Dr. Sansom,

In an effort to support Cypress Creek watershed protection planning and better characterize instream *E. coli* bacteria sources near downtown Wimberley, TX, the Meadows Center for Water and the Environment (Meadows Center) has entered into a partnership with the Texas A&M AgriLife Research – Soil & Aquatic Microbiology Laboratory (SAML) to conduct a short-term Bacterial Source Tracking (BST) study of Cypress Creek.

*BST is a valuable tool that can identify, and also rule-out, significant sources of *E. coli* pollution in a watershed. Using DNA fingerprints and bacterial markers, fecal pollution sources are identified by comparing *E. coli* DNA to those in a statewide library of known sources.*

The purpose of this memorandum is to provide a brief summary of the Cypress Creek BST study and to present initial laboratory results from the first round of sampling conducted on August 7, 2017.

**Summary of Cypress Creek BST Study**

**Goal:** Collection and effective interpretation of high quality *E. coli* bacteria source data toward the purpose of implementing more strategic management practices for addressing nonpoint source pollution in the Cypress Creek watershed.

SAML will perform *E. coli* bacterial enumeration and BST analysis of raw water samples collected once per month from Cypress Creek by Meadows Center staff from August 2017 through October 2017. Meadows Center staff will collect standard field data during each sampling event. Findings will be reported to stakeholders on a monthly basis as data becomes available and will be summarized in a final report.

Beginning in August 2017, duplicate samples will be collected on a monthly basis from two locations on Cypress Creek. Site #1 is located on Cypress Creek approximately 0.33 mile upstream of the RR12 bridge located in downtown Wimberley. Site #1 was selected to provide data on upstream *E. coli* sources with minimal potential impact from septic systems or other *E. coli* sources in the downtown Wimberley area. Site #2 is located on Cypress Creek approximately 0.10 mile downstream of the RR12 bridge and was selected to provide data on Cypress Creek *E. coli* sources including the downtown Wimberley area.

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1 From Texas Water Resources Institute website, http://texasbst.tamu.edu/
2 Meadows Center staff utilized for this study have been trained and certified in standard industry water quality sampling methods and collect data under multiple state and federal quality assurance plans. A copy of the approved Quality Assurance Project Plan for this study is on file at both the Meadows Center and SAML.
To provide a more representative sample set of routinely occurring *E. coli* sources in Cypress Creek, the project sampling plan specifies that one sampling event be conducted during “wet weather” conditions with an additional two sampling events to be conducted during “dry weather” conditions. BST analysis will be performed by SAML as follows:

- 3 events (2 dry, 1 wet - if possible)
- 6 isolates for each event from 2 sample sites (3 isolates per raw sample)
- 3 events x 6 isolates = 18 total isolates

In the Cypress Creek BST study, *E. coli* bacterial isolates from fecal pollution will be “fingerprinted” and their DNA compared to those in a statewide library of known sources. The bacterial isolates will be selected randomly and identified using both a 3-way categorization and a 7-way categorization protocol. The calculated “rate of correct classification” for SAML is 100% for the 3-way split and 91% for the 7-way split. More information on the Texas Statewide BST Library and BST classification can be obtained from the attached BST laboratory report provided by SAML on September 12, 2017 for sampling conducted on August 7, 2017.

### Bacterial Source Tracking Results for August 7, 2017 Sampling Event (Wet Weather Event)

For a more thorough analysis of the BST results, please see the attached BST laboratory report referenced above.

#### Table 1. Classification of *E. coli* isolates from sample 572572 collected on 08/07/17 (Site #1 – Upstream)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>3 way id</th>
<th>7 way id</th>
<th>Closest Match*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cypress Creek-582572-8/7-A</td>
<td>Livestock and Domesticated Animals</td>
<td>Cattle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cypress Creek-582572-8/7-C</td>
<td>Wildlife</td>
<td>Wildlife, Non-Avian</td>
<td>Mouse</td>
<td></td>
</tr>
<tr>
<td>Cypress Creek-582572-8/7-D</td>
<td>Livestock and Domesticated Animals</td>
<td>Cattle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cypress Creek-582572-8/7-E</td>
<td>Wildlife</td>
<td>Wildlife, Non-Avian</td>
<td>Feral Hog</td>
<td></td>
</tr>
</tbody>
</table>

#### Table 2. Classification of *E. coli* isolates from sample 582571 collected on 08/07/17 (Site #2 – Downstream)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>3 way id</th>
<th>7 way id</th>
<th>Closest Match*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cypress Creek-582571-8/7-A</td>
<td>Human</td>
<td>Human</td>
<td>Raw Sewage</td>
<td></td>
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<tr>
<td>Cypress Creek-582571-8/7-B</td>
<td>Unidentified</td>
<td>Unidentified</td>
<td>Cattle</td>
<td></td>
</tr>
<tr>
<td>Cypress Creek-582571-8/7-C</td>
<td>Livestock and Domesticated Animals</td>
<td>Cattle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cypress Creek-582571-8/7-E</td>
<td>Wildile</td>
<td>Wildlife, Non-Avian</td>
<td>Feral Hog</td>
<td></td>
</tr>
</tbody>
</table>

* The ID of the closest library match for each isolate is provided for informational purposes only.

SAML *E. coli* bacterial enumeration results for the August 7, 2017 sampling event using EPA Method 1603 were reported as follows:

- Site #1 (Upstream) = 6,400 CFU/100mL
- Site #2 (Downstream) = 18,000 CFU/100mL

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3 Bacterial isolates are defined as a pure strain of bacteria that has been separated from a mixed bacterial culture.
Notes and observations

The August 7, 2017 sampling event was conducted during a storm event under wet weather conditions.

SAML provided data on 4 isolates per sampling site at no additional cost.

The BST results in this report should be interpreted cautiously since they represent only 4 E. coli isolates from each sample and the samples were collected at only a single time-point. Analysis of additional E. coli isolates from multiple sampling events may strengthen and further validate these initial results. Further, the August 7, 2017 sampling event was conducted during a storm event yielding much higher concentrations of E. coli bacteria than would be expected during baseflow conditions in Cypress Creek. Nonpoint source pollution carried by stormwater may also yield different E. coli bacterial sources carried over longer distances than would be expected under dry conditions.

Thank you for the opportunity to provide the first round of results from the Cypress Creek BST study. I look forward to continuing this effort with the Meadows Center and SAML to enhance the characterization of E. coli bacteria sources in the Cypress Creek watershed.

Sincerely,

Nick Dornak

Attachments:

1. Cypress Creek BST Report from SAML for August 7, 2017 Sampling Event
2. Cypress Creek SAML Test Results for August 7, 2017 Sampling Event, EPA Method 1603
3. 2017 Cypress Creek BST Project Update #1 (includes field conditions, notes and project map)