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Using Integrated Environmental Modeling to Assess Sources of Microbial Contamination in Mixed-Use Watersheds

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Abstract

Microbial fate and transport in watersheds should include a microbial source apportionment analysis that estimates the importance of each source, relative to each other and in combination, by capturing their impacts spatially and temporally, under various scenarios. A loosely configured software infrastructure was used in microbial source-to-receptor modeling by focusing on animal- and human-impacted mixed-use watersheds. Components include data collection software, a microbial source module that determines loading rates from different sources, a watershed model, an inverse model for calibrating flows and microbial densities, tabular and graphical viewers, software to convert output to different formats, and a model for calculating risk from pathogen exposure. The system automates, as much as possible, the manual process of accessing and retrieving data and completes input data files of the models. The workflow considers land-applied manure from domestic animals on undeveloped areas; direct shedding (excretion) on undeveloped lands by domestic animals and wildlife; pastureland, cropland, forest, and urban or engineered areas; sources that directly release to streams from leaking septic systems; and shedding by domestic animals directly to streams. The infrastructure also considers point sources from regulated discharges. An application is presented on a real-world watershed and helps answer questions such as: What are the major microbial sources? What practices contribute to contamination at the receptor location? What land-use types influence contamination at the receptor location? Under what conditions do these sources manifest themselves? This research aims to improve our understanding of processes related to pathogen and indicator dynamics in mixed-use watershed systems.

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Keywords

Integrated environmental modeling; QMRA; risk assessment; pathogens; manure; watershed modeling; source apportionment

Introduction

Determining the microbial quality of recreational, drinking, irrigation, and shellfish-harvesting waters is important to ensure compliance with health-related standards and associated legislation (Oliver et al., 2016). Levels of microbial pollution in environmental matrices are often measured by quantifying fecal indicator organisms (FIOs). Understanding fate and transport of FIOs and pathogenic organisms has become key to evaluating FIOs as indicators of potential microbial contamination of waters. Such understanding is currently supported by modeling as an essential component of explanatory research.

Two modeling technologies were historically applied to simulate microbial fate and transport at the watershed scale (Cho et al., 2016): single models and integrated modeling systems. Single models specialized in water quality at the watershed scale, consuming environmental management variables and providing water quality-related outputs such as microbial concentrations and fluxes. Examples include Soil and Water Assessment Tool (SWAT) (Arnold et al., 1998) and Hydrologic Simulation Program – FORTRAN (HSPF) (Bicknell et al., 1997). Integrated Environmental Modeling (IEM), on the other hand, uses models of individual processes and constructs a workflow in which they seamlessly exchange data and generate outputs relevant to the problem at hand (Whelan et al., 2014a). Examples include Framework for Risk Analysis in Multimedia Environmental Systems (FRAMES) (Whelan et al., 2014a; Johnston et al., 2011), Object Modeling System (OMS) (David et al., 2013), Community Surface Dynamics Modeling System (CSMDS) (Peckham et al., 2013), and OpenMI (Moore and Tindall, 2005) (see Whelan et al., 2014a; Laniak et al., 2013).

It was amply demonstrated that pathogen or indicator loads are the most sensitive input of such models. Historically, most research into waterborne disease has focused on human fecal sources such as sewage and storm water (McBride et al., 2013). There is a growing recognition, however, that animal feces also represent a significant human health risk (Soller et al., 2015; Dufour et al., 2012; Till et al., 2008). Thus, microbial modeling for mixed-use watersheds requires information on microbial loads from many sources.

Tools to estimate and use microbial loads in a form suitable for modeling have been developed over the last 20 years. The U.S. Environmental Protection Agency (EPA) Bacterial Indicator Tool (BIT) estimated microbial loadings from domestic animals, wildlife, and human activities to a mixed-use watershed (EPA, 2000). Zescocki et al. (2005) developed the Bacteria Source Load Calculator (BSLC) that aids in the source characterization and Total Maximum Daily Load (TMDL) allocation scenario process. The BSLC uses externally generated inputs, such as land use distribution and livestock, wildlife, and human population estimates, to calculate monthly bacterial land loadings and hourly bacterial stream loadings. Teague et al. (2009) presented SELECT (Spatially Explicit Load

Enrichment Calculation Tool) to identify potential *E.coli* sources in watersheds that estimate the daily average potential *E.coli* production from livestock, wildlife, wastewater treatment plants, septic systems, and pet sources. Taken together, these findings reveal that microbial fate parameters along with the load parameters appear to be critical for modeling results. Whelan et al. (2018, 2017a) developed the Microbial Source Module (MSM) to be used as a component of an IEM workflow. They described its mathematical formulations, coupled it within a quantitative microbial risk assessment (QMRA) IEM workflow that automates input data collection, and presented the cumulative results of an application on a mixed-use watershed in the United States.

The availability of reliable microbial source estimates allows one to evaluate the relative importance of different sources of fecal organisms across the watershed with respect to microbial water quality in specific locations for recreation, irrigation, or other activities. Objectives of this work were to 1) use an IEM workflow with MSM as means for evaluating the relative importance of diverse sources of fecal bacteria in a mixed-use watershed and 2) demonstrate the efficiency of the IEM workflow with MSM in providing inputs for the QMRA in such watersheds. Limitations of the approach and assessment are documented in Whelan et al. (2018).

Materials and Methods

Watershed characteristics

The Manitowoc River basin is located in eastern Wisconsin in the United States, on the western shore of Lake Michigan and was earlier described in the water quality modelling work of Whelan et al. (2018). It has an area of 1128 km², mainly rural and agricultural, and has been delineated into nine subwatersheds (or subbasins) (Supplemental Figure S.1). Table S1 provides a summary of subwatershed characteristics, including area by land-use type, number and type of domestic animals, and number of septic systems. Information on domestic animals and wildlife (numbers or densities, types, production or shedding rates, number of grazing days per month, manure application rates) were obtained from the State of Wisconsin and documented in the Supplemental Material. Whelan et al. (2018) also listed more than 20 databases automatically accessed and used in the modeling exercise that described additional watershed characteristics (e.g., slope, soil type, land-use type and cover, meteorology, etc.).

Five land-use types were considered: cropland, pasture, forest, urban (Builtup), and stream. Six source types were assessed: land-applied manure on cropland and pasture (Manure Application); domestic animals shedding on pasture due to grazing [Grazing (Pasture) or just “grazing”]; direct shedding by cows instream [Grazing (In-Stream)]; leaking septic systems (Septic); direct shedding by wildlife to forest, cropland, and pasture (Wildlife); and urban runoff (Builtup). Assumptions associated with watershed modeling are summarized in Table S.1 which also summarizes other supporting data: fecal coliform production rates (Table S.2), number of grazing days and fraction of time cows are allowed instream (Table S.3), fraction of the available domestic-animal manure applied to the land (Table S.4), enterococci die-off rates (Table S.5), support data for leaking septic systems (Table S.7), and density of wildlife in the Manitowoc Basin (Tables S.8, S.9, and S.10).

Integrated environmental modeling

Five software components that compose an IEM workflow were used in this assessment: Site Data Manager Project Builder (SDMPB), Data for Environmental Modeling (D4EM), MSM, HSPF, and Better Assessment Science Integrating point and Nonpoint Sources (BASINS). D4EM (EPA, 2013; Wolfe et al., 2007) manages, accesses, retrieves, analyzes, and caches web-based environmental data. SDMPB (Whelan et al., 2017b) leverages D4EM and provides geographical information system capabilities. MSM (Whelan et al., 2018, 2017a) organizes, analyzes, and supplies data that calculates microbial loading rates within subwatersheds. HSPF (Bicknell et al., 1997) simulates watershed hydrology and microbial fate and transport. BASINS (EPA, 2001a) provides graphical and tabular viewers of input data, and flow and concentration output.

Data collection for model calibration and validation

Daily flow data were obtained from the U.S. Geological Survey National Weather Information System (USGS 2017) gage station 04085427 for years 2000–2012. The first seven years of discharge data (2000–2006) were used for model warm-up, and the final five years (2007–2012) for calibration. Stream water was sampled and analysed for enterococci and *Cryptosporidium* during the summers of 2011 and 2012 at three locations (pour point and the outlets of subwatersheds 4 and 5 in Figure S.1), resulting in 41, 40, and 40 detectable grab samples, respectively. Enterococci enumeration was performed following EPA Method 1600, and enumeration of *Cryptosporidium* oocysts was performed using EPA Method 1623. Depending on the type of sample, 10–200L were analysed for *Cryptosporidium* enumeration. Recovery for Method 1600 was 45% using laboratory-prepared standards, and the average percentage difference between duplicate samples was 29%. Recovery for Method 1623 using *Cryptosporidium parvum* oocysts was 37%.

Microbial samples at the Manitowoc River Basin outlet were used to calibrate the watershed model, while samples from the outlets of Subwatersheds 4 and 5 were used to validate it. Using initial loading rates from the MSM model as input to HSPF, the Parameter ESTimation (PEST) (Doherty, 2005) model calibrated HSPF input parameter values. Parameters used by HSPF to model microbial fate and transport include monthly instream deposition (loading) rate by stream reach (MONTH-DATA; cells/day), monthly loading rate per unit area by subwatershed by land-use type (MON-ACCUM; cells/acre/day), monthly maximum microbial storage per unit area (MON-SQOLIM; cells/acre), rate of surface runoff which will remove 90 percent of stored microbes per hour (WSQOP; in/hr), microbial concentrations in interflow and groundwater (IOQC and AOQC, respectively; cells/ft³), first-order instream die-off rate (FSTDEC; day⁻¹), and temperature correction coefficient for the first-order die-off (THFST; dimensionless).

MSM estimated microbial loading rates (MONTH-DATA, MON-ACCUM) based on literature values and local information (see Tables S.1 through S.10). Initial calibration only adjusted key parameters with proportional rates (i.e., DATA_rate, ACCUM_rate), while maintaining monthly relationships; for example, values of MON-SQOLIM were adjusted by ACCUM_rate to keep the functional relationship between MON-ACCUM and MON-SQOLIM, as defined in Eq. (15) in Whelan et al. (2018). Based on local well data which

indicated an absence of enterococci (WDNR, 2017), microbial concentrations in interflow and groundwater flow (IOQC and AOQC, respectively) were set to zero and excluded from parameter calibration. As a result, five parameters (DATA_rate, ACCUM_rate, WSQOP, FSTDEC, THFST) were automatically calibrated. PEST performed a sensitivity analysis, along with the parameter calibration.

Scenarios to assess microorganism sources

Two management scenarios are investigated: cows allowed in and cows restricted from streams. During the non-freezing months of April through October, 50% of cows graze, although there is evidence that some are allowed on pasture during freezing months (i.e., November to March); see Table S.2 for domestic animal shedding rates and Table S.3 for number of grazing days per month. Although cows are restricted from streams by a Manitowoc County Code (2011), the fraction of time they are in streams from June through August is assumed to be 10% (see Table S.3). Impacts of the two management scenarios are evaluated as a function of source type (e.g., land-applied manure, grazing, leaking septs, etc.) by interrogating

- microbial source loadings to the basin,
- microbial concentrations as they vary in time at the watershed outlet,
- microbial concentrations as they vary in time at the watershed outlet as a function of subwatershed, and
- microbial concentrations at the watershed outlet as a function of flow discharge;

and by comparing indicator and pathogen microbial concentrations at the watershed outlet to recreational water quality criteria. The basin-wide analysis investigated the impacts of allowing and not allowing cows in streams during summer months.

Results and Discussion

Sensitivity analysis and calibration results

Microbial loadings and die-off rates were obtained from the literature and local information (Tables S.1 through S.10) and used as the starting point in the calibration. PEST performed a parameter sensitivity analysis using the Jacobian matrix estimated at each iteration (Doherty, 2005) indicating WSQOP and THFST as most sensitive, DATA_rate and ACCUM_rate as moderately sensitive, and FSTDEC as least sensitive. Calibrated values were 22.5 for ACCUM_rate, 0.099 in/hr for WSQOP, 2.0 day⁻¹ for FSTDEC, 1.0 for THFST, and 0.0077 for Data_rate. Calibrated values for ACCUM_rate and DATA_rate indicate that MSM initially underestimated MON-ACCUM and overestimated MONTH-DATA, which could be caused by initial assumptions for cows directly shedding instream (Table S.1), direct loading of septs to streams (Table S.1), estimated microbial production rates (Table S.2), and uncertainty in overland microbial fate and transport modeling. For example, overestimated microbial loadings (MON-ACCUM or MON_DATA) could be compensated for by high die-off rates (FSTDEC) and vice-versa, although output results are not particularly sensitive to FSTDEC.

Calibrations of flow and microbial concentration at the watershed outlet (i.e., pour point) resulted in correlation coefficients (r) of 0.86 and 0.45, respectively (Whelan et al. 2018). Based on the pour point calibration, validations of microbial fate and transport simulations were performed at the outlets of Subwatersheds 4 and 5, by comparing 40 grab samples from each to simulated results, which resulted in correlation coefficients of 0.23 and 0.32, respectively, as defined in Figure 1. These microbial validations included correlations at very low concentrations. Data in Figure 1 reflect the fact that PEST minimizes the squared-error by not weighting the smaller values as heavily; hence, there is better correlation at higher, more important, concentrations.

Model simulations for scenario assessment

Simulated microbial source loadings to the basin by month—Figure 2 presents the average simulated enterococci loadings by source and land-use type by month for cows allowed and not allowed instream. Results are presented by loading rate (Cells/d) and fraction of the total concentration. Loadings from leaking septics, wildlife shedding, and urban (Builtup) runoff are assumed constant throughout the year, while other loadings vary by month. Manure produced by domestic animals is stored in holding ponds during the winter months (November through March), then applied to the land during warm-weather months. Seasonal trends dominate. The largest microbial loadings occur from April to October and result from land-applied manure and domestic animals shedding on pasture and cropland due to grazing (“grazing” in the text). Grazing cannot be ignored because, for example, the typical manure shedding rate for a dairy cow is between 8 – 150 kg/d (ASAE, 2005). During winter months, the largest enterococci loading by fraction within the basin is due to shedding from wildlife. Stream loadings from urban runoff, direct shedding by cows, and leaking septics represent relatively insignificant enterococci loadings to the basin. Similar results occurred for cows allowed and not allowed instream (Figures 2a and 2b, respectively).

Simulated microbial concentrations by time at the watershed outlet—Flow and microbial fate and transport simulations are computed at an hourly time step, which allows source types to be analyzed and presented on an hourly basis (Figure 3), and rolled-up for monthly (Figures 4a and 4c) and annual (Figures 4b and 4d) analyses, where cows are and are not allowed instream. Figure 3 provides time-varying hourly values for 2008 and 2009 at the watershed outlet for precipitation and air temperature (Figure 3a), simulated discharge and enterococci concentrations for cows instream (Figure 3b), and source apportionment results for cows allowed in (Figure 3c) and restricted (Figure 3d) from the stream. These years are typical for 2007 – 2012. Year 2008 (Figure 3’s left panels) illustrates a wet year with numerous precipitation events that resulted in two pronounced hydrographs in April and June. The first event in April coincides with land-applied manure in the spring and results in overland runoff of land-applied manure and shedding during grazing as the dominant sources of contamination at the watershed outlet (Figure 3b). Year 2009 (Figure’s 3 right panels) represents a year with more moderate precipitation, allowing the land surface to assimilate the water which resulted in smaller, more evenly distributed hydrographs (Figure 3b). It also illustrates the impact of cows shedding directly in streams, especially

during low-flow (end of July 2009, Figure 3b) when the highest enterococci concentrations occur at the watershed outlet.

Hourly smear diagrams presented in Figures 3c and 3d correlate the importance of environmental conditions (e.g., precipitation, land-use type) with source type (land-applied manure, grazing, instream shedding, etc.) and management practice (cows allowed in and restricted from streams). When manure is applied to the land and rains occur during the spring, runoff of land-applied manure dominates concentrations levels at the watershed outlet (> 80%), followed by runoff due to grazing (see April, Figures 3c and 3d). During intermittent periods of no runoff, leaking septs and urban runoff are significant contributors to concentrations at the watershed outlet. When cows are allowed instream during the summer months which are generally low-flow periods (e.g., < 100 ft³/s) with less dilution by surface flow, they represent the largest fraction of enterococci concentration at the watershed outlet (Figure 3c). If their access is restricted, leaking septs and runoff of land-applied manure, shedding due to grazing, and urban runoff become important, depending on environmental conditions (Figure 3d). Leaking septs and urban runoff dominate winter months (>50 – 60%).

Figure 4 presents simulated monthly and yearly averaged enterococci concentrations at the watershed outlet for cows allowed and not allowed instream for the six source types over the entire simulation period of 2007–2012. Results are presented by concentration and fraction of the total concentration. Figures 4a and 4c and Figures 4b and 4d present results of averaged monthly and annually concentrations, respectively. Cows shedding instream during the summer months (Figure 4a) contribute most to enterococci concentrations, while septic and urban runoff dominate the winter months; there is little to no runoff during the winter, and significant microbial die-off has occurred since the spring application of manure. During the spring, land-applied manure and grazing contribute most to the concentration. On an annual basis (Figure 4b), cows shedding instream tend to be a consistently important contributor to the concentration at the watershed outlet, followed by land-applied manure, then grazing. There was a large spike in 2009 concentrations because it was a low-flow year – the same loading rate with lower flow rate resulted in higher concentrations. Low flow signifies less rainfall and runoff, maximizing the importance of direct releases to the stream (i.e., direct shedding by cows). Higher rainfall and runoff (years 2010–2012) increased contributions from land-applied manure and grazing.

When cows are not allowed instream (Figures 4c and 4d), the results change dramatically especially during summer months; peak concentrations are only one-fourth as high (Figure 4a versus Figure 4c), with most of the value due to land-applied manure, followed by grazing (Figure 4c). During the late fall and winter, leaking septs and urban runoff dominate. Sources that dominate on a monthly average (land-applied manure, followed by grazing) also dominate when averaged over the year, although urban runoff and leaking septs are also important contributors (Figure 4d).

Averaged microbial concentration at the watershed outlet by month by subwatershed—The Manitowoc River Basin was delineated into nine subwatersheds (see Figure S.1 and Table S1). Subwatersheds contain many characteristics that can impact

microbial concentrations at the watershed outlet. For example, larger areas associated with a subwatershed contribute more runoff (flow and microbes) but also more dilution. More cows contribute to more instream shedding during the summer months. Larger numbers of animals (domestic and wildlife) contribute to higher levels of microbial loadings from runoff. More leaking septic systems contribute to more contamination entering streams. The importance of a subwatershed's contribution to the outlet of the basin reflects a combination of these factors; hence, the importance of a subwatershed's contribution may change with time, as contributions change. In other words, no one subwatershed, time of year, land-use type, source type, or land-use practice can singly represent the primary contribution to contamination at the outlet of the watershed. Whelan et al. (2010) drew a similar conclusion from their modeling exercise, noting that the manure application method (e.g., shedding, spreading, pond leakage, etc.); pathogen rate of release; timing of the manure loading; sequence and type of transporting media; pathogen characteristics (e.g., prevalence, excretion density, inactivation rate, and distribution coefficient); timing of rainfall events; duration and intensity of rainfall; antecedent moisture conditions; and landscape characteristics all play important roles in identifying which source contributes to the contamination and pathogen density at the receptor location, and to what degree.

Figure 5 presents the contribution of each subwatershed to the average monthly enterococci concentration at the instream location adjacent to the subwatershed; therefore, Subwatersheds 1, 2, 5, 6, 7, and 8 (see Figure 1) are headwater subwatersheds, and those instream concentrations directly reflect only contributions from those subwatersheds. Subwatersheds 3, 4 and 9 also include contributions from upstream flows. Subwatersheds 1, 6, 7, and 8 flow into 3; Subwatersheds 2 and 3 flow into 4; and Subwatersheds 4 and 5 flow into 9. Figures 5a and 5b present scenarios where cows are allowed in and restricted from streams respectively.

When cows are allowed instream (Figure 5a), the highest contributions to the outlet concentration are during the summer months (June – August) and from Subwatersheds 1 and 5 (see Figure S.1) followed by Subwatersheds 2, 9, 7, and 4, although 4 and 9 are not headwater subwatersheds and include inputs from other upstream areas. Subwatershed 1's loading area (i.e., area of Cropland, Pasture, Forest, and Builtup) is less than half of Subwatershed 5's loading area (105 km² versus 247 km²), with only 60% of the number of Subwatershed 5's dairy cows (5748 versus 9663) (see Table S1), resulting in densities of 55 and 39 cows/km², respectively. A higher density suggests more cows shedding directly to the stream per unit area (and per volume of runoff). Both subwatersheds have similar densities of swine per cropland and pasture area (2 swine/km² versus 3 swine/km²), although the density of poultry and number of leaking septs are significantly higher in Subwatershed 5 than Subwatershed 1 (468 poultry/km² versus 7 poultry/km², and 101 septs versus 40 septs, respectively), helping to offset the effect of the higher density of dairy cows in Subwatershed 1. Dilution from upstream inputs contributes to the reduced concentration levels associated with Subwatersheds 4 and 9.

When cows are restricted from streams (Figure 5b), peak concentrations decrease by 4.5 times, overland runoff of land-applied manure and grazing during spring and summer contribute the most to concentrations at the watershed outlet, and Subwatershed 2

contributes the most to the peak concentrations followed by Subwatershed 5, with peaks occurring during different months. Even though Subwatershed 5 has more land-applied manure from domestic animals, domestic animal grazing, wildlife shedding, urban runoff, and leaking septics, it has fewer dairy cows per cropland and pasture area (46 cows/km² versus 84 cows/km²).

Microbial concentrations by flow discharge at the watershed outlet—Figure 6 presents the average enterococci concentrations by flow discharge at the pour point for cows allowed in and restricted from streams. It clearly shows the importance of restricting cows from streams during low-flow periods, which mostly occur during late spring and summer months, since >80% of enterococci concentration contribution is due to instream shedding of cows at flows below 100 ft³/s (Figure 6a). In fact, instream shedding continues to influence concentrations at the outlet of the watershed at flows between 100 – 500 ft³/s. Research demonstrates the importance of restricting cows from streams during summer months. For example, Nader et al. (1998) reported that direct shedding of cattle feces and urine to streams represented the primary water quality concern. Above 500 ft³/s, land-applied manure and grazing become important due to runoff, until they eventually dominate with >80% share. In the modeling exercise, density of cows per unit area is important because it can offset the impact of subwatersheds with more land-applied manure from domestic animals, domestic animal grazing, wildlife shedding, urban runoff, and leaking septics, as illustrated by Figure 5 and Table S1.

In the case of cows being restricted from streams (Figure 6b), the concentration significantly decreases by a factor of five as compared to allowing cows instream; leaking septics and urban runoff become the major contributors (>75%) during low flow (below 100 ft³/s), while contributions by land-applied manure and grazing dominate (>80%) at flows greater than 100 ft³/s. Increased flow corresponds to increased runoff (e.g., spring runoff).

Risk assessment

EPA (2012) recommendations for primary contact recreational uses are based on bacterial indicators such as enterococci and *E.coli*, not pathogens. Most strains of enterococci and *E.coli* do not cause human illness; rather, the basis for EPA's recommendation is that pathogens often co-occur with indicators of fecal contamination. EPA (2012) recommends that states make risk management decisions regarding illness rates during recreational activities, and the designated use of primary contact recreation will be protected if the Geometric Mean (GM) and related Statistical Threshold Value (STV) are adopted for either enterococci or *E.coli* in fresh waters. The GM corresponds to samples (or values) in any 30-day interval. The STV approximates the 90th percentile of the water quality distribution. For our analysis, the EPA standards are a culturable enterococci at a GM of 35 cfu/100 mL and an STV of 130 cfu/100 mL, measured using EPA Method 1600 (EPA, 1997), or any other equivalent method that measures culturable enterococci (EPA, 2012). The recommendation corresponds to an estimated illness rate of 3600 per 100,000 people (EPA, 2012). This section compares the simulation time series of enterococci concentrations at the outlet of the basin to EPA's GM and STV standards. In addition, a quantitative microbial risk model is applied to the most prevalent pathogen (*Cryptosporidium parvum*) sampled at the outlet of

the watershed to estimate risk of exposure to pathogens (Haas et al., 1999; Whelan et al., 2014b; Soller et al., 2015).

Comparison of microbial concentrations at the watershed outlet with recreational water quality criteria recommendations

Figure 7 presents a comparison of average enterococci concentrations simulated at the watershed outlet for a moving 30-day interval from 2007 – 2012 for the GM (top, blue curves) and STV at the pour point for cows allowed in and restricted from streams with EPA standard GM (35 cfu/100 mL) and STV (130 cfu/100 mL) recommendations. Even though the concentration time series for the scenario where cows are restricted from streams is significantly less than for cows allowed instream, simulated results for both scenarios regularly exceed the recommended GM and STV criteria. In the case of the GM, exceedances are 95% and 94%, respectively; for the STV, they are 85% of the entire period in both cases.

Comparison of simulated microbial risk calculations at the watershed outlet with recreational water quality criteria recommendations

A number of dose-response models have been published to compute the probability of infection from acute exposure to pathogens, such as inadvertent ingestion during recreational activities: exponential, beta-Poisson, hypergeometric, and Gompertz-log (Conlan et al., 2011; EPA, 2010; Haas, 2002; Haas et al., 1999; Soller et al., 2008, 2004). The recommended dose-response model for *Cryptosporidium* exposure is based on the exponential function (Whelan et al., 2014b; Soller et al., 2004, 2008):

$$P(d) = 1 - \exp(-r C I) \quad (1)$$

where $P(d)$ is the probability of infection and subsequent illness; r is the exponential constant (Cells^{-1} or oocysts^{-1}); C is the microbial concentration (Cells/L or oocysts/L), and I is the intake volume (L). Dose (e.g., Cells or oocysts) is a multiple between the concentration and intake. Whelan et al. (2014b) and Soller et al. (2004, 2008) report a range for “ r ” between 0.04 – 0.16. Recreational water incidental ingestion rates were highest for children, with EPA (2016) reporting the 90th percentile at 330 mL/d. Of the grab samples collected at the watershed pour point and outlets of Subwatersheds 4 and 5, 12 were analyzed for pathogens, of which *Cryptosporidium* had the largest number of detectable samples (4 of 12) and highest concentrations: 1.84×10^{-2} , 4.76×10^{-2} , 4.55×10^{-2} , and 6.11×10^{-2} oocysts/L. By assuming a single-event exposure, a conservative estimate for intake of 330 mL per event, the maximum recorded concentration of 6.11×10^{-2} oocysts/L, and the upper value for “ r ” of $0.16 \text{ oocysts}^{-1}$, the probability of infection is 3.22×10^{-4} or 322 infections per 100,000 people. This value is significantly below EPA’s recommended limit of 3600 infections per 100,000 people and well below the national total number of infections reported by the Centers for Disease Control and Prevention of 9313 and 8008 cases of cryptosporidiosis per 100,000 people for 2011 and 2012, respectively (Painter et al., 2015). This exemplifies the difficulty of using only indicator levels to suggest illness due to pathogen exposure during recreational events because pathogens often co-occur with indicators of fecal contamination. Here, the GM and STV of enterococci were exceeded by 94% and 85%, respectively, during the simulation period; yet, EPA’s recommended limit on

infection rate was 53 times higher than a worst-case scenario using *Cryptosporidium*, which suggests a possible *de minimis* health impact.

Conclusions

A software infrastructure, which automates many manual steps associated with a Quantitative Microbial Risk Assessment (QMRA), was implemented on the Manitowoc River Basin near Manitowoc, Wisconsin USA. It considered land-applied manure from domestic animals on undeveloped areas; direct shedding (excretion) on undeveloped lands by domestic animals and wildlife; pastureland, cropland, forest, and urban or engineered areas; sources that directly release to streams from leaking septic systems; and shedding by domestic animals directly to streams. This source-allocation assessment compares impacts of allowing and restricting cows access to streams; stream access is restricted by a local ordinance.

Simulation results suggest that multiple factors contribute to whether a source of contamination affects downstream concentration levels. For example, larger areas contribute more runoff (flow and microbes) but also more dilution. More cows contribute to more instream shedding during the summer months. Larger numbers of animals (domestic and wildlife) contribute to higher levels of microbial loadings from runoff. More leaking septic systems contribute to more contamination entering streams, especially during winter months. The importance of a subwatershed's contribution to the outlet of the basin reflects a combination of these factors and may change with time, as the factors change. No single subwatershed, time of year, land-use type, source type, or land-use practice alone can represent the primary contribution to contamination at the outlet of the watershed.

The largest microbial loadings occur from April to October and are due to land-applied manure and domestic animals shedding on pasture and cropland while grazing. During winter months, the largest enterococci loading by fraction within the basin is from shedding by wildlife. Stream loadings from urban runoff, direct shedding by cows, and leaking septic systems represent relatively insignificant enterococci loadings to the basin. Similar results occur for cows allowed and not allowed instream.

Simulation results, however, indicated that the sources contributing the most microbial loadings to the basin did not necessarily correlate to sources with the greatest influence on concentrations at the watershed outlet. Cows shedding instream during summer months contribute the most to enterococci concentrations, while leaking septic systems and urban runoff dominate winter months. During the spring, land-applied manure and grazing contribute most to the concentration. When cows are not allowed instream, peak concentrations are only one-fourth as high, mostly due to land-applied manure followed by grazing. During the late fall and winter, leaking septic systems and urban runoff dominate concentration levels at the watershed outlet. Finally, simulation results indicate that restricting cows during low-flow periods (summer months) can reduce downstream concentration levels by a factor of five.

Simulated enterococci concentrations at the watershed outlet were compared to the U.S. Environmental Protection Agency's (EPA) recreational water quality criteria and exceeded

the Geometric Mean and Statistical Threshold Value recommendations 94% and 85% of the time, respectively. A risk assessment on the pathogen *Cryptosporidium* was also performed because indicators, such as enterococci, do not cause human illness, only pathogens. Results of a worst-case risk-assessment scenario indicated that the risk of infection of 322 per 100,000 was more than 10 times lower than EPA's recommended infection rate of 3600 per 100,000. Assessment of predicted indicator concentrations from modeling could, therefore, be used in determining the appropriateness of waivers to criteria and standards concentration numbers on the basis of site-specific environmental settings and source conditions (Whelan et al., 2018). It informs how criteria can be interpreted (e.g., human versus non-human sources, land-use types, management practices, microbes, source locations, and timing) by linking modeling, monitoring, and methods development. Likewise, it can integrate data obtained from new alternative indicators – such as bacteriophages which are believed to behave more similarly to human pathogenic viruses – with sources of contamination to obtain more precise risk assessment information. Future work could combine this source-allocation assessment with microbial source tracking and more refined manual calibration to produce more accurate representations of the origins of microbial contamination and their importance to downstream health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Glossary

BASINS	Better Assessment Science Integrating point and Nonpoint Sources
BIT	Bacterial Indicator Tool
BSLC	Bacteria Source Load Calculator
CSMDS	Community Surface Dynamics Modeling System
D4EM	Data for Environmental Modeling
EPA	U.S. Environmental Protection Agency
FIO	fecal indicator organisms
FRAMES	Framework for Risk Analysis in Multimedia Environmental Systems
GM	Geometric Mean
HSPF	Hydrologic Simulation Program – FORTRAN
IEM	Integrated Environmental Modeling
MSM	Microbial Source Module
OMS	Object Modeling System
PEST	Parameter ESTimation

QMRA	Quantitative Microbial Risk Assessment
SDMPB	Site Data Manager Project Builder
SELECT	Spatially Explicit Load Enrichment Calculation Tool
STV	Statistical Threshold Value
SWAT	Soil and Water Assessment Tool
TMDL	Total Maximum Daily Load
USGS	U.S. Geological Survey

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Figure 1. A process-based integrated environmental modeling workflow for quantitative microbial risk assessment.

Figure 2.
Calibration (*a*) and validation (*b*, *c*) results for enterococci concentrations at the pour points of the watershed and Subwatersheds 4 and 5, respectively.

Figure 3.

Average enterococci loadings by source and land-use type by month for (a) cows allowed instream and (b) no cows allowed instream. Left y axis refers to sources (histograms); right y axis refers to land uses.

Figure 4.

Hourly results for 2008 and 2009 for (a) monitored precipitation (left y axis) and temperature (right y axis), (b) simulated flow discharge and enterococci concentrations, (c) source apportionment at the pour point for cows allowed instream, and (d) source apportionment at the pour point for no cows allowed instream.

Figure 5. Average enterococci concentration at the pour point for cows allowed instream by (a) month and (b) year, and for no cows allowed instream by (c) month and (d) year.

Figure 7.

Average enterococci concentration by flow discharge at the pour point for (a) cows allowed instream and (b) no cows allowed instream.

Figure 8.

Average enterococci concentrations at the pour point for a moving 30-d interval from 2007 to 2012 for the geometric mean (GM) and statistical threshold value (STV) at the pour point for (a) cows allowed instream and (b) no cows allowed instream.