**Stream Biodiversity and Function**

# Learning objectives

By the end of this lesson, students should be able to:

* Recall specific chemical, physical, and biological indicators of stream health
* Calculate stream health using a biotic index
* Compare local sites in terms of stream health
* Plot stream health from different EPA regions using R
* Compare, contrast, and summarize patterns of stream health locally and nationally

## Introduction

A mere 3% of the earth’s water occurs as fresh water, and less than 1% is the relatively free flowing surface waters of lakes, rivers, and streams. The physical characteristics of streams or lakes, such as particle size of sediment, flow velocity and volume, temperature, oxygen content, and nutrient concentrations, combine to create more or less favorable habitat for organisms.

Monitoring programs are often concerned with changes to these physical characteristics and how they affect the biodiversity of the flora and fauna. Rivers and streams offer a unique habitat to aquatic organisms because the constant flow of water provides a source of food and nutrients, a ready disposal system for waste, renewal of oxygen content, and influences the microhabitat characteristics for organisms. Streams typically contain fewer species of plants compared to terrestrial or lake ecosystems, in part because lower levels of sunlight result in less primary production and because the water flow minimizes the nutrients available within the water column for plant life. Lakes are most strongly defined by the light penetration (which can be affected by factors such as silt and phytoplankton growth), temperature differentials from the surface to greater depths, and the oxygen content (which is increased by wind and internal currents, and decreased by respiration and decomposition).

**Ecosystem ecology** focuses on the movement of energy and nutrients through organisms and their environment. Energy from the sun is fixed into carbon-based molecules in primary producers and then transferred to herbivores, carnivores, and detritivores in multiple and overlapping pathways. What are other pathways in which energy enters a biotic food web?

In terrestrial systems, much of the incoming energy is fixed by green plants. In freshwater streams, the biomass of aquatic plants is much smaller and consequently makes up a minor portion of the total energy input to the stream ecosystem. Energy inputs are from outside the stream ecosystem (called “allochthonous inputs”) and can include leaf litter, woody debris, animal carcasses, and wind- or tree-fall organisms (e.g. caterpillars). This material can make up to 99% of the total energy input to woodland streams, and close to a third of this material can be leaf litter (Fisher and Likens 1973). Thus, understanding how this leaf litter and other debris are processed by stream organisms is important for ecosystem ecologists who want to quantify how energy and nutrients are moving in the stream ecosystem.

To characterize your study site, we will collect data on the primary producers and benthic invertebrates which indicate ecosystem health (**bioindicator species**), as well as the chemical composition of the site that allows for organic growth (**chemical indicators**). The combination of chemical indicators and biological indicator species will allow us to determine the 1) specific types of pollution at a snapshot in time, as well as measure the 2) overall ecosystem health and average water conditions over time. Importantly, these measurements allow for comparisons over time and between sites, which allows us to say when and where conditions are better—or not.

References

Fisher, S.G. and G.E. Likens. 1973. Ecological Monographs 43:421-439.

Sparkes, T.C., C.M. Mills, L.A. Volesky, J.A. Talkington, J.S. Brooke. 2008. The American Biology Teacher, 70(2): 90-94.

## Chemical and Physical Indicators of Ecosystem Health:

Water Pollution in the United States and the Clean Water Act

The Federal Water Pollution Control Act of 1948 was the first major law to address water pollution in the United States. Growing public concern for water quality and highly publicized events, such as the Cuyahoga River in Cleveland, Ohio catching fire, led to a restructuring of this law and in 1972 it was renamed The Clean Water Act. Under the Clean Water Act, laws were added which made it illegal for any person to discharge any pollutant from a point source into navigable waters (without permit), and also recognized the need for planning to address non-point source pollution into waterways.

Point vs. Non-point Source Pollution

**Point source pollution** is pollution that comes from a single source, such as a factory, storm drainage pipe, or wastewater treatment plant. Basically, if you can identify one specific point where pollution enters water it can be considered a point source of pollution. Historically, point source pollution was the biggest threat to water quality in the United States. However, the Clean Water Act and laws making it illegal to discharge pollutants into water has reduced what once was our biggest threat to water quality. While this has not eliminated industrial or domestic waste from entering our waters completely, it has reduced the amount and kinds of pollutants that can be dumped directly into rivers and lakes.

**Non-point source pollution** does not have one specific source, such as a drainage pipe dumping pollutants into a river. Non-point source pollution comes from the cumulative effect of a community’s residents going about their everyday activities, such as fertilizing a lawn, adding pesticides to a garden, pet wastes, and household hazardous wastes.

As a case study, consider lawn fertilizers as a non-point source pollution. Fertilizers contain nitrogen compounds called nitrates. When fertilizer is applied to a lawn or garden, and there is a rain event, some of the fertilizer washes off the lawn and enters the surface water. This surface water can directly enter a river or lake, or a storm drain system, and bring along with it the nitrates in the fertilizer. Nitrates in the water enhance aquatic plant growth, just as they were intended to enhance your grass or garden to grow. Although plants are an important part of aquatic ecosystems, too many can potentially block sunlight from reaching plants that are lower in the water column. Additionally, when plants die the associated decomposers may consume significant quantities of oxygen within the water. Thus, non-point source pollution can have effects on aquatic communities that are widespread and difficult to manage.

Monitoring Water Quality and Water Chemistry

Water chemistry plays an important role in the health, abundance, and diversity of aquatic life in a waterbody. Excessive amounts of some chemicals such as nitrates, or the lack of others such as dissolved oxygen, can result in imbalances in water chemistry. Imbalances can degrade aquatic conditions and harm aquatic life. An imbalance in chemical measurements can also make water unsuitable for human consumption or use, and greatly increase the cost of water treatment before it can be used. The following are a few physical and chemical measurements that are commonly used to monitor water quality and overall health of aquatic ecosystems. Note that there are several different types of instruments to collect these measurements, with the majority of measurements ideally taken via specific probes immediately at the stream site, rather than on-site collection for later measurement in a lab.

*Water Temperature*

Water temperature directly affects most of the physical, chemical and biological characteristics of bodies of water. For example, **temperature directly affects the amount of dissolved oxygen in water; in general, colder water can hold more dissolved oxygen than warmer water.** So simply adding warm water, which is not regulated the same as adding other pollutants, can deplete dissolved oxygen levels in water and kill aquatic organisms. Similarly, the metabolic (respiration and digestion) rates of organisms, such as fish, aquatic insects and aerobic bacteria, increases with higher temperatures, meaning they need more oxygen to survive. Additionally, although water temperatures will fluctuate naturally throughout the year, humans can also increase water temperature by altering the plant coverage (shade) along waterbodies and by adding warmer water from industrialized areas to bodies of water. Thus, water temperature changes happen and can have large effects on ecosystems.

*pH (Parts Hydrogen)*

Water (H2O) contains both H+ (Hydrogen ions) and OH- (hydroxyl ions). “pH” is an

abbreviation for the French expression, “Pouvoir Hydrogene,” meaning “the power of

Hydrogen.” It measures the H+ ion concentration of substances and gives results on a scale

from 0 to 14. Water that contains equal numbers of H+ and OH- ions is considered neutral and will have a pH 7 (e.g., distilled drinking water). If a solution has more H+ than OH- ions, it is considered acidic and has a pH less than 7. If a solution contains more OH- ions than H+ ions, it is considered basic with a pH greater than 7. The pH scale is logarithmic, which means that every one-unit change on the pH scale is a ten-fold change of the sample.

In the United States, the pH of rivers is usually between 6.5 and 9.0, and because rain water is naturally acidic (pH~ 5.6), rain can turn rivers and lakes slightly acidic, but usually within tolerable ranges. However, **acid rain,** produced from byproducts of either natural geological formations or industrial processes, can have a pH ranging from 4.5 to as low as 2.0. Because most organisms have adapted to life in water of a specific pH, slight pH changes can harm the organisms and occasionally lead to death, but at extremely high or low pH values (12.0 or 3.5), water becomes lethal to most organisms.

*Nitrates*

Nitrogen is an essential nutrient required by all living plants and animals for building proteins. Excess amounts of nitrogen compounds in rivers and lakes can result in unusually large populations of aquatic plants and/or organisms that feed on plants. For instance, some algal blooms are a result of excess nitrogen entering the waterbody. For this reason, excess nitrogen can be considered a chemical pollutant. We will be measuring the levels of nitrates (NO3) as an indication of nitrogen-based pollution entering the river, and as a measure of increased rates of decomposition within the river. Decomposition is a natural process and healthy process in aquatic ecosystems, but recording nitrates in higher levels than expected or in different areas of the same water body can be an indication of an unbalanced ecosystem.

*Phosphorus*

Phosphorus is also an essential plant nutrient and is most readily available to aquatic plants in the form of phosphate (PO4). In many instances, phosphate can be the nutrient that limits or prevents plant growth, although in nature, phosphate it is generally present in very low levels measured in tenths or hundredths of a mg/L. So, even small increases in the amount of phosphorus entering a stream can have a large impact on plant growth. If point source or nonpoint sources of pollution are high in phosphate, they can over-stimulate the growth of all types of aquatic plants. Increased plant growth due to excess phosphorus can lead to higher decomposition rates and ultimately higher levels of nitrogen in the water, and such growth can also cover water surfaces and physically block sunlight from reaching plants that are lower in the water column. Without sunlight, these plants no longer conduct photosynthesis or produce dissolved oxygen in the water.

*Turbidity*

Turbidity is the measure of relative clarity of a liquid, measured as the amount of light that is scattered by material in the water when a light is shined through the water sample. The higher the intensity of scattered light, the higher the turbidity. Light scatters due to the material, or particulates, found in water, such as clay, silt, inorganic and organic matter, algae, soluble colored organic compounds, and microscopic organisms. High particulate concentrations affect light penetration, and thereby the productivity and habitat quality of an area, and are also know to provide attachment places for other pollutants, notably metals and bacteria. For this reason, turbidity readings can be used as an indicator of potential pollution in a water body.

*Conductivity*

Specific conductance (shorthand ‘conductivity’) is a commonly-measured water quality parameter than can indicate change in water systems. Most bodies of water maintain a fairly constant conductivity that can be used in comparisons between sampling sites or through time. Differences, whether due to natural flooding, evaporation or man-made pollution, can be very detrimental to water quality and are best detected early using conductivity.

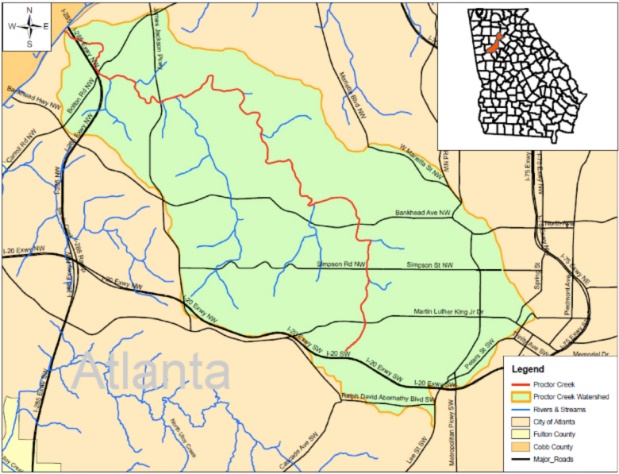
*Available Light*

Photosynthesis provides the primary production necessary to sustain ecosystems. However, photosynthetic reactions (and the organisms reliant on them) can only function with adequate light. Photosynthetic Active Radiation (PAR) is measured to quantify the amount and potential effects of available light on ecosystems. This information is highly-depth specific, as light penetration through water decreases with depth, but even shallow areas can have low light availability due to contaminants in the water and canopy coverage—both of which may change over time.

Sanitary Sewers and Storm Sewer Systems

Many people incorrectly believe waste that is dumped into the storm sewer is treated at a wastewater treatment plant. In fact, many metropolitan and suburban areas have separate storm and sanitary sewer systems. Sanitary sewers collect wastewater from dorms, academic buildings and residential homes and treat it before discharging it back into waterways. Storm sewers, on the other hand, are a direct connection to the city's waterways. Issues can arise when heavy rains flood an area overloading these systems.

**A Short History on Proctor Creek and The Emerald Corridor of Atlanta**

Proctor Creek, which starts in West Atlanta near I-20 and winds its way to the Chattahoochee, was historically a place of importance for locals. It has gone from a fishing and swimming hole where local churches baptized generations of families, to a polluted and largely neglected urban creek. The health of the surrounding community has followed this decay.

During the construction of many of the nearby sports arenas, the natural water table was disrupted. This caused massive flooding in the surrounding area through water diverted into overflowing sanitary and sewage systems. Combined with litter dumping, these overflows have damaged the ecological health of the streams, which is only now recovering.

Recent efforts have begun to revitalize the creek and community. Volunteers and members of the Chattahoochee Riverkeeper and West Atlanta Watershed Alliance, as well as USFW, USDA, and the EPA, have been working together to monitor Proctor Creek and assess its health. The nonprofit Emerald Corridor Foundation has also begun plans on Proctor Park, a 9.2-acre greenspace including a natural wetland to help to control and buffer the creek to pollution.

In 2013, the Urban Waters Federal Partnership designated Proctor Creek to be a priority Urban Waters location. Through this program, there is increased coordination and focus among federal agencies and partners on problems in the watershed. Solving these problems will lead to economic, social, and ecological revitalization in the area; ***your data through the ecological experiments we conduct in this course will contribute to these efforts.***

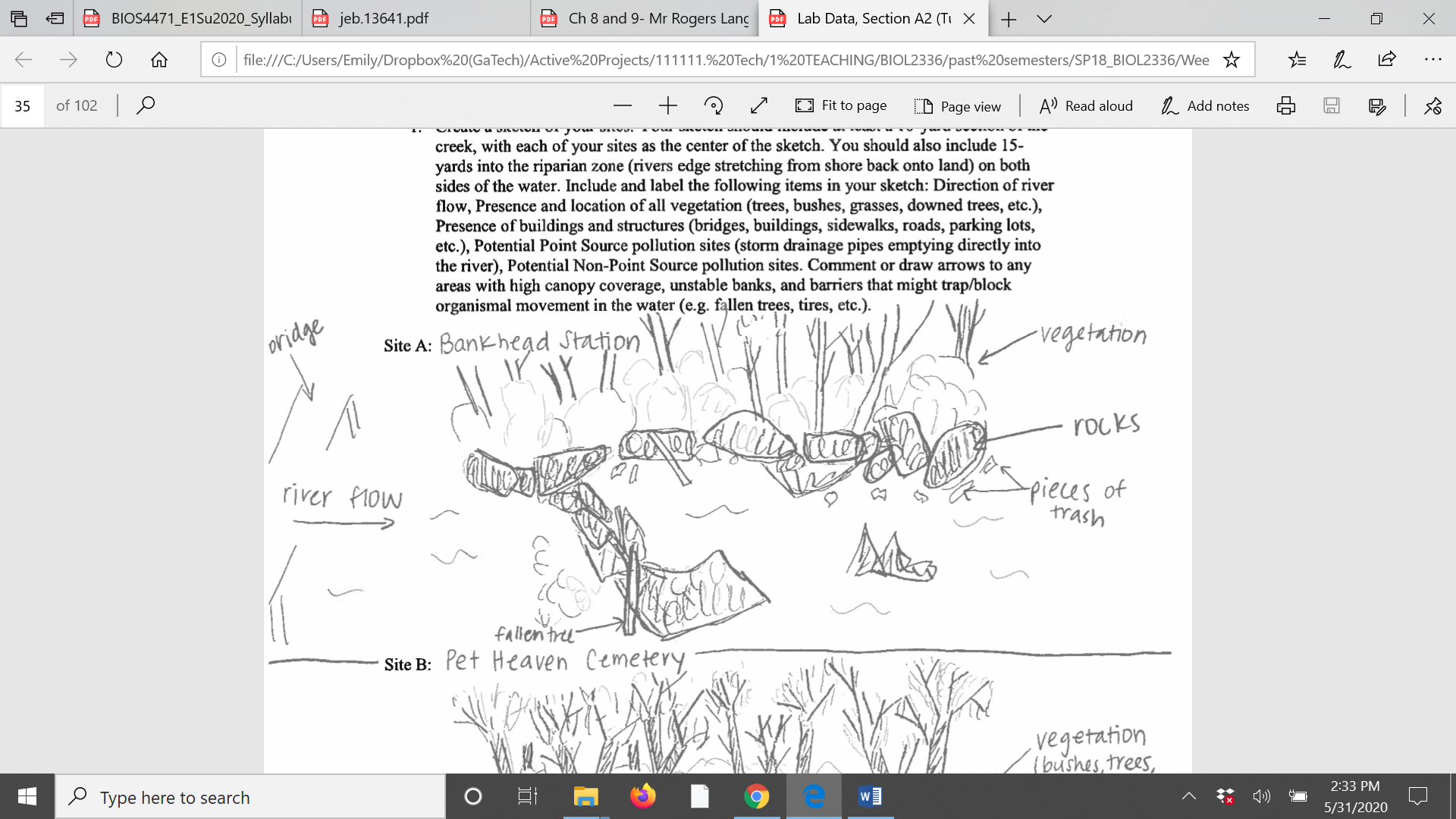
*For more information on the creek and surrounding watershed, see here:*

* <http://www.atlantawatershed.org/inside-dwm/offices/watershed-protection/atlantas-watersheds/the-proctor-creek-watershed/>
* <http://www.proctorcreek.org/creek-data.html>

**Data to Collect in the Field**

You will visit two sites (Site A: Bankhead Station and Site B: Pet Heaven Cemetery) over the next two weeks of lab. Each week, you will be recording a bit about these sites to understand more about Proctor Creek’s Ecology

1. Create a sketch of your sites. Your sketch should include at least a 10-yard section of the creek, with each of your sites as the center of the sketch. You should also include 15-yards into the riparian zone (rivers edge stretching from shore back onto land) on both sides of the water. Include and label the following items in your sketch: Direction of river flow, Presence and location of all vegetation (trees, bushes, grasses, downed trees, etc.), Presence of buildings and structures (bridges, buildings, sidewalks, roads, parking lots, etc.), Potential Point Source pollution sites (storm drainage pipes emptying directly into the river), Potential Non-Point Source pollution sites. Comment or draw arrows to any areas with high canopy coverage, unstable banks, and barriers that might trap/block organismal movement in the water (e.g. fallen trees, tires, etc.).

Example: 

Site A:

Site B:

1. Conduct tests for chemical and physical characteristics of each site. Follow the directions in each kit/probe closely as this will influence your results. Record results below. Additional space is provided below, should you wish to use the sensors or other equipment in your investigations of ecosystem health.

Site A:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Measurement | Replicate 1 | Replicate 2 | Replicate 3 | Average |
| Temperature (C°) |  |  |  |  |
| pH |  |  |  |  |
| Nitrates (NO3-) ppm (mg/L) |  |  |  |  |
| Light (µmol s-1m-2) |  |  |  |  |
| Conductivity (SPC) |  |  |  |  |
| Turbidity (FNU) |  |  |  |  |
| Phosphates (PO4-3) ppm (mg/L)\* |  |  |  |  |

Site B:

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| --- | --- | --- | --- | --- |
| Measurement | Replicate 1 | Replicate 2 | Replicate 3 | Average |
| Temperature (C°) |  |  |  |  |
| pH |  |  |  |  |
| Nitrates (NO3-) ppm (mg/L) |  |  |  |  |
| Light (µmol s-1m-2) |  |  |  |  |
| Conductivity (SPC) |  |  |  |  |
| Turbidity (FNU) |  |  |  |  |
| Phosphates (PO4-3) ppm (mg/L)\* |  |  |  |  |

\*Note: You will only need to conduct to conduct one of these tests, given the low variation in this measurement at a given field site.

Other Notes:

## Biological Indicators of Ecosystem Health:

Calculating the Biotic Index of River Health (BI)

Because macroinvertebrates have different sensitivities, or tolerances, for growth and development in the presence of different forms of pollution, these **bioindicators** can be used by ecologists to assess and compare ecosystem health through time and space.

Each macroinverterbrate has a tolerance value (**a**) that indicates level of pollution tolerance. The tolerance scale is on a 0-10 ranking system, where 0 means the organism does not tolerate pollution at all and a 10 is given to an organism that can survive in very polluted waters. Thus, a macroinvertebrate with a **low** tolerance value is **not tolerant** to pollution, and a macroinvertebrate with a **high** tolerance value **can tolerate** pollution. The higher your overall Biotic Index, the more polluted the system.

*This is how to calculate a General Biotic Index using macroinvertbrates:*

1. You counted 24 Mayflies in your sample (n)
2. Mayflies have a Tolerance Value of 4 (a)
3. Multiply n (24) X a (4) = 96 *(see the top row of the table below)*
4. Repeat for each Macroinvertebrate in the entire sample
5. Add the values in the n\*a column and divide by the total number of organisms

Calculate the Historic Biotic Index for Proctor Creek (below). Complete before lab; will be checked

Site: **Proctor Creek** Date: October 17, 1963

Sum (n\*a): \_\_\_\_\_\_\_\_\_\_\_ / Total n: \_\_\_\_\_\_\_\_\_ = Biotic Index (BI) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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| --- | --- | --- | --- |
| Macroinvertebrate Taxa | Number (n) | Tolerance Value (a) | n\*a |
| Ephemeroptera (mayflies) | 24 | 4 | 96 |
| Plecoptera (stoneflies) | 35 | 1 |  |
| Trichoptera (caddisflies) | 46 | 4 |  |
| Odonata (dragonflies) | 18 | 3 |  |
| Coleoptera (beetles) | 52 | 5 |  |
| Megaloptera (dobsonflies) | 2 | 3 |  |
| Chironomidae (flies) | 25 | 7 |  |
| Amphipoda (scuds) | 39 | 4 |  |
| Isopoda (sowbug) | 12 | 7 |  |
|  | Sum n: |  | Sum: |

The Biotic Index (BI) generated is then compared to a range of values that correspond to overall Water Quality and Pollution Estimates (below).

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| **BI** | **Water Quality** | **Pollution Estimate** |
| <3.75 | Excellent | No pollution |
| 3.75-5.0 | Good | Low pollution input |
| 5.01-6.5 | Fair | Moderate pollution input |
| >6.5 | Poor | Heavy pollution input |

From your BI, what are the water quality and pollution estimates of the area historically?

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**Data to Collect in Lab**

1. Use dichotomous keys and other resources to identify the macroinvertebrates found at your site. Collect your invertebrate sample in each focal habitat using D-nets and other tools to sample the benthic community. Continue analysis by identifying macroinvertebrate organisms (take pictures for later identification/confirmation, if necessary).
2. Calculate the Biotic Index for your sample. Record your data in the blank table below. Use the above table & online references as sources for tolerance values.

Site A: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_** Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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| Macroinvertebrate Taxa | Number (n) | Tolerance Value (a) | n\*a |
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|  | Sum n: |  | Sum: |

Sum (n\*a): \_\_\_\_\_\_\_\_\_\_\_ / Total n: \_\_\_\_\_\_\_\_\_ = Biotic Index (BI) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Site B: **\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_** Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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| --- | --- | --- | --- |
| Macroinvertebrate Taxa | Number (n) | Tolerance Value (a) | n\*a |
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|  |  |  |  |
|  | Sum n: |  | Sum: |

Sum (n\*a): \_\_\_\_\_\_\_\_\_\_\_ / Total n: \_\_\_\_\_\_\_\_\_ = Biotic Index (BI) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Average Biotic Index of Proctor Creek: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Interpreting Your Results**

1. Given your chemistry samples, what can you say about the health of Proctor Creek? *Justify your answer using the range of values that might be expected in urban waters.*

1. What was the average Biotic Index of Proctor Creek, and what does this indicate about the water quality and pollution estimate of the site? How does it compare to the site years ago?
2. Explain two potential limitations to using the following methods to assess water quality:
   1. Chemical Tests
   2. Biotic Index
3. When assessing water quality, under what circumstances might it be more appropriate to use chemistry techniques? Biotic index? *In your answer, make comparative statements between biotic and abiotic techniques to assess pollution, and state factors (such as specific time and space variables) which might influence either or both types of tests.*
4. Explain at least one way that ecology of local streams ties into local health and activity of human communities.
5. Imagine you had unlimited time, funding, and people to help you execute a stream monitoring and recovery program. What experiments or restoration activities would you prioritize?

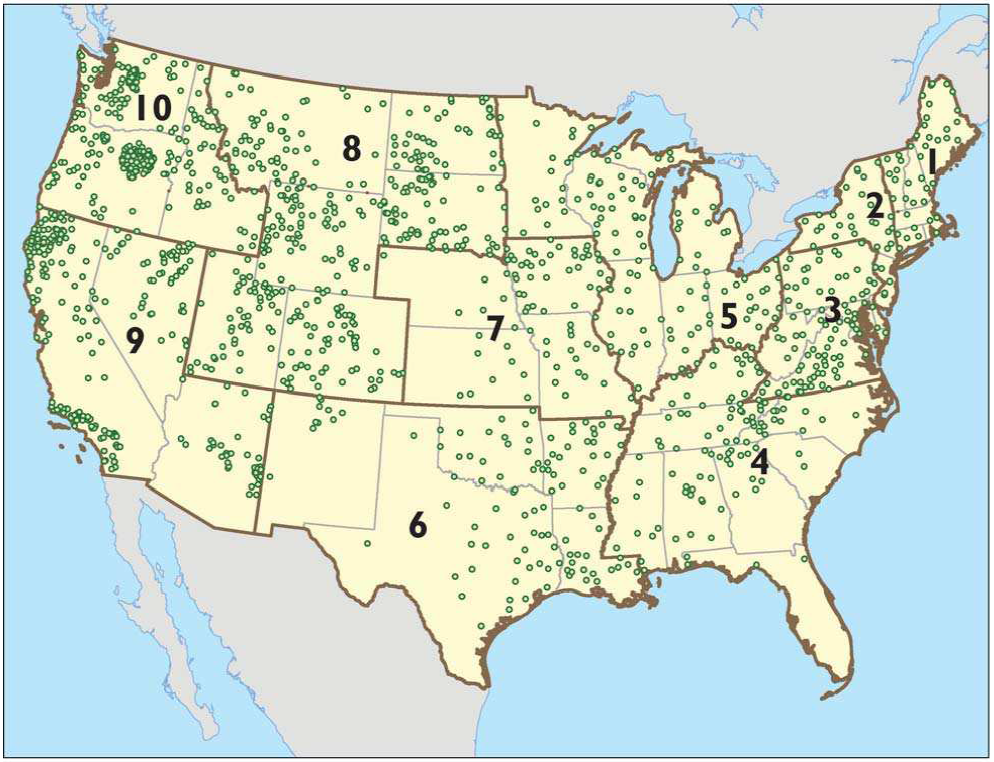
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***Going further: Examining Stream Health at a National Scale***

Adapted from: Nuding, A. and Hampton, S. (2012). Investigating human impacts on stream ecology: locally and nationallyTeaching Issues and Experiments in Ecology, Vol. 8: Practice #1.

We will now expand our view to address total nitrogen and total phosphorus levels nationwide. These two nutrients are among the most common pollutants in streams, lakes and coastal waters and high levels of these nutrients result in degraded water quality (EPA 2007).

## Wadeable streams



**Figure 1**. Wadeable streams that were sampled within each of the ten designated EPA regions.

In 2002, the US Environmental Protection Agency (EPA) set out to characterize the health of all the waterways throughout the continental US. The EPA is required by law as set forth in the Clean Water Act to report to Congress on the health of the nation’s waters. This survey of “wadeable” streams – streams shallow enough to sample without a boat – is the EPA’s largest effort to make a scientifically and statistically defensible claim about how healthy, or unhealthy, the nation’s waters are (EPA 2007). Wadeable streams provide a strong link between land use and water quality, and they contribute to larger river systems, so they are a good indicator of the health of waters throughout the entire US (EPA 2007). Even though wadeable streams are relatively small and shallow, they comprise about 90% of the length of all perennial waterways.

A total of 1,392 sites were sampled in the 48 states and grouped into 10 regions (Figure 1). The sampling was designed to ensure that the site selection was representative and random.

Just as you did locally, we will now will be looking at concentrations of total nitrogen (µg/L), total phosphorus (µg/L), and macroinvertebrate levels across regions of the IS.

The EPA developed a method of characterization that worked best for the Wadeable Stream Assessment and called it “MMI” for “Multi Metric Index” (EPA 2007). It includes measures of macroinvertebrate taxonomic richness, composition, and diversity, as well as feeding groups, habits, and pollution tolerance and assigns a MMI score to each stream. More information about these groups and measures can be found in the WSA report (Ch 2, pgs. 27 – 29). In all cases, a higher value indicates a greater diversity of organisms and is considered to be indicative of a healthier stream. A high MMI score (max = 100) tends to indicate a healthy stream, and low score (min = 0) tends to indicate an impaired stream.

## Boxplot refresher

A common first step in analyzing the distribution of data points is through the use of box plots. Box plots show you where the median of your data is – the point at which half (50%) of the data points are below that value, and half of them are above. The median is shown as the thick black line in the “middle” of the box (it doesn’t have to be centered though!). The lower quartile, Q1, is the point below which 25% of the data is contained. The second quartile (Q2) is the same as your median. The upper quartile, Q3, is the point below which 75% of the data is contained. The upper “extreme” is the largest value that is less than Q3+ 1.5\*(Q3-Q1), where Q3-Q1 is called the “inter-quartile range”. The lower “extreme” is the smallest value that is greater than Q1- 1.5\*(Q3-Q1). Potential “outliers” are points far outside the range of the majority of the data - specifically, if their value is larger than the upper extreme, Q3+ 1.5\*(Q3-Q1), or less than the lower extreme, Q1- 1.5\*(Q3-Q1).



**Exploring Data Sets**

1. **Land Cover**

Many agencies and other organizations that have a lot of ecological data, like the U.S. Geological Survey, provide those data online with some tools to explore the data. Look at the map of NLCD land cover ([Supplement 1](http://tiee.esa.org/vol/v8/issues/data_sets/nuding/resources/supplement1.pdf)), or on a website provided by your instructor, to answer the following questions. You may find it helpful to print out a color copy of [Supplement 1](http://tiee.esa.org/vol/v8/issues/data_sets/nuding/resources/supplement1.pdf), or view it on your screen at a larger size (e.g., 200%) to see more detail.

1. **What are the dominant types of land cover in your region? How do these things compare to what you observed locally?**
2. **Based on your knowledge of sources of nutrients, which EPA Region(s) do you predict will have the highest and lowest nutrient values? If you are using an online tool to determine land cover of vegetation types you can refer to** [**Supplement 2**](http://tiee.esa.org/vol/v8/issues/data_sets/nuding/resources/supplement2.pdf) **for a map of the EPA Regions.**
3. Now look at the table of mean (average) values of total nitrogen (NTL) and total phosphorus (PTL) in each EPA region, located in [Supplement 3](http://tiee.esa.org/vol/v8/issues/data_sets/nuding/resources/supplement3.pdf). **Which regions have the lowest and highest mean nutrients, and how does this compare with your prediction? Are the mean values useful at characterizing streams in a region? Why or why not? Offer some suggestions about what additional information would be useful to more fully characterize nutrient levels within an EPA region.**
4. **Nutrients**

A common first step in analyzing the distribution of data points is through the use of box plots.

1. **Open the data sheet in R. After opening the file, find the median, quartile, extreme and outlier values of total nitrogen for Regions 7 and 10 separately, as indicated in Table 1. The samples code is provided for Regions 7 and 10, but we are in Region 4. Modify the given code to achieve this data.**

1. Using these data, create box plots for each region (4, 7, and 10). What percent of the data lies within the box? Describe the distribution of the data outside the box, e.g. are the points relatively close or spread widely, where are outliers, etc. **Given what you have learned so far, would you expect an “outlier below Q1” to have more or less algae than other streams?**
2. **Based on the data and maps you’ve looked at, can you assert whether the waters in EPA Regions 4, 7 and 10 are “clean” or “polluted”?** Explain your answer using data from parts I and II. If you feel more information is needed to make an assertion, state what information you would need to have. Note: there is not one “right” answer here, so what is important is how well you explain your answer.
3. How does the region in which you live (Region 4) compare to other regions? Briefly hypothesize factors that may influence nutrient levels in your region.
4. **Macroinvertebrates**

The EPA developed a method of characterization that worked best for the Wadeable Stream Assessment and called it “MMI” for “Multi Metric Index” (EPA 2007). It includes the following six categories:

1. Taxonomic richness – the number of distinct taxa (e.g., species)
2. Taxonomic composition – a measure of the abundance of the ecologically important taxa in the sample
3. Taxonomic diversity – the distribution of the numbers of organisms in different taxa
4. Feeding groups – the distribution of macroinvertebrates that have different feeding habits (e.g. leaf shredders vs. algal feeders)
5. Habits –the distribution of macroinvertebrates that burrow, cling, crawl and/or swim
6. Pollution tolerance – a measure of how many taxa present are pollution tolerant and intolerant

More information about these groups can be found in the WSA report (Ch 2, pgs. 27 – 29). In all cases, a higher value indicates a greater diversity of organisms and is considered to be indicative of a healthier stream. A high MMI score (max = 100) tends to indicate a healthy stream, and low score (min = 0) tends to indicate an impaired stream.

1. Make a Prediction. **If we plotted the total nitrogen values (for the entire nation) on the x-axis, and the benthic macroinvertebrate index values on the y-axis, what do you think the graph would look like?**
2. Using actual WSA data**, create a scatter plot of MMI vs. NTL and MMI vs. PTL**, with a trend line for each. When you do a linear regression of MMI with NTL, and MMI with PTL, the R2 can tell you how well the trend line fits the data (how strong is the relationship between the x and y variables).
   1. How does it appear that the macroinvertebrate community changes at low and high levels of total nitrogen and phosphorus?
   2. What are the similarities and differences between the two graphs? Is this what you expected?
3. Because the nitrogen data have such a wide range of values (over three orders of magnitude) and many data points are clustered at one end, we may want to use the logarithm (log) of the nitrogen data to plot on the x-axis instead. **Create this plot with MMI on the y-axis and Log NTL on the x-axis, and add a trend line.**

**Comment on the results.** Explain why the numbers on the x-axis changed as they did - please give a sample calculation. How did the log transformation change the distribution of the points and the fit of the trend line (the R2 value)? Would you also perform this operation on the total phosphorus data? Why or why not?

1. **Construct a regional comparison of data.** Create one plot of MMI vs. Log NTL for each region. Be sure your axes on both graphs have identical value ranges to allow for easier comparison. In a paragraph, discuss differences in the slopes of the lines, and the distribution of points between the two plots. **Do these graphs demonstrate what you expected to see? Why or why not?**
2. **Synthesis Activities**

One of the primary purposes of the Wadeable Stream Assessment was to be able to make a statement about the condition of the nation’s waters. In this exercise you used the same data to look at distributions of nutrient data, to create charts of ecological trends both nationally and regionally, and connected these concepts to land use patterns. Write a 2-page response paper based on the following questions. Tables and diagrams are expected, and they do not count toward the page limit. Also, cite all your sources you use in your investigation (e.g. peer-reviewed papers, published reports, or datasets).

* 1. Give the nation a grade (A+ - F) based on its level of nutrient pollution in the waters. Your explanation for the grade should be based in part on the results and analyses done in this exercise, in part from external resources and in part from your original ideas. At the end, make suggestions about how the nation can improve its grade. *Be sure to include suggestions that can be implemented locally and ‘scaled up’ across the region.*
  2. Compare macroinvertebrate populations and stream conditions using EPA’s on-line WSA data plotter: <http://www.epa.gov/bioiweb1/statprimer/ProbabilisticSampling/index.html>. Water chemistry, habitat and watershed characteristics can affect macroinvertebrate populations. As a starting point you may want to think about the grade you gave the nation, and the EPA Regions that seem to be pushing that grade higher or lower, according to your explanation.

**Moving Forward:**

Atlanta, specifically Proctor Creek, has made some strides in recent years for restoration. But the creek’s health has a long history. Take a look at the two posted pdf files addressing historic EPA and other governmental responses to the creek’s pollution. Then reflect on making changes on the national scale. What have we learned from our local example? What measures do you think we can take to make water quality improvements nationwide?

**ADDITIONAL RESOURCES**

Benthic Macroinvertebrate Indices:

Stoddard, J.L., A.T. Herlihy, D.V. Peck, R.M. Hughes, T.R. Whittier, E. Tarquinio. 2008. A process for creating multimetric indices for large-scale aquatic surveys. Journal of the North American Benthological Society *27:* 878-891.

Klemm, D.J., K.A. Blocksom, F.A. Fulk, A.T. Herlihy, R.M. Hughes, P.R. Kaufmann, D.V. Peck, J.L. Stoddard, W.T. Theony, M.B. Griffith. 2003. Development and evaluation of a macroinvertebrate biotic integrity index (MBII) for regionally assessing Mid-Atlantic highland stress. Environmental Management. 31:656-669.

U.S. Environmental Protection Agency. 2001. Ambient water quality criteria recommendations: information supporting the development of state and tribal nutrient criteria. (<http://water.epa.gov/scitech/swguidance/waterquality/standards/criteria/aqlife/pollutants/nutrient/rivers_index.cfm>). Accessed April 17, 2010.

U.S. Environmental Protection Agency. 2009. Invertebrates as indicators. (<http://www.epa.gov/bioiweb1/html/invertebrate.html>) Accessed June 2010.