**Investigating human impacts on stream ecology – locally and nationally**

**Introduction**

Small streams are vital to the United States' major rivers because they deliver water and provide pathways for the movement of fish and other aquatic organisms. Humans rely on both large and small waterways for drinking, irrigation, industrial uses and transportation. Thus, if the water quality of any particular stream is impacted, it affects not only *local* fish, aquatic organisms and plants, but also non-local organisms, in other parts of the state or country, and ultimately the livelihood of humans. While the term “environmental health” is a scientifically controversial term (Simberloff 1998), we may find it useful to think about stream “health” as describing the conditions that humans find desirable such as water with low levels of pollutants and pathogens, high levels of oxygen, and providing otherwise good habitat for fish and wildlife (Meyer 1997; Gordon et al. 2004). A variety of factors affect which aquatic organisms are present, such as the stream's size and morphology, geographic location, stream flow (volume and speed of water), available light, temperature, and water quality (Vannote et al. 1980; Poff 1997).

In this exercise we will examine two water quality parameters of great importance in streams: nitrogen and phosphorus. These two nutrients are among the most common pollutants in streams, lakes and coastal waters, resulting in degraded water quality (EPA 2007). Nitrogen and phosphorus generally come from fertilizer applied to farm lands (and from lawns, to a lesser extent), as well as sewage, and they are carried by ground water, rain water, or irrigation water from the land into streams (Vitousek et al. 1997; Smith 2003; Dodds 2006; EPA 2007). In addition, these nutrients may be deposited on land or water through the air after the combustion of fossil fuels or other industrial operations (Driscoll et al. 2006; Pepper et al. 2006). Specifically in this activity you will be looking at values of “total nitrogen” and “total phosphorus,” two very commonly measured water quality parameters. Total nitrogen includes all organic and inorganic nitrogen-containing compounds in the water. Inorganic forms are nitrate (NO3-), nitrite (NO2-), ammonia (NH3), and ammonium (NH4+). Organic forms include proteins, peptides, nucleic acids, urea and synthetic organic materials (Pepper et al. 2006). Total phosphorus, similarly, includes all phosphorus-containing compounds in the water, which includes orthophosphate (PO4) and organically bound phosphate.

When these nutrients become very high, *algae* (photosynthetic organisms) can grow extremely quickly and the waters can become cloudy, reducing the light availability in the stream. Importantly, when these algal blooms die, this large amount of dead algae fuels bacterial growth in the water. The bacteria decompose the algae and in doing so they consume much of the oxygen available in the water (Mallin et al. 2006). In many cases the waters become uninhabitable by aquatic organisms because of the lack of dissolved oxygen in the water. An extreme example of this is the “dead zone” in the Gulf of Mexico, which is an area of the ocean about as large as Connecticut, in which few aquatic organisms can survive, primarily due to the input of nutrients from the Mississippi River (USGS 2010).

Aquatic *benthic* *macroinvertebrates* are insects and other small invertebrates (like crustaceans, mollusks and aquatic worms) that live in streams and other aquatic habitats. “Benthic” refers to the lowest level of a water body, which in this case is the stream bed, and this is where these animals reside. “Macro” means that these animals are large enough to be seen with the naked eye, and “invertebrates” means that they have no spine. Many common flying insects have larval stages in streams and lakes, such as mayflies, stoneflies and dragonflies. These stream macroinvertebrates play very important roles in the stream ecosystem. For example, by shredding leaves and other detritus that falls into streams, they convert terrestrial carbon and other nutrients into forms available to other stream organisms (Vannote et al. 1980; Wallace and Webster 1996). Some macroinvertebrates eat algae, and others are predators on small invertebrates. Most macroinvertebrates eventually become an important food resource to fish and birds (Vannote et al. 1980; Wallace and Webster 1996).

Because benthic macroinvertebrates are such important members of the food web and they respond relatively quickly to ecological changes, they can be very useful to humans as indicators of the health of a stream. Accordingly, they are called “bioindicators.” Some macroinvertebrates require high levels of oxygen and low pollutant levels, and so they are useful as indicators of good water quality, while other groups of macroinvertebrates can tolerate low oxygen and high pollutant levels, thereby indicating lower water quality (EPA 2007). They are not highly mobile (especially compared with fish) and so they are susceptible to the water quality conditions around them and whatever pollutants may have accumulated in the sediment of the stream bed.

**Data Set Description**

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).

A statistically sound sampling design was necessary for the EPA to be able to detect major trends in stream quality across the nation. Ideally the EPA would take samples from every waterway in the US, but that is totally infeasible; it would be a huge, expensive, and very time-consuming endeavor. Therefore, the EPA devised a sampling regime of wadeable streams. Wadeable streams provide a strong link between land use and water quality, and they contribute to larger rivers 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 Lower 48. Pilot data was collected in Alaska and Hawaii to inform inclusion in future assessments. In Alaska, the pilot data was collected by the Alaska DEC and the University of Alaska Anchorage in the Tanana River basin, around Fairbanks (Rinella et al. 2009). The type of sampling design selected for any ecological study or experiment is key to making more general assertions about the status of waterways throughout the nation. The sampling was designed to ensure that the site selection was representative and random. More information about the sampling design can be found in Chapter 1in the section of the WSA Report entitled “How Were Sampling Sites Chosen?” (pgs. 15 – 17, <http://www.epa.gov/owow/streamsurvey/pdf/WSA_Assessment_May2007.pdf>). More information about the pilot program in Alaska can be found in its final report (<https://dec.alaska.gov/media/16835/tanana-wadeable-streams-ecological-condition-2004-2005-adec.pdf>)

**Literature Cited**

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**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)) to answer the following questions. You may find it helpful to 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 Region 10 (Note: Pacific Northwest Region also includes Alaska, which isn’t shown on this map, so just answer this question for the portion of Region 10 shown on this map)? Based on your knowledge of sources of nutrients, which EPA Region(s) (numbered in white) do you predict will have the highest and lowest nutrient values?
2. Now look at the table of mean (average) values of total nitrogen (NTL) and total phosphorus (PTL) in each EPA region, located in [Supplement 2.](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.

3. Look at the map of Alaska’s NLCD land cover ([Supplement 3](https://landsat.gsfc.nasa.gov/wp-content/uploads/2015/02/20150218_nu1.png), note: the legend for this map can be found here - <https://www.mrlc.gov/data/legends/national-land-cover-database-2011-nlcd2011-legend>). Then look at the map of where the pilot data was collected for Alaska ([Supplement 4](https://dec.alaska.gov/media/9057/2004-streams-survey-sites.pdf)). What are the dominant types of land cover in that region where pilot data was collected in Alaska? How does this compare to what you found for Region 10 (Question 1)?

1. **Nutrients**

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 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. To find Q1, you take the median of the data points that lie between your lowest value and Q2. To find Q3, you take the median of the data points that lie between your highest value and Q2. The upper extreme is the largest value that is less than Q3+ 1.5\*(Q3-Q1). The lower extreme is the smallest value that is greater than Q1- 1.5\*(Q3-Q1). “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).

4. Open the WSA data table (WSA\_data\_for\_students.xls). After opening the file, find the median, quartile, extreme and outlier values of total nitrogen for Regions 7 and 10 and Alaska separately, as indicated in Table 1. The data for Alaska are in a separate tab “Alaska WSA Pilot Data”. The median and quartiles can be found using the following Excel functions.

The parentheses should contain the column or row of data you wish to analyze. Here we use cells E1:E70, just as an example.

To calculate the median, type (without the quotation marks) “=MEDIAN(E1:E70)”

To calculate the Q1, type “=QUARTILE(E1:E70,1)”

To calculate Q3, type “=QUARTILE(E1:E70,3)”

The remainder of the boxplot data points can be obtained from the equations provided, and from inspection of the data.



Figure 1. An example of a box plot table for total nitrogen (NTL).

5. Using these data, sketch box plots for each region on the one chart, using Excel or R. Copy/paste the plot below along with a few sentences describing the data. 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? If you need help with figuring out how to make a box plot in Excel, see these instructions (<https://support.office.com/en-us/article/create-a-box-plot-10204530-8cdf-40fe-a711-2eb9785e510f>) or send your instructor an email to set up a time to talk.

**Table 1. Levels of total nitrogen (NTL) for EPA Regions 7 and 10, and Alaska. Put the values you calculate into this table.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Median  (µg/L) | Q1  (µg/L) | Q3  (µg/L) | Upper  Extreme  (µg/L) | Lower  Extreme  (µg/L) | # outliers  above Q3  (upper  extreme) | # outliers  below Q1  (lower  extreme) |
| EPA  Region 7 7 7 |  |  |  |  |  |  |  |
| EPA  Region  10 |  |  |  |  |  |  |  |
| Alaska |  |  |  |  |  |  |  |

In a separate nutrient study by the EPA (EPA 2001), the lower quartile (Q1) value from water samples was recommended as the level below which nutrients in streams should be maintained in each *ecoregion* - note this is not the same as an *EPA Region*, as you can see in the map provided in [Supplement 5](http://tiee.esa.org/vol/v8/issues/data_sets/nuding/resources/supplement4.pdf). Below are the **recommended total nitrogen values** for the ecoregions within EPA Regions 7 and 10. The full data set and a map of the ecoregions are available in [Supplement 5](http://tiee.esa.org/vol/v8/issues/data_sets/nuding/resources/supplement4.pdf).

EPA REGION 7:

Ecoregion IV (Great Plains Grass and Shrublands) – 560 ug/L

Ecoregion V (South Central Cultivated Great Plains) – 880 ug/L

Ecoregion VI (Corn Belt and Northern Great Plains) – 2180 ug/L

EPA REGION 10:

Ecoregion II (Western Forested Mountain) – 120 ug/L

Ecoregion III (Xeric West) – 380 ug/L

Compare the information you generated in Table 1 with the EPA recommended nutrient criteria.

1. How do the values you calculated for Region 7 and Region 10 compare to EPA’s recommendations for various ecosystem types, or ecoregions, within these two regions?
2. Based on the data and maps you’ve looked at, can you assert whether the waters in EPA Regions 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 Alaska compare to other regions? Briefly hypothesize factors that may influence nutrient levels in Alaska.
4. **Macroinvertebrates**

Streams often contain a large number and a wide variety of benthic macroinvertebrates. Stream ecologists often look for the pollution-sensitive insects, because they serve as bioindicators of stream health. The following taxonomic orders of pollutant-intolerant insects are collectively referred to as “EPT” because they are such a useful grouping of insects that react strongly to pollution: Ephemeroptera (mayflies), Plecoptera (stoneflies) and Trichoptera (caddisflies). These insects begin their lives in the water, and later emerge as adults to live on the land.

Macroinvertebrates can be sampled by shuffling a net along the bottom of the stream bed and counting the number and types of insects brought up in the net. Ecologists have developed sophisticated methods of characterizing these benthic macroinvertebrate communities – rather than just counting the number of insects, we consider things like the diversity of the species present, and how many of those species are known to be pollution intolerant (e.g., EPT).

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. MMI data was not collected for the Alaska pilot project, so for this next portion of the lab, you will be working only with the data collected in the Lower 48.

9. 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? Draw a line that expresses your prediction on the graph below (Hint: Use the SmartArt tab to add a line).

Total Nitrogen (ug/L)

0

50,000

MMI

100

0

MMI vs. Total Nitrogen

1. 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). Display the R2 and the linear regression equation on each chart.
   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?
2. 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. On the Log chart you just plotted, draw circles over the areas where you would expect data from Region 7, Region 10, and Alaska to be located.
2. Construct a regional comparison of data. Create one plot of MMI vs. Log NTL for Region 7 and one for Region 10. Make 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?

**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.

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