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Microbial investigations of water, sediment, and algal mats in the mixed use watershed of Saginaw Bay, Michigan

Marc P. Verhougstraete *, Joan B. Rose [1]

Michigan State University, Department of Fisheries and Wildlife, 480 Wilson Road, Natural Resources Building, Room 13, Michigan State University, East Lansing, MI 48824, USA

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ABSTRACT

Beach monitoring often includes testing for a single fecal indicator organism in the swimmable waters. Here, sediment, algae mat, shallow water, and deep water samples collected from four Saginaw Bay (Michigan, USA) beaches were tested for multiple fecal indicator organisms (*Escherichia coli*, enterococci, *Clostridium perfringens*, F+amp coliphage, and CN-13 coliphage) and molecular markers (human and bovine *Bacteroides* and enterococci surface protein) to determine the occurrence and sources of fecal indicator bacteria across beachscapes and characterize the environmental parameters which influence microbial water quality. Results show algae mats and sediment had higher levels of bacteria compared to surrounding water column. Higher concentrations of fecal indicators in shallow waters compared to deep water were attributed in part to sediment and algae bound bacteria and potential regrowth. Fecal indicator organisms were primarily influenced by wind, waves, and precipitation and partially identified as human specific using the enterococci surface protein gene. This project suggests the potential for sediment and algal mats to act as non-point sources of pollution in the nearshore zone. Future beach protection measures should focus on shallow water monitoring of multiple fecal indicators and beach grooming during calm morning hours.

Introduction

A single fecal indicator is generally used during routine beach monitoring to characterize water quality and protect human health (United States Environmental Protection Agency (USEPA), 2012). *Escherichia coli* (*E. coli*) and enterococci are traditionally used for beach monitoring because they were linked to gastrointestinal illnesses through epidemiological studies (Cabelli et al., 1982; Wade et al., 2006, 2010). However, other fecal indicators are available and can be used. For example, *Clostridium perfringens* (*C. perfringens*) and coliphage viruses have also been suggested as fecal indicator organisms in recreational waters. *C. perfringens* form spores that do not regrow in the environment and are resistant to high temperatures and disinfection treatments (Payment and Franco, 1993). Coliphage viruses infect *E. coli* and, like *C. perfringens*, do not regrow in the environment and are used to indicate the presence of enteric pathogens in water (Allwood et al., 2003).

Molecular source tracking methods are finding widespread application for defining the cause of fecal pollution in water as the previously mentioned indicators (above) cannot identify the source associated with the contamination. Scott et al. (2005) and Masago et al. (2011) demonstrated *Enterococci faecium* surface protein (esp) gene was highly specific to human feces and confirmed the presence of disinfected wastewater. Bernhard and Field (2000) developed two commonly used *Bacteroides–Prevotella* ribosomal DNA markers specific to cow and human feces. In addition to fecal indicator monitoring and source tracking, environmental surveys capture surrounding parameters at beaches not identified during microbial and molecular analyses (Field and Samadpour, 2007).

Current recreational water quality monitoring practices also focus only on swimmable water (i.e. one meter deep). This practice fails to address the multitude of pollution sources threatening the nearshore including reservoirs such as sediments and algal mats. Many studies have shown algal mats provide a suitable habitat for the growth and persistence of enteric bacteria such as *E. coli*, enterococci, *Shigella*, *Campylobacter*, and *Salmonella* (Byappanahalli et al., 2009; Ishii et al., 2006; Verhougstraete et al., 2010). As a result of wind and wave action, microorganisms can detach from the algae, enter the surrounding water column, and influence water quality (Englebert et al., 2008b). Pathogen occurrence in algal mats or the impact on surrounding water quality and public health is not well understood (Verhougstraete et al., 2010). Similarly, sediments are being increasingly implicated as a cause of beach water quality impairments. *E. coli* were reported at concentrations up to 40 times higher in nearshore sediment than the overlying water (Alm and Burke, 2003; Whitman and Nevers, 2003). Phillips et al. (2011) identified sediments as a major reservoir of enterococci that impacted beach water quality. Despite previous studies, a strong

* Corresponding author at: University of North Carolina, Institute of Marine Sciences, 3431 Arendell Street, Morehead City, NC 28557, USA. Tel.: +1 252 726 6841.
E-mail addresses: verhoug3@msu.edu (M.P. Verhougstraete), rosejo@msu.edu (J.B. Rose).
[1] Tel.: +1 517 432 4412.

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understanding of bacterial impacts from sediment and algal mats continues to elude water quality managers.

There exists a need to better characterize microbial occurrence in multiple nearshore zones because no microbial standards exist for sediment and algae mats. Using multiple microbial analyses across beach transects in a mixed use watershed (Saginaw Bay, Michigan, USA), this investigation aimed to: 1) determine the occurrence and relationship of fecal indicator bacteria in water, sediment, and algae at beaches; 2) identify human and bovine sources of fecal contamination using microbial source tracking and; 3) characterize the environmental parameters influencing water quality degradation. Application of this approach and corresponding evolution of analytical methods will help to better characterize pollution impacts on shorelines of freshwater systems.

Materials and methods

Sample collection and processing

Saginaw Bay averages 9.8 m in depth, drains over 22,000 km^2 (USEPA, http://epa.gov/glnpo/aoc/sagrivr.html, August 21, 2012), and hosts 43 public beaches. Four Saginaw Bay beaches were chosen for this study based on their proximity to rivers with large drainage basins and historically poor beach water quality with undefined sources of pollution (Michigan Department of Environmental Quality, http://www.deq.state.mi.us/beach/, August 21, 2012) (Table 1 and Fig. 1). Furthermore, nearshore algal mass accumulation had been recorded at two of the beaches, SB1 and SB2 (S. Singh and J. Rose, unpublished report, http://cws.msu.edu/projects/documents/Saginaw_bayReportwithmaps2007.pdf, August 21, 2012). Each beach was sampled eight times from June to September, 2008. Transect samples (sediment, shallow water, deep water, and algae mats in the shallow water when present) from three equally spaced points parallel to the shoreline were monitored at each beach. Using sterile one liter bottles, water column grab samples were collected at depths of 15–20 cm (shallow) and 100 cm (deep) above lake bottom. Sediment and algal samples were collected in the swash zone by inverting a Whirl-Pak®, grabbing a handful of material from three horizontal points along the beach, and then compositing all subsamples in one bag. All samples were placed on ice (4 °C), stored in a cooler, and processed within 24 h.

Table 1
Site description, water quality exceedances, and potential pollution influences.

Site ID	Location	Site description (latitude/longitude)	Water quality standard exceedances [a]	Suspect pollution source(s)
SB1	Caseville County Park beach	(Huron County) Adjacent to the Pigeon River which has been subject to fish kills from sediment and agricultural runoff (43.98964, −83.27540)	5	Sewage agriculture algae
SB2	Bay City State Recreation Area beach	(Bay County) North of the Saginaw River which receives significant inputs from CSOs and urban runoff (43.67407, −83.90903)	9	Sewage algae
SB3	Whites Beach	(Arenac County) Surrounded by dense residential homes relying on septic systems for wastewater management (43.92861, −83.89051)	21	Septage
SB4	Port Crescent State Park-day use	(Huron County) Southwest of the Pinnebog River which receives inputs from multiple agricultural drains (44.00246, −83.06981)	3	Agriculture

[a] Closures reported since the creation of Michigan Beach Guard database (circa 2001).

Water analysis

Microbial analysis of water and sediment included *E. coli*, enterococci, *C. perfringens*, and coliphages (CN-13 and F+ amp). Undiluted water samples were filtered through 0.45 μm hydrophilic mixed cellulose esters filters (Pall Corporation 66278). *E. coli*, enterococci, and *C. perfringens* were analyzed using cultivation and selective media, mTEC (USEPA, 2005), mEI (USEPA, 2002), and mCP (Bisson and Cabelli, 1979; USEPA, 1995), respectively, and reported as CFU/100 mL. Double agar layers were utilized to detect two coliphage strains on selected hosts (*E. coli* F+amp and *E. coli* CN-13) following EPA methods 1601 (USEPA, 2001). Clearings in the host lawn were counted and reported as plaque forming units (PFU)/100 mL. *E. coli* C-3000 (ATCC 15597), *E. faecium* (ATCC 35667), *C. perfringens* (ATCC 3624), and ΦX-174 coliphage were used as positive controls for verification of medium integrity. Sterile reagent water was used as negative controls for verification of method integrity (APHA et al., 2005).

Sediment and algal analyses

Sediment and algal samples were diluted with sterile Phosphate Buffered Water (PBW) to a final wet weight/volume ratio of 10% and 1%, respectively, to obtain quantifiable results. Algal mat samples were weighed and then underwent an initial pulse blending in a sterile blender until homogenized. Each sample and PBW solution was vigorously hand shaken (10 cm radius) for 2 min, allowed to settle for 2 min, and the eluent was decanted into a sterile bottle (Boehm et al., 2009; Shibata et al., 2004). An additional volume of PBW was added to the sediment or algae sample, swirled for 10 s, allowed to settle for 30 s, and the eluent was added to the sterile bottle from the first rinse. *E. coli*, Enterococci, *C. perfringens*, and coliphage were assayed from the diluted sediment or algae solution using the same methods described in the Water analysis section and reported as CFU or PFU/100 g wet weight.

Molecular analysis

Water, sediment, and algae samples were analyzed for the presence of the esp gene and *Bacteroides* human and bovine specific markers using polymerase chain reaction (PCR). Marker, primer sequence, and product size for each assay are described in Table 2. Analysis for esp was performed on colonies grown on filters during the enterococci cultivation process. For the *Bacteroides* markers, water and eluted sediment and algae were filtered through 0.45 μm hydrophilic mixed cellulose esters filters. For all molecular assays, each filter was placed into a 50 mL centrifuge tube containing 20 mL of sterile PBW, vortexed on high for 10 min, and then centrifuged (30 min; 4000 ×*g*) to pellet the cells. Eighteen milliliters was decanted from the tube and the remaining eluent and pellet were stored at −80 °C until DNA could be extracted. After thawing, approximately 50 μL of DNA was extracted from 200 μL of the pellet using a QIAamp® DNA mini kit.

The esp gene was assayed using PCR amplification according to Scott et al. (2005). Briefly, master mix was prepared with forward and reverse primers (3 μM), HotStarTaq Master Mix (Qiagen 203443), and molecular grade water to make up a final volume of 19 μL. The analysis was carried out using a Bio-Rad PCR thermocycler (iCycler) under the following conditions: 15 min at 95 °C, 35 cycles of 1 min at 94 °C, 1 min at 58 °C, and 1 min at 72 °C, and a final extension step for 5 min at 72 °C.

Bacteroides were assayed using PCR amplification according to Bernhard and Field (2000). Briefly, the analyses were carried out using forward and reference primers (0.4 μM), $MgCl_2$ (3 mM), HotStarTaq Master Mix, and molecular grade water to make up a final volume of 22 μL. The bovine and human *Bacteroides* assays were processed using a Bio-Rad PCR thermocycler (iCycler) with a 15 minute initial warming step (95 °C), followed by 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 60 s, followed by a final extension step of 8 min at 72 °C.

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