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Monitoring and understanding stream water quality

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Abstract

Have you ever wondered how clean your local streams are? Just like you need clean air to breathe, aquatic ecosystems need clean water to function. Throughout the country there are numerous watersheds, which are areas of land containing a set of streams and/or rivers that all drain through a specific location. Healthy watersheds are important because they improve water quality, allow for indigenous species to out-compete invasive species, are better adapted to extreme weather patterns, and reduce drinking water treatment costs. During this workshop you will learn how to monitor the health of stream systems using bacterial, chemical, and macroinvertebrate indicators. Participants will walk to a nearby creek to collect samples for bacterial and chemical monitoring. Due to the depth of the stream, macroinvertebrate sampling will be demonstrated in the laboratory. After learning how to interpret the data from the local stream, workshop participants will be led through a case study created using data from a stream in Athens, Georgia that was damaged by runoff from a chemical fire. They will evaluate data that was gathered by citizen scientists before and after the chemical fire and learn about the events impact on water quality. Participants will also compare water quality parameters between an impacted and relatively pristine stream. Participants will gain the knowledge needed to set-up a monitoring site close to their home campus that will allow their students to participate in water quality monitoring.

Keywords: Stream Water Quality, Bacterial Testing, Chemical Testing, Macroinvertebrate Testing, Watershed

Link to Supplemental Materials: In-field Monitoring Protocols, AAS Chemical/Bacteria Form, AAS Macroinvertebrate Form, AAS Macroinvertebrate Field Guide

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INTRODUCTION

Introducing students to water quality testing protocols and how they can participate in stream water quality monitoring can provide lasting impacts and habits of mind regarding the natural environment. Training in water monitoring protocols and this case study can be applied in middle school through college level biology, chemistry, and environmental science courses. If there is no stream close to campus, the case study can be used on its own to expose students to water quality parameters and the environmental impacts to a stream following an ecological crisis using monitoring data, as well as analyzing differences in water quality parameters for an urban (impacted) and rural (relatively pristine) stream. If a stream is close to campus, students can engage in hands-on activities to learn how to monitor the stream through bacterial, chemical, and macroinvertebrate testing that will strengthen the concepts they learned in the case study. In addition, the data provided can reinforce skills related to reading and interpreting graphs and the understanding of acute and chronic ecosystem responses to environmental change.

Case Study

The case study provides students the opportunity to examine data from a stream that is located near Athens, Georgia that experienced a chemical spill in 2010. Through a volunteer organization, the Upper Oconee Watershed Network (https://uown.org) we have data before and after a chemical spill that shows the impact on conductivity, pH, biological score, turbidity, and *E. coli* levels. We are also able to compare longer term data from the impacted stream to a nearby stream that had no impact from the chemical spill and is comparatively healthier. This will allow the students to learn how long it takes a stream to recover from a major ecological crisis and allow the teacher to incorporate types of pollution events that can impact our streams. The streams being compared also exhibit the differences between an impacted and relatively pristine stream. Students will learn how to read graphs and interpret data to identify pollution events. The raw data for the two streams in the case study have also been provided, which would allow students to learn how to create graphs from the data before interpretation. If the instructor decides to have the students produce graphs, the graphs can be removed from the case study before distributing to the students. The questions below the graphs in the case study could still be used to help the student interpret the data. This should take roughly an hour of class time with students. If students are creating graphs from the data, I would expect it would take two hours to go through the case study.

Monitoring of Local Streams

If your school is fortunate enough to have a stream within walking distance you can allow the students hands-on experience monitoring the stream for the same parameters that are in the case study. Monitoring of chemical and bacterial parameters should be done monthly during the school year while the macroinvertebrates should be done quarterly (seasonally) throughout the school year. Chemical and bacterial monitoring will take approximately thirty minutes at the stream. Macroinvertebrate sampling will take approximately one hour which includes identifying the macroinvertebrates in the samples collected. This will allow you as a class to record and graph data from your own stream over time and notice how weather and seasonal change can affect the different parameters. During the visual stream survey, students can also map the changes from weather events over time by comparing to previous years visual surveys. It should be noted that if data is out of normal (acceptable) range, a potential pollution source should be investigated in addition to notifying your local water authority.

Using real-time data from Georgia Adopt-A-Stream

If your school does not have a stream within walking distance to monitor, data can be used from the Georgia Adopt-A-Stream website (https://adoptastream.georgia.gov/). Once on the website, navigate to the Data views and then select view data by region. You can look at data by city, county, watershed, coastal, or water plan region. Using a map of Georgia, you could find a city or region that is similar to your location for analysis using the website. Once you select a site, you can download the data from their monitoring events and use that data to teach students how to analyze real-time data.

STUDENT OUTLINE

Objectives

Which indicators vary most between impacted and pristine streams?
    Compare Trail and Bear Creek
Which indicators reflect impact from the spill?
    Compare Trail Creek before and after
How long did it take for the stream to recover?
    Compare time after

Introduction

Just like you need clean air to breathe, aquatic ecosystems need clean water to function. Throughout the country there are numerous watersheds, which is an area of land that contains a set of streams and/or rivers that all drain into a single larger body of water or through a chosen location. Watersheds are important because they improve water quality, allow for indigenous species to out-compete invasive species, they are better adapted to extreme weather patterns, and reduce drinking water treatment costs (Healthy Watersheds Protection [updated 2021]).

There are countless watersheds in the world, but we will focus on a watershed in Georgia, called the Upper Oconee Watershed. A group of citizen scientist volunteers have been monitoring the Upper Oconee Watershed since 2000 in response to concerns about the rapid growth of Athens and its surrounding area (UOWN Mission [updated 2017]).

What is the color of water? Most people would respond by saying clear or colorless and they would of course be describing drinking and/or treated water. Are natural waters clear, transparent, opaque, colored? The answer is yes to all of these options. How do we know when the color observed in natural waterways is an indicator of hazardous conditions or a contamination event? First and foremost, it's important to know what is 'normal' or healthy for a particular stream. Natural streams can vary in color due to the presence of organic material, sediment, or algae and can vary from almost black to vibrate reds, oranges, greens, and yellows, but when a stream dramatically changes colors, it is likely the result of a contamination event. Chemical and oil spills and constant non-point surface contamination have caused surface waters to also be black, rainbow colored, muddy brown, and a variety of vibrant colors.

In downtown Athens, there is a little creek that meanders its way behind the restaurant, Mama's Boy, and eventually empties into the North Oconee River. This creek is called Trail Creek and without knowing the creek's background, not many would guess it had an extreme ecological crisis in the past. In 2010, a fire broke out at J&J Chemicals, a company that mainly produces toilet-bowl cleaners. To put out the blaze, Athens firefighters dumped 700,000 gallons of water on the chemical company. This water mixed with the janitorial chemicals and carried it out into a nearby stream, Trail Creek (Black 2013). This mix of chemicals dyed the water bright blue and killed an estimated 15,000 fish and almost all wildlife that lived within the creek's waters.

Figure 1: Pictures of Trail Creek taken the day after the contamination event. Notice the neon blue water.

Even before the 2010 incident, Trail Creek was not an extremely healthy stream. It had had previous occurrences of sewage leaks and trash dumping, which impacted its water quality. However, with the massive amount of chemicals dumped into Trail Creek, this stream went from decent water quality to extremely toxic for both animals and humans. The water turned bright blue, allowing the spill to be easily noticed.

What happened? What were the impacts of the event? How was the event recorded? Has the creek recovered from the event? In this study you will analyze water quality data to assess the impact and recovery of a stream that has experienced a colorful contamination event. In addition, long term water quality parameters between the impacted and a relatively pristine stream system can be evaluated.

Questions:

1. What would you expect to see in a healthy stream?
2. How would you expect an impacted stream to look different than a pristine stream?
3. How would you expect a stream before a contamination event to look compared to a stream after a contamination event?

Indicators of Water Quality

Monitoring within the watershed includes measurements of conductivity, pH, biological score, turbidity, and *E. coli* counts. Collection of this type of data can be used to monitor both long term and acute changes within a watershed as well as comparing impacted and non-impacted streams.

Figure 2: Citizen Scientist sampling a stream

Variables indicating water quality:

Conductivity

Conductivity refers to the ability of water to pass an electrical current. It indicates the number of dissolved ions in the water. It is typically measured with a handheld meter in the field and reported in scientific units of either milli- or micro-siemens per centimeter (mS/cm or µS/cm). Field measurements can be (a) a single observation or (b)

multiple (averaged) observations. Conductivity can be affected by runoff from mining operations, agriculture, sewage effluent, and urban runoff. (Georgia Adopt-A-Stream [updated 2015])

Figure 3: Citizen scientist measuring conductivity in a stream.

pH

pH is the measure of hydrogen ions in water, and it is measured on a logarithmic scale between 0 and 14. Aquatic organisms are sensitive to pH fluctuations, therefore minor changes in pH can impact the environment. Natural waters are typically slightly acidic with pH a little below 7. pH can be increased by photosynthesis and calcium carbonate from limestone. Respiration and mine drainage can decrease the pH of a stream. (Georgia Adopt-A-Stream [updated 2015])

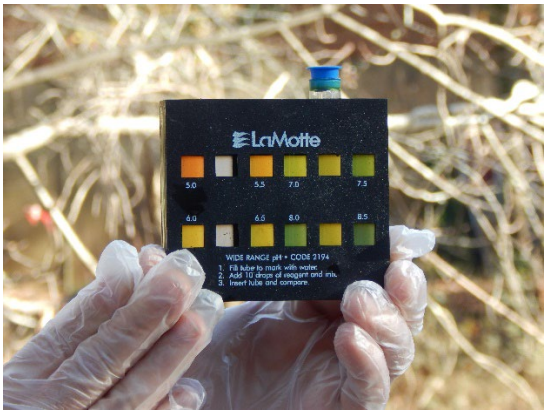


Figure 4: Determining the pH of the stream water. Note the blue capped tube that is being compared to the colored squares.

Biological Score

The biological score reflects the number and diversity of macroinvertebrates in a stream and the surrounding riparian buffer. It reflects the water quality and habitat quality of the environment. Macroinvertebrates are separated into three groups: pollution sensitive, somewhat sensitive, and tolerant. The weighted score depends upon the diversity and sensitivity of the organisms sampled. A higher biological score translates to a more diverse habitat and better water quality. Macroinvertebrates are used as an indicator of water quality because they are present during all stream events, and they are affected by the physical, chemical, and biological conditions of the stream. (Georgia Adopt-A-Stream [updated 2015])

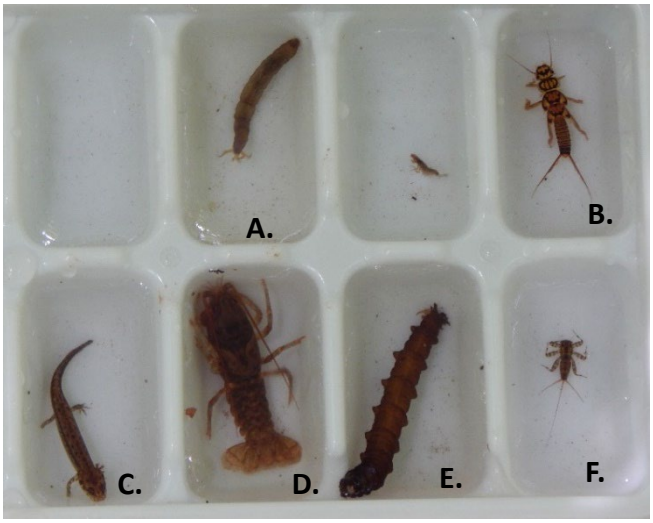


Figure 5: Examples of aquatic macroinvertebrates collected from a stream. A. Caddisfly B. Stonefly C. Salamander (not a macroinvertebrate) D. Crayfish E. Cranefly F. Mayfly

Turbidity

Turbidity is an indicator of how clear water is. It measures how much light is scattered by the material suspended in the water when light is passed through the water. The higher the intensity of scattered light, the higher the turbidity. Materials suspended in the water, such as clay, silt, organic matter, and microscopic organisms can all affect the turbidity. Increased turbidity negatively impacts photosynthetic organisms. The higher the turbidity the greater the harm to the habitat for fish and other aquatic life. High turbidity is an indicator of potential pollution in a water body and low values of dissolved oxygen. (Turbidity and Water [updated 2019])

Figure 6: Showing the turbidity of water. The jar on the right is much more turbid than the jar on the left.

E. coli

Measurement of *E. coli* indicates the level of health risk due to bacterial contamination of surface waters. If there are high amounts of *E. coli*, most likely there is a high number of fecal coliforms found in the water. Fecal coliforms are bacteria that is found in the intestinal tract of humans and other warm-blooded animals. These bacteria can be

pathogenic, meaning they can cause harm to humans, animals, or plants. Not all bacteria are bad, some can be beneficial such as those that help with decomposition of plants in the water, nutrient cycling, and pollution control. Certain bacteria can also help with pollution control by remediating contaminants from oil spills and sewage treatment. Sources of *E. coli* in streams can come from wildlife, livestock, urban storm runoff, leaking pipes, and failing septic systems. (Georgia Adopt-A-Stream [updated 2014])

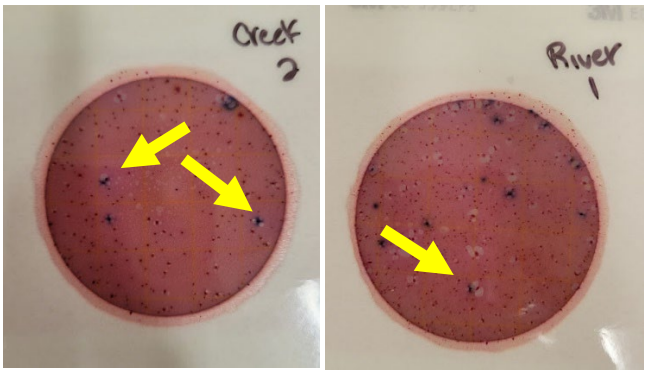


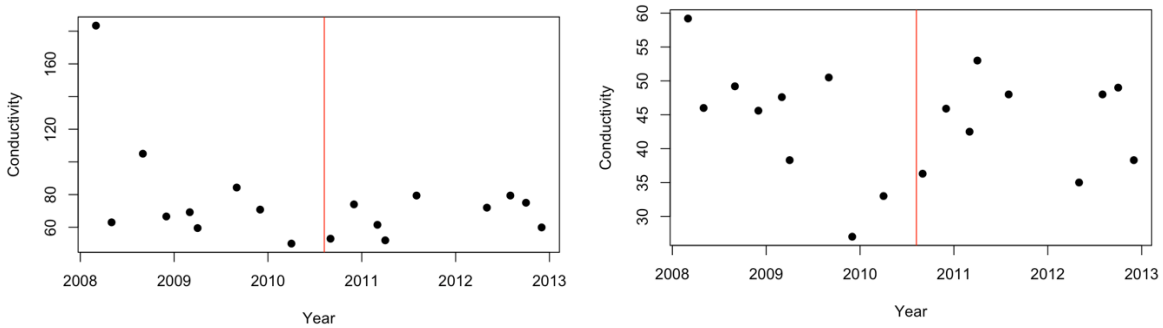
Figure 7: Examples of Petrifilm™ to measure *E. coli*. The *E. coli* positive colonies are the blue colonies with gas bubbles surrounding them. The yellow arrows are pointing to some of the positive colonies.

Questions.

4. Given these indicators, what specific differences would you expect to see between pristine and impacted streams?
5. Given these indicators, what specific differences would you expect to see before and after a contamination event?
6. How long do you think it would take a stream to recover from a contamination event?
7. What factors would affect the rate of recovery?

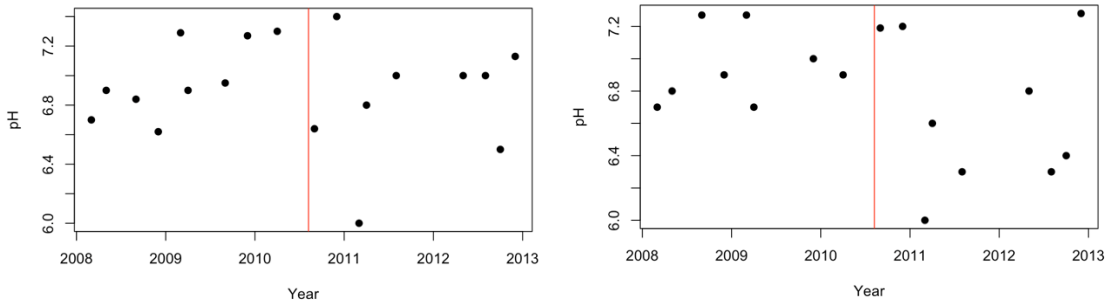
Watershed Data

Below are data from UOWN's quarterly water quality monitoring events. Data is shown for 2 creeks: (1) Trail Creek, downstream of where the spill occurred and (2) Bear Creek, a generally healthy stream unaffected by the spill. On each plot, the red line denotes the date of the spill. Plots from each creek are shown for conductivity, pH, biological score, turbidity, and *E. coli*. Pay attention to differences on the y axes and changes in data before and after the spill. Also provided is a data table pre and post spill for Trail Creek, the impacted stream, including the acceptable range for the water quality indicators discussed. (Science and Monitoring [updated 2017])



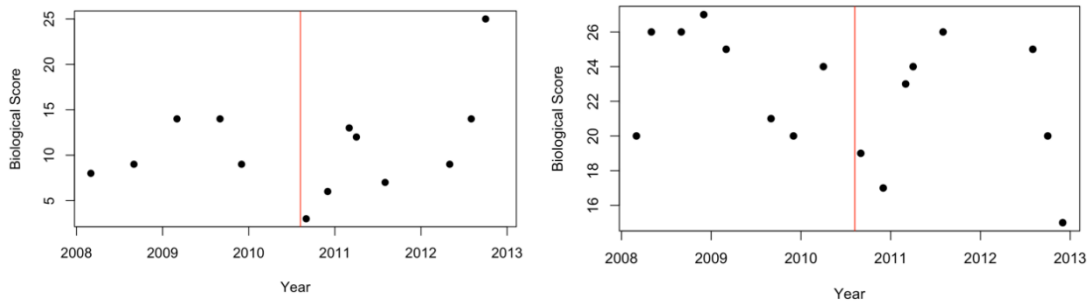
Trail Creek Bear Creek

Figure 8: Graphs of the Conductivity at Trail Creek and Bear Creek overtime. The red line indicates the time of the chemical spill.



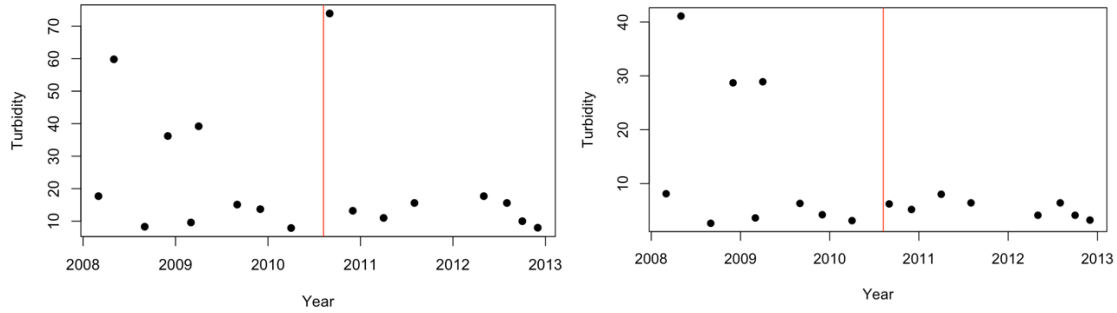
Trail Creek Bear Creek

Figure 9: Graphs of the pH at Trail Creek and Bear Creek over time. The red line indicates the time of the chemical spill.



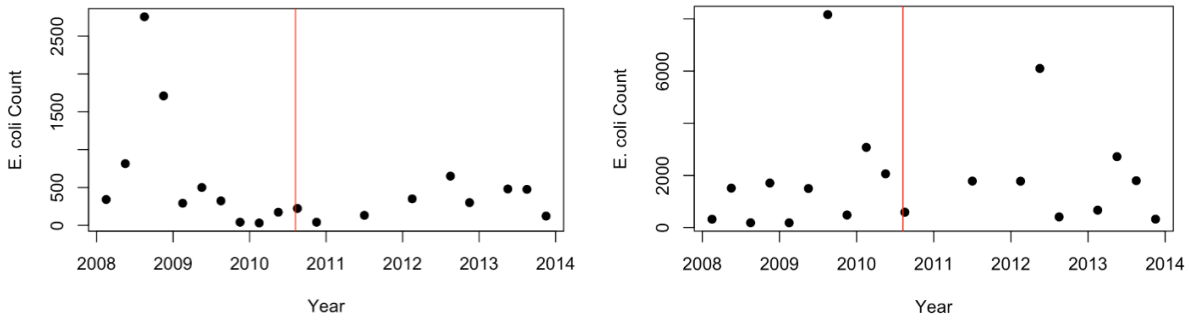
Trail Creek Bear Creek

Figure 10: Graphs of the Biological Score at Trail Creek and Bear Creek overtime. The red line indicates the time of the chemical spill.



Trail Creek Bear Creek

Figure 11: Graphs of the Turbidity at Trail Creek and Bear Creek overtime. The red line indicates the time of the chemical spill.



Trail Creek Bear Creek

Figure 12: Graphs of the *E. coli* count at Trail Creek and Bear Creek overtime. The red line indicates the time of the chemical spill.

Table 1: Comparison of Normal Values to Trail Creek pre- and post-spill

Variable	Normal Range	Trail Creek pre-spill	Trail Creek post-spill
Conductivity	50 to 1500µS/cm	50 µS/cm	53 µS/cm
pH	6 – 8.5	7.3	6.64
Biological score	Less than 11 = poor Between 11 - 16: fair Between 17 - 22: good Greater than 22: excellent	14	3
Turbidity	Less than 10 NTU Drinking water: minimum less than 5 NTU	7.9 NTU	73.9 NTU
E. coli	Designated swimming areas: ≤235 CFU/mL Moderate swimming areas: ≤298CFU/mL Light swimming areas: ≤ 410 CFU/mL Infrequent swimming areas: ≤576 CFU/mL	3075 CFU/mL	2063 CFU/mL

(Georgia Adopt-A-Stream [updated 2014])

Questions:

8. Observe the y-axis for each water quality indicator between the pristine and impacted stream, list the maximum and minimum values observed for each variable.
9. Which variables are higher for the pristine stream versus the impacted stream?
10. Which variables are similar between pristine and impacted stream?
11. Which variables are notably different in the impacted stream pre and post contamination event?
12. How quickly did the impacted stream recover from the contamination event?

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MATERIALS

Cooler with ice
*Gloves – needed for students who will handle collection of water for bacterial and chemical analyses
*Whirl-pak®bags (one per site monitoring and blank) (Cole-Parmer mfr: B01065| Item: EW-06499-80)
Markers
P1000 pipettes
*P1000 filtered pipette tips
*3M™Petriflim™*E. coli*/Coliform Count Plates (4 plates per site plus one blank) – come in packs of 24 or 50 (Neogen: SKU No. 700002271, Catalog No. 6404)
Incubator
Min/Max Thermometer
*Disinfectant
*LaMotte®dissolved oxygen kit (Forestry supplies item # 77191)
*LaMotte®pH Test Kit (Forestry supplies item # 77150)
Conductivity Meter (Forestry supplies item # 76101)
Enviro-safe thermometer (Forestry supplies item # 77141)
Safety Glasses
Waste Jug
Clipboard
Pen/Pencil
D-frame net or kick seine net (depending on stream type)
Plastic spoons
Magnifying Glasses
Collection tray (white) (Grainger Item 24AN11 Mfr. Model 209295-0003)
Ice cube tray
Jug for rinsing out macros from nets
Boots or waders for going into the stream

*Indicates supplies that would be consumable and need to be replaced as needed

For up-to-date information on the supplies, please visit the Georgia Adopt-A-Stream supply website: https://adoptastream.georgia.gov/data-forms-2/equipment-purchasing-information

NOTES FOR THE INSTRUCTOR

Introduction/Background

The case study is an exercise in comparing and analyzing data from an impacted and pristine stream and the impacted stream pre and post contamination event. It is suitable for middle grades through undergraduate lower-level division environmental science, ecology, microbiology class or any introductory science class with an environmental component. Students are introduced to what a watershed is and learn what variables are tested to monitor water quality. In the case study, students are given the background information needed but some background knowledge on how to interpret graphs might be beneficial. Students will analyze data from the impacted stream versus a pristine stream pre and post contamination event of the impacted stream. To increase the level of the case study, the raw data has also been provided, so students can learn how to create graphs, as an alternative to analyzing the data provided graphically.

Objectives

By the end of the case, students should be able to analyze graphical data from water quality monitoring. They should also become familiar with the variables used to monitor water and how those generated variables change quality during a contamination event as well as compare a pristine stream to an impacted stream.

- Describe a watershed and the water quality indicators, conductivity, pH, biological score, turbidity, and *E. coli* counts
- Identify potential differences in water quality indicators between 1. Pristine stream compared to an impacted stream and 2. Pre and post contamination event for impacted stream.
- Analyze water quality data graphs for differences between 1. Pristine streams versus an impacted stream and 2. Pre and post contamination event.
- Compare graph scales to recognize differences between 1. Pristine streams compared to an impacted stream and 2. Pre and post contamination event.
- Interpret and discuss water quality indicators responsive to degradation.
- Create graphs with data between 1. Pristine streams compared to an impacted stream and 2. Pre and post contamination event.

Classroom Management

This case study begins with giving students a lesson in water quality parameters displaying pictures of a stream pre and post contamination event. During this you would discuss watershed health and how you can tell if a stream is healthy or not. Discussion can also occur on what activities you would guess are the most common in an impacted watershed. This first part would take between 10 to 15 minutes in class.

During the second part of the case study, students will learn about the different indicators of water quality. The students will hypothesize what similarities and differences they would expect between a pristine stream and an impacted stream as well as pre and post contamination event for each indicator discussed. As the teacher, you can provide visual examples of what each parameter looks like. This part takes between 10 to 15 minutes in class.

During the third part of the case study, students will analyze graphs of real data from two creeks, one a pristine creek (Bear Creek) and one an impacted creek (Trail Creek). The contamination event is noted on the graphs by a red line so the students can compare the indicators before and after the contamination event. While Bear Creek did not have a contamination event, the red line is still indicated on the graph so students can match the timeline between each creek. You can break the students into groups and have them analyze in detail two of the indicators between the pristine stream and a contaminated stream. Students might struggle with realizing that the axes are not the same so that might need to be pointed out to them. There is also a table of normal ranges for each indicator. The data from before and after the contamination event is to help students interpret the data in the graphs. Questions you can use to help the students get started are 1. What do the graphs tell us about what happened in terms of water quality?, 2. How long is it before the impact of the spill lessens?, 3. Does it continue to improve up until today?, and 4. Would you say it is safe to enter either or both streams? Students can take up to 15 to 20 minutes to analyze the graphs. Then bring the students back as a whole to discuss all the water quality indicators, reporting

back on the discussions they had in the groups, and make conclusions as a class on the data. This part can take 10 to 15 minutes. If you would prefer for students to learn how to create graphs, raw data from the pristine and impacted stream has been provided. This would take 20 to 30 minutes per parameter to graph. If you have access to a stream, you may even want to measure some of these water indicators in a future class period or set-up monthly monitoring of that stream.

ACKNOWLEDGEMENTS

Thank you very much to Georgia Adopt-A-Stream who has developed the quality control/quality assurance methods we are using for stream water quality. Thank you also to Bruno Giri and Reilly Farrell who helped in development of the case study used in this publication. Finally, thank you to the Upper Oconee Watershed Network (UOWN) whose volunteers have collected the data provided in the case study.

About the Authors

Julia Schmitz has been a professor at Piedmont University (Piedmont College until 2021) since 2011, where she teaches microbiology for majors as well as the science requirement courses (Human Anatomy & Physiology and Medical Microbiology) for the nursing and health science majors. Julia is a member of the education committee of the Upper Oconee Watershed Network. She actively participates in education and monitoring initiatives to raise community awareness of local water resources.

Deb Dooley has been a professor at Piedmont University (Piedmont College until 2021) since 1996, where she teaches geology, environmental science, and hydrology to the department majors. Deb is the chair of the education committee and monitoring committee for the Upper Oconee Watershed Network, where she participates in education and stream monitoring initiatives to raise community awareness of local water resources.

APPENDIX A

Student Version of the Laboratory Handout – Infield Monitoring Protocols
Bacterial Monitoring (Monthly)

Materials

- Cooler with ice
- Gloves
- Whirl-pak® bags (one per site monitoring and blank)
- Marker
- P1000 pipettes
- P1000 filtered pipette tips
- 3M™ Petriflim™ *E. coli*/Coliform Count plates
- Incubator
- Min/Max Thermometer
- Shoes that can get wet (boots, waders, old tennis shoes)
- Disinfectant
- Bacteria data sheet (AAS)

Methods

1. Label your Whirl-pak® bags with sites to be sampled.
2. Put on gloves and remove the perforated portion on the top of the bag.
3. Use the two white tabs to open the bag without touching the inside.
4. Fill the Whirl-pak® bag with distilled water to serve as your control. If sampling more than 10 sites, do one blank per 10 sampling sites.
5. Grab the ends of the twist ties and twist to close the bag. Invert the bag to make sure it is sealed.
6. Place the Whirl-pak® bag in the cooler.
7. Repeat steps 1 – 6 for each sampling site with water from the stream. Also make sure to face upstream when sampling.
8. Samples can be stored on ice or refrigerated for up to 24 hours prior to plating.
9. Turn your incubator on to 35°C prior to plating your samples.
10. Clean the benchtop with disinfectant and let dry.
11. Put on gloves.
12. Label the Petriflim™ (1 for the blank and 3 for each site sampled) with stream name, date, and start time.
13. Invert the Whirl-pak® bag to mix the sample and then place in a cup to keep the bag upright.
14. Lift the film on the Petriflim™ and pipette 1mL of sample onto the circle. Slowly roll down the film to prevent formation of air bubbles. Do not touch the film that is on top of the circle. Repeat two more times for a total of three plates per sample site.
15. Place the Petriflim™ into the incubator with top film side up. Incubate for 24 hours.
16. After 24 hours, while wearing gloves remove the plates from the incubator. Record the minimum and maximum temperatures on the thermometer. Record the data on the bacterial data form. *E. coli* positive colonies will be blue with gas bubbles nearby.
17. To calculate colony forming units (CFU) count the total number of blue colonies with gas per plate. Add those three numbers together and then find the average. Multiply the average by 100 to determine your CFU per sample site.
18. Ensure that the blank has no colonies on its Petriflim™. If there are colonies on the blank, the data is invalid for the experiment.
19. Clean-up the Petriflim™ by spraying with disinfectant, sealing in a Ziploc bag and throwing in the trash. Wipe the benchtop down with disinfectant.

Chemical Monitoring (Monthly)

Materials

- LaMotte® dissolved oxygen kit
- LaMotte® pH Test Kit
- Conductivity meter
- Conductivity standard
- Enviro-safe thermometer
- Gloves
- Safety glasses
- Waste jug
- Pen/pencil
- Clipboard
- Physical/Chemical Data Form (found on the AAS website)

Methods

Calibrating the conductivity meter

1. Within 24 hours of sampling, calibrate the conductivity meter by removing the cap and turning the meter on, then set in calibration mode.
2. Rinse the probe with deionized water.
3. Submerge the probe in Conductivity standard.
4. Adjust your meter until it matches the value of the standard.

Stream Survey

5. At the sample site, fill out the stream visualization survey.

Temperature

6. Measure air temperature in the shade and record on the sheet.
7. Measure the water temperature in the shade and record on the sheet.

Conductivity

8. Measure the water conductivity by submerging the sensor in the water at the sample site. Once the reading has stabilized, record the data.

pH

9. Rinse the two small glass test tubes for pH with sample water.
10. Fill the test tubes to the line indicated on the test tubes. If there is excess water, flick it out.
11. Add 10 drops of the pH indicator to each tube.
12. Cap and gently mix the tube.
13. Determine the pH using the color comparator boxes. The two samples should be within 0.25 of each other for duplicate precision. Record the data.
14. Dump the water into the waste bottle.

Dissolved oxygen

15. Wearing gloves, using the glass bottle in the dissolved oxygen kit, rinse twice with the stream water. In a well-mixed area of the stream hold the bottles underwater and allow to completely fill. There should be no air in the bottle – this can be done by capping the bottle underwater. Note this test needs to be done two times for duplicate precision.
16. Add 8 drops of Manganous Sulfate Solution to each sample bottle.
17. Add 8 drops of Alkaline Potassium Iodine Azide to each sample bottle.

18. Cap the bottles and invert to mix. Wait until the precipitate settles towards the bottom of the bottle before proceeding.
19. Add 8 drops of sulfuric acid. Cap and invert the bottles until the brown flakes dissolve. After this step is done, the solution is "fixed" and won't keep reacting.
20. Place 20 mL of the fixed sample in the glass titration vial.
21. Using the pink tip, fill the syringe to the 10mL line with Sodium Thiosulfate. Ensure there are no bubbles trapped in the syringe.
22. Place the syringe into the hole of the titration vial and slowly add Sodium Thiosulfate drop by drop. Make sure to mix between each drop. Repeat until the solution is a pale-yellow color.
23. Carefully remove the syringe and do NOT disturb the syringe. Add 8 drops of Starch Solution to the titration vial and mix. Solution will turn a dark blue.
24. Continue titrating Sodium Thiosulfate drop by drop. Make sure to mix between each drop. Repeat until the solution turns clear.
25. Record the number on the syringe to indicate the amount of dissolved oxygen in your sample. For duplicate precision, the two samples should be within 0.6ppm of each other.

Macroinvertebrate Monitoring (Quarterly)

Materials

- D-frame net or kick seine net (depending on stream type)
- Plastics spoons
- Magnifying glasses
- Collection tray (white)
- Ice cube tray
- Jug for rinsing out macros from nets
- Boots or waders for going into the stream
- Macroinvertebrate sampling sheet (found on the AAS website)

Methods

Table 2: Methods Used based on Stream Type

	Methods		**Habitat Type**		
	Net Used	**Area Sampled per net (square feet)**	**Vegetative Margins**	**Organic Matter**	**Substrate**
Rocky Bottom	Kick Seine	2x2	0	4	3
Muddy Bottom	D-Frame	1x1	7	4	3

1. Determine the type of stream you have and use the table above to collect your samples.

<u>Rocky Bottom</u>

2. Find three different riffle areas to sample. Place the kick seine in water and use rocks to hold the bottom of the net down. Wash off rocks into the kick seine to collect samples.
3. Then kick the streambed with your feet doing the "macro boogie" to loosen samples from the stream bed and collect them with the kick seine net.
4. Lift the kick seine and wash the contents into a bucket.
5. Repeat steps 2 – 4 three times.
6. Look for decayed leaf packs and add 4 handfuls to your sample bucket.
7. Continue to step 13.

Muddy Bottom

8. Use the D-frame to sample different habitats that include vegetated margins, woody debris with organic material, and streambed material. Make sure to move the net one foot forward with each collection.
9. Collect 7 scoops of vegetated margins and place them in bucket.
10. Collect 4 scoops of organic matter and place them in bucket.
11. Collect 3 scops of substrate and place in bucket.
12. Continue to step 13.

Macroinvertebrate Sorting (regardless of stream site)

13. Use the macroinvertebrate identification chart to sort the macroinvertebrates that you find in your sample.
14. Use the Macroinvertebrate sheet from the Adopt-A-Stream website to calculate the biotic index of the stream based on the diversity of the macroinvertebrates you have found.
15. Return all samples to the stream.

Protocols adapted from the following sources:

Georgia Adopt-A-Stream. 2014 *Bacterial Monitoring*. Georgia Environmental Protection Division. [accessed 2025 Aug 17]. Available from: https://adoptastream.georgia.gov/data-forms-2/aas-manuals

Georgia Adopt-A-Stream. 2015 *Macroinvertebrate & Chemical Stream Monitoring*. Georgia Environmental Protection Division. [accessed 2025 Aug 17] Available from: https://adoptastream.georgia.gov/data-forms-2/aas-manuals

APPENDIX B

Answers to Case Study

Part I

Questions:

1. What would you expect to see in healthy stream?
   Answers will vary but would expect to get answers including clear water, fish, vegetation along the banks, etc.
2. How would you expect an impacted stream to look different than a pristine stream?
   Answers will vary but would expect to get answers including cloudy water, slight odor, eroded banks, etc.
3. How would you expect a stream before a contamination event to look versus a stream after a contamination event?
   Answers will vary but would expect to get answers including dramatic change in color, fish kills, strong odor, etc.

Part II

Questions:

4. Given these indicators, what specific differences would you expect to see between pristine and impacted streams?

	Pristine	Impacted
Conductivity	Lower	Higher
pH	Near neutral	Lower or higher
Biological score	Higher	Lower
Turbidity	Lower	Higher
E. coli	Lower	Higher

5. Given these indicators, what specific differences would you expect to see before and after a contamination event?

	Pre-event	Post-event
Conductivity	Lower	Higher – ions in solution
pH	Near neutral	Lower or higher – depends on the contamination
Biological score	Higher	Lower – loss of living organisms
Turbidity	Lower	Higher – contaminate clouding water
E. coli	Higher	Lower – loss of bacteria

6. How long do you think it would take a stream to recover from a contamination event?
   Recovery should be relatively quick on the order of hours to days.

7. What factors would affect the rate of recovery?
   Factors that might influence the rate of recovery include stream discharge (cross sectional area times the flow velocity), quality of the stream margins, stream bed material, etc.

Part III

Questions:

8. Observe the y-axis for each water quality indicator between the pristine and impacted stream, list the maximum and minimum values observed for each variable. (values are approximate based on graphs provided)

	Pristine	Impacted
Conductivity (µS/cm)	25 – 60	40 – 180
pH	6 – 7.4	6 – 7.4
Biological score	14 – 27	1 – 25
Turbidity (NTU)	1 – 40	5 – 75
E. coli (CFU/mL)	2 – 85	8 – 85

9. Which variables are higher for the pristine stream compared to the impacted stream?

Higher for pristine stream: biological score

Higher for impacted stream: conductivity, turbidity

10. Which variables are similar between pristine and impacted stream?

Similar between streams: pH, E. Coli

11. Which variables are notably different in the impacted stream pre and post contamination event? (refer to table provided for values)

Post contamination event the pH dropped, the biological score dropped, and the turbidity increased.

12. How quickly did the impacted stream recover from the contamination event?

The impacted stream recovered within the year, many of the indicators improved by the second water sampling event after the spill.

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