THERMAL RESPONSE OF RESPIRATION: FROM LEAVES TO FORESTS
LABORATORY 7: APRIL 10TH OR 11TH, 2013
ENVIRONMENTAL BIOLOGY II/LIFE SYSTEMS

The Big Picture:
Respiration is thought to be the oldest of all metabolic pathways and is shared by all living organisms. It is through respiration that organisms produce energy and carbon skeletons that support growth, differentiation, metabolic activity and the maintenance of life. In the most basic sense, respiration is enzymatically controlled and regulated by the supply of substrates (from autotrophy or ingestion) and is sensitive to environmental conditions, particularly temperature. Respiration is a cellular process, however a basic understanding of the regulation of respiration at these small scales provides useful insights in the functioning of ecosystems.

Synopsis:
In this lab, you will study the effects of substrate and temperature on the physiological process of respiration. During this you will make some predictions about how different tree species and environmental temperature affects carbon exchange. We will first consider enzyme kinetics by using a model system with marbles. Next, we will use an online tool to examine the effect of temperature on respiration of oak and maple leaves. Finally we will scale these results to the level of an entire forest and consider the effects of a change in species composition under a warming climate.

We will go through sections I and II of the lab as a class, and your answers for those sections will be in addition to your lab report. Section III will introduce the topics for your report.

Background reading (a classic paper on the temperature response of respiration):
Forward DF (1960) Effect of temperature on respiration. *In*: Handbuch der Pänzenphysiologie (eds Ruhland W et al.), Vol. 12, Part 2, pp. 234-258. Springer, Berlin.

Primary research paper:
Bolstad PV, Davis KJ, Martin J, Cook BD and Wang W (2004) Component and whole-system respiration fluxes in northern deciduous forests. *Tree Physiology* **24**: 493-504.

Introduction:
Respiration is a series of enzymatically driven reactions that together oxidize glucose to CO_2 and release energy that was formerly stored in the covalent bonds holding the glucose together. All living organisms use respiration to create energy and the carbon building blocks of complex molecules. The enzymes involved in the many steps of respiration (more than 20 distinct reactions occur) regulate the rate of the overall process, so we will begin this lab considering enzyme kinetics using a heuristic model. In this model your hand will be the enzyme and marbles will be the substrate. Of course enzymes are not sentient and cannot actively search out substrates, so to make this fair you will need to be blindfolded. You will work through this part of the lab by gathering and posting the data, and then analyzing it together.

Section I. Enzyme kinetics

Enzymes catalyze biochemical reactions, that otherwise would proceed very slowly. They are essential to life, as without them, the chemistry that maintains life out of equilibrium from the environment would not be possible. It has been observed that a typical enzyme (E) converts a substrate (S) into a product (P) according to the chemical formula $S + E \rightarrow E + P$. Assuming that we are dealing with a single-step reaction, at any time t , $P(t) = kS(t)E(t)$, where k is a rate constant. This is due to the law of mass action, which defines the rate at which a single-step chemical reaction proceeds as being proportional to the product of the concentration of reactants. Thus increasing S_o , the initial concentration of substrate, and keeping the enzyme concentration constant, we could increase without limits the initial rate v_o at which the product is formed. This conclusion is not supported by observations; rather v_o saturates, reaching a value beyond which the addition of more substrate does not increase the rate of initial formation of the product. Therefore, it has been postulated that an intermediate compound (C) is formed, rapidly comes to equilibrium with the reactants, and then decomposes more gradually producing a molecule of the product and regenerating a molecule of enzyme.

That is to say, $S + E \leftrightarrow C \rightarrow E + P$.

If we consider v_o as a function of S_o (keeping E constant), the following graph can be drawn:

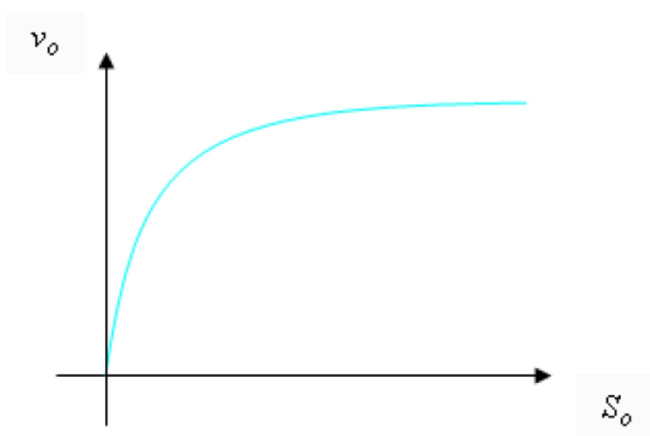

Figure 1. Initial rate as a function of initial concentration of substrate

This curve demonstrates the observed phenomenon of saturation: no matter how much substrate is added, the initial rate cannot surpass the limiting rate $V_{\max}$ (the maximum velocity).

Rate or velocity (v) of the reaction can now be calculated by the following equation, where K_m is the rate constant:

$$v = \frac{V_{\max} S}{K_m + S}$$

This relationship is known as the **Michaelis-Menten equation**, honoring Leonor

Michaelis and Maud Menten, who in 1913 published a groundbreaking paper on enzyme kinetics. We will use this equation to think about how properties of the enzyme affect metabolism.

Experiment 1a:

Note: This experiment will be done jointly by the whole lab

Start by calculating **Michaelis-Menten** values for our hypothetical reaction (soy bean transfer). We will run an experiment where a student's hand is the enzyme and the soy beans are the substrate. We will make a graph on the board and discuss all of the variables involved. From this graph, estimate V_{max} (the maximum velocity of the reaction if there were an unlimited substrate supply), and then calculate K_m (the rate constant, also know as the half saturation constant or the amount of **substrate** required to get *half* of the maximum rate of the reaction). So once you estimate V_{max}, simply determine the amount of substrate needed to get half that rate visually by using the information on the graph).

Regulation of glucose uptake

Now lets consider an actual enzymatic reaction related to glucose oxidation in respiration. The number of glucose transporters on the cell surface and the affinity of the transporters for glucose limit the rate of entry of glucose into a cell. Blood glucose levels typically hover around 5 mM, but can go as high as 12 mM immediately after a rich meal. The basal glucose transporters present in nearly all cells have a K_m for glucose of approximately 1 mM, much less than the average blood glucose concentration. For most tissues, then, glucose uptake proceeds at a fairly constant rate, regardless of the amount of glucose present in the blood. The first enzyme involved is hexokinase and it converts glucose into glucose 6-phosphate. Lets assume following the consumption of some chocolate your blood glucose level increases from 5 to 12 mM.

Experiment 1b:

Before you begin, *<u>start by forming a hypothesis</u>* about how the reaction rate will be affected by increasing and decreasing both K_m and V_{max}.

You will test your hypothesis by analyzing three graphs. All three graphs will plot blood glucose levels (concentrations) from 0-30 mM. The reaction velocity will need to be calculated based on the provided information and the **Michaelis-Menten** equation (above). First, use the **Michaelis-Menten equation** to make a plot of hexokinase reaction rate (v) vs. glucose concentration (S) and then calculate the change in the rate of the reaction (v, or velocity) before and after you ate the chocolate. We will then use this graph to explore how the two variables in the equation (K_m and V_{max}) affect the rate of the reaction.

1. Make a graph of the reaction rate (v) vs. substrate concentration (S)
    a. K_m = 1 mM (Glucose)
    b. V_{max} = 15 reactions s^{-1}
2. Make one graph demonstrating the influence of changing the K_m. It should have three

lines demonstrating each of the three K_m values.

   a. K_m = 0.5, 1, and 2 mM
   b. V_{max} = 15 reactions s^{-1} for all three scenarios

3. Make a graph demonstrating the influence of changing the V_{max} It should have three lines demonstrating each of the three V_{max} values.
   a. V_{max} = 7.5, 15 and 30 reactions s^{-1}
   b. K_m = 1 mM (Glucose) for all three scenarios

***Note, results from Experiment 1b will not be included in your normal lab report, but will be included in your appendix.**

Section II. Forest Tree respiration and climate change

As discussed above, respiration is highly sensitive to temperature. This temperature sensitivity originates at the enzyme level but is realized at higher levels of organization through CO_2 and/or O_2 exchange. As temperatures increase, the added energy in the system leads to an increase in molecular motion. Generally things become much more fluid and diffusion rates increase. Imagine the marbles in the container representing the substrate are moving faster, making them more likely to come in contact with your hand. Eventually if the temperatures get too high, the enzymes also become highly fluid and may lose their shape reducing the efficiency with which the reactions occur, or they may even lose their shape completely and denature. As this happens and the biological membranes that are so important to cellular function can also become compromised and the cells may die. Over ecologically relevant temperatures (~ 0 to 45 °C) respiration tends to increase exponentially with temperature. The precise rate of increase however is dependent on many biological and physical variables. Next we will consider how species and the environment regulate respiration, scaling up our knowledge of enzymes to leaves and forests.

In this part of the lab we will change our focus to specifically consider tree and canopy respiration, and how it might respond to global warming and/or changes in the species distribution brought on by human activities. Plant respiration in many ways is identical to human respiration: it's enzyme mediated, sugar consuming and energy producing. We most often measure respiration as CO_2 flux from the surface of a leaf, stem, soil or forest canopy. Respiration is strongly regulated by temperature and both the absolute rate of respiration and the temperature response can vary from one plant species to the next. Most vegetation and ecosystem models incorporate simple temperature coefficients to consider the impact of climate on this physiological process.

The equation we will use is a modified Arrhenius function described by Lloyd and Taylor (1994): where R_0 is respiration rate at the base temperature, T_0 (15 °C), T_a is actual leaf temperature (K), R_g is the gas constant (8.314 J mol^{-1} K^{-1}) and E_o is a parameter related to the energy of activation. You will need to convert T_0 into units of K (°C + 273.15 = K)

$$R = R_0 e^{\frac{Eo}{R_g}\left(\frac{1}{T_o} - \frac{1}{T_a}\right)},$$

For this next exercise you will use real data collected here in NY at **Black Rock Forest**. Located in the Highlands Physiographic Province, the Black Rock Forest (BRF) is a 1550-ha oak-dominated forest preserve. The topography is rocky with steep slopes and elevations ranging from 110 to 450 m above sea level. Mean annual precipitation is 1.2 m and air temperature is strongly seasonal, with monthly averages ranging from –2.7 °C in January to 23.4 °C in July.

Oaks dominate the forest, but there are relatively few oaks in the understory (see Figure 2), suggesting the composition forest is likely to change over the next 50 years. **The purpose of this section of the lab is to consider how environmental variables drive physiological processes and then make some simple predictions regarding how the forest may function in the future**. At a temperature of 15°C oaks and maples respire at similar rates, however they have very different temperature responses (E_o above).

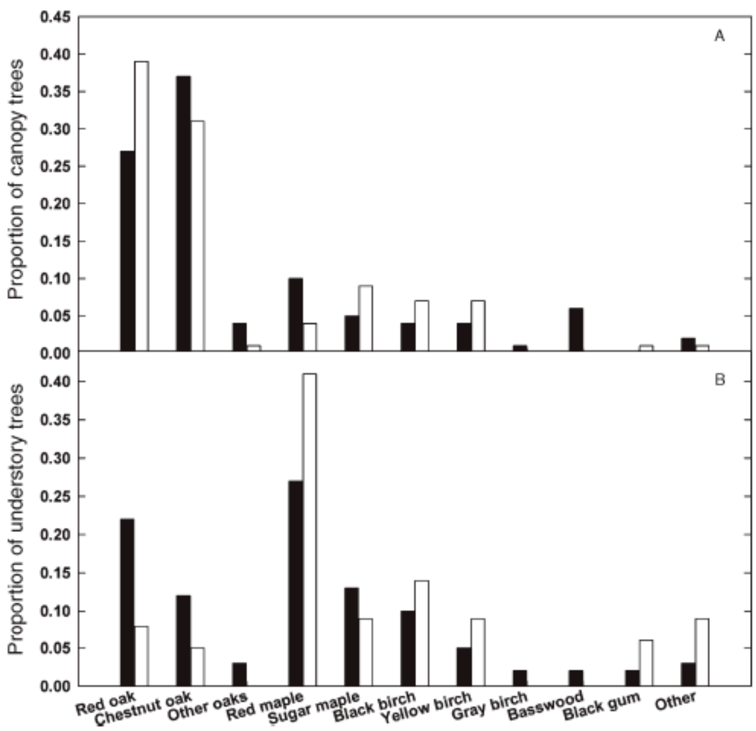

Figure 2. (A) Canopy and (B) understory tree species composition on eight long-term plots in the Black Rock Forest in 1936 (open bars) and 2006 (filled bars).

Experiment 2a: Leaf-level respiration

Note: For the next experiment, you should read through the scenario (above) and write down your hypothesis before collecting any data.

1. **Use the Leaf-level tool in the *tree respiration module* of the Virtual Forest Initiative tool** to plot the response of respiration to temperature for the two main types of trees, oaks and maples (*note this tool works best in Firefox and we strongly recommend you use this browser to complete this exercise*). Use the following information from our publication (*Turnbull et al 2001, Tree Physiology 21:571-578*) to create the graph of *R*

vs. T_a from 0 to 30 ºC. (T_o = 15)

a. Oak: R_{15} = 0.86 μmol m^{-2} s^{-1}, E_o = 40000 J mol^{-1}
b. Maple: R_{15} = 0.84 μmol m^{-2} s^{-1}, E_o = 27000 J mol^{-1}

Questions: *Be sure you understand and are comfortable with the Arrhenius equation and the effect each variable has on the temperature response. Understanding these responses will be useful as you interpret your results in the rest of the lab. Experiment with changing variables and observing the effects so that you can answer the following questions:*

a. What is the effect of a doubled R_{15} vs. a halved E_o?
b. Is the rate of change constant across the entire temperature range?
c. How do Oaks compare to Maples?
d. How does the response of the two species compare at low temperatures vs. high temperatures? – (note: moving the vertical line on the graph tool lets you see the calculated rate of respiration at any temperature)
e. The base rate of respiration (R_{15}) is very similar in these two species; do you think it is even worth worrying about this small difference?
f. At what temperature is the respiration of the two species equal and how does this relate the ambient air temperature in NY?

Experiment 2b: Forest-level respiration

2. Use the background information provided by the graph you plotted in experiment 3a to formulate hypotheses for the following:
    a. ± 5°C change in air temperature, assuming the growing season average is 15°C.
    b. A change in species composition favoring:
        i. Oaks
        ii. Maples

Now lets make some calculations using actual climate data from Black Rock Forest to test your hypotheses about variations in respiration. It is recommended that you read through the scenarios and assumptions before you start to make any calculations. In this section we are scaling the results from the leaf module to longer time frames and larger spatial scales. Essentially from experiment 3a, we have a good understanding of the leaf response, now we need to consider how many leaves of each type (Oak vs. Maple) there are in the forest, and how the actual air temperature varies over a growing season. We will do this in the **canopy module of the virtual forest initiative tool**. Clicking on the tab near the top of the page will take you to this module and the information you have entered in the leaf-level tool should be forwarded.

3. For each scenario (a-d) plot and report the total amount (moles) of carbon respired from a m^{-2} of forest. Use the data from the "open lowland" station in 2005 (an average year)
    a. Assume the forest is all oak (species composition (SC) = 100%)
    b. Assume the forest is all maple (species composition (SC) = 100%)
    c. Assume the forest is 70% oaks and 30% maples

d. Compare today's forest with 70% oaks and 30% maples with a potential future scenario when the forest might be 70% maples and only 30% oaks, and temperatures might be 5°C warmer than they are today

To do this you will need to make the following assumptions:

i. Leaf temperature = air temperature
ii. The amount of leaf area in the forest does not change and equals 2.5 m^2 of leaf area per m^2 of ground area.
iii. Respiration continues 24 hours a day
iv. The growing season last from May 15 to September 15

Questions: *Please comment on the validity of these assumptions in your lab report. Be sure you understand and are comfortable with all the variables in the canopy level module and the effect each variable has on the overall response. Experiment with changing variables and observing the effects, then run the four scenarios (a-d) above so that you can answer the following questions:*

a. How do a hypothetical Oak forest compare to a Maple forest?
b. How do mixed forests relate to monocultures?
c. For the final test scenario (the forest changes to be 70% maple and 30% oak at a time in the future when the temperatures are 5°C warmer) please discuss if you think the change in species composition had a greater or lesser effect on the amount of CO_2 respired than the warming, and indicate how you might test this opinion (with further calculations).

***Note, results from Experiments 2a and 2b will not be included in your normal lab report, but will be included in your appendix.**

Section III. Create your own experiment! (NOTE: this is the material for your actual lab report)

Experiment 3.

1. Use the virtual forest tool to explore a relationship between temperature and respiration at Black Rock Forest. You may ask any question you like focused on how respiratory responses scale across space and or time, and will be graded on the process of testing a hypothesis. The required steps are:

   a. *Clearly state a testable prediction*
   b. *Test this prediction with virtual forest tool*
   c. *Interpret your results*

There are many appropriate questions that are easily testable with this tool, so formulate one that you find interesting.

Please choose one of the following questions to base your testable prediction on for your lab report (if you would like to address a question that is not on this list, please run it by your TA first):

How does respiration change through the season – is the total respiration the same in May vs. June, July, August and September?
How much does respiration change from one day to the next?
How does respiration change from one year to the next (for example with in the virtual forest module you have data available from 1996 – a wet year and 2002/2205 average rainfall years)?
The climate has warmed by 1ºC in the last 100 years, has this had an effect on the amount of respiration? How might climate change affect forest respiration?
Gypsy moths can dramatically defoliate the forest, what would happen if moths consumed half the leaf area?

Remember using the Virtual Forest tool you can alter:

Leaf level variables:

- R_{15} & E_o for as many species as you like
- T_o - the base temperature

Canopy level variables:

- Number of species and their respiratory characteristics (R_{15}, E_o & T_o)
- The relative species composition of the forest (sc)
- The Leaf area index of the forest
- The station and year of the climate data
- The length of the field season to be considered (the integration time)
- A step change in the temperature (*e.g.* global warming – ΔT_a)

Lab Report Format

Your official lab report will be based on section III of the protocol (where you formulate your own prediction based on the list of questions given).

Your answers (including any figures) to sections I and II will be include in an appendix in addition to your lab report.

Your lab report will consist of the following parts:

- Abstract
- Results (including figures and tables)
- Discussion (including the relationship to the primary research paper)
- References
- Appendix (includes answers to other questions in the lab protocol)

Note that we do not ask you to write an introduction or methods section. This is because we explain the basis for the lab report (which usually goes in the introduction) and provide the methods, so there's less value in asking you to repeat those in your report. This modified format involves less writing, but emphasizes the analysis of your own results.

The ***Abstract*** should concisely summarize the point of the lab, the results and the conclusions from the discussion. The abstract should include quantitative results. Limit to 250 words

The ***Results*** should report a summary of your data and the results of your statistical tests and comparisons. It should include both a narrative of your key findings and figures and/or tables (see below for details). These should include at a minimum:

1. At least one graph presenting the results of your experiment from experiment 3 in section III.

Figures and tables should all have numbers and captions so you can easily refer to them in your text. Table numbers and captions should be located above the table, and figure numbers and captions below the figure. Basically, a table or figure should be self-explanatory and be able to stand alone with just the caption and still make sense. Do not interpret your findings in this section; save that for the discussion.

Check out any scientific journal article (like the one you'll use to write this report) to get an idea of how this is done and what sort of information should be in the caption and how a results section's text is laid out.

The ***Discussion*** needs to interpret your results (From the Virtual Forest portion of the lab section III) and relate them to other literature. These should include at a minimum:

Experiment 3:

a. *Why your prediction is interesting/important?*
b. *How you were able to test this hypothesis with the virtual forest tool?*
c. *What is your Interpretation of these results?*

In general you should discuss your predictions and whether your results supported these predictions. Interpret any differences you find in light of the natural history of these two species. Comment on whether these data provide a valid test of your predictions. You should also generally discuss how you think environmental variation will affect respiration.

Remember that variation has both a genetic (species) component and an environmental component. Read the primary research paper (Bolstad et al 2004) to get some idea of how this variation may be expressed in contrasting environments and how respiratory fluxes (e.g. soils and stems – remember all living tissues respire!) contribute to forest carbon exchange with the atmosphere. Relate your findings to the key issues in this paper.

The ***Reference*** section will include full citation information for any sources that you use in your report. At a minimum, you should cite the two papers provided with the lab.

The ***Appendix*** for this lab should include answers for sections I and II of the lab (answers to the following questions). These answers should only be one or two sentences (NOT more). And include figures where appropriate.

Experiment 1b:

1. Make a graph of the reaction rate (v) vs. substrate concentration (S)
   a. $K_m = 1$ mM (Glucose)
   b. $V_{max} = 15$ reactions s^{-1}

2. Make one graph demonstrating the influence of changing the K_{m}. It should have three lines demonstrating each of the three K_m values.

c. $K_m = 0.5$, 1, and 2 mM
d. $V_{max} = 15$ reactions s^{-1} for all three scenarios

3. Make a graph demonstrating the influence of changing the V_{max} It should have three lines demonstrating each of the three V_{max} values.

e. $V_{max} = 7.5$, 15 and 30 reactions s^{-1}
f. $K_m = 1$ mM (Glucose) for all three scenarios

Experiment 2a:

a. What is the effect of a doubled R_{15} vs. a halved E_o?
b. Is the rate of change constant across the entire temperature range?
c. How do Oaks compare to Maples?
d. How does the response of the two species compare at low temperatures vs. high temperatures
e. The base rate of respiration (R_{15}) is very similar in these two species; do you think it is even worth worrying about this small difference?
f. At what temperature is the respiration of the two species equal and how does this relate the ambient air temperature in NY?

Experiment 2b:

a. The validity of the canopy level assumptions made:
    i. Leaf temperature = air temperature
    ii. The amount of leaf area in the forest does not change and equals 2.5 m^2 of leaf area per m^2 of ground area.
    iii. Respiration continues 24 hours a day
    iv. The growing season last from May 15 to September 15
b. How do a hypothetical Oak forest compare to a Maple forest?
c. How do mixed forests relate to monocultures?
d. For the final test scenario (the forest changes to be 70% maple and 30% oak at a time in the future when the temperatures are 5°C warmer) please discuss if you think the change in species composition had a greater or lesser effect on the amount of CO_2 respired than the warming, and indicate how you might test this opinion (with further calculations).