (Fig. 20).

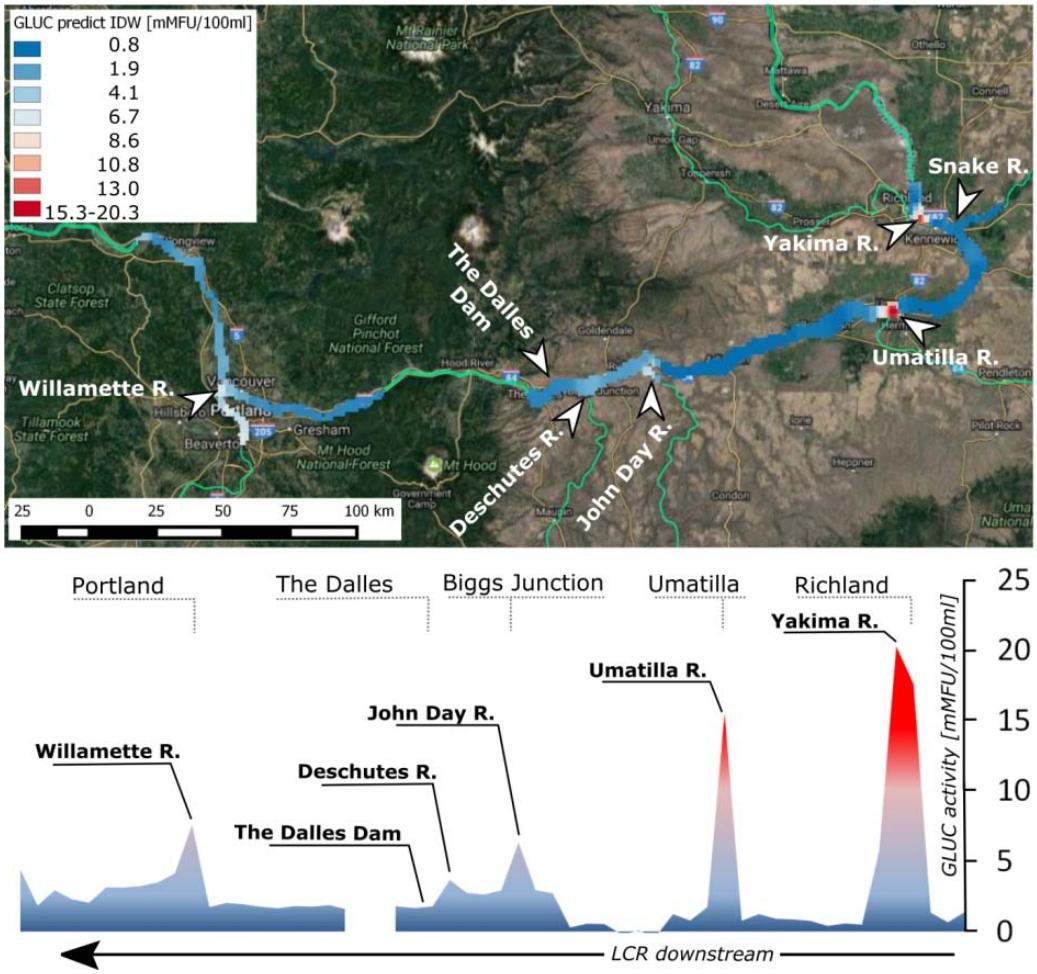

Fig. 20: Results of survey on the Lower Columbia River (LCR). On the top the GLUC activity screening map, generated using inverse distance weighting, shows low GLUC activity for the upper LCR stretch upstream of the John Day River, with GLUC hotspots at the confluences of the Yakima River and the Umatilla River. Increased GLUC activity in the main channel of the LCR could be observed downstream of the John Day River and downstream of the Willamette River confluence. The graph on the bottom shows the corresponding course of GLUC activity, disclosing peaks of GLUC activity due to tributaries, a decrease of GLUC values within the reach of the The Dalles Dam and an increase of GLUC activity in the LCR going downstream.

5.6.6. Survey 4: Effects of land use on the GLUC activity in river water, Upper Mississippi River

GLUC values in the Pool 8 of the UMR and at confluences (Fig. 21) ranged between 5.0 and 15.0 mMFU/100ml, with a mean of 8.0 mMFU/100ml and a median of 7.5 mMFU/100ml (Table 7). Maximum GLUC signals were captured at the confluences of the La Crosse River (15.0 mMFU/100ml) and the Root River (13.7 mMFU/100ml) both draining agricultural catchments. Corresponding to the land use, the UMR shows increased GLUC values, compared to the LCR (Fig. 20 and Fig. 21). Furthermore a higher spatial variability of GLUC activity was observed in the 30 km section surveyed at the UMR (amplitude of GLUC values excluding tributaries: 5.6 mMFU/100ml), compared with the almost 500 km section surveyed at LCR (amplitude of GLUC values excluding tributaries: 4.1 mMFU/100ml).

The higher spatial heterogeneity of GLUC values in the UMR can be a consequence of the braided fluvial network of backwaters and side channels in the UMR, while the LCR represents a fairly unbranched and homogenous river with a distinct main channel.

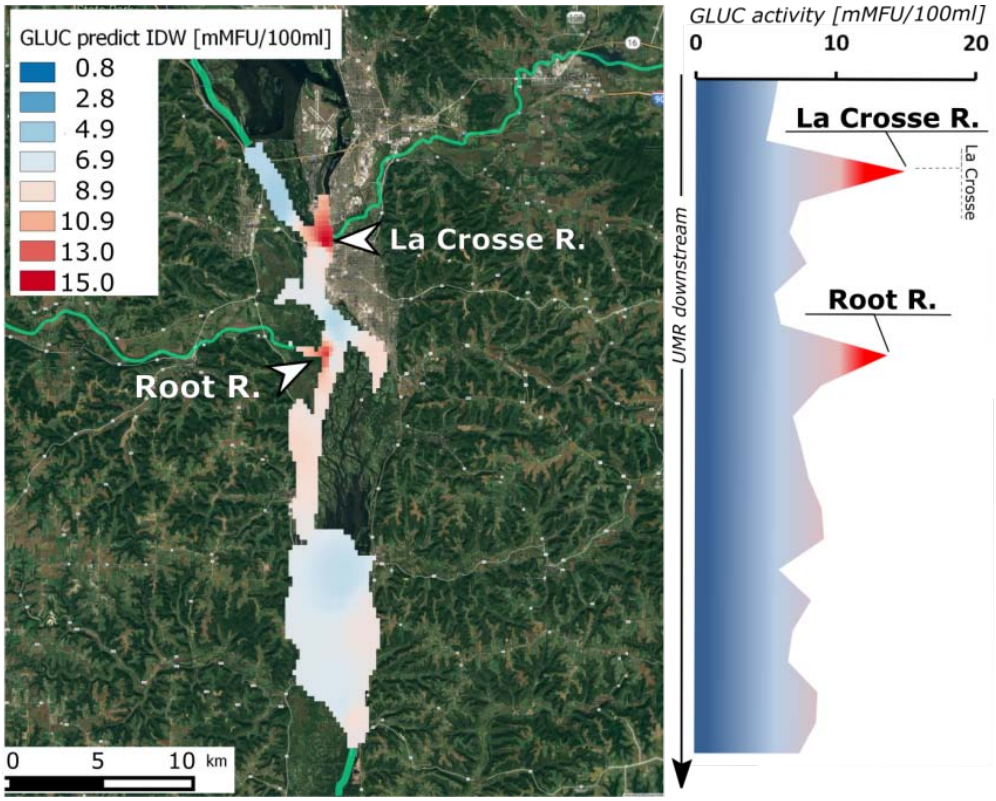

Fig. 21: On the left, GLUC activity screening map of Pool 8 of the Upper Mississippi River (UMR). It shows the influence of the La Crosse River and the Root River (drainage basins are marked with dashed lines), both discharging agricultural catchments, on the GLUC activity of the UMR. The UMR shows a significant higher GLUC activity, compared to the LCR. The graph on the right shows the corresponding course of GLUC activity, disclosing peaks of GLUC activity due to tributaries (La Crosse River, Root River).

5.6.7. Comparison of GLUC signals with limnological parameters

In Lake Mendota GLUC activity is strongest correlated with turbidity (R^2=0.60) and fDOM (R^2=0.58) (Table 8), which is interpreted as the dominant influence of the Yahara River on the GLUC activity of lake water. Draining a catchment with agricultural land use (including live stock) the river water is characterized by increased suspended sediment and nutrient loads, and may contain fecal pollution from livestock waste. Nutrients in the slow flowing river water are for the most part consumed by primary production once the Yahara River reaches the lake. The described correlations of GLUC and limnological parameters indicate an increased primary production along with a potential input of fecal microbial contamination at the north shore.

Along the chain of lakes and along a gradient of urbanization, respectively, the GLUC activity showed strongest correlations with chlorophyll (R^2=0.86) and NO_3-N (R^2=0.80) (Table 8). Nutrients may origin e.g. from urban and sub urban lawns("Nutrients in the Nation's Waters: Identifying Problems and Progress, USGS Fact Sheet FS218-96," n.d.) reaching the lake with uncompleted denitrification promptly in storm drainages and promote algae growth in the lake. Potential fecal pollution due to pet waste, animals living in drains and suburban drainage areas as well as leaking sewer lines are assumed to have the same pathway (storm drains) into the lake as nutrients, leading to this tight correlations. Compared to the other surveys, measurements along the gradient of urbanization showed the strongest association of GLUC with chlorophyll. Therefore this is seen as a consequence of related pathways leading to a parallel signal of both parameters, rather than an influence of cross sensitivity between algae and GLUC. Additionally the occurrence of algae can promote growth and survival of *E. coli* in water (Byappanahalli et al., 2003; Englebert et al., 2008; Whitman et al., 2003).

Regression analysis from data yielded from the LCR survey showed that GLUC activity was mostly correlated to Chlorophyll (R^2=0.55) and fDOM (R^2=0.38) (Table 8). The LCR was the only surveyed water body were a statistically significant correlation between GLUC activity and electrical conductivity (R^2=0.32, $p<0.001$) has been observed (Table 8). In the fairly homogenous stretch of the LCR, GLUC activity is bond to parameters indicating point-inlets of potential fecal pollution due to tributaries. Anthropogenic influences can be highlighted by the association of GLUC activity to nutrients, fDOM and consequently chlorophyll. Association of GLUC activity and electrical conductivity in the LCR is interpreted as a consequence of tributaries, draining hydrogeologically diverse sub-catchments and causing an input of GLUC activity organisms from population centers and agricultural areas, respectively.

Contrary to the other surveyed water bodies, at the UMR GLUC activity has been found only significantly correlated to two of the selected limnological parameters, namely fDOM (R^2=0.65) and turbidity (R^2=0.48), where GLUC activity and fDOM are negatively correlated (r= -0.81). The Pool 8 of the UMR, as a sink for nutrients and organic matter, is illustrated by the negative correlation of GLUC and fDOM, where tributaries show lower fDOM values than the UMR but cause an input of GLUC. The association of GLUC to turbidity is interpreted as the

influence of tributaries, draining agricultural catchments which are susceptible for soil erosion and account for an in-put of fecal contamination most likely from livestock waste.
The correlations of GLUC signals with selected limnological parameters showed distinct differences for the respective water body (Table 8). These differing correlations between GLUC activity and limnological parameters are to some degree expected, referring to the range of parameters characterizing the respective water body and the waters contributed into the water body by tributaries that are relatively different regarding their physico-chemical and microbiological properties. However it allowed a complementary interpretation of survey data, in respect to in-put and transport processes of potential fecal pollution (Table 8).

Table 8: Correlation (linear regression) R^2 between GLUC activity and limnological parameters 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). As shown, the association of GLUC activity to limnological parameters was characteristic for each surveyed water body and enabled a complementary data interpretation.**

	Lake Mendota			Chain of 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 (r=-0.81)	***
Chlorophyll a [µg/l]	*38*	0.35	***	*23*	0.86	***	*80*	0.55	***	*23*	0.17	*
NO3-N [mg/l]	*24*	0.16	*	*23*	0.80	***	*80*	0.26	***	*23*	0.00	
CO2-D [ppm]	*25*	0.05		*23*	0.33	**	*80*	0.00		*23*	0.01	
CH4-D [ppm]	*25*	0.14		*23*	0.12		*80*	0.46	***	*23*	0.06	

5.7. Evaluation of the developed approach and future perspectives

The presented work shall serve as a pilot study pointing out the possibilities and perspectives of ship-borne measurements conducted by following a combined approach of both microbiological and limnological methods. The study´s examples and results may encourage follow up studies to bridge the spatial gab of microbiological research in aquatic habitats. 181 ship-borne measurements of GLUC activity have been conducted during all surveys on various water bodies without technical failures. The prototype and the developed on-board set-up proved to be reliable and technically robust during the various and challenging field conditions it was objected to. The temporal resolution of these measurements proved to be sufficient for both large scaled screening surveys (Fig. 16, Fig. 19, Fig. 20, Fig. 21) and purposeful localization of point-inlets of potential microbiological contamination into fresh water bodies (Fig. 17). The described approach of a rapid screening of microbial water quality by means of ship-borne GLUC measurements yielded results with an unmatched spatial extend and –resolution, with decisively lower efforts in time, staff and resources, compared to microbiological standard assays.
The capability of the mobile and rapid enzymatic activity assay as a quantifying proxy for culture-based standard assays (required correlation: R^2>0.95 (Stadler et al., 2010)) was not confirmed, nevertheless the results demonstrate the indicator

applicability (R^2=0.71, $p<0.001$) of rapid GLUC measurements (Fig. 18). Moreover the potential of a water quality screening by means of ship-borne enzymatic measurements has been presented. Such endeavors can significantly enhance the monitoring of water resources, as well as our understanding of the spatial heterogeneity of fecal associated microbiological contamination in water bodies. The use of this biochemical indicator as a rapid screening tool on large fresh water bodies can be the basis for a more purposeful and resourceful selection of sample points for further elaborate microbiological analyses. Additional research is needed to disclose the site specific correlation of rapid GLUC measurements and *E. coli* analyses. The presented results suggest that it is feasible to define a site specific threshold of GLUC depending on the utilization of water resources. This has great potential to be implemented into early warning systems, such as the surveillance of bathing water quality.

Enhancement of available substrates as well as the preparation and the application of different substrates may also enable a more diverse assessment of enzymatic activities in waters, going beyond fecal associated microbiological contamination to ask different ecological questions. For example, xylanase or invertase can be evaluated as enzymes involved in C transformation, urease or amidase can be evaluated as enzymes involved in N transformation, arylsulfatase can be used as an enzyme involved in organic S transformation, and alkaline phosphatase and acid phosphatase can be evaluated as enzymes involved in organic P transformation (Burns and Dick, 2002; Chrost, 2012; Hoppe, 1991, 1983). Developments regarding buffer and reagents are underway, that enable the operation of automated enzymatic activity assays also in saline environments, suitable for coastal water quality assessment. Consequently the integration of such prototypes into well-established systems for ship-borne measurements of physico-chemical parameters, such as the FLAMe (Crawford et al., 2015), paves new ground for data interpretation and process understanding within the fields of health related water quality and water resource management.

Chapter 6

Conclusions

This thesis presents a comprehensive assessment of rapid determination of enzymatic activities in water, a novel bio-chemical on-site parameter. The thesis demonstrates that prototype apparatuses for fully automated on-site measurements of enzymatic activity are technically realized successfully, in regards to long-term operation and function. The thesis discloses the potentials and limits of rapid enzymatic assays in regards to indicator applicability, early warning systems and microbial transport dynamics. Additionally, an innovative application of the enzymatic assay is presented, that disclosed spatial patterns of enzymatic activity on large surface water resources and highlights new perspectives in water quality screening and -monitoring.

By designing and constructing a programmable sampling devise (SAMP-FIL) that features filtration of water samples together with an effective self-cleaning procedure, it was possible to conduct rapid monitoring of enzymatic activity at technically very challenging locations, such as sediment laden streams. It was shown that low-cost microcomputers, together with open-source software can be used by non IT-specialists to control custom-built monitoring- or sampling equipment. By installing the SAMP-FIL, error-free running time of connected measurement devices has been considerably enhanced and measurement accuracy has been increased to an up-to-now unmatched quality. The results of the field tests also point out the important role of adequate sample pretreatment on the quality of on-site measurements: Technically functional pretreatment set-ups can still lead to significant bio-film and debris accumulation within the sampling device. Such depositions of organic and inorganic matter may not directly lead to a failure of the connected measurement device, but can cause a fouling of the sampling- or measuring device that is indicated by damped, delayed or implausible signals. This thesis showed that by establishing high flow velocities during the sampling procedure and by back-flushing the apparatus with pressurized air before idle, depositions within the apparatus can be reduced to a negligible degree. Although the SAMP-FIL has been constructed to be used as pretreatment for on-site enzymatic activity measurements, it is designed in a way that other measurement equipment can be connected with simple modifications. It can therefore benefit any other on-site monitoring procedure that cannot handle coarse sediment fractions and therefore requires a filtered water sample.

Furthermore the thesis outlines a hypothesis driven methodology to test novel on-site monitoring techniques, such as automated GLUC measurements, for their measurement robustness, comparability of measurement results and impact of environmental factors on the measurement signal. By following this methodology,

prototype apparatuses for on-site GLUC determination were tested long-term at a technically challenging monitoring location. It was shown that results yielded with the same construction design are highly consistent over long periods of on-site operation. Results yielded with different prototype constructions are somewhat lower correlated, but still follow the same range and dynamics. An elaborated reference sampling campaign compared on-site generated GLUC data with culture-based *E.coli* analyses. This showed that rapid GLUC measurements cannot serve as a quantifying proxy for standard microbiological assays, but that the rapid enzymatic assay has significant capabilities as a qualitative indicator for microbial water quality. GLUC signals in stream water were found to be tightly correlated to *E.coli* analyses during event-run off conditions, but showed a weaker correlation for longer observation periods (e.g. seasonal). The potential regarding indicator applicability of rapid GLUC measurements was supported by findings that disclosed a better over-all correlation between GLUC and *E.coli* in stream water than GLUC to turbidity or suspended solids, respectively. The assessment of the impact of environmental parameters, such as ambient temperature, suspended solid concentrations in stream water or discharge, on the quality of GLUC signals, were the basis for important technical improvements. The thesis showed, that the fluorogenic substrate for on-site GLUC activity measurements showed degeneration effects when objected to ambient temperatures higher than 25°C for a longer period (>24 h). Following these findings a pelitier cooler solved that issue. These findings are also relevant for operators using devices for automated enzymatic measurements that are not equipped with cooling options. Using only small portions of substrate state an adequate approach to omit issues regarding temperature induced substrate degradation. The series of field tests presented in this thesis described for the first time dynamics of GLUC activity measured in stream water, draining an agricultural headwater catchment. The detailed descriptions of captured GLUC dynamics, ranging from event- over diurnal- to seasonal fluctuation, are essential for follow up studies assessing microbial transport processes and pathways by means of rapid enzymatic assays.

Another important finding of this thesis is the habitat specific correlation of automated GLUC measurements with culture-based *E.coli* analyses. These results stay in contrast to previously published studies, based on laboratory enzymatic assays. This has great relevance in regards to future applications of automated GLUC measurements at differing water resources and early warning systems. This thesis advises that GLUC data interpretation and GLUC threshold definition shall be conducted on a catchment- and site specific basis. These findings point out the role of the viable but non culture-able (VBNC) subpopulation of fecal indicator bacteria (FIB) in respect to the indicator applicability of FIB. These results can benefit follow up studies focusing on the persistence and fate of FIB, such as *E.coli* in natural systems. It is concluded that the correlation of on-site generated GLUC data and culture-base *E.coli* depends on the source, type and age of fecal contamination, as well as on the site specific run-off patterns, which are determined by soil properties and local hydrogeology.

Furthermore, this thesis delineates a novel approach for quality screening of large surface water resources, by means of ship-borne automated GLUC measurements. It was shown that GLUC measurements from a mobile research vessel can be used to localize potential in-lets of fecal pollution into lakes and rivers in near-real time. The generation of GLUC activity screening maps is advised to gain a rapid bio-chemical snapshot of surface waters at large-scales. Moreover, the thesis advises to use an interdisciplinary approach by combining microbiological methods with hydrological-, or limnological research to enhance data interpretation. Ship-borne GLUC measurements as a qualitative indicator can be of great use at under-studied sites. It also allows a more purposeful selection of sample points for elaborate microbiological studies. This will benefit a more resourceful and target-oriented design of future field studies, where points of interest can be localized during the screening procedure and project resources can be concentrated on these.

Modern emerging technologies, such as online flow-cytometry or automated enzymatic assays, that will enhance the spatial and temporal resolution of microbiological measurements in water resources are of increased interest and the awareness regarding such on-site methods will likely rise in the near future. It is therefore essential to test such novel methods, but also to give perspectives how and for what research questions they can be used effectively. This thesis offers both, a technical assessment and an outline for an innovative application of automated enzymatic activity measurements. The overall goal of this thesis is to introduce new research in the field of monitoring and microbial transport processes but also to serve as a consolidated basis for new applications of rapid enzymatic assays, such as early warning systems or process control.

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Appendix A:
Script for the SAMP-FIL auto sampler

```
import time
import logging


#Sample IN:
gpio1 = '#insert here GPIO pin no.#'
#Sample OUT:
gpio2 = ''#insert here GPIO pin no.#'
#Pressured AIR:
gpio3 = ''#insert here GPIO pin no.#'
#PUMP
gpio4 = ''#insert here GPIO pin no.#'

#Config Logging
logging.basicConfig(filename='samp_fil.log',level=logging.IN
FO,format='%(asctime)s %(message)s')

#=========================================================

#Make sure resources are ready to use (unexport)

    f= open ('/sys/class/gpio/unexport','w')
    f.write(str(gpio1))
    f.close()
except IOError as e:
    lol=0
#_____________________________________________________

    f= open ('/sys/class/gpio/unexport','w')
    f.write(str(gpio2))
    f.close()
except IOError as e:
    lol=0
#_____________________________________________________

    f= open ('/sys/class/gpio/unexport','w')
    f.write(str(gpio3))
    f.close()
except IOError as e:
    lol=0
#_____________________________________________________

    f= open ('/sys/class/gpio/unexport','w')
    f.write(str(gpio4))
    f.close()
except IOError as e:
```

```
    lol=0
#============================================================

#Export GPIO numbers
f= open ('/sys/class/gpio/export','w')
f.write(str(gpio1))
f.close()
#____________________________________________________
f= open ('/sys/class/gpio/export','w')
f.write(str(gpio2))
f.close()
#____________________________________________________
f= open ('/sys/class/gpio/export','w')
f.write(str(gpio3))
f.close()
#____________________________________________________
f= open ('/sys/class/gpio/export','w')
f.write(str(gpio4))
f.close()
#============================================================

#Define GPIO Direction as OUTPUT
path = '/sys/class/gpio/gpio' + gpio1 + '/direction'
f= open (path,'w')
f.write('out')
f.close()
#____________________________________________________
path = '/sys/class/gpio/gpio' + gpio2 + '/direction'
f= open (path,'w')
f.write('out')
f.close()
#____________________________________________________
path = '/sys/class/gpio/gpio' + gpio3 + '/direction'
f= open (path,'w')
f.write('out')
f.close()
#____________________________________________________
path = '/sys/class/gpio/gpio' + gpio4 + '/direction'
f= open (path,'w')
f.write('out')
f.close()
#============================================================

#Switch AIR VALVE -ON-
print "FILTER CLEANING started"
logging.info("FILTER CLEANING started")
path = '/sys/class/gpio/gpio' + gpio3 + '/value'
f= open (path,'w')
f.write('1')
f.close()
```

```
        ############SET TIME FOR FILTER CLEANING############
#=============#
time.sleep(17)
#=============#

#_____________________________________________________
#Switch AIR VALVE -OFF-

path = '/sys/class/gpio/gpio' + gpio3 + '/value'
f= open (path,'w')
f.write('0')
f.close()

#f= open ('/sys/class/gpio/unexport','w')
#f.write(str(gpio3))
#f.close()
print "#### FILTER CLEANING completed ####"
logging.info("FILTER CLEANING completed")
time.sleep(30)
#============================================================

#SWITCH SAMPLE IN VALVE -ON-
print "FLUSHING started"
logging.info("FLUSHING started")
path = '/sys/class/gpio/gpio' + gpio1 + '/value'
f= open (path,'w')
f.write('1')
f.close()

#SWITCH SAMPLE OUT VALVE -ON-
path = '/sys/class/gpio/gpio' + gpio2 + '/value'
f= open (path,'w')
f.write('1')
f.close()

#SWITCH PUMP -ON-
path = '/sys/class/gpio/gpio' + gpio4 + '/value'
f= open (path,'w')
f.write('1')
f.close()

            ##############SET TIME FOR
FLUSHING###############
#============#
time.sleep(10)
#============#

#_____________________________________________________
#Shut OFF
path = '/sys/class/gpio/gpio' + gpio1 + '/value'
f= open (path,'w')
```

```
f.write('0')
f.close()

path = '/sys/class/gpio/gpio' + gpio2 + '/value'
f= open (path,'w')
f.write('0')
f.close()

path = '/sys/class/gpio/gpio' + gpio4 + '/value'
f= open (path,'w')
f.write('0')
f.close()

print "#### FLUSHING completed #####"
logging.info("FLUSHING completed")
time.sleep(1)

#============================================================
#SWITCH SAMPLE OUT VALVE -ON-
print "EMPTYING BOTTLE started"
logging.info("EMPTYING BOTTLE started")
path = '/sys/class/gpio/gpio' + gpio2 + '/value'
f= open (path,'w')
f.write('1')
f.close()

            #######BOTTLE EMPTYING AFTER FLUSHING###########
#=============#
time.sleep(20)
#=============#

#Shut OFF
path = '/sys/class/gpio/gpio' + gpio2 + '/value'
f= open (path,'w')
f.write('0')
f.close()
print "Bottle is empty - ready for sampling"
logging.info("Bottle is empty - ready for sampling")
#============================================================
==
path = '/sys/class/gpio/gpio' + gpio2 + '/value'
f= open (path,'w')
f.write('0')
f.close()


#SWITCH SAMPLE IN VALVE -ON-
print "BOTTLE FILLING started"
logging.info("BOTTLE FILLING started")
path = '/sys/class/gpio/gpio' + gpio1 + '/value'
f= open (path,'w')
```

```
f.write('1')
f.close()

#SWITCH PUMP -ON-
path = '/sys/class/gpio/gpio' + gpio4 + '/value'
f= open (path,'w')
f.write('1')
f.close()

            ##########SET TIME FOR BOTTLE FILLING###########
#============#
time.sleep(8)
#============#

#_____________________________________________________
#Shut OFF
path = '/sys/class/gpio/gpio' + gpio1 + '/value'
f= open (path,'w')
f.write('0')
f.close()

path = '/sys/class/gpio/gpio' + gpio4 + '/value'
f= open (path,'w')
f.write('0')
f.close()

print "#### BOTTLE FILLING completed #####"
logging.info("BOTTLE FILLING completed")
print "#### DEVICE READY FOR MEASUREMENT #####"
logging.info(" # DEVICE READY FOR MEASUREMENT # ")

           #######SET TIME FOR MEASUREMENT
IDLE###############
time.sleep(3300)
#============================================================
#SWITCH SAMPLE OUT VALVE -ON-
print "EMPTYING BOTTLE started"
logging.info("EMTYING BOTTLE started")
path = '/sys/class/gpio/gpio' + gpio2 + '/value'
f= open (path,'w')
f.write('1')
f.close()

            #########SET TIME FOR BOTTLE EMPTYING##########
#=============#
time.sleep(30)
#=============#

#Shut OFF
#path = '/sys/class/gpio/gpio' + gpio2 + '/value'
#f= open (path,'w')
```

```
#f.write('0')
#f.close()
#print "EMPTYING BOTTLE completed"
logging.info("EMTYING BOTTLE completed")
#============================================================
            ############### CLEANING DEVICE  ###############
print "DEVICE CLEANING started"
logging.info("DEVICE CLEANING started")
#SWITCH SAMPLE IN VALVE -ON-
path = '/sys/class/gpio/gpio' + gpio1 + '/value'
f= open (path,'w')
f.write('1')
f.close()

#SWITCH AIR VALVE -ON-
path = '/sys/class/gpio/gpio' + gpio3 + '/value'
f= open (path,'w')
f.write('1')
f.close()

#SWITCH PUMP -ON-

path = '/sys/class/gpio/gpio' + gpio4 + '/value'
f= open (path,'w')
f.write('1')
f.close()

#SWITCH SAMPLE OUT VALVE -ON-
path = '/sys/class/gpio/gpio' + gpio2 + '/value'
f= open (path,'w')
f.write('1')
f.close()

          #########SET TIME FOR DEVICE CLEANING ############
#=============#
time.sleep(10)
#=============#

#SWITCH ALL DEVICES -OFF-
path = '/sys/class/gpio/gpio' + gpio1 + '/value'
f= open (path,'w')
f.write('0')
f.close()
path = '/sys/class/gpio/gpio' + gpio2 + '/value'
f= open (path,'w')
f.write('0')
f.close()
path = '/sys/class/gpio/gpio' + gpio3 + '/value'
f= open (path,'w')
f.write('0')
f.close()
```

```
path = '/sys/class/gpio/gpio' + gpio4 + '/value'
f= open (path,'w')
f.write('0')
f.close()
print "#### DEVICE CLEANING completed #####"
logging.info("DEVICE CLEANING completed")
#Unexport all GPIOS
f= open ('/sys/class/gpio/unexport','w')
f.write(str(gpio1))
f= open ('/sys/class/gpio/unexport','w')
f.write(str(gpio2))
f= open ('/sys/class/gpio/unexport','w')
f.write(str(gpio3))
f= open ('/sys/class/gpio/unexport','w')
f.write(str(gpio4))
f.close()
print "#### BOTTLE is EMPTY ####"
logging.info("BOTTLE is EMPTY")
print "######## CYCLE COMPLETED ########"
logging.info("CYCLE COMPLETED")
```

Appendix B: Authorship

Chapter 2 of this thesis is based on the publication "*Development and evaluation of a self-cleaning custom-built auto sampler controlled by a low-cost RaspberryPi microcomputer for online enzymatic activity measurements*" by Philipp Stadler, Andreas Farnleitner and Matthias Zessner. Talanta - International Journal of Pure and Applied Analytical Chemistry (2017). The contribution of Philipp Stadler to this paper was:

- Literature review
- Concept development
- Construction and design
- Programming
- Field tests
- Laboratory analyses
- Analysis and interpretation of the results
- Elaboration of graphs, figures and tables
- Paper writing

Chapter 3 of this thesis is based on the publication "*Real-time monitoring of beta-d-glucuronidase activity in sediment laden streams: A comparison of prototypes"* by Philipp Stadler, Günter Blöschl, Wolfgang Vogl, Juri Koschelnik, Markus Epp, Maximilian Lackner, Markus Oismüller, Monika Kumpan, Lukas Nemeth, Peter Strauss, Regina Sommer, Gabriela Ryzinska-Paier, Andreas Farnleitner and Matthias Zessner. Water Research (2016). The contribution of Philipp Stadler to this paper was:

- Literature review
- Concept development
- Field work
- Laboratory analyses
- Data collection
- Analysis and evaluation of data
- Interpretation of results
- Elaboration of graphs, figures and tables

- Paper writing

Chapter 4 of this thesis is based on the publication "*Automated near-real-time monitoring of enzymatic activities in water resources*" by Philipp Stadler, Gabriela Ryzinska-Paier, Thomas Lendenfeld, Wolfgang Vogl, Paul Blaschke, Peter Strauss, Hermann Stadler, Maximilian Lackner, Matthias Zessner and Andreas Farnleitner. Handbook of Online and Near-real-time Methods in Microbiology (2017). The contribution of Philipp Stadler to this paper was:

- Literature review
- Concept development
- Analysis and evaluation of data
- Interpretation of results
- Elaboration of graphs, figures and tables
- Paper writing

Chapter 5 of this thesis is based on the publication (final draft) "*Spatial variability of enzymatic activity on large water bodies: Ship-borne measurements of beta-D-glucuronidase as a rapid indicator for microbial water quality*" by Philipp Stadler, Luke Loken, John Crawford, Paul Schramm, Kirsti Sorsa, Catherine Kuhn, Domenico Savio, Rob Striegl, David Butman, Emily Stanley, Andreas Farnleitner and Matthias Zessner. Environmental Science and Technology (to be submitted 2017). The contribution of Philipp Stadler to this paper was:

- Literature review
- Concept development
- Field work
- Laboratory analyses
- Analysis and evaluation of data
- Interpretation of results
- Elaboration of graphs, figures and tables
- Paper writing