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Fenway Water Quality

Purpose: To compare several water quality parameters between upstream and downstream locations in the Muddy River in the Back Bay Fens and to examine the relationships between these water quality parameters.

Water quality – Water quality is a composite of the physical, chemical, and biological characteristics of water.

The chemistry of the water impacts the life it can support (think limiting factors). We will look at certain water quality parameters to determine how the organisms living in the Fens are affected and affect these parameters.

In the Field, we will measure:

- Temperature
- Nitrates
- Ammonium
- Salinity
- pH

In the Lab, we will measure:

- Turbidity
- Dissolved Oxygen

Field Sample Sites:

1) Water quality measurements and sample collection will be made at 3 upstream (Fig. 1) and 3 downstream (Fig. 2) sites.

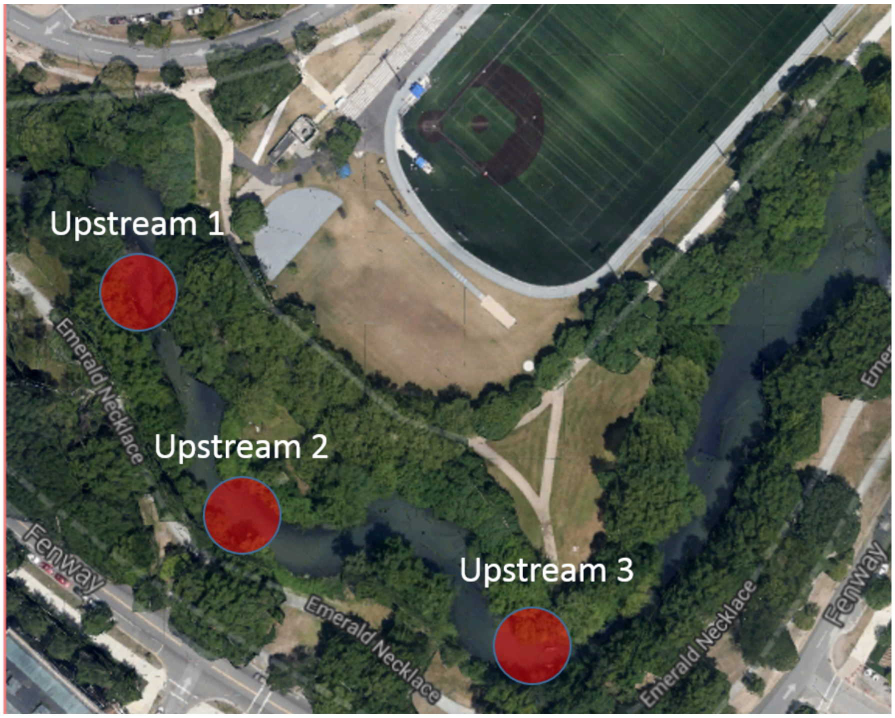

Figure 1. Upstream sampling locations at Back Bay Fens.

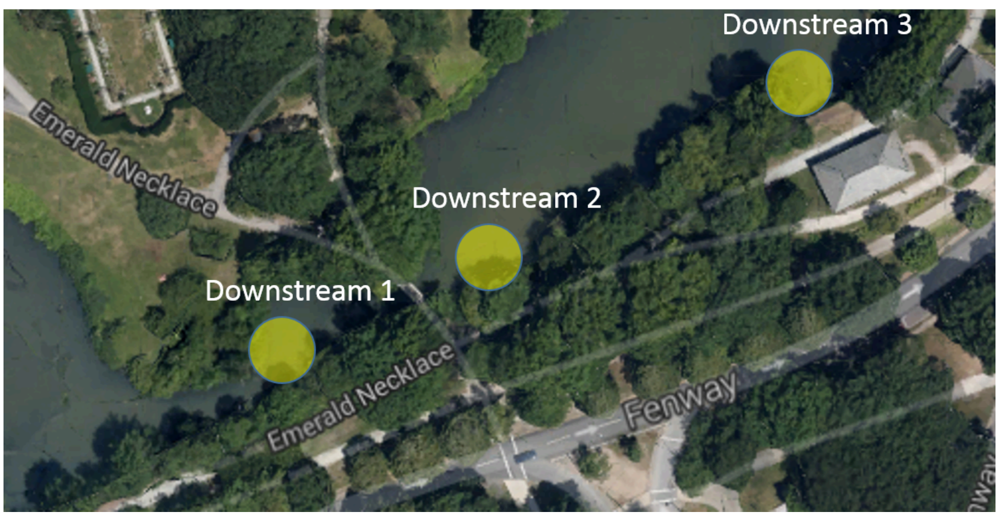

Figure 2. Downstream sampling locations at Back Bay Fens.

2) Each group will take measurements at the three upstream and three downstream sampling sites. Group members will rotate tasks so that each member gets experience recording data and employing field sampling techniques.

Background: Fens Water Quality

Dissolved Oxygen (DO): The amount of dissolved Oxygen gas in water is vital to the health and survival most aquatic organisms. The maximum amount of dissolved oxygen a body of water can contain depends upon temperature and air pressure. At 0°C and 760mmHg pressure, water can hold up to 14.57mg/L of DO, but at 30°C and the same pressure, water can only hold 7.67mg/L of DO. Organisms differ in their tolerances to low levels of dissolved oxygen. For example, Trout and Smallmouth Bass cannot survive with DO levels below 6.5mg/L, but Catfish and Carp can persist until DO levels drop below 2.5mg/L. Bodies of water with low levels of DO are said to be **hypoxic** and those that are completely deficient in DO are said to be **anoxic**. Sources of DO include diffusion from the air, aeration due to water movement and photosynthesis of aquatic plants. DO is used up when aquatic organisms respire, when organic material decays or decomposes and when oxygen diffuses into the air.

Temperature: Water temperature can vary based upon many factors including climatic region, season, time of day, etc., and can influence many aspects of water quality. Larger bodies and deeper bodies of water tend to have less fluctuation in temperature than do smaller, shallower bodies of water. Anthropogenic (human caused) changes in temperature such as warm water run-off or discharge or removal of vegetation that provides shade are considered to be **thermal pollution**. Temperature can impact the solubility of oxygen and other gases, as well as the growth rates of plants, and metabolic rates of organisms.

Nitrates: The concentration of nitrogen in the form of nitrate ions (NO_3^-) in a stream comes from by both natural and manmade sources. In the nitrogen cycle, **nitrification** produces nitrate from ammonium. Nitrogen is essential to plant growth and therefore, the amount of available nitrogen can impact both photosynthesis and DO levels. Nitrate is often called the **usable** form of nitrogen. Many plants can only take-up and utilize nitrogen in the form of nitrate, and thus, many fertilizers include high concentrations of industrially converted nitrate. Run-off of these fertilizers and other nitrogen laden chemicals can contribute to excessively high levels of nitrogen, often referred to as **eutrophication.** Unpolluted freshwater areas typically have nitrate levels below 1mg/L.

Ammonium: Nitrogen in the form of ammonium ions (NH_4^+) is often called an **unusable** form of nitrogen, because although some plants can utilize this ion as a source of nitrogen, most plants can only use nitrate. Ammonium in streams can come from the conversion (usually by bacteria) of either atmospheric nitrogen (N_2) or the nitrogen in animal waste or decaying plants and animals. High levels of ammonium can be toxic, but moderate levels are usually beneficial because they increase the rate of nitrification (and thus available nitrate) in the system. Typically ammonium levels are low (<0.5mg/L)

Turbidity: Turbidity is the measure of the *lack* of clarity of body of water (high turbidity = unclear waters, low turbidity = clear waters). Sources of turbidity include: soil erosion, run-off, bottom-dwelling organisms that stir up sediments, decaying plants and animals, and living microorganisms. High turbidity reduces water clarity and therefore, decreases photosynthetic rates. Also, because turbid waters absorb (rather than reflect) more light, unclear waters can increase water temperature.

Salinity: Salinity is the total of all non-carbonate salts in water and is typically measured in mg/L in freshwater systems (compared to parts per thousand in seawater). Seawater has a salinity of approximately 35ppt or 35,000 mg/L and is driven by high concentrations of sodium and chlorine. In freshwater, sodium and chlorine are less abundant and calcium and bicarbonate are more common. The mineral content of the streambed as well as the run off from salted roads can influence freshwater salinity. Freshwater salinity can range from 1 to 250mg/L (or 0.001 to 0.25 ppt).

pH: pH measures the acidity on a log scale (it is the negative log the concentration of H^+ ions). pH ranges from 0-14, with 0 the most acidic, 14 the most basic and 7 as neutral. Because pH is measured on a log scale, a pH of 5 indicates 10x more H^+ ions than a pH of 6. Freshwater streams and lakes are typically basic with pH values between 7 and 8. pH values between 6.5 and 8.2 are optimal for most freshwater organisms, but individual species' tolerances for pH values outside that range can vary widely. Rainfall is typically acidic (pH between 5 and 6.5), but soils with high mineral concentrations can increase the pH of run-off. Carbonic acid from respiration and decomposition can also create more acidic waters.

In the Field Procedure:

1) In groups of 3 or 4, you will sign up to measure one of the water quality metrics we will measure in the field.
2) Temperature: Use a handheld thermometer to measure temperature
   Nitrates: Use nitrate testing kit to measure concentration
   Ammonium: Use ammonium testing kit to measure concentration
   Salinity: Use handheld refractometer to measure salinity (0/00).
   pH: Use litmus paper to measure pH to the nearest whole number (log scale)
3) Group members should rotate between data collection and data recording positions.

In Lab: While you are in the field collecting measurements, you will be collecting water samples to be analyzed upon your return to the lab. When you return to lab:

1) Turbidity will be measured using the spectrophotometer. Cuvettes will be filled with each water sample and measured for transmittance. Transmittance will be standardized to a light wavelength of 450 nm.
2) The spectrophotometer is expensive and extreme care must be used to ensure the sample does not spill in the machine. Transmittance reference will be 100% transmittance for distilled water.
3) Dissolved oxygen will be measured in ppm (mg/L) using D.O. probes and Vernier software on lab computers.
4) Repeat measurements for all replicate water samples
5) **ENTER YOUR GROUP'S DATA FOR ALL SAMPLES INTO THE SPREADSHEET PROVIDED BY YOUR TA**

FENS ASSIGNMENT

All questions should be answered in detail and justified using your ecological knowledge. Statistical tests must be written out in full. Each statistical test should include a null hypothesis and interpretation of your results. Questions that ask for an explanation require a thoroughly explained, logically reasoned justification for predictions. If results contradict your prediction, you should reevaluate your hypothesis and formulate new hypotheses based on your findings.

Explanation of **t-test shortcut in Excel

You need two columns of data, one for each parameter you are examining, in an Excel spreadsheet. Then, in an empty cell in the same spreadsheet type '=test(' (without the quotes). The equals sign tells Excel that you want to perform a mathematical function, the t-test tells them what function you want to perform and it is always followed by an open parenthesis. Excel will then tell you what it wants from you to perform the t-test. It should say 'array1,array2,tail,type). Array 1 is your first column of data, Array 2 is your second column. We are conducting a 2 tailed, type-2 t-test. In the end, it should look like this:
=ttest(A2:A7, B2:B7,2,2) (those cell names are just examples—select all the data for both parameters).

When you press enter, it will give you a value. This is the p-value, NOT the t-value. Interpret as necessary.

1) **Which parameters of water quality that we measured do you expect to differ between upstream and downstream sites in the Muddy River? List all.**

   I expect Nitrates (mg/L), Ammonium (mg/L), pH (negative log the concentration of H^+ ions), and Turbidity (nm) to differ between upstream and downstream sites in the Muddy River.

2) **For _one_ parameter above, explain why you expect it to differ between the sites. Do you expect this parameter to be higher or lower at the upstream site? This should be an ecological explanation, not a mathematical one.**

   For the parameter, nitrates (mg/L), I expect it to differ between the upstream and downstream sites in the Muddy River because upstream sites might have higher levels of nitrates due to agricultural runoff, urban runoff carrying nitrogen-rich pollutants, and organic matter decomposition. Downstream sites, on the other hand, may experience dilution effects as water flows downstream and also potential assimilation or denitrification processes occurring within the river system, which could reduce nitrate levels. I expect nitrate measurements to be higher at the upstream site due to the influx of pollutants and organic matter from upstream sources.

3) **Conduct a t-test to determine whether there is a significant difference between that parameter at the upstream and downstream sites. You should AVERAGE the replicates for a given site so that you have 3 upstream values and 3 downstream values. Show ALL work and include: Null hypothesis, means, variances, t-Values, degrees of freedom, p-values, and interpretation of the p-value.**

Sample	Nitrate (mg/L)
Fens Upstream Site 1 Rep 1	5
Fens Upstream Site 1 Rep 2	5
Fens Upstream Site 1 Rep 3	5
Average of Upstream Reps Site 1	5
Fens Upstream Site 2 Rep 1	5
Fens Upstream Site 2 Rep 2	5
Fens Upstream Site 2 Rep 3	5
Average of Upstream Reps Site 2	5
Fens Upstream Site 3 Rep 1	5
Fens Upstream Site 3 Rep 2	5
Fens Upstream Site 3 Rep 3	5
Average of Upstream Reps Site 3	5
Fens Downstream Site 1 Rep 1	5
Fens Downstream Site 1 Rep 2	5
Fens Downstream Site 1 Rep 3	5
Average of Downstream Reps Site 1	5
Fens Downstream Site 2 Rep 1	2
Fens Downstream Site 2 Rep 2	2
Fens Downstream Site 2 Rep 3	2
Average of Downstream Reps Site 2	2
Fens Downstream Site 3 Rep 1	2.5
Fens Downstream Site 3 Rep 2	2.5
Fens Downstream Site 3 Rep 3	3

Average of Downstream Reps Site 3	2.667

- Null Hypothesis (H_0): There is no significant difference in nitrate levels between the upstream and downstream sites.
- Alternative Hypothesis (H_A): There is a significant difference in nitrate levels between the upstream and downstream sites.

T-test calculations:

Mean ($\overline{x}$) = $\frac{sum\ of\ terms}{number\ of\ terms}$

- Upstream mean = $\frac{(5 + 5 + 5)}{3}$ = 5.000 mg/L
- Downstream mean = $\frac{(5 + 2 + 2.667)}{3}$ = 3.223 mg/L

Variance (s^2) = $\frac{\Sigma(x_i - \overline{x})^2}{n-1}$

- Upstream variance = $\frac{((5-5) + (5-5) + (5-5))^2}{3-1}$ = $0\ mg/L^2$
- Downstream variance = $\frac{((5-3.223) + (2-3.223) + (2.667-3.223))^2}{3-1}$ = $2.480\ mg/L^2$

Standard deviation (s) = $\sqrt{variance}$

- Upstream standard deviation = $\sqrt{0}$ = 0 mg/L
- Downstream standard deviation = $\sqrt{2.4796333}$ = 1.575 mg/L

Standard error (SE) = $\frac{s}{\sqrt{n}}$

- Upstream standard error = $\frac{0}{\sqrt{3}}$ = 0 mg/L
- Downstream standard error = $\frac{1.5746852}{\sqrt{3}}$ = 0.909 mg/L

t- value (t) = $\frac{\left|\overline{x}_1 - \overline{x}_2\right|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$

where

$\bar{x}_1$ = mean of group 1

$\bar{x}_2$ = mean of group 2

s_1^2 = variance of group 1

s_2^2 = variance of group 2

n_1= number of samples in group 1

n_2= number of samples in group 2

(Group 1 = upstream, Group 2 = Downstream)

- t-value = $\frac{|5-3.223|}{\sqrt{\frac{0}{3}+\frac{2.4796333}{3}}}$ = 1.955

Degrees of freedom (df) = $n_1 + n_2 - 2$

- Degrees of freedom = 3 + 3 - 2 = 4

- p-value: 0.1223

- Interpretation of the p-value: Because our computed p-value is greater than 0.05 (p>0.05) at 0.1223, we fail to reject the null hypothesis that there is no significant difference in nitrate levels between upstream and downstream sites.

  In other words, we do not have enough evidence to conclude that there is a significant difference in nitrate levels between upstream and downstream sites based on the sample data. Interpreting this result ecologically, it suggests that, within the context of this study, the upstream and downstream sites do not exhibit a statistically significant difference in nitrate levels. It could indicate that factors affecting nitrate concentrations, such as agricultural runoff or local land use, are relatively consistent along the river's course. However, it's essential to consider other factors and conduct further research to fully understand the drivers of nitrate levels in the ecosystem.

4) Do the results of your t-test fit your prediction? Why or why not? This should be an ecological explanation, not a mathematical one. Use your observations from each site to support your argument.

The results of my t-test do not perfectly fit my prediction that nitrate measurements would be higher at the upstream site due to factors such as agricultural runoff, urban pollution, and organic matter decomposition. The t-test results indicated that the differences between the upstream and downstream sites were not statistically significant. While agricultural runoff and urban pollution can contribute to higher nitrate levels upstream, the actual distribution and impact of these sources may vary spatially within the river system. It's possible that the specific locations chosen for sampling did not capture the full extent of upstream nitrate inputs. Nitrate levels in rivers can fluctuate seasonally due to factors such as rainfall, temperature, and agricultural activities. This sampling period (Spring, 3/18/24, ~11:30-12:30am, Boston, MA) might not have represented the peak of nitrate input from upstream sources. Additionally, variations in flow rates and water residence times could influence the dilution and processing of nitrate downstream.

5) **The Ecology lab completes water sampling at these same sites in the fall (mid-October). The data for Upstream Site 1 from the fall are below:**

	Nitrate	Ammonium	pH	Temp	Sal	DO	Turbidity
Fens Upstream Site 1 Rep 1	**2.5**	**1**	**7**	**18**	**10**	**2.9**	**0.047**
Fens Upstream Site 1 Rep 2	**5**	**0.5**	**7**	**18**	**11**	**2.9**	**0.040**
Fens Upstream Site 1 Rep 3	**5**	**0.5**	**7**	**17**	**10**	**2.8**	**0.038**

Choose *one* parameter that you think might vary between spring and fall sampling (our sample) at Upstream Site 1 and explain why you expect it to differ. In which season do you expect it to be higher or lower?

One parameter that might vary between spring and fall sampling at Upstream Site 1 is turbidity (nm). Turbidity can be affected by various factors such as rainfall, which can lead to increased soil erosion and runoff, resulting in higher turbidity levels. In the spring, increased precipitation can wash sediments and debris into the river, potentially increasing turbidity levels compared to the fall when there might be less rainfall and reduced erosion.

6) **Conduct a t-test to determine whether there is a significant difference between that parameter in the spring and the fall. Since you are only comparing one site, use all the values in your t-test. In addition, you can use the Excel shortcut to find your p-value, but must include: Null hypothesis, means, p-value, and interpretation of the p-value.**

Sample	Turbidity (nm)
Fens Upstream Site 1 Rep 1 (Fall)	0.047
Fens Upstream Site 1 Rep 2 (Fall)	0.040
Fens Upstream Site 1 Rep 3 (Fall)	0.038
Fens Upstream Site 1 Rep 1 (Spring)	0.040

Fens Upstream Site 1 Rep 2 (Spring)	0.038
Fens Upstream Site 1 Rep 3 (Spring)	0.034

- Null Hypothesis (H_0): There is no significant difference in turbidity levels between spring and fall at upstream site 1.
- Alternative Hypothesis (H_A): There is a significant difference in turbidity levels between spring and fall at upstream site 1.

T-test calculations:

Mean ($\bar{x}$) = $\frac{sum\ of\ terms}{number\ of\ terms}$

- Upstream site 1 mean (fall) = $\frac{(0.047+ 0.040 + 0.038)}{3}$ = 0.042 nm
- Upstream site 1 mean (spring) = $\frac{(0.040+ 0.038 + 0.034)}{3}$ = 0.037 nm

Variance (s^2) = $\frac{\Sigma(x_i - \bar{x})^2}{n-1}$

- Upstream variance (fall) = $\frac{((0.047-0.042) + (0.040-0.042) + (0.038-0.042))^2}{3-1}$ = $2.233\ x\ 10^{-5}\ nm^2$
- Upstream variance (spring) = $\frac{((0.040-0.037) + (0.038-0.037) + (0.034-0.037))^2}{3-1}$ = $9.333\ x\ 10^{-6}\ nm^2$

Standard deviation (s) = $\sqrt{variance}$

- Upstream standard deviation (fall) = $\sqrt{2.233\ x\ 10^{-5}}$ = 0.004 nm
- Upstream standard deviation (spring) = $\sqrt{9.333\ x\ 10^{-6}}$ = 0.003 nm

Standard error (SE) = $\frac{s}{\sqrt{n}}$

- Upstream standard error (fall) = $\frac{0.0047258}{\sqrt{3}}$ = 0.003 nm
- Upstream standard error (spring) = $\frac{0.003055}{\sqrt{3}}$ = 0.002 nm

t- value (t) = $\frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$

where

$\bar{x}_1$ = mean of group 1

$\bar{x}_2$ = mean of group 2

s_1^2 = variance of group 1

s_2^2 = variance of group 2

n_1= number of samples in group 1

n_2= number of samples in group 2

(Group 1 = fall, Group 2 = spring)

- t-value = $\frac{|0.0416667 - 0.0373333|}{\sqrt{\frac{2.2333333 \times 10^{-5}}{3} + \frac{9.3333333 \times 10^{-6}}{3}}}$ = 1.334

Degrees of freedom (df) = $n_1 + n_2 - 2$

- Degrees of freedom = 3 + 3 - 2 = 4

- p-value: 0.253

- Interpretation of the p-value: Because our computed p-value is greater than 0.05 ($p>0.05$) at 0.253, we fail to reject the null hypothesis that there is no significant difference in turbidity levels between spring and fall at upstream site 1.

  This data suggests that there is no statistically significant difference in turbidity levels between spring and fall at upstream site 1. In other words, the observed difference in turbidity between the two seasons could likely be due to random variation rather than a true difference in turbidity levels. The seasonal variations in turbidity levels at this site are likely within the range of normal fluctuations and do not represent a consistent pattern of change between the two seasons.

7) **Do the results of your t-test fit your prediction? Why or why not? This should be an ecological explanation, not a mathematical one. Use your observations from each site to support your argument.**

The results of my t-test for turbidity levels between spring and fall at upstream site 1 do not align with my initial prediction. My prediction was that turbidity levels would likely be higher in the spring due to increased rainfall and subsequent runoff carrying sediments into the river; the t-test did not find a statistically significant difference between the two seasons. This may be because the flow rate and velocity of the river can also play a significant role in turbidity levels. While increased rainfall in the spring may lead to higher runoff, the flow rate of the river itself may vary seasonally, affecting sediment transport and turbidity. Additionally, biological processes, such as the growth of aquatic vegetation or algae blooms, can also influence turbidity levels. These processes may be influenced by seasonal factors such as temperature and nutrient availability, rather than solely by rainfall. Considering these ecological factors, the lack of a significant difference in turbidity levels between spring and fall at upstream site 1 suggests that other complex interactions are at play beyond just seasonal rainfall patterns. While rainfall and runoff may contribute to turbidity, they are likely not the only driving factors, and a more comprehensive understanding of the local ecosystem dynamics is necessary to fully interpret turbidity variations over time.

8) **Which pairs of parameters of water quality that we measured do you expect to be *correlated* with one another? List all.**

I expect temperature and dissolved oxygen as well as nitrates and ammonium to be paired parameters in this case.

9) **For *<u>one</u>* pair of parameters listed above (your answers to #8), <u>explain</u> whether you expect a positive or negative correlation between those factors. Provide an ecological explanation for why you would expect this relationship.**

For the paired parameters, nitrates and ammonium, I expect a negative correlation between nitrates and ammonium levels. My prediction here is due to my limited knowledge of the nitrogen cycle dynamics in aquatic ecosystems. High levels of ammonium may indicate an abundance of organic matter or waste input, which can lead to increased nitrification processes converting ammonium to nitrate. Therefore, as ammonium levels increase, nitrates may decrease due to utilization in nitrification processes.

10) **Calculate (by hand) the coefficient of correlation to determine whether your expectation is supported by the data. Use all data points (replicates) for both upstream and downstream locations unless there are an unequal number of replicates for your two parameters (e.g., if one parameter has 18 data points and the other has 17, exclude a replicate from the same site for the parameter with 18 data points). Show all work for full credit.**

Sample	**(A) Nitrate (mg/L)**	**(B) Ammonium (mg/L)**	ΔA	ΔB	ΔA^2	ΔB^2	ΔAxΔB
Fens Upstrea	5	0.15	-0.889	0.078	0.790	0.006	-0.069

m Site 1 Rep 1							
Fens Upstream Site 1 Rep 2	5	0.25	-0.889	-0.022	0.790	0.000494	0.020
Fens Upstream Site 1 Rep 3	5	0	-0.889	0.228	0.790	0.052	-0.203
Fens Upstream Site 2 Rep 1	5	0.25	-0.889	-0.022	0.790	0.000494	0.020
Fens Upstream Site 2 Rep 2	5	0.25	-0.889	-0.022	0.790	0.000494	0.020
Fens Upstream Site 2 Rep 3	5	0.25	-0.889	-0.022	0.790	0.000494	0.020
Fens Upstream Site 3 Rep 1	5	0.25	-0.889	-0.022	0.790	0.000494	0.020
Fens Upstream Site 3 Rep 2	5	0.25	-0.889	-0.022	0.790	0.000494	0.020
Fens Upstream Site 3 Rep 3	5	0.25	-0.889	-0.022	0.790	0.000494	0.020
Fens Downstream Site 1 Rep 1	5	0.25	-0.889	-0.022	0.790	0.000494	0.020

Fens Downstream Site 1 Rep 2	5	0.25	-0.889	-0.022	0.790	0.000494	0.020
Fens Downstream Site 1 Rep 3	5	0.25	-0.889	-0.022	0.790	0.000494	0.020
Fens Downstream Site 2 Rep 1	2	0.25	2.111	-0.022	4.456	0.000494	-0.046
Fens Downstream Site 2 Rep 2	2	0.25	2.111	-0.022	4.456	0.000494	-0.046
Fens Downstream Site 2 Rep 3	2	0.2	2.111	0.028	4.456	0.000784	-0.046
Fens Downstream Site 3 Rep 1	2.5	0.25	1.611	-0.022	2.595	0.000494	-0.035
Fens Downstream Site 3 Rep 2	2.5	0.25	1.611	-0.022	2.595	0.000494	-0.035
Fens Downstream Site 3 Rep 3	3	0.25	1.111	-0.022	1.234	0.000494	-0.024
	A = 4.111	B = 0.228			Σ = 29.273	Σ = 0.066179	Σ = -0.105

Coefficient of correlation, r, calculations:

Mean $(\overline{x}) = \frac{sum\ of\ terms}{number\ of\ terms}$

Nitrate (A) Mean:

- Nitrates Mean = $\frac{(5+5+5+5+5+5+5+5+5+5+5+5+2+2+2+2.5+2.5+3)}{18}$ = 4.111 mg/L

Ammonium (B) Mean:

- Ammonium Mean = $\frac{(0.15+0.25+0+0.25+0.25+0.25+0.25+0.25+0.25+0.25+0.25+0.25+0.25+0.25+0.2+0.25+0.25+0.25)}{18}$ = 0.228 mg/L

Coefficient of Correlation (r) = $\frac{\Sigma(\Delta B\ x\ \Delta B)}{\sqrt{\Sigma\Delta A^2\ x\ \Sigma\Delta B^2}}$

where

ΔA = A (mean of variable 1 - each value of variable 1)
ΔB = B (mean of variable 2 - each value of variable 2)
(Variable 1 = Nitrate, Variable 2 = Ammonium)

r = $\frac{\Sigma(\Delta A\ x\ \Delta B)}{\sqrt{\Sigma\Delta A^2\ x\ \Sigma\Delta B^2}} = \frac{-0.10542}{\sqrt{29.27348\ x\ 0.06618}}$ = - 0.0757

which means that Nitrate and Ammonium are negatively correlated and that this correlation is fairly weak (because of r's distance from -1 and +1).

11) Does the co-efficient of correlation support your expectation? Why or why not? This should be an ecological explanation, not a mathematical one. Use your observations from each site to support your argument.

The negative coefficient of correlation for nitrates and ammonium in the fall supports the expectation of a negative correlation. This negative correlation suggests an inverse relationship between nitrates and ammonium, consistent with the understanding of nitrogen cycling in aquatic ecosystems. As ammonium levels increase, nitrates decrease, indicating utilization of nitrogen in nitrification processes.