SEEing SCIENCE IN APPALACHIA

TATES CREEK WATERSHED PROJECT – Overview and Course Materials

The Tates Creek project is designed to give both college and 7th-grade students an opportunity to do science. Project goals are:

(1) To introduce potentially disadvantaged middle school students to science and to the idea of a college education;
(2) to gain familiarity with streams and watersheds;
(3) learn about the relationship between human activities, stream contaminants, and water quality;
(4) to measure contaminants (nutrients and fecal microbes) within a local stream system;
(5) to collect and identify stream biota;
(6) to analyze data with the goal of determining water quality and contaminant sources; and
(7) to identify any ways to limit entry of contaminants into Tates Creek.

We have conducted the course three times now.

Tates Creek is a secondary stream that enters the Kentucky River (see map below). Its watershed (~14.5 mi², 37.5 km²) drains mostly agricultural land that is used for cattle grazing, although small settlements (~5%), small to large housing developments (~5%), and urban areas also occur in the watershed (~5%). Anthropogenic contaminants are dependent upon land use, so the major contaminants in the Tates Creek watershed are dissolved nutrients (ammonium, nitrate, phosphate) and fecal microbes from multiple sources. Students learn: how to sample the stream for these contaminants; how to correctly count macroinvertebrates living the stream; how to accurately count both macroinvertebrates and fecal microbes; how to measure the concentration of dissolved nutrients; how to construct graphs that summarize these data, and how to analyze data to draw useful conclusions. The project culminates with group presentations. Honors college students give a group PowerPoint presentation in class, whereas the 7th graders come to campus and display and explain group posters.

Our honors students serve as science mentors to middle school students. Our course involves all the 7th graders at a local school (Madison Middle School) that typically number at about 110 to 120 students in any given year. Our college class accommodates up to 24 students, so honors college students in the course mentor 5 to 8 middle schools students each, working with the same group of students throughout the term. We lead both sets of students through the project by mimicking the steps of any scientific investigation (background information, project design, data collection, data analysis, conclusions) and the presentation of its results. Our strategy is to first instruct our college students in the project module of the day, and soon thereafter the college students instruct their middle-school students. Of 45 class days of a typical semester, our honors students spend 16 class days (35%) on the project, and we meet with the 7th graders eight times, either at the middle school or on the Eastern Kentucky University (EKU) campus. The 7th graders work on the project throughout the term.
<table>
<thead>
<tr>
<th>Day</th>
<th>Activity</th>
<th>Materials</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Introduction to the watershed – Honors students</td>
<td>Worksheet, map of watershed, Reading – Dobson &amp; Beck</td>
<td>EKU</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Introduction to the watershed – 7th graders</td>
<td>Worksheet, map of watershed, Reading – Dobson &amp; Beck</td>
<td>Madison Middle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Planning the project – honors students</td>
<td>Worksheet, map of watershed, overview of sampling sites</td>
<td>EKU</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Planning the project – 7th graders</td>
<td>Worksheet, map of watershed, overview of sampling sites</td>
<td>Madison Middle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Tates Creek field sampling – honors students</td>
<td>Worksheets, field equipment (see list)</td>
<td>Tates Creek</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Tates Creek field sampling – 7th graders</td>
<td>Field equipment (see list)</td>
<td>Tates Creek</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Water chemistry – honors students</td>
<td>Worksheets, laboratory equipment (see list)</td>
<td>EKU</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Laboratory work – water chemistry &amp; macroinvertebrates – 7th graders</td>
<td>Worksheets, laboratory equipment (see list)</td>
<td>EKU</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Data analysis – honors students</td>
<td>Worksheet, tabulated data</td>
<td>EKU</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Data analysis – 7th graders</td>
<td>Worksheet, tabulated data</td>
<td>Madison Middle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Workday for presentations – honors students</td>
<td>Watershed information, tabulated data, outside sources</td>
<td>EKU</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Preparing presentations – 7th graders</td>
<td>Watershed information, tabulated data, outside sources</td>
<td>Madison Middle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Workday for presentations 2 – honors students</td>
<td>Watershed information, tabulated data, outside sources</td>
<td>EKU</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Preparing presentations 2 – 7th graders</td>
<td>Watershed information, tabulated data, outside sources</td>
<td>Madison Middle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Group PowerPoint presentations – honors students</td>
<td>Projector</td>
<td>EKU</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Group poster presentations</td>
<td>Projectors poster easels</td>
<td>EKU</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Equipment lists and worksheets appear in the Appendix.**
Map of the Tates Creek watershed with sampling sites. The site identification codes are tied to the table of sampling sites below.
Table of project sampling sites in the Tates Creek watershed.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Sampling Site</th>
<th>KY 169 Mileage</th>
<th>Effluent Type</th>
<th>Likely Contaminants</th>
<th>Sampling Type</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCH</td>
<td>Creek headwaters</td>
<td>0.1</td>
<td>urban</td>
<td>Metals?</td>
<td>Creek only</td>
<td>2</td>
</tr>
<tr>
<td>MP</td>
<td>McCready pond</td>
<td>0.3</td>
<td>Residential</td>
<td>N, M</td>
<td>Inflow, pond, outflow</td>
<td>3</td>
</tr>
<tr>
<td>AC</td>
<td>Arlington confluence</td>
<td>1.3</td>
<td>Golf course</td>
<td>Metals</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>I75*</td>
<td>Interstate I-75</td>
<td>1.35</td>
<td>Roadway</td>
<td>Metals?</td>
<td>Creek only</td>
<td>1</td>
</tr>
<tr>
<td>SPU</td>
<td>Sewage plant</td>
<td>1.5</td>
<td>Sewage</td>
<td>N, M</td>
<td>Upstream</td>
<td>1</td>
</tr>
<tr>
<td>SPD</td>
<td>Sewage plant</td>
<td>1.8</td>
<td>Sewage</td>
<td>N, M</td>
<td>Downstream</td>
<td>1</td>
</tr>
<tr>
<td>SKC</td>
<td>South Keeneland confluence</td>
<td>2.0</td>
<td>Urban, Residential</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>ILC*</td>
<td>Irvine Lick confluence</td>
<td>2.2</td>
<td>Urban, sewage Residential septic</td>
<td>N, M</td>
<td>Metals?</td>
<td>3</td>
</tr>
<tr>
<td>SC</td>
<td>Substation confluence</td>
<td>2.3</td>
<td>Residential septic</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>WC</td>
<td>Wellington confluence</td>
<td>3.0</td>
<td>Residential septic</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>TCEC*</td>
<td>Tates Creek Estates con.</td>
<td>3.1</td>
<td>Residential septic</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>FCC</td>
<td>Finney Creek confluence</td>
<td>3.5</td>
<td>Pasture</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>CFC</td>
<td>Crutcher Fork confluence</td>
<td>4.9</td>
<td>Pasture</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>HBC*</td>
<td>Honest Branch confluence</td>
<td>6.2</td>
<td>Pasture</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>SFC</td>
<td>Shallow Ford confluence</td>
<td>6.4</td>
<td>Pasture</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>7.8C</td>
<td>Mile 7.8 confluence</td>
<td>7.8</td>
<td>Pasture</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>BC</td>
<td>Baldwin confluence</td>
<td>8.2</td>
<td>Pasture</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>BCC*</td>
<td>Buffalo Creek confluence</td>
<td>8.5</td>
<td>Pasture</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>8.9C</td>
<td>Mile 8.9 confluence</td>
<td>8.9</td>
<td>Pasture</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>SEC</td>
<td>Stringtown east confluence</td>
<td>10.3</td>
<td>Pasture</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>SWC</td>
<td>Stringtown west confluence</td>
<td>10.3+</td>
<td>Pasture</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>LBC*</td>
<td>Long Branch confluence</td>
<td>11.3</td>
<td>Pasture</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>1156C</td>
<td>KY 1156 confluence</td>
<td>12.0</td>
<td>Pasture</td>
<td>Residential septic</td>
<td>N, M</td>
<td>3</td>
</tr>
<tr>
<td>VV</td>
<td>Valley View</td>
<td>12.5</td>
<td>Pasture</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
<tr>
<td>KRC</td>
<td>Kentucky River confluence</td>
<td>12.6</td>
<td>Pasture</td>
<td>N, M</td>
<td>Inflow, up, down</td>
<td>3</td>
</tr>
</tbody>
</table>

Total samples: 68

**KEY:** N = nutrients, M = microbes, up = upstream, down = downstream, *KRWW location
APPENDIX

COURSE MATERIALS

AND

WORKSHEETS
NAME _________________________________


This excerpt from Dobson & Beck (targeted for younger audiences) will do nicely in getting us thinking about the Tates Creek project and planning our investigation of the water quality of Tates Creek.

Please answer the questions below.

1. After reading pages 28-33, briefly explain the concept of biogeochemical (or nutrient) cycling. What are some chemicals whose cycles play important roles in ecosystems?

2. Draw an illustration of the phosphorus cycle as it may operate in the Tates Creek watershed.

3. The sewage treatment plant on Tates Creek formerly provided secondary treatment to sewage piped into the plant before discharge into the creek. (It now serves only as a holding/pumping station.) Diagram or list the steps of primary and secondary sewage treatment below. What components of potential water pollution do secondary plants mitigate effectively? What potential pollutants do they not remove effectively?
4. Can there be too much of a good thing in aquatic ecosystems? Explain what eutrophication is.

5. What are possible sources of nutrients entering Tates Creek? Keep in mind how the land of the Tates Creek watershed is used. How would we recognize eutrophication in Tates Creek?

6. How might eutrophication affect the Tates Creek ecosystem?
Directions for Project Notebooks

You project notebooks are very important as all activities, data, and information regarding the Tates Creek Project will be recorded in the notebook, as well as a record of your hypotheses, expectations, and thoughts. Your notebook thus serves as evidence of your work and contains documentation of same, analogous to any laboratory notebook kept by researchers. Your notebook is golden – do not lose it!

We will give you assignments to be completed in your notebook as you also document your project work. Periodically, we will also collect your notebooks and grade your work, so please keep up to date! Obviously, at project’s end we will collect your notebook for final grading.

Ink is preferable to pencil, but ink should not run if wet.

1. Write your name and contact information on the front and back covers in indelible marker. Repeat this information on the first page.

2. Leave the next page of your notebook blank; on the third page begin a table of contents keyed to page numbers (see below) for easy reference. Be sure to leave room to begin content entries for later additions.

3. Outside of class, number each page of the notebook from front to back. The first page is of course “1” and the its left-side back will be “2” and so on.

4. NEVER tear a sheet out of your notebook, and NEVER erase anything. Errors should be lightly crossed-out or X-ed-out, corrected, and explained but still remain easily legible.

   Documenting errors is important and is not considered a negative in a scientific notebook or in your project notebook (you will be only praised for keeping errors in plain sight, and never penalized). Often field and laboratory activities do not go smoothly because of initial errors and/or other circumstances and if the errors are documented they can be quickly corrected with minimum energy lost. Similarly, any hampering circumstances can be identified and factored into data interpretation.

   Your notebook is an absolutely honest record of your activities. NEVER “fudge” anything within it!

5. Keep a NEAT notebook that can be understood and assessed by any reader (including your instructors!). It is not useful to cram all your notebook’s contents into the smallest space. When completing a task or entry, skip a page or so in case you want to add more information at a later time (date all entries).

6. Any notebook activity or entry should be dated. As suggested above, it is perfectly fine to add material to notebook at a later date as long as it is documented. Your notebook after all documents your thought processes as well as activities and data. It is amazing of what one will think of in the shower, when exercising, or taking a long drive!

End
Tates Creek Project – Introductory Questions

Refer to your Tates Creek poster in answering these questions. Groups will “report out” their answers for questions labeled with asterisks (***). during the all-class discussion.

1. Locate Richmond on the map. Can you locate your house on the map? Your school?

2. Have you ever been down Tates Creek road (Hwy 169) before? Ever seen Tates Creek?

3. Have you floated across the Kentucky River on the Valley View ferry?

4. Which way does all water run?

5. Follow Tates Creek from Richmond to the Kentucky River. In what direction does the creek flow?

6. Do other creeks join with Tates Creek? Do these tributaries flow into or out of Tates Creek?

***7. Notice the bold red line surrounding Tates Creek. What does this line show? Why is this area on the map important?

8. What percentage of the watershed and stream lies in an urban (Richmond) setting? What percentage is non-urban (rural or farm)? A rough estimate is fine!!

9. How do you think most of the land is used along the creek? For farming? For livestock? For houses? Other?

10. People living in Richmond have their sewage treated by a sewage treatment plant. How is human waste treated in rural areas?
11. How is animal waste treated in rural areas?

12. What stuff in poop could be considered a pollutant or contaminant? Can this stuff enter stream water?

***13. How might contaminants from poop affect a stream?

***14. Can you think of other pollutants that might enter stream waters in rural settings? [Remember the slide show.] What are the sources of these pollutants?

15. Knowing the percentage of watershed land in rural/farm setting, what factors probably affect the water quality of Tates Creek?

***16. What types of organisms do you think can be found in Tates Creek? Make a list!

***17. What do you want to discover about Tates Creek through our work at the stream and in the laboratory?

End
Meeting 2 - PLANNING THE TATES CREEK PROJECT

Please discuss and answer the questions below.

1. To refresh our memory, what are the main land uses in the Tates Creek watershed?

2. The sewage treatment plant on Tates Creek formerly provided secondary treatment to sewage piped into the plant before discharge into the creek. (It now serves only as a holding/pumping station.)

   What are the stages of treatment at secondary sewage treatment plants? What components of potential water pollution do secondary plants remove effectively? What potential pollutants do they not remove effectively?

3. How might excess nutrients affect organisms in Tates Creek?

4. Given the information above, what contaminates should we sample for in Tates Creek?
5. Now that we know the land use within the watershed and what contaminates we are sampling for, we must come up with a plan of sampling the creek to determine patterns of contamination.

   Where should we definitely take samples? Why do you want to sample there?

6. How should we get a sense of the make-up of organisms within the stream community within Tates Creek?
Tates Creek Sampling Information and Directions

Water measurements, water samples, and microbe samples

Several preparations have already occurred in getting ready for the field work.

- Calibration of the YSI probe.
- Preparation of sampling kits for water chemistry and fecal microbes.
- Pre-taping of water sample containers, now ready for labeling with permanent marker.
- Addition of a few drops of concentrated sulfuric acid (H$_2$SO$_4$) to water sample vessels. This preserves the samples by killing any microbes and preventing the precipitation of any chemicals that may change the ambient concentration of nutrients (ammonium, nitrate, phosphate).
- Collection of equipment and reagents for sampling and preservation of stream biota.

We will sample today at four different points along Tates Creek:

- Irvine Lick confluence (station ILC)
- Finney Creek confluence (station FCC)
- At KY 1985 (station BC)
- At Perkins-Ashcraft road (station PAR, a new station!)

Your instructors will also get a complete set of water and microbial samples along Tates Creek later in the day.

DIRECTIONS

- For water and microbial samples, and YSI probe measurements - sample the tributary and also upstream and downstream of the confluence within Tates Creek.
- Pick a portion of the stream that has good flow.
- Stay downstream of the sampling or measurement point, so that you don’t kick-up mud and compromise the sample.

Physical Parameters

- Turn the YSI instrument on.
- Attach the flow-through sleeve to the probe.
- Carefully place the probe into the stream flow and allow a minute or so for the instrument to settle down.
- Read off and record temperature (°C), conductivity (mS/cm), dissolved oxygen (mg/L), and pH.
- Go to the next measurement place.
- When finished with all measurements, carefully re-pack the YSI instrument.
Water

- Pull the plunger out of a 60-cc syringe and rinse both items thoroughly with stream water at the sampling point.
- Fill the syringe with stream water, quickly inserting the plunger and inverting the syringe.
- Screw-in a syringe filter to the lure-lock fitting on the syringe.
- Label TWO of the pre-acidified, 20-cc sample bottles with the station name and date.
- Fill each bottle to the top with filtered stream water by depressing the plunger.
- When finished cap the bottles firmly and place them in the dishpan.

Microbes

- Find the provided sterile, 100-cc plastic sample cups.
- Label the cup with permanent marker – station and date.
- Dip the sample vessel in the creek water and fill it.
- Pour out the water to the 100 mL line scored on the sample cup (the bottom of the meniscus should be touching the fill line).
- Cap the sample cup firmly and place in the dishpan.
Instructions for Field Work

Macroinvertebrate Samples

Quantitative Samples

1. You will need to do this 4 times, so look your site over. Select areas in riffles to sample. You want quickly moving water that is not too deep.

2. Work you downstream sample first, then progress upstream to leave successive sampling areas as undisturbed as possible.

3. Two students with boots need to hold the kick net.

4. Two students with boots need to hold/use the quadrat (0.25m²).

5. Students with the kick net position the net perpendicular to the stream current. Be sure that the bottom of the net is at the bottom of the stream so that organisms cannot be washed under it.

6. Place the quadrat just upstream of the kick net.

7. While one student holds the quadrat in place, the other student should disturb (with feet) the rocks and substrate in the quadrat so that loosened material is swept into the net.

8. When done, carefully raise the kick net so that samples are not lost.

9. At the stream edge, place bottom of kick net into 5-gallon bucket (bend net as necessary). Using another bucket, wash material from the kick net into the 5-gallon bucket. Repeat as necessary to dislodge material from the kick net.

10. Pour water from the 5-gallon bucket through a D-frame net to capture biological material. Rinse bucket and pour through D-Frame net as necessary. Do not collect any vertebrates.

11. Place material from D-frame net into the bottom of a garbage bag. Dislodge as much material as possible by tapping the net inside the bag. Rinse material from the sieve into the bag with 95% alcohol from a squeeze bottle.

12. Add additional 95% alcohol to the bag so that the sample is completely covered.

13. Place a label identifying the site, date, type of sample (kick net), and sample number (1, 2, 3, or 4) into the bag. Label must be filled out IN PENCIL. It goes into the alcohol with the sample.

14. Tie a firm knot in the bag just above the sample so that very little air is in the bag. Trim away excess bag material above the knot.

15. Place bag into the plastic storage container.

16. Gather three additional samples using this procedure.
**Qualitative Samples**

1. Start qualitative sampling after quantitative sampling has been done.

2. Students should share boots and D-frame nets.

3. Students can sample wherever they think they might find organisms in the stream. They can scoop along the bottom, under overhangs of the bank, among branches. Students can pick up rocks and rinse them into nets.

4. Remove large objects such as rocks and sticks (inspect them for organisms before discarding). Qualitative samples can be consolidated by emptying nets and rinsing them into a 5-gallon bucket. Then pour the bucket contents through a D-frame net to remove the water. Do not collect any vertebrates.

5. Qualitative samples can be combined into one or two garbage bags. Rinse from D-frame net with 95% alcohol and top up sample with additional alcohol.

6. Place a label identifying the site, date, type of sample (dip net), and sample number (1, or 2) into the bag. Label must be filled out IN PENCIL. It goes into the alcohol with the sample.

7. Tie a firm knot in the bag just above the sample so that very little air is in the bag. Trim away excess bag material above the knot.

8. Place bag into the plastic storage container.

*End*
USING THE SPECTROPHOTOMETER

ThermoFisher Evolution 201 UV-visible spectrophotometer

1. Turn on the machine by pushing the power button on the right-hand side of the instrument panel (it may also be necessary to turn on the switch located on the back left.)

2. Allow spectrophotometer to warm up and initialize (this will ~ 2-3 minutes). Be sure that the top of the machine is closed. Press “ok” to have the accessories initialize.

3. Choose the one of the methods listed on the display by using the stylus or mouse. We’ll be using method “PO4-885” for phosphate that is measured at a wavelength of 885 nanometers (nm).

4. Open the hatch on the top-back of the machine and place a cuvette containing a blank solution (reagents plus deionized water) in the slot. Close the hatch.

5. From the next screen choose the tab “measure” (upper left).

6. The machine will prompt you to present the blank solution, which means that you place the thin piece of tubing in your test tube containing more blank solution. Press “ok” on the screen and fluid will be sucked out of the test tube.

7. Hit the “Run” button with the green arrow on the top instrument panel and the machine will suck-up the solution into the reading cell. Remove the test tube after the pump stops, wiping the tubing with a kim-wipe.

8. The absorbance will be appear on the display (upper bar next to wavelength) – write down the standard or sample identifier and its absorbance value.

9. The machine will now prompt you to “rinse”, so place the tubing in a vessel that contains deionized water and hit “Run” and the machine will flush out the tubing and reader cell with distilled water. After the pump stops, remove your test tube and wipe the tubing.

10. The machine will prompt you for another sample, so repeat steps 6 through 9 until all samples and standards have been processed.

11. Flush the tubing and cell several times with distilled water by repeating steps 6 through 9 without noting the absorbance.

12. Turn the machine off by pressing the “Power” button. Do NOT save any changes.

End
MEASURING PHOSPHATE VIA COLORMETRY

1. Mix up reagents following the directions below:

- **AMMONIUM MOLYBDATE** solution: Dissolve 0.2 g \((NH_4)_6Mo_7O_{24}\cdot4H_2O\) in a total volume of 100 mL using nanopure water. The solution is stable indefinitely if stored in a plastic bottle.

  Alternate recipe: 2 g \(NH_4Mo\) in 1000 mL

- **SULFURIC ACID** solution: Dilute 2 mL concentrated \(H_2SO_4\) (specific gravity, 1.82 g cm\(^{-3}\)) in a total volume of 200 mL using nanopure water.

  Alternate recipe: 10 mL \(H_2SO_4\) in 1000 mL

- **ASCORBIC ACID** solution: Dissolve 0.35 g ascorbic acid in a total volume of 100 mL using nanopure water. The solution must be refrigerated and should not be stored for more than 1 week.

  Alternate recipe: 3.5 g ascorbic in 1000 mL

- **POTASSIUM ANTIMONYL-TARTRATE** solution: Dissolve 0.009 g of \(KSB_{6}C_{4}H_{4}O_{7}\cdot0.5H_2O\) in a total volume of 100 mL using nanopure water. This solution is stable for many months.

  Alternate recipe: 0.09 g KSB in 1000 mL
• MIXED REAGENT: Mix:
  o 50 mL ammonium molybdate solution
  o 125 mL sulfuric acid solution
  o 50 mL ascorbic acid solution
  o 25 mL potassium antimonyl-tartrate

  This solution should be mixed immediately before use.

2. Mix up the standard and samples in a test tube using repeatable pipettes and the following recipe:
   • 1 mL standard or sample
   • 1 mL nanopure water
   • 2 mL mixed reagent

3. Place finished standards and samples in the dark for 1 hour.

4. Measure at 885 nm using a spectrophotometer.
Processing of Macroinvertebrate Samples from Tates Creek

Locations
NSCB 3119: Irvine Lick (IL)
NSCB 3120: Finney Creek (FC)
NSCB 4119: Junction 1985 (BC)
NSCB 4122: Perkins/Ashcroft Road (PA)

Overview
1. Your most important task today is to separate all macroinvertebrates from algae and debris in the samples. By the end of today, you want to be able to confidently say, “We got all the critters out.” A secondary task will be to start identifying things, but this is not as important as “picking” the “bugs”.
2. Remember that we have two types of samples: the kick samples for which we used the 1/4m² quadrats and the dip net samples. The kick samples are quantitative samples. So, if we do this work carefully, we will be able to compare the density of invertebrates among sites. The other sample(s) (the dip net samples) are qualitative. These samples will not allow us to compare numbers of critters among sites, but will allow us to see if other kinds of critters than what we picked up in the kick samples are present at each site. You may combine quantitative samples that you pick into vials, and you may combine qualitative samples, but please keep quantitative separate from qualitative.

Procedures
1. Cut open bags and empty into a tray. You may decant the liquid through a sieve down the drain. Rinse the sample in the tray with water, and decant the water through the sieve. Rinse anything in the sieve back into the tray and add additional water to the tray so that you can sort the sample more easily.
2. The sieving part of the procedure will be the bottleneck since we have limited numbers of sieves. So, encourage students to move quickly through that. I would not dump everything into the sieve – that will really slow things down. Just pour liquid from your trays through the sieve, and use the sieve to catch any stuff that wants to slide out with the liquid.
3. “Pick” through the sample. Remember that some organisms are small. Have students put small amounts of sample in a watch glass and “pick” it under a microscope. Other students can “pick” larger organisms by naked eye. Ideally, at some point, the entire sample will be looked at by microscope.
4. Place macroinvertebrates into small vials in 70% alcohol.
5. Place other material that has been “picked” into a larger jar with 70% alcohol.
6. If you have any sample that is “unpicked” at the end of the period, place it in its own jar and cover it with 7% alcohol.
7. Every jar or vial needs to be labeled (use pencil and place a label inside each jar or vial).
8. As you begin accumulating organisms, some students may wish to work on identifying them. (But be sure that a team of students continues to “pick” so that we process the whole sample). There is a notebook containing the Guide to Aquatic Invertebrates of the Upper Midwest in and also a copy of A Guide to Common Freshwater Invertebrates of North America each room. The former has a good key that begins on page 12 that will get you into the right ballpark with organisms. The latter has some good pictures in it and descriptions of different invertebrate groups.
9. If possible, group similar kinds of organisms into separate vials.
10. At the end of the period, be sure that all vials and jars have alcohol in them and that tops are on securely. Place all vials and jars into the large plastic container that formerly held your samples from the field.
# Kentucky Water Watch Biological Monitoring Assessment Report

<table>
<thead>
<tr>
<th>River Basin</th>
<th>Date</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stream Name</th>
<th>Supervising Sampler</th>
<th>Organization</th>
<th>Mailing Address</th>
<th>Phone #</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sampling Site</th>
<th>Flow Rate</th>
<th>Area Sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Flow Rate:** 1. Ponded 2. Low 3. Normal 4. Bank Full 5. In Flood

**Area Sampled In Square Feet:**

<table>
<thead>
<tr>
<th>Participants</th>
<th>Number of Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## General Description of Water Conditions

## Macroinvertebrate Tally

<table>
<thead>
<tr>
<th>Group 1 Taxa</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Penny Larvae</td>
<td></td>
</tr>
<tr>
<td>Mayfly Nymphs</td>
<td></td>
</tr>
<tr>
<td>Stonefly Nymphs</td>
<td></td>
</tr>
<tr>
<td>Dobsonfly Larva</td>
<td></td>
</tr>
<tr>
<td>Caddisfly Larvae</td>
<td></td>
</tr>
<tr>
<td>Riffle Beetle Adults</td>
<td></td>
</tr>
<tr>
<td>Other Snails</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 2 Taxa</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damselfly Nymphs</td>
<td></td>
</tr>
<tr>
<td>Dragonfly Nymphs</td>
<td></td>
</tr>
<tr>
<td>Crane Fly Larvae</td>
<td></td>
</tr>
<tr>
<td>Beetle Larvae</td>
<td></td>
</tr>
<tr>
<td>Crayfish</td>
<td></td>
</tr>
<tr>
<td>Scuds</td>
<td></td>
</tr>
<tr>
<td>Clams</td>
<td></td>
</tr>
<tr>
<td>Sow Bugs / Isopods</td>
<td></td>
</tr>
</tbody>
</table>

**Number of taxa present**

**Times index value of (1) =**

**Cumulative Index Value**

**Biological Quality Assessment Scale**

**Send Report Form To:** WATER WATCH BIOLOGICAL STREAM ASSESSMENT TEAM KENTUCKY DIVISION OF WATER 14 REILLY ROAD FRANKFORT, KY 40601 502-564-3410 ATT: Ken Cooke
DATA ANALYSIS - TATES CREEK PROJECT

(add these directions to your project notebook)

Now that we’ve come up with some snazzy and effective ways to display our data, we will begin to analyze same in class.

Please:

- Complete graphs of *E. coli* counts for all three sample dates using *MS Excel*.

The IDEXX method we use to enumerate microbes (see IDEXX; Borowski et al., 2012) counts both Total Coliform and *E. coli*. However, it has been shown *E. coli* counts more faithfully record entry of fecal material into surface waters (e.g., Borowski et al., 2012), so we will look only at *E. coli* counts in this project.

- Complete graphs for all three nutrient species (PO$_4$, NO$_3$, and NH$_4$) showing data for all three samples dates (31 May 2011, 15 May 2012, 1 March 2013).

- Be sure your graphs have labeled axes (e.g. station, [PO$_4$] mg/L) and titles (e.g., Phosphate 15 May 2012) so that they can be distinguished from each other.

- You’ll turn paper copies of these materials on Monday. Place all your graphs for each contaminant on one page (paste into *MS Word* from *Excel*) for easy comparison. In that way you’ll have only 4 pieces of paper for the respective microbe, phosphate, nitrate, and ammonium data.

- We’ll have some questions prepared for y’all that start our process of data analysis, and you’ll use your graphs to address those questions.

*End*
1. Complete graphs of *E. coli* counts for all three sample dates (31 May 2011, 15 May 2012, 1 March 2013) using *MS Excel*.

2. Complete graphs for all three nutrient species (PO$_4$, NO$_3$, and NH$_4$) showing data for all three sample dates.

3. All graphs should be on a single sheet for each contaminant.

4. All graphs should be the same size.

5. Be sure your graphs have labeled axes (e.g. Station, [Phosphate] mg/L) and titles (e.g., Phosphate - 15 May 2012) so that they can be distinguished from each other.

6. *E. coli* should be in italics; subscripts for NO$_3$, PO$_4$, etc.

7. Graphs of each contaminant should have the same scale for easy comparison.
   - Nitrate – 0 - 60 mg/L – label every 10, tick marks every 2
   - Ammonium – 0 - 3 mg/L – label every 1, tick marks every 0.2
   - Phosphate – 0 - 8 mg/L – label every 2, tick marks every 0.5
   - *E. coli* – 0 - 2500 cfu/100 mL – label every 500, tick marks every 100

8. Font, font size, and notation should be consistent between all graphs for titles, axes labels.

9. Your graphs should be continuous with respect to stations axis.

10. Excise extraneous stuff like keys, lines in the x-axis legend

End

Additional information about this study is available at http://water.usgs.gov/nawqa/nutrients/pubs/circ1350

This publication is suitable as a reference for your project report. Also, please answer the questions below concerning this paper.

1. Looking on page 21, what are the major findings of this study regarding nutrient levels in surface waters (streams and rivers)? What is the trend of nutrient concentrations in streams?

2. Note Fig. 1-1. When you write your reports concerning the water quality of Tates Creek, you should compare your finding to the data shown in the figure. [ For our purposes, total nitrogen = nitrogen in nitrate + nitrogen in ammonium; total phosphate = orthophosphate (that’s what we measured). ]

3. What are the major sources of nitrogen to streams?

4. What problems can elevated nutrient concentrations cause in surface waters?

5. Define and distinguish between point sources and non-point sources.
6. What is the major source for nutrients in Kentucky (see Fig. 2-6)?

7. Fill in the table below concerning background levels of nitrogen and phosphorus in streams.

Background Nutrient Levels in Streams of the United States

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Concentration (milligrams per liter, mg/L, ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia</td>
<td></td>
</tr>
<tr>
<td>Nitrate</td>
<td></td>
</tr>
<tr>
<td>Total Nitrogen</td>
<td></td>
</tr>
<tr>
<td>Phosphate (=orthophosphate)</td>
<td></td>
</tr>
<tr>
<td>Total Phosphorus</td>
<td></td>
</tr>
</tbody>
</table>

End
DATA ANALYSIS - TATES CREEK PROJECT

(add questions, with answers, to your project notebook)

Answer the questions below using our data and the graphs you produced for today’s homework. Feel free to collaborate with your colleagues.

Recall that the sewage treatment plant was operating until 19 July 2011, therefore the plant was operating on 31 May 2011, but not on our other sample dates of 15 May 2012 and 1 March 2013. Consult the graph below that shows discharge from the sewage treatment plant (in millions gallons per day, mgd; filled circles) and rainfall (columns) for 2011.

Conditions on sampling day:
- 31 May 2011: No recorded rain for 5 days before sampling
- 15 May 2012: Warm, rainfall 3 of 5 days before sampling
- 1 March 2013: Cold, persistently wet on prior days

Probe data

Our probe data show us water temperature, well oxygenated waters, and slightly alkaline waters (usually pH>7). We won’t use these data explicitly, but please do display the data in your project report as a table appearing either in the text or in the Appendix.

Microbe data

Look at all your E. coli data for all three sample dates.

1. On which sample date(s) are E. coli generally most numerous?

2. What are possible reasons for such a difference between sample dates?
Look at the data for 31 May 2011 near the sewage treatment plant. Compare the counts upstream of the discharge point (those upstream and including SP-u), to that immediately downstream of the discharge (station SP-d), and at station ILD-bridge (where Goggins lane crosses Tates Creek upstream of station ILC).

3. Explain these data. [Hint: there is a well-used cow pasture between stations SP-d and ILC-bridge.] Recall that the Richmond is connected to city sewer while domiciles in the watershed downstream of the STP are not. What have our data unequivocally demonstrated here?

Look at the *E. coli* data on March 1, 2013 for the PAC samples. Draw a schematic map of the PAC tributary entering Tates Creek, note the sample points, and write the *E. coli* counts on their corresponding map position.

4. What do you notice? What is happening here? Does this happen elsewhere? Nutrients as well?

5. Are there any consistent “hotspots” for fecal microbe pollution in any of the data?

6. What are you conclusions about the most likely sources of fecal microbes in Tates Creek? Is this point source or non-point-source pollution? Explain.

**Nutrient data**
7. On which sample date(s) are nutrients more abundant?

Look at the nutrient data for 31 May 2011.

8. Where are the concentrations highest?

What happens to nutrient concentration downstream of the sewage plant discharge (station SP-d)?

Are there any large sources of nutrients downstream of SP-d? What are possible nutrient sources upstream of SP-d?

Please form a hypothesis to explain the data.

Is this evidence for point source or non-point-source pollution? Explain.

Now compare the nutrient data for all sampling dates.

9. What are possible reasons for such a difference between sample dates?

Look at the ammonium data.

10. How are patterns of ammonium concentration different from that of nitrate and phosphate?

11. How can one explain this pattern? [Hint: most organisms absorb nitrogen from the environment in the form of ammonium.]
It will now be necessary to look at our nutrient data at a more highly-resolved scale. Be sure that your original graphs of nutrients at higher-concentration scales are saved, then copy the graphs for 15 May and 1 March, and paste them elsewhere on the spreadsheet. For the newly-created graphs, expand the nutrient scale but be sure to use the same scale for each nutrient.

Do a tally for each nutrient graph for each sample date that compares the nutrient concentration of tributaries to that of Tates Creek (for stations like AC, TCEC, WC compare them to the nearest value within Tates Creek). Please display your data tables in the Appendix of your project report. If the results are worthy, please consider making graphs that illustrate your findings in the most compelling manner.

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Tributary &gt; Tates Creek value</th>
<th>Tates Creek &gt; Tributary value</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 May 2011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 May 2012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 March 2013</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12. Are nutrient concentrations generally highest within the trunk stream of tributaries?

Is this condition dependent on the sample day or other factors?

Please explain.

13. At some point, perform this same exercise for our microbe data. What are your results?

14. Are there any consistent “hotspots” for fecal microbe pollution in any of the data? After the plant shutdown, would you consider fecal microbe and nutrient pollution as point source or non-point-source? Please give evidence supporting your hypothesis.

15. Considering Tates Creek with fecal microbe and nutrient pollution, what steps would be effective in eliminating or negating point source pollution? Non-point-source pollution?
Macronvertebrate data

16. What is the nature and quality of our invertebrate data? (You will need to confer among groups to decide if data labeled “quantitative” are truly quantitative. That is, were they carefully collected using the 0.25m² quadrats? Were four replicates collected so that the combined data for a site are for 1m²? Were the bags and vials labeled accurately?) If you cannot say for sure that you have a bona fide quantitative sample, then you will need to treat the data as a qualitative sample.

17. For quantitative data, consider comparing the density (# per m²) of all macroinvertebrates combined and of different taxonomic groups among sample sites.

18. For qualitative data, compute Biological Monitoring Assessment values for each site. (Remember that you can combine kick sample data with dip net sample data for this qualitative assessment.)

19. There are samples on two dates from the ILC site. How do the Biological Assessment values compare from the two dates?

20. How do Biological Assessment values compare as one moves from upstream to downstream sites?

21. How can you organize the data and display them (or summaries of the data) succinctly in your paper?

End
GROUP PRESENTATIONS - TATES CREEK PROJECT

Presentation Introduction: Wednesday, 9 April 2014, in class

Presentation Workdays: Monday, 14 April 2014, in class
Wednesday, 16 April 2014, in class

Presentations: 30 April 2014, in class
PowerPoint files emailed to instructors by 7 AM, presentation day

Length: 15-20 minutes.

Content: Each group member must present. Many pertinent materials are posted on BlackBoard.
- Context for the Tates Creek Project - maps, stations, etc.
- Land use characterization
- Findings using fecal microbe, nutrient, and macroinvertebrate data
- Discrete sources for nutrient and/or E. coli contamination
- Determination of water quality using above
- Illustrations demonstrating findings of noted species.

Organization: Introduction, Methods, Results, Interpretations, Summary

Sources: Readings posted on BlackBoard are listed below.

Grade:
- Possible 100 points.
- See the grading rubric below.

REFERENCES


Presenter ______________________________ Title Keywords ______________________________
Evaluation of Oral Presentation

_____ (25 pts) I. Content
- Is the topic well-defined?
- Has the presenter uncovered, used, and cited appropriate literature sources? (Has the presenter located and used appropriate sources?)
- Has the presenter integrated source material into the presentation?
- Does the presenter demonstrate understanding of the topic?
- Comments:

_____ (25 pts) II. Organization
- Does the presentation include a clear introduction and statement of the problem?
- Is the content of the talk presented in a logical and coherent manner?
- Does the presenter include an effective summary of the topic?
- Comments:

_____ (25 pts) III. Oral Presentation
- Do the presenters speak with a clear, expressive voices?
- Are the speakers’ deliveries paced appropriately?
- Do the presenters use proper grammar and vocabulary?
- Do the presenters make clear statements?
- Comments:

_____ (25 pts) IV. Visual Presentation
- Are visuals used large, clear, uncluttered, and appropriate to the topic?
- Is written text in visuals readable, uncrowded, and appropriate to the topic?
- Has the presenter used an effective color scheme?
- Has the presenter used a consistent and effective slide layout?
- Comments:

_____ (100 pts) TOTAL

Critic ___________________
List of Field Materials

**Water properties**

- YSI 556 MPS probe – simultaneous measurement of temperature, conductivity, oxygen concentration, pH

**Water sampling for nutrients**

- Indelible markers
- Water tight, glass scintillation vials (26 mL) – water samples for nutrients
- Concentrated sulfuric acid (H₂SO₄) - used to preserve water samples for nutrients
- 60 mL syringes, lure-lock – used to collect water samples
- 0.45 μm, lure-lock syringe filters – used to filter water samples for nutrient measurements
- Cooler to transport samples on ice

**Water sampling for fecal microbes**

- Indelible markers
- IDEXX sterile sampling cup (120 mL) – water sampling for fecal microbes

**Macroinvertebrate sampling**

- Ethanol for organism preservation (95% ethyl alcohol, EtOH)
- Sample identification slips
- Pencils
- Fine-mesh dip nets
- Fine-mesh seine nets
- 0.25-m² quadrats
- Several standard buckets
- Small plastic garbage bags for sample storage (double bag)
List of Laboratory Materials

**Nutrient measurements**

- ThermoFisher Evolution 201 UV-visible spectrophotometer.
- Set of pipettes and pipette tips (0.1, 1, 5, 10 mL)
- 10 mL test tubes and test-tube racks – for ammonium and phosphate measurements
- Centrifuge tubes or 25 mL test tubes and racks – for nitrate measurements
- Supply of deionized water (resistivity = 18 mega-ohms)
- Reagents for ammonium measurements (sodium hypochlorite method)
  - Phenol
  - Ethanol (ethyl alcohol) [95% EtOH]
  - Sodium nitroprusside
  - Trisodium citrate
  - Sodium hydroxide [NaOH, solid]
  - Sodium hypochlorite (bleach)
- Reagents for nitrate measurements (cadmium reduction method)
  - Hach NitraVer 5 packets
- Reagents for phosphate measurements (ascorbic acid method)
  - Ammonium molybdate [(NH₄)₆Mo₇O₂₄·4H₂O]
  - Sulfuric acid
  - Ascorbic acid
  - Potassium antimonyl-tartrate [K₃SbC₄H₄O₇·0.5 H₂O]

**Fecal microbe measurements**

- IDEXX Coliert-18 media – for Total Coliform and *E. coli* counts
- IDEXX quanti-trays - for Total Coliform and *E. coli* counts
- Incubator
- Black light – for *E. coli* counts

**Macroinvertebrate counts**

- Index for water quality
- Keys for macroinvertebrates
- Aluminum trays
- Forceps and dissecting needles
- Binocular microscopes and associated light sources
- Storage bottles for organisms
- Ethanol (95% ethyl alcohol, EtOH)