Water on the Web Stream Ecology Lab
Unit 1 Modules 4/5 Lab #2

Detecting Nutrient Impacts on Stream Water Quality

Context: Lab #2 is one of four labs devoted to fundamental concepts in stream ecology. It is designed to give students an opportunity to make simple comparisons of their stream to existing data (Directed Procedure) or go through the process of selecting appropriate indicators to identify and assess the impacts of nutrient enrichment in streams (Investigative Procedure). Lab #1 focuses on measuring flow and suspended sediments, #3 on benthic invertebrate sampling and analysis, and #4 on aquatic habitat assessment.

Objectives: Following this lab you will be able to:

1. Select, sample, and measure selected indicators of nutrient runoff and primary production in multiple streams or at multiple sites within a stream;

2. Hypothesize the relationship between sources of nutrients, in-stream concentrations of nutrients, primary productivity, and basic water quality changes at your sampling site(s);

3. Hypothesize why streams in different watersheds may show differing responses to nutrient enriched runoff;

4. Interpret and explain the results you obtained from the stream(s) you sampled within a local, regional and/or national perspective.

Introduction: The loading of nutrients and sediment from domestic wastewater, agricultural runoff (from fields and feedlots), urban stormwater runoff, and unwise forestry practices is a major cause of water pollution to our lakes and streams.
Excessive loadings impact aquatic organisms by degrading water quality, habitat and food resources. Beneficial uses of these aquatic ecosystems by humans are also negatively affected, such as recreation (swimming, fishing and aesthetics) and source water for domestic supply (i.e., drinking water). The ability of a stream to assimilate nutrients before its ecological health or integrity is damaged varies widely among and within geographic regions depending upon the climate, the geomorphology and geochemistry of its watershed, and its biological communities.

The principal nutrients that cause problems are phosphorus and nitrogen, both of which are critical to the survival and growth of all organisms. However, their biologically available forms are relatively scarce in aquatic environments, generally occurring at concentrations in water that are tens of thousands of times lower than in biological organisms. In general, the impact of excessive nutrient rich runoff on streams is to increase algal and higher plant growth (primary productivity), leading to high inputs of particulate material and organic matter. These can physically smother bottom habitats and cause oxygen depletion from both high nighttime plant respiration and from the bacterial decomposition of the excess plant material. When this occurs, stream reaches may be created that are nutrient rich and oxygen poor, with biological communities characterized by species tolerant of low oxygen levels and much lower biodiversity overall. In extreme cases, low (hypoxic) or near-zero (anoxic) conditions may be associated with increased concentrations of chemically reduced compounds that further impair water quality, habitat and beneficial uses. These include: un-ionized ammonia (NH₃ and NH₄OH which are toxic to aquatic organisms), hydrogen sulfide gas (H₂S which is toxic and smells bad) and ferrous iron (black slime).

Nitrogen (N) and phosphorus (P) exist in a number of chemical forms. Depending on the form, N and P may be dissolved in water (soluble) or appear as particulate matter. A filter is used to separate the latter two fractions and care must be taken to select the correct filter type and nominal pore size (see details in Module 9 –
Lake Sampling Lab slides). N and P are also classified into organic and inorganic fractions, the latter being the form most available for algal and higher plant nutrition. From a water quality perspective, the most important inorganic forms are nitrate-N ($\text{NO}_3^-$-N), ammonium-N ($\text{NH}_4^+$-N) and phosphate-P ($\text{PO}_4^{3-}$-P; also called orthophosphate).

Sources and transformations of nitrogen are complex and are generally described as a global nitrogen cycle in biology and limnology textbooks (see references and N-cycling figure). Phosphorus transformations and the "phosphorus-cycle" (P-cycle) are conceptually simpler (see P-cycling figure). Phosphate is an extremely adsorptive molecule (it "sticks" to mineral and detrital particles). Higher concentrations in streams tend to be associated with higher levels of suspended sediments. However, in some cases there may be direct sources of domestic wastewater (such as from leaky or broken pipes) with extremely high levels of phosphate (most streams have values < 100 µgP/L, or < 0.1 mgP/L) in relatively clear water. In general, since ammonium-N tends to adsorb to soil relatively well, high levels of ammonium in streams suggest inputs of domestic wastewater or fertilizer. In contrast, nitrate-N is very soluble in water and moves freely through soil. Its largest sources are also typically from wastewater and fertilizer. It also may derive from ammonium that has been oxidized by nitrifying bacteria in the soil or water. All of these nutrients are pollutants when they occur at high levels and are contributed by various industrial processes, automotive emissions, deposits from oils and tires, and other sources in addition to those described earlier.

In this lab you will select appropriate indicators to detect the presence or absence, and severity of nutrient rich runoff at locations within a single or multiple watersheds determined by your instructor. If the stream(s) you sample are urban or suburban the source of the nutrients is likely to be from road runoff enriched with nutrients from eroded soils, automotive wastes, lawn fertilizers, pet wastes and possibly leaky sanitary sewer pipes. If the streams you sample are in an
agricultural area the source of the nutrients is more likely to be from field runoff of fertilizer, animal feedlots, or faulty septic systems. If feedlots or septic systems are the source it is likely that relatively high levels of fecal coliform bacteria will be present in the runoff and, therefore, in the stream(s) you are sampling. Fecal coliform bacteria are a traditional indicator of human feces and may indicate sanitary sewer leaks and faulty on-site septic systems; general indicator of urban runoff (human and pet waste). They are also contributed by wildlife including birds (all warm-blooded creatures). If erosion from construction or logging activities, or from agricultural fields is an important source, you might expect relatively high levels of total suspended sediments (TSS).

In high gradient watersheds (steep slopes), the residence time of nutrients may be too short to allow much nutrient assimilation by primary producers and so impacts on water quality may be minimal. However, excess nutrients will ultimately end up in a receiving body of water such as a lake, reservoir or estuary where the nutrient concentrations and total load may degrade the resource. In slower moving streams negative effects from enriched runoff may occur at relatively short distances downstream from the nutrient source. In short, the lag time between the introduction of nutrients and the response by algae and plants is an important consideration in assessing potential nutrient impacts, and one that can vary widely among watersheds.

Because nutrient rich runoff may cause increased primary production (usually algae is the problem), it is of value to attempt to link nutrients and algal growth, remembering that they may be separated in time (days/weeks) and space (upstream/downstream). Depending upon sampling and analytical equipment availability a number of indirect or direct measures of nutrients and accumulated algal growth measures can by used to detect the sources and effects of nutrient runoff on streams.
Table 1. Nutrient Runoff Indicators

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate-P (ortho-P or OP)</td>
<td>colorimetric by kit or spectrophotometry LINKS to slides</td>
</tr>
<tr>
<td>Total- P (TP)</td>
<td>needs digestion with hot plate or pressure cooker</td>
</tr>
<tr>
<td>Nitrate-N (NO₃-N or [NO₃⁻ +NO₂⁻]-N)</td>
<td>colorimetric by kit; spectrophotometry more difficult than for P (nitrite-N typically automatically included in nitrate-N analysis)</td>
</tr>
<tr>
<td>Ammonium-N (NH₄-N or NH₃-N )</td>
<td>colorimetric by kit or spectrophotometry</td>
</tr>
<tr>
<td>Total Kjeldahl-N (TKN)</td>
<td>$TKN = total\ organic-N + (NH_4-N) ; needs distillation and titration\ apparatus</td>
</tr>
<tr>
<td>Total- N (TN)</td>
<td>By spectrophotometry after digestion (specialized technique); or calculate as $TN = TKN + (NO_3^- +NO_2^-)-N$</td>
</tr>
</tbody>
</table>

Related Water Quality Parameters

<table>
<thead>
<tr>
<th>TSS and turbidity</th>
<th>TP often correlates with TSS because of phosphate adsorption.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Link to duluthstreams.org/ WQ primer</td>
</tr>
<tr>
<td>Organic matter measures:</td>
<td>BOD can be estimated easily in the lab but needs a DO meter or Winkler titration kit and takes 5 days; relatively insensitive VSS measured by ashing a TSS filter at 475-550°C; TOC usually requires a $10-20,000 instrument; sensitive</td>
</tr>
<tr>
<td>Biological Oxygen Demand (BOD)</td>
<td>Link to duluthstreams.org/ WQ primer</td>
</tr>
<tr>
<td>VSS (volatile suspended solids)</td>
<td></td>
</tr>
<tr>
<td>Total organic carbon (TOC)</td>
<td></td>
</tr>
<tr>
<td>Fecal coliform bacteria</td>
<td>Assays can be done in lab several ways</td>
</tr>
<tr>
<td></td>
<td>Link to duluthstreams.org/ WQ primer</td>
</tr>
<tr>
<td>Dissolved oxygen (DO)</td>
<td>DO meter or Winkler titration kit. Direct measure of the potential impact associated with increased organic matter</td>
</tr>
<tr>
<td></td>
<td>Link to duluthstreams.org/ WQ primer</td>
</tr>
</tbody>
</table>

The blue denotes an eventual link to another website section with more detailed information
Table 2. Primary Production Indicators

<table>
<thead>
<tr>
<th>Algal accumulation (natural or artificial substrates)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet weight (WW)</td>
<td>Gravimetric (by weight) of standard area scraped from rock or other substrate  LINKS to slides</td>
</tr>
<tr>
<td>Dry weight (DW)</td>
<td>WW dried overnight at 103°C; less variable than WW</td>
</tr>
<tr>
<td>Organic matter = AFDW (ash-free dry weight)</td>
<td>After recording DW, the sample is ashed at 475-550°C to determine the volatile (organic) fraction; AFDW is a cheap, easy measure but includes fungi, bacteria, invertebrates, etc</td>
</tr>
<tr>
<td>Chlorophyll-a</td>
<td>Spectrophotometric or fluorescence techniques used on solvent extract to quantify chlorophyll-a; algae specific</td>
</tr>
<tr>
<td>Total organic carbon (TOC)</td>
<td>Determined on a DW sample using a specialized $20,000 instrument (usually a Carbon-Nitrogen Analyzer)</td>
</tr>
<tr>
<td>Microscopic ID</td>
<td>Qualitative enumerations to identify major groups</td>
</tr>
</tbody>
</table>

Other Biological Indicators

<table>
<thead>
<tr>
<th>Benthic invertebrate IDs</th>
<th>Oxygen tolerance classification  LINK to WOW UNIT III Module 10/11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benthic indices</td>
<td>e.g., Indices of Biotic Integrity (IBIs); many formulas exist in the literature  LINK to DuluthStreams/RiverWatch</td>
</tr>
<tr>
<td>Fish community structure</td>
<td>Various - IBIs</td>
</tr>
</tbody>
</table>

Other

The blue denotes an eventual link to another website section with more detailed information
**Directed Lesson:**
This field and lab lesson is intended to allow simple comparisons between your stream’s current basic nutrient chemistry and primary production and one or more of the following:

1. Appropriate state/federal criteria;
2. Historical data from other seasons or sites for your stream;
3. Data from other local or regional streams or other national streams;
4. Data for other national streams (e.g., data from WOW, USGS, USEPA).

**Tasks:**
1. Select nutrient and algal accumulation indicator(s) (Tables 1 and 2);
2. Select sampling site;
3. Collect water and periphyton (attached algae) samples;
4. Perform analyses in the field and/or the lab;
5. **Write-up:** Compare to reference data of your choice. Represent your results in tables and/or graphs. Summarize each of your results in a short paragraph. Include answers to the additional “questions” listed below after consulting with your instructor for specific instructions. You should also read the WOW Lesson “Investigating Data Interpretation” at [http://wow.nrri.umn.edu](http://wow.nrri.umn.edu) in the STUDENT-INVESTIGATING section of the website.

**Investigative Lesson:**
This field and lab lesson is intended to allow a more in-depth assessment of the potential nutrient inputs to a stream through multiple site sampling on a single stream, multiple dates on a single stream or comparisons among streams. Class data for your local stream’s current basic nutrient chemistry and primary production (measured as accumulated algal growth) should be compared to one or more of the following:

1. Appropriate state/federal criteria;
2. Historical data from other seasons or other sites for your stream;
3. Data from other local or regional streams or other national streams;
4. Data for other national streams (e.g., from WOW, USGS, USEPA).

Tasks:
1. Develop a set of testable hypotheses
2. Design a sampling program to assess the potential impact of a nutrient enriched input to a stream or streams. Examples include:
   - Sampling upstream and downstream of a nutrient source (try to include the source tributary as a third sampling site
   - Time sequence – i.e., comparing before and after a particular point in time or event or comparing current to historical data
   - Comparing your data to other local or regional streams that may be considered as “reference” or “control” sites
3. Select one or more nutrient and algal accumulation indicator (Tables 1 and 2);
4. Collect water and periphyton (attached algae) samples
5. Perform analyses in the field and/or the lab;
6. Write-up: Interpret results, draw conclusions and prepare a report formatted as if you were conducting the project for an agency, environmental consulting firm or as university research. This would typically include the sections outlined in the box below:

| I. Introduction (Background) |
| II. Objectives               |
| III. Methods & Site Description |
| IV. Results                  |
| V. Discussion (can combine with Results) |
| VI. Summary & Conclusions    |

7. Optional Small Group Exercise- Hurricane Bonnie made landfall off the coast of North Carolina on August 26, 1998. Stations along the Cape Fear River from the coast to ~80 kms upstream were sampled at ~monthly intervals for a
variety of water quality parameters (collection dates for the suite of stations were Aug 3-6, Aug 31-Sep 3, and Oct 5-8, 1998). Making use of the River Run Data Visualization Tool and Lower Cape Fear River Monitoring Program Report or other data (see Website Links and References below)

- Describe the major water quality differences observed in the Lower Cape Fear River that occurred from August through September 1998 by contrasting mid-reach station NAV (Navassa) with upstream station NC11 and downstream station M18. Focus your analysis on DO, salinity, TN, fecal coliform bacteria, and turbidity. You may use other data as needed.

- Summarize your findings in the form of a short report using tables and figures as needed. Develop a PowerPoint-style slide show to present your findings to the class, pretending that you represent the North Carolina Department of Environment and Natural Resources.

**Materials:**

**Sampling**

- Hip boots or waders
- Sample bottles for water (“clean” as per standardized de-contamination methods). Different bottles may be required for different analyses, depending on the volumes required; e.g., sterile bottles for fecal coliform sampling; BOD bottles, and others.
- Sampling pole to extend out from shore if water is unsafe for wading
- Jars, Ziplok baggies, Whirlpak bags for periphyton algae and cobble-sized rocks if necessary
- Periphyton sampling equipment (e.g., standard area template, toothbrushes, squirt bottle for rinsing)
- Chemistry test kits if analyzed in field (e.g., nutrients, DO, and other water quality parameters)
- Bucket for carrying supplies and miscellaneous uses
- Clipboard with waterproof paper, pencils, indelible marker pens, etc
• Thermometer, DO meter (usually has a temperature sensor but may also have electrical conductivity (EC) or EC25 and sometimes pH). NOTE – sensors must be calibrated on the sampling day and preferably within a few hours of sampling.

**Laboratory Analyses:** Select instructions matched to the parameters you will measure (see attached NRRI lab manual instructions and Tables 1 and 2).

*NOTE: Simplified sets of instructions for spectrophotometric nutrient analyses and basic water quality parameters are being drafted. These are not complete as of 5/1503 but some are included. Hach kits will be specified where applicable.*

**Additional Questions for Inclusion in Write-ups:**

1. Provide a rationale to explain why you selected the specific indicators you used for determining nutrient levels and assessing their impacts in the stream(s) you sampled.

2. Based on your knowledge of the flow rate or gradient in your stream(s), explain the relationship you observed between nutrient inputs and observed changes in primary productivity. Hypothesize how this relationship might change seasonally in your stream(s), if at all.

3. Based on your sampling results, do you believe that the macroinvertebrate communities in your stream(s) are being affected by nutrient enrichment? If not, why not? If so, what is your evidence and how would you propose to find out if there are differences in the macroinvertebrate communities among your sites or streams that are attributable directly or indirectly to nutrient enrichment?

4. a) If you detected nutrient enrichment, what are its likely sources? Suggest methods to reduce nutrient delivery to your stream.
b) If you did not detect nutrient enrichment in your stream, identify features and practices in the stream’s watershed that may be protecting it from the effects of excess nutrients.

5. a) Discuss the potential methodological design shortcomings and limitations of your study. How might they have affected your conclusions?

b) You just found out that a lawsuit was filed. The resolution of the lawsuit will depend on the accuracy of your data and the validity of your conclusions. You decide that you should re-do your study. What would you do differently?

**Additional Information:**
The following downloadable PDF files are available on the website (also as .doc files) and PowerPoint slide lecture (as .ppt files).

- Figures illustrating the nitrogen and phosphorus cycles in aquatic systems
- Nomenclature for analyzing the physical and chemical forms of N and P in water
- Cape Fear River nutrients background PowerPoint presentation slides
- *DuluthStreams* nutrients background PowerPoint presentation slides (may be up after May 23, 2003).

**Website Links:**
1. *Water-on-the-Web*, *LakeAccess*, and *DuluthStreams* – Limnology and Stream Primers (eventually they will all be on the WOW website)
   - [http://wow.nrri.umn.edu/wow/under/russ.html](http://wow.nrri.umn.edu/wow/under/russ.html)
   - [http://lakeaccess.org/lakedata/datainfo.html](http://lakeaccess.org/lakedata/datainfo.html)
   - [http://duluthstreams.org/understanding/primer.html](http://duluthstreams.org/understanding/primer.html)
   - [http://duluthstreams.org/understanding/WQprimer.html](http://duluthstreams.org/understanding/WQprimer.html)
2. *River Run* – This is part of the *Students as Scientists* website developed by Dr. Rich Huber and others at the University of North Carolina at Wilmington. It features a data visualization tool (DVT) developed at NRRI that animates monthly Cape Fear River water quality data from 1995-2002 for about 15 sites along a gradual salinity gradient as the river flows into the Atlantic Ocean. It also includes GIS data and other educational sections and tools.
   - [http://www.uncwil.edu/riverrun/index.html](http://www.uncwil.edu/riverrun/index.html)

3. The Ecology of the Cape Fear River System. Dr. Michael A. Mallin, Center for Marine Science, University of North Carolina at Wilmington.
   - [http://www.uncwil.edu/riverrun/river_tutorial/CFRSystem.htm](http://www.uncwil.edu/riverrun/river_tutorial/CFRSystem.htm)

4. Lower Cape Fear River Program – contains annual reports with data and interpretation
   - [http://www.uncwil.edu/cmsr/aquaticecology/lcfrp/](http://www.uncwil.edu/cmsr/aquaticecology/lcfrp/)

5. Medicine Lake Lawn Fertilizer Study. The last 2 years of the *Lake Access* project focuses on a large field study of stormwater runoff into a eutrophic lake from two residential subwatersheds. Homeowners in one subwatershed used P-free lawn fertilizer while in the other there were initially no phosphorus restrictions.
   - [http://www.lakeaccess.org/lakedata/lawnfertilizer/mainlawn.htm](http://www.lakeaccess.org/lakedata/lawnfertilizer/mainlawn.htm)

6. USEPA Watershed Academy Website. There are many useful modules on this website related to watershed science.
   - [http://www.epa.gov/watertrain/nonpoint.htm](http://www.epa.gov/watertrain/nonpoint.htm)

   In particular there is a link to a report by a panel of experts:

   - [http://www.epa.gov/watertrain/rbp]

References (see also module slide notes for specific methods references)


Nutrient analysis literature contains an alphabet soup of acronyms for various forms of nitrogen and phosphorus. Without delving into the reasons for all of the classifications, the following represents the standard nomenclature used in most laboratories.

**Nitrogen**

1. dissolved = soluble = filtered (“filtrate” water)
2. total = unfiltered = filtered + particulate (“raw” water”)
3. total-N (TN) = particulate-N (PN) + dissolved-N (DN)
4. total-N (TN) = inorganic-N + organic-N
5. total-N (TN) = dissolved inorganic-N (DIN) + dissolved organic-N (DON) + particulate organic-N (PON); assumes particulate inorganic-N always = 0 therefore, TN = DIN + DON + PN , where:
   6. DIN = nitrate-N + nitrite-N + ammonium-N (DIN = [NO₃⁻ + NO₂⁻]-N + [NH₄⁺-N])
   7. “nitrate-N” usually refers to a combined analysis of [nitrate + nitrite] - N
   8. ammonium-N (NH₄⁺-N) is the predominant form of “ammonia” at the typical pH’s encountered in natural waters (it is also often referred to as “NH₃-N”).
   9. DON is determined by digesting and analyzing a filtrate as per TN and then subtracting DIN.
   10. Total Kjeldahl Nitrogen (TKN) = PN + DON + NH₄⁺-N and so we can also calculate TKN as TN -[NO₃⁻ + NO₂⁻]-N . The term “Kjeldahl” refers to a method that converts organic-N to ammonia that is removed via steam distillation and then analyzed to estimate all chemically reduced N. It is mostly used in wastewater laboratories.
   11. Similarly, we can estimate total-N as TN = TKN + [NO₃⁻ + NO₂⁻]-N
   12. dissolved-N (DN) = DON + DIN (but not very common nor very useful since DON is often >> DIN and most of it is not useable by algae or plants.
Phosphorus

1. dissolved = soluble = filtered (“filtrate” water)
2. total = unfiltered = filtered + particulate (“raw” water)
3. dissolved inorganic-P (DIP) = ortho-P (OP) = soluble reactive-P (SRP) = dissolved reactive-P (DRP as in Standard Methods, 1995) = molybdenum reactive-P (MRP) - *Lots of names for the same thing*
4. Total-P (TP) = dissolved-P (DP) + particulate-P (PP)
5. Dissolved-P = filtrate digested as per TP for analysis
6. TP = OP + DOP + PP (total = ortho + dissolved organic + particulate)
7. Particulate-P can be analyzed directly from the residue on a glass fiber filter (Whatman GF/C or GF/F, Gelman A/E, RA 984 or equivalent), adding deionized water and digesting it as as TP sample; or by calculation as TP – DP but this may be problematic in unpolluted water because PP is often a small difference between two larger number