## **Quantitative Microbial Risk Assessment Tutorial**

Pour Point Analysis of Land-applied Microbial Loadings and Comparison of Simulated and Gaging Station Results

Gene Whelan
Kurt Wolfe
Rajbir Parmar
Michael Galvin
Marirosa Molina
Richard Zepp
U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Athens, GA

### **Keewook Kim**

University of Idaho Idaho National Laboratory Center for Advanced Energy Studies Idaho Falls, Idaho

Paul Duda Mark Gray AQUA TERRA Consultants, a Division of RESPEC, INC. Decatur, GA 30030

## **Summary**

This tutorial demonstrates a pour point analysis

- Initiates execution of the SDMPB.
- Navigates the SDMPB.
- Chooses a pour point within a watershed, delineates the sub-area that contributes to that pour point, and collects data for it.
- Considers land application of microbes.
- Develops necessary input files to execute HSPF.
- Develops necessary input file to view results from the SDMPB and HSPF.
- Views simulation results for flows and microbial densities with BASINS.
- Retrieves observed daily USGS flows and compares to simulated daily flows at the pour point.

# Pour Point Analysis of Land-applied Microbial Loadings and Comparison of Simulated and Gaging Station Results

#### **PURPOSE**

Automate data acquisition for input data requirements of a confederation of models

#### **OBJECTIVE**

Pre-populate input data files of models automatically. Importation of point source data will be performed in the context of an assessment of microbial fate and transport, within a pour-point delineated watershed, by capturing contextual data for the watershed model WinHSPF (a.k.a. HSPF) and pre-populating its input data files to account for:

- Overland runoff;
- Snow accumulation/melt;
- Hourly simulations;
- Remote-sensing NLDAS, coupled with monitoring, meteorological data;
- Land-applied microbial loadings;
- Instream point source microbial loadings; and
- Microbial fate and transport.

#### **DEMONSTRATION**

This tutorial reviews screens, icons, and basic functions of the SDMProjectBuilder (SDMPB), performs a Pour Point simulation for Microbial Fate and Transport using HSPF, and analyzes and visualizes results at multiple locations in the watershed using BASINS. It demonstrates how to

- Initiate execution of the SDMPB.
- Navigate the SDMPB.
- Choose a pour point within a watershed, delineate the sub-area that contributes to it, and collect data for it.
- Consider land application of microbes.
- Develop necessary input files to execute HSPF.
- Develop necessary input files to view results from the SDMPB and HSPF.
- View simulation results for flows and microbial densities with BASINS.
- Retrieve observed daily USGS flows and compare to simulated daily flows at the pour point.

#### SOFTWARE ACCESS, RETRIEVAL, AND DOWNLOAD

Instructions for access, retrieval, and download of the SDMProjectBuilder, HSPF, and BASINS software products are provided by Whelan et al. (2015a). When installed, three desktop icons appear.







#### **TUTORIAL - TABLE OF CONTENTS**

## EXECUTE THE SDMProjectBuilder

- Identify a Project
- Identify a HUC-8 and Watershed of Interest

## MODIFY AND IMPORT LOCAL DATA FILES

- Identify and Modify Local Source-term Data
  - MonthlyFirstOrderDieOffRateConstants.csv
  - WildlifeDensities.csv
- Import Local Data Files

## **EXEUCUTE THE PROJECT BUILDER**

## EXECUTE HSPF, SIMULATING FLOW AND MICROBIAL TRANSPORT

## VIEW HSPF SIMULATION RESULTS WITH BASINS

- Register Flow and Microbial Densities with BASINS
- View the Discharge Time Series
- View the Microbial Density Time Series

RETRIEVE OBSERVED DAILY USGS FLOWS AND COMPARE THEM TO SIMULATED DAILY FLOWS AT THE POUR POINT

#### **EXECUTE THE SDMProjectBuilder**

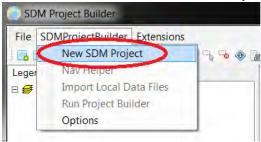
## **Identify a Project**

Whelan et al. (2015b) provide a detailed tutorial for identifying an 8-digit HUC. Whelan et al. (2015c) extend this tutorial and review screens, icons, and basic functions of how to take output from the SDMProjectBuilder (SDMPB) and perform a fate and transport analysis of land application of microbes within a HUC-12. This section follows the instructions of Whelan et al. (2015b, 2015c) without going into similar detail, describes creation of a working folder, and briefly identifies the HUC-8 and a watershed based on a pour point.

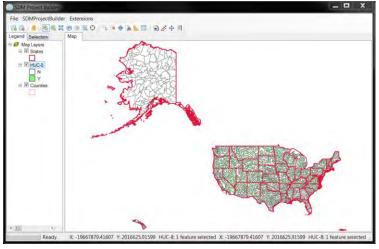
1. Execute the SDMProjectBuilder (SDMPB) by clicking on the shortcut icon displayed on the computer screen.



- 2. Create a folder where you have administrative rights.
- 3. From the Menu Bar, select "SDMProjectBuilder", then "New SDM Project".

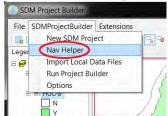


4. Create a new file within the working folder. A map of the Unites States including Alaska, Hawaii, and Puerto Rico should appear.



## **Identify a HUC-8 and Watershed of Interest**

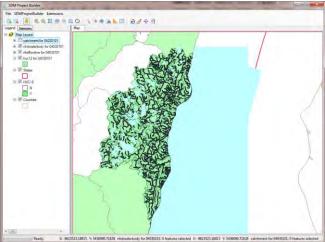
5. Under "SDMProjectBuilder", select "Nav Helper".



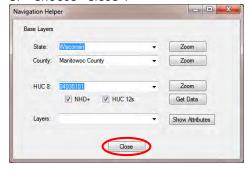
6. Fill out the screen as below, choose "Zoom", then "Get Data".



7. A screen similar to the following will appear.



8. Choose "Close".



9. In this example, we assess only the four uppermost 12-digit HUCs in the Manitowoc watershed (highlighted in the following figure). The pour point does not have to coincide with any particular location or HUC, although in this case it will match four 12-digit HUCs.



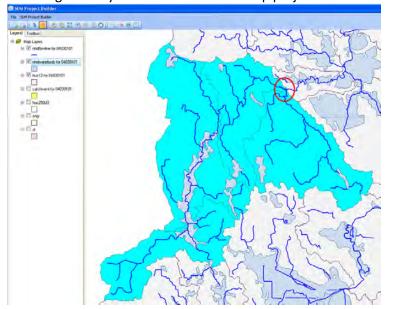
10. Using Zoom In, zoom to this area. Check the "nhdflowline" Map Layer. If it is not at the top of the listing, move it there to ensure it is the outermost projection and will appear on the map.



11. To see where all ponded waters are located, check the Map Layer titled "nhdwaterbody for 04030101". This provides a picture of all water sources and their locations.



12. For this example, you will identify the pour point that includes only the four uppermost HUC-12s, as designated by the red circle on this map projection.



#### **MODIFY AND IMPORT LOCAL DATA FILES**

This section identifies and modifies local data files, imports local data files to the SDMPB, executes the project builder, and collects map layers describing environmental characteristics.

## **Identify and Modify Local Source-term Data**

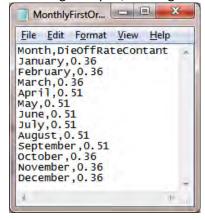
The user can modify certain source-term parameters and influence the degree of resolution of the watershed through 12 local-data files that are installed when a user begins a new project. They are located in the "LocalData" folder within the working folder. Descriptions of the local source-term data are in Whelan et al. (2015d). Whelan et al. (2015c, 2015e) provide additional examples identifying and modifying the local source-term data example default files. Metadata associated with the parameters contained within each file, including definitions and units, are summarized in Table 1. Five of the 12 files denote locations such as the location of point sources, animal locations, septic systems, etc. For this example problem:

- **MonthlyFirstOrderDieOffRateConstants.csv** Different first order die-off rates will be used for land-applied microbes. They will be changed from 0.36 and 0.51 d<sup>-1</sup> to 0.064<sup>-1</sup>.
- WildlifeDensities.csv Wildlife densities will be updated to 29 deer/mi² from 5 deer/mi² to more accurately reflect densities in this area (WDNR, 2015).
- AnimalLL.csv, FCProdRates.csv, GrazingDays.csv, ManureApplication.csv, SepticsLL.csv, SepticsDataWatershed.csv – Animal numbers and locations and septic-system locations will use default values, since they are applicable to the Manitowoc River basin; no change to these files is required.
- PointSourceLL.csv, PointSourceData.csv, BoundaryPoints.csv, OutputPoints.csv There are no
  point sources or intermediate points. Since these default locations are outside the watershed,
  no modifications to these files are necessary.

The only files that will be modified will be MonthlyFirstOrderDieOffRateConstants.csv and WildlifeDensities.csv.

MonthlyFirstOrderDieOffRateConstants.csv

13. Using Notepad, the original file includes die-off rate constants by month with units of d-1:



**Table 1.** Names of default support files and input types to which the user has access for modifications

FILE NAME	d input types to which the user has access INPUT DATA AND DEFINITION	UNITS
Domestic Animals and Wildlife		
AnimalLL.csv	Domestic animal locations by Latitude and Longitude	Degree (by fraction)
	Domestic animal numbers by type and location	Number
FCProdRates.csv	Production or shedding rate of microbes from the domestic animal, which equals the multiple of the 1) Domestic animal shedding rate in mass of waste (wet weight) per time and 2) Microbial concentration based on mass of waste shed by the domestic animal	Counts/d/animal
	Typical microbial production or shedding rate per wildlife per area	Counts/d/ac
Grazing Days.csv	Number of grazing days per domestic animal per month	Number
	Fraction of the number of grazing days that beef cattle spend in a stream per month	fraction
Manure Application.csv	Fraction of manure applied to soil each month per domestic animal	fraction
	Fraction of amount of manure shed by the domestic animal incorporated into soil	fraction
MonthlyFirstOrderDieOffRateConstants.csv	First-order microbial inactivation/die-off rate on the land surface per month	1/d
WildlifeDensities.csv	Typical number of wildlife per unit area by land use type	Number/mi <sup>2</sup>
Point Sources		
PointSourceLL.csv	Point source locations by Latitude and Longitude	Degree (by fraction)
PointSourceData.csv	Annual-average flow for each point source	ft <sup>3</sup> /s
	Annual-average microbial loading rate for each point source	Counts/yr
	Annual-average chemical loading rate for each point source	Lbs/yr
Septic Systems		
SepticsLL.csv	Septic system locations by Latitude and Longitude	Degree (by fraction)
SepticsDataWatershed.csv	Number of people per septic unit	Number
	Average fraction of septic systems that fail	fraction
	Average septic overcharge rate per person	gal/d/person
	Typical microbial density of septic overcharge reaching the stream	Counts/L
Intermediate Points		
BoundaryPoints.csv	Boundary point locations by Latitude and Longitude	Degree (by fraction)
OutputPoints.csv	Output point locations by Latitude and Longitude	Degree (by fraction)

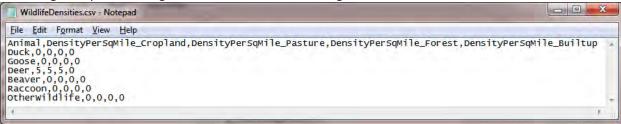
14. Change the rate constants to 0.064 d<sup>-1</sup>, so the file becomes:



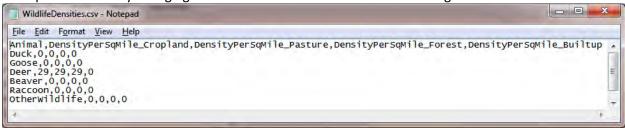
15. Save as a csv file, and exit.

WildlifeDensities.csv

16. Using Notepad, the original file includes the following locations:



17. Update the file by changing the "5" to "29" so it looks like the following:



- 18. Save as a csv file, and exit.
- 19. Ensure that all revised files have replaced the original files contained in the "LocalData" folder under the working directory.

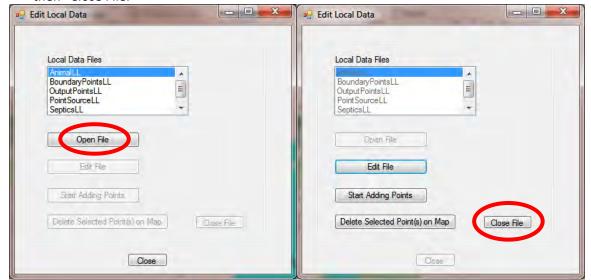
#### **Import Local Data Files**

After replacing files in the "LocalData" folder within the working folder, these data can be registered with the SDMProjecBuilder (SDMPB).

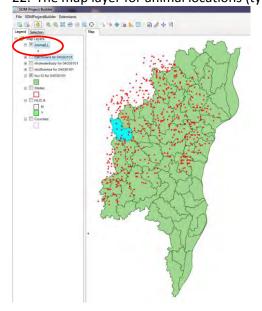
20. From the Menu Bar, choose "SDMProjectBuilder", then "Import Local Data Files". AnimalLL, SepticsLL, and PointSourceLL will be registered using the procedure outlined by Whelan et al. (2015c, 2015d).



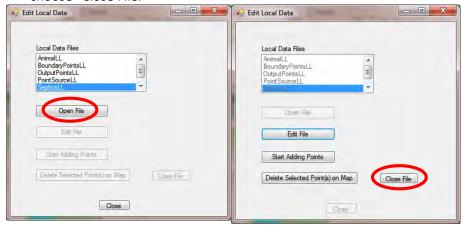
21. To register "AnimalLL", highlight "AnimalLL" and choose "Open File". Wait until the screen changes, then "Close File."



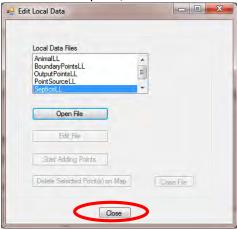
22. The map layer for animal locations (typically farms) will appear on the map, like that below:



23. To register "SepticsLL", highlight "SepticsLL" and choose "Open File". When the screen changes, choose "Close File."



24. When complete, "Close" the screen.



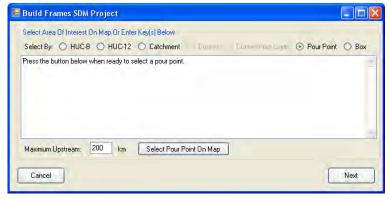
#### **EXEUCUTE THE PROJECT BUILDER**

The project builder delineates the watershed of interest and collects environmental map layers that support the microbial assessment.

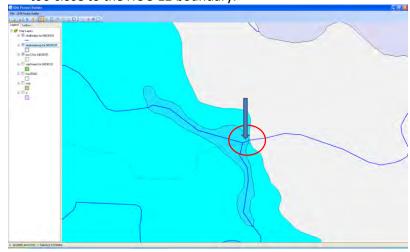
25. From the Menu Bar, choose "SDMProjectBuilder", then "Run Project Builder".



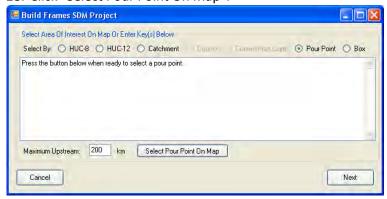
26. Choose "Pour Point". For "Maximum Upstream", use an upstream distance that reflects the distance to the upstream divide. Because this HUC-8 is an upstream HUC and has a boundary that is not totally adjacent to another HUC, a large "Maximum Upstream" distance can be used, such as "200" km.



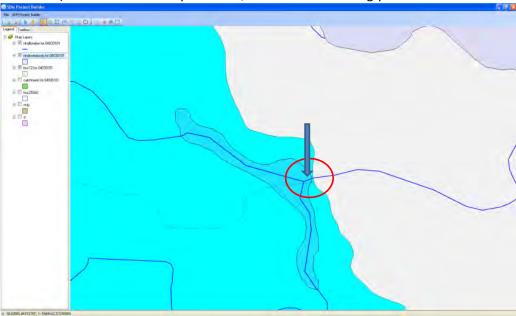
27. Go back to the map and zoom in on the pour point location, using the Zoom icon to clearly see the location which has been highlighted with the red circle and arrow. Zoom in because identifying the location is crucial to placing the pointer in the next step. If this location is incorrectly identified, you may inadvertently include more area than is needed for the assessment by getting too close to the HUC-12 boundary.



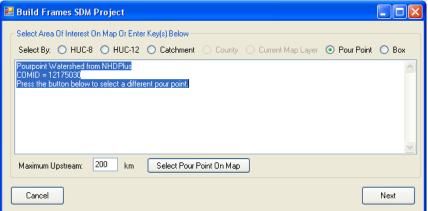
28. Click "Select Pour Point On Map".



29. On the map, click on the pour point location which should be just downstream of the junction (see arrow). The location is very sensitive, so zoom in accordingly.



30. Click "Next" on the "Build FRAMES SDM Project" interface.

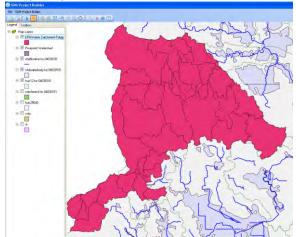


31. The aqua square is the pour point and the delineated area is blue in the figure below.

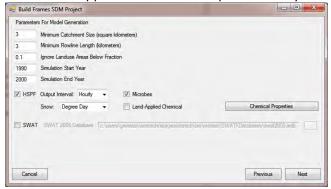


32. When you Zoom Out, you see the red highlighted area associated with the assessment, corresponding to the four upstream HUC-12s that comprise the watershed draining through the

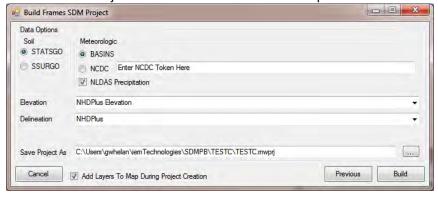
pour point.



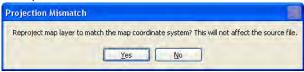
33. After choosing "Next", complete the "Build FRAMES SDM Project" as shown. Use values included in this figure. To ensure adequate sizes, define the "Minimum Catchment Size" and "Minimum Flowline Lengths" as "3" and "3", respectively. Land areas less than 10% of the total have been factored into other Map Layers, as noted by the "Ignore Landuse Areas Below Fraction" of "0.1". Check "HSPF"; the "Degree Day" method is chosen for snow accumulation and melt and "Microbes" for land applied simulation. Output is "Hourly".



34. Click "Next" and the following screen appears. Change the screen to that below; make no changes to the "Save Project As" text box unless there is a special folder location:



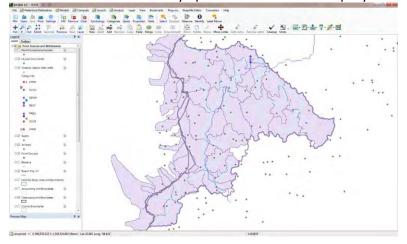
- 35. Now choose "Build" which takes approximately 25 minutes to complete for a pour point with four HUC-12s, depending on the computer.
- 36. Some computers may ask several times if the re-project map layer should match the map coordinate system; choose "Yes" each time.



37. When the SDMPB has finished running, choose "Open in BASINS".



38. BASINS will automatically open with the SDMPB map layers for this assessment:



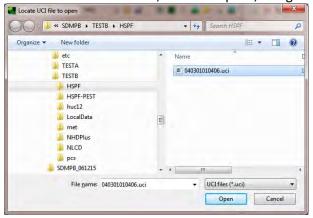
39. Go to the BASINS menu, choose "File," then "Save". Choose "File", then "Exit" to close BASINS.

## **EXECUTE HSPF, SIMULATING FLOW AND MICROBIAL TRANSPORT**

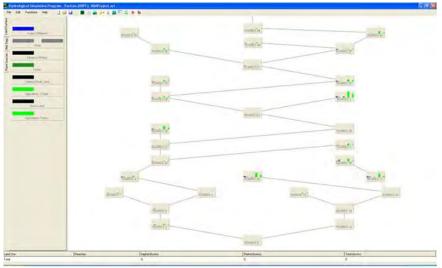
40. To open the HSPF project, activate the WinHSPF3.0 icon on the Windows desktop:



41. From the "File" menu, choose "Open", navigate to the UCI file and click "Open".



42. The new HSPF project will appear in the HSPF GUI, with the watershed workflow schematic shown below.



43. To run the HSPF simulation, click the "Run Simulation" icon. Save the HSPF project by clicking "File", then "Save" on the main menu bar. Leave the HSPF workflow schematic screen open since you may want to refer to it when operating in the BASINS interface.



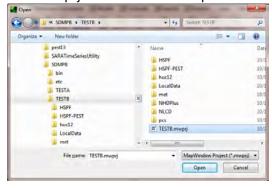
#### VIEW HSPF SIMULATION RESULTS WITH BASINS

## **Register Flow and Microbial Densities with BASINS**

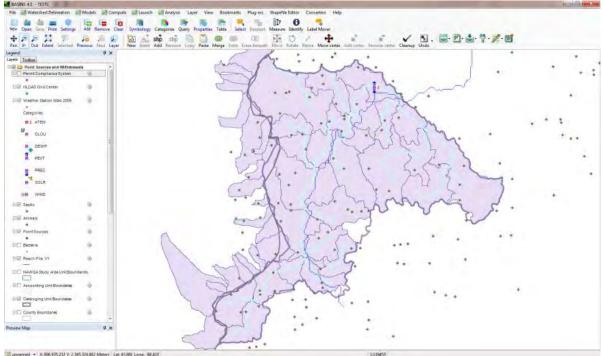
44. If BASINS is not already open, start BASINS with the BASINS 4.1 icon on the desktop. At the "Welcome" window, open your existing project.



45. Navigate to the project folder (e.g., TESTB) and select the \*.mwprj file. Note that the name of the file reflects your selection. Be certain the file name you assigned to your assessment matches the \*.mwprj extension. An example:



46. Click "Open" so the SDMPB project will open in BASINS 4.1. The following screen, showing domestic animal and septic locations, which overlay each other and NLDAS stations,, will appear:



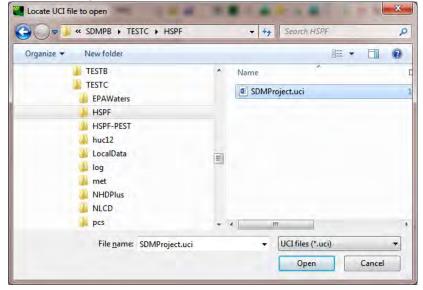
47. Go to the "File", "Manage Data" menu in the BASINS menu bar. With the "Data Sources" window open, see that no times series data sources are pre-loaded except, possibly, a met.wdm file. Time series data sources from the HSPF simulation are needed to view the simulation results, so they will be added.



48. To add time series data source, select "File", then "Open" from the "Data Sources" window. A selection window appears:



49. Select "WDM Time Series", then "OK". Navigate to the HSPF project folder, and select the file "SDMProject.wdm". It contains the output time series written from HSPF to WDM. [Note: For the pour point simulation, the wdm name should be SDMProject. Although an equivalent \*.wdm file name may be there, do not select met.wdm file.]



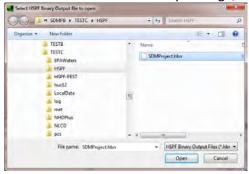
50. With this data source open, the file name appears in the "Data Sources" window. [Note: Other WDM files may already be registered in the list.]



51. The HSPF Binary file (\*.hbn) only exists after executing HSPF; hourly data associated with the "RO" designation (Reach Outflow) are associated with the \*.hbn file. To view results of the Microbial simulation, the HSPF binary output file must be added to the current BASINS project. From the "Data Sources" window, choose "File", then "Open". The window below will appear to select a data source. Select "HSPF Binary Output", then "OK".



52. Navigate to the HSPF project folder and select the file with the HBN extension. Click "Open". Because the HBN file may be large, the registration may take time to read.

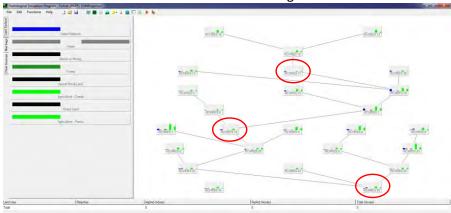


53. After the file has been read, it appears in the "Data Sources" window. Click "File", then" Exit".

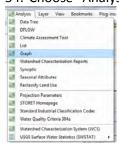


#### **View the Discharge Time Series**

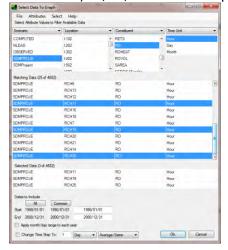
This sections views discharge time series based on hourly data by graphing the results at multiple locations. The locations will be Reaches (RCHRES) 11, 19, and 25 since they represent junctions in the waterbody network. The ID numbering scheme correlates to the site layout in HSPF. Leave HSPF open with this diagram in the background to allow viewing of the reach locations, numbering scheme, and ID numbers if you are not using BASINS to number these locations. See Whelan et al. (2015c) for instructions on how to label the watershed using the BASINS interface.



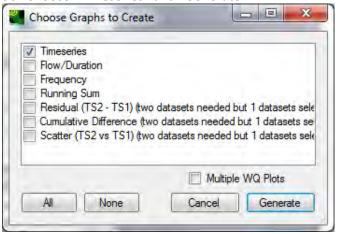
54. Choose "Analysis", then "Graph".



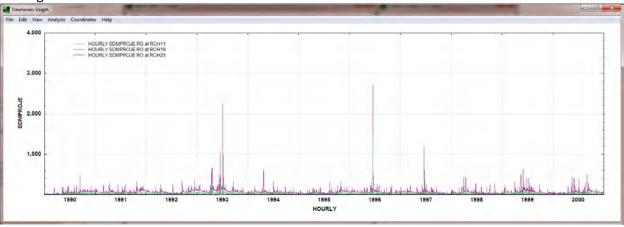
55. See Whelan et al. (2015c) for guidance on selecting data to graph. Under "Constituent", scroll down to "RO" and a list of reaches with flow data appears under "Matching Data". Choose reaches (i.e., RCHRES) 11, 19, and 25 which appear under "Select Data". Click "Ok".



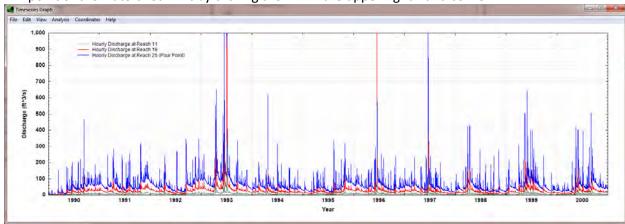
56. Choose "Timeseries" and "Generate".



57. The following graph appears. The discharge is defined as cfs (ft³/s). All three hydrographs are plotted together.

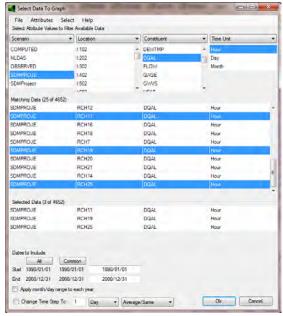


58. Using the editor, as described in Whelan et al. (2015c), the graph can be modified to provide a better view of the results. See how the discharge changes as one moves downstream to the pour point of the watershed. Exit by clicking the "X" in the upper right-hand corner.



## **View the Microbial Density Time Series**

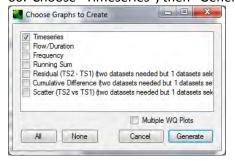
59. Select "Analysis", then "Graph" again. Under "Constituent", choose "DQAL" which provides microbial densities in Counts/L at various reaches. Under "Matching Data", choose reaches corresponding to 11, 19, and 25 (RCH11, RCH19, and RCH25, respectively). After they appear under "Selected Data", click 'Ok'.



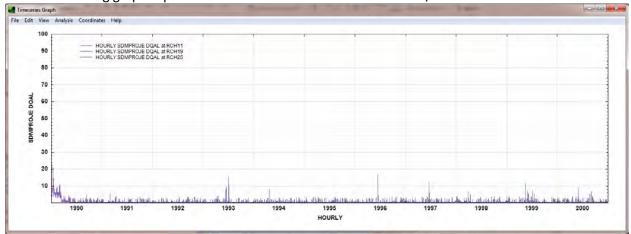
## The definitions of the microbial parameters are:

Parameter	Units	HSPF-12.2 Manual (http://water.epa.gov/scitech/datait/models/basins/bsnsdocs.cfm)
Microbe-DDQAL-BIODEG	counts/time interval	amount of parent material decayed by process BIODEG
Microbe-DDQAL-GEN	counts/time interval	amount of parent material decayed by process GEN
Microbe-DDQAL-HYDROL	counts/time interval	amount of parent material decayed by process HYDROL
Microbe-DDQAL-OXID	counts/time interval	amount of parent material decayed by process OXID
Microbe-DDQAL-PHOTOL	counts/time interval	amount of parent material decayed by process PHOTOL
Microbe-DDQAL-TOT	counts/time interval	amount of parent material decayed by process TOT
Microbe-DDQAL-VOLAT	counts/time interval	amount of parent material decayed by process K VOLAT
Microbe-DQAL	counts/L	concentration of "dissolved" microbe (i.e., microbe in water)
Microbe-IDQAL	counts/time interval	input of microbe in water column from upstream reach
Microbe-RDQAL	counts	total storage of microbe in water column
Microbe-RODQAL	counts	microbial outflow quantity
Microbe-RRQAL	counts	microbial storage in reach
Microbe-TIQAL	counts	total inflow of microbes
Microbe-TROQAL	counts	total outflow of microbes
Time interval' is hourly in this case.		

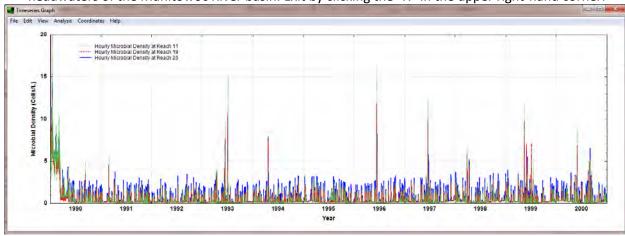
60. Choose "Timeseries", then "Generate".



61. The following graph is produced with microbial densities of Counts/L:



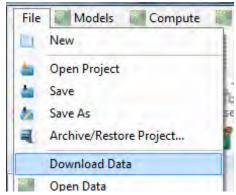
62. Using the editor described in Whelan et al. (2015c), the graph can be modified to provide a better view of the results. See how the densities change as one moves downstream to the pour point of the watershed. As the uncalibrated results indicate, microbial densities are very low in this area, the headwaters of the Manitowoc River basin. Exit by clicking the "X" in the upper right-hand corner.



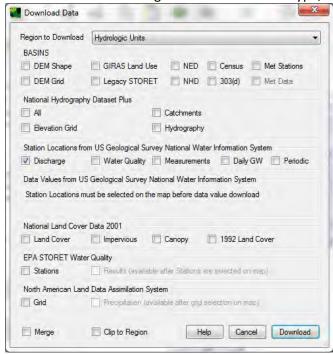
## RETRIEVE OBSERVED DAILY USGS FLOWS AND COMPARE THEM WITH SIMULATED DAILY FLOWS AT THE POUR POINT

BASINS can also be used to add data to the project. The objective of this exercise is to retrieve the Observed Daily USGS Flows at the pour point and compare them to uncalibrated daily discharge simulations at the pour point, Reach 25.

63. From the main BASINS menu, select "File, then "Download Data".



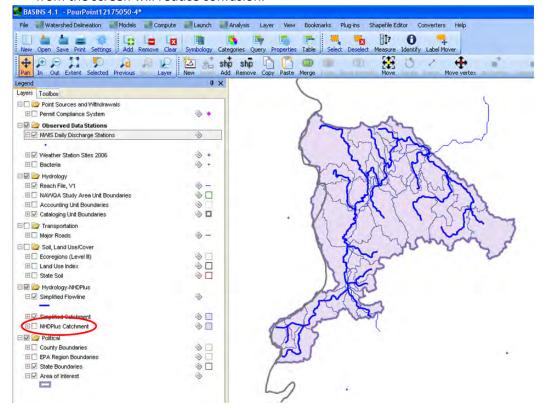
64. Choose USGS Discharge stations as the data type, and click "Download".



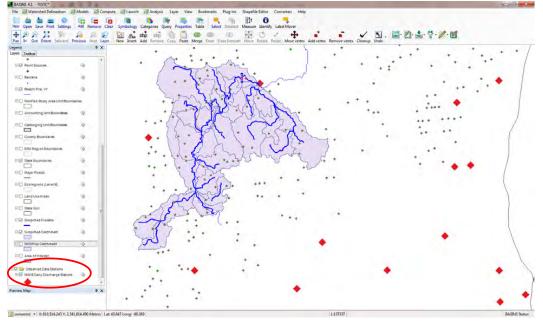
## 65. Click 'OK'.



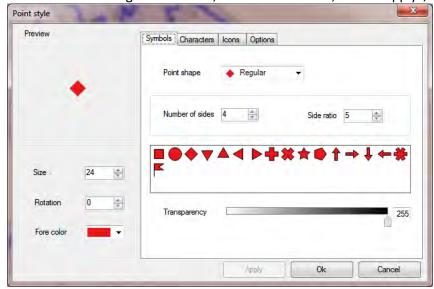
66. The NWIS Daily Discharge Stations layer will appear on the map. De-select "NHDPlus Catchment" (red circle in figure below) since these catchments are outside the simulation area; removing them from the screen will reduce confusion.



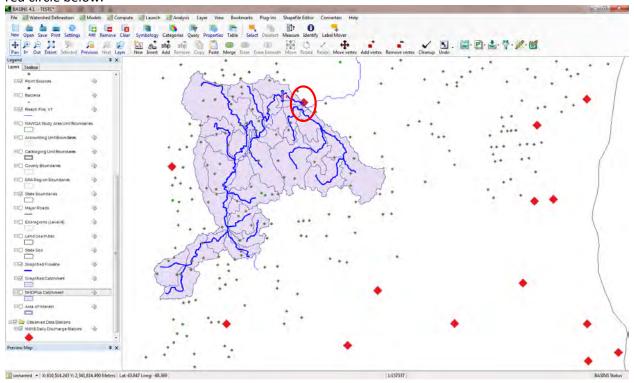
67. Highlight the "NWIS Daily Discharge Stations" (one click), then click on the symbol below this Map Layer.



68. The size of the symbol for the discharge stations to download data for the correct station is increased. Change "Size" to 24, "Fore color" to red, select "Apply", then "Ok".

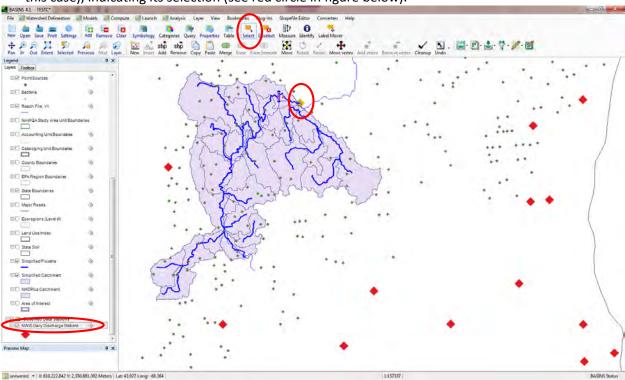


If you can see stations on the map, there is no need to zoom in near the outlet of the study area to select the nearby USGS station. The USGS flow station nearest the outlet (Reach 25) is identified by the red circle below.

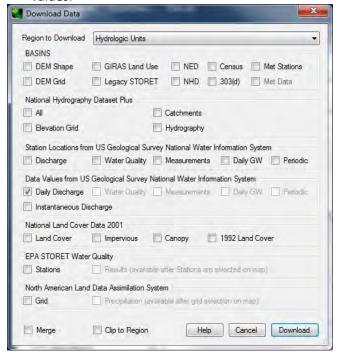


69. Highlight the "NWIS Daily Discharge Stations" map layer using the BASINS Select button on the tool

bar, then select the gaging station at the outlet. The symbol will change color (orange in this case), indicating its selection (see red circle in figure below).



70. Choose "File", then "Download Data" again, this time specifying download "Daily Discharge" data values.



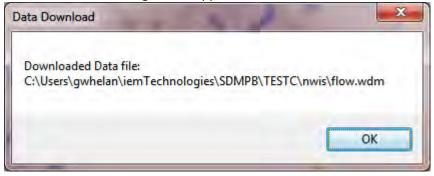
71. Click "Download". A prompt will ask where the data should be saved. Click "Ok".



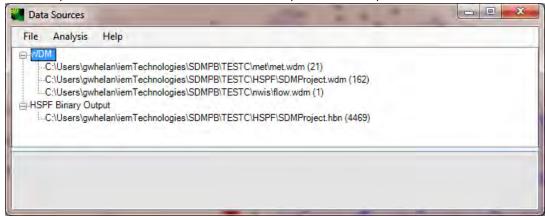
72. When the download is complete, this message appears. Click "OK":



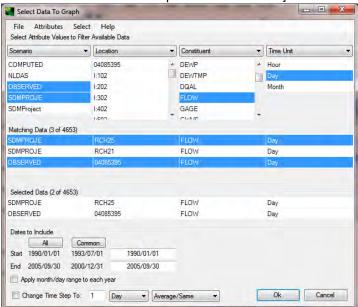
73. When the following screen appears, click "OK".



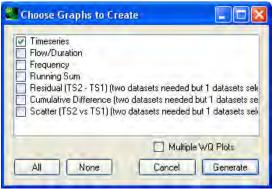
74. The "Data Sources" window appears, indicating NWIS data have been added to the project. Choose "file", then "Exit". In the main BASINS menu, choose "File", then "Save".



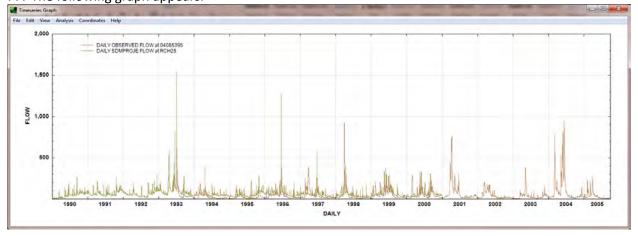
75. To generate a graph of the observed and simulated discharge time series, go to the main BASINS screen, select "Analysis", then "Graph" on the tool bar. Make the following choices [Note: The lower-case "observed", if available, refers to observed instantaneous discharge data that were added and that are not part of this tutorial.]:



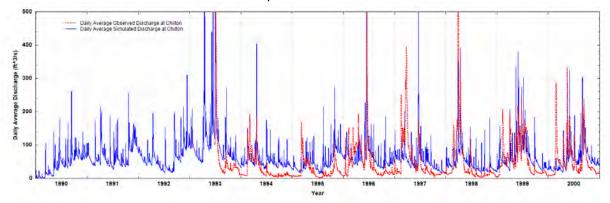
76. Select "Timeseries", then "Generate".



77. The following graph appears.



78. Using the editor described in Whelan et al. (2015c), the graph can be modified to provide a better view of the results. The daily average observed discharge at Chilton (Reach 25) is in red and the daily average simulated discharge is in blue. The simulated results are uncalibrated and appear to overestimate base flow. The trends are captured well.



#### **DISCLAIMER**

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication.

#### REFERENCES

WDNR (Wisconsin Department of Natural Resources). 2015. Deer abundance and densities in Wisconsin deer management units. <a href="http://dnr.wi.gov/topic/hunt/maps.html">http://dnr.wi.gov/topic/hunt/maps.html</a> (Last accessed 23.09.15).

Whelan, G., K. Kim, K. Wolfe, R. Parmar, M. Galvin. 2015a. Quantitative Microbial Risk Assessment Tutorial: Installation of Software for Watershed Modeling in Support of QMRA. EPA/600/B-15/276. U.S. Environmental Protection Agency, National Exposure Research Laboratory, Athens, GA.

Whelan, G., K. Kim, K. Wolfe, R. Parmar, M. Galvin, M. Molina, R. Zepp. 2015b. Navigate the SDMPB and Identify an 8-Digit HUC of Interest. EPA/600/B-15/273. U.S. Environmental Protection Agency, Athens, GA.

Whelan, G., K. Kim, R. Parmar, K. Wolfe, M. Galvin, M. Gray, P. Duda, M. Molina, R. Zepp. 2015c. Quantitative Microbial Risk Assessment Tutorial: Land-applied Microbial Loadings within a 12-Digit HUC. EPA/600/B-15/298. U.S. Environmental Protection Agency, Athens, GA.

Whelan, G., R. Parmar, K. Wolfe, M. Galvin, P. Duda, M. Gray. 2015d. Quantitative Microbial Risk Assessment Tutorial – SDMProjectBuilder: Import Local Data Files to Identify and Modify Contamination Sources and Input Parameters. EPA/600/B-15/316. U.S. Environmental Protection Agency, Athens, GA.

Whelan, G., K. Wolfe, R. Parmar, M. Galvin, M. Molina, R. Zepp, P. Duda, M. Gray. 2015e. Quantitative Microbial Risk Assessment Tutorial: Point Source and Land-applied Microbial Loadings within a 12-Digit HUC. U.S. Environmental Protection Agency, Athens, GA.