Environmental Biology II/Life Systems – Spring 2010

Lab 1: Energy & Respiration

This lab is designed to explore some concepts related to respiration, an important physiological process regulating life on Earth. Your TA will begin the lab with some comments about respiration in general, enzyme kinetics, energy equivalents and the temperature response of respiration.

I. Energy

This chocolate bar has a net weight of **99**g, how much energy is in it? ***Put your results on the answer sheet-no formulas.***

To get started:

1. Convert the mass of the candy bar to moles of glucose (assume the entire bar is sugar - *not* a bad approximation).

How do you do this?
If chocolate were made of water –
1 mol of chocolate would have a mass of 18g
Hydrogen has an atomic mass of 1 and there are two atoms of hydrogen= 2g
Oxygen has an atomic mass of 16 and there is one oxygen = 16g
One mol of H_2O therefore has a mass of 18g
Of course we are assuming the chocolate is glucose - $C_6H_{12}O_6$
2. Glucose contains 686 kcal mol^{-1}. How many kcal of energy does the chocolate bar contain?

Now for the reward - show your *CORRECT* answer to your TA and consume the 30 kcal of energy they will give to you in form of a Dove Chocolate - hopefully this should be enough energy to complete the remainder of the lab!)

3. If that reward (the small chocolate) was 30 kcal, how much did it weigh?

To complete the remainder of the calculations you will need this information.

The complete oxidation of glucose during aerobic respiration leads to:

All 6 Carbons being converted to 6 molecules of CO_2

4 ATP molecules are formed by substrate level phosphorylation

10 NADH are formed

2 FADH2 are formed

The theoretical yield from this is 36 mol ATP mol^{-1} glucose

The actual yield from this is closer to 30 mol ATP mol^{-1} glucose

4. Now, back to the **large chocolate bar** –

How many moles of ATP could an organism THEORETICALLY harvest through respiration (assuming the complete oxidation of the entire mass of chocolate)?

If each mol of ATP contains 7.3 kcal of energy, how much energy is produced?

How many moles of NADH were produced and then converted to ATP?

How many moles of $FADH_2$ were produced and then converted to ATP?

How many moles of ATP were made directly from substrate-level phosphorylation?

5. Try as you might, none of you were able to gain the *theoretical yield* from the **small chocolate** your TA gave you, not even you overachievers.

How much energy (kcals) did you harvest (the actual yield)?

What percentage of the total energy available were you able to recover?

How many moles of CO_2 did you exhale from chocolate?

What was the mass of CO_2 that you exhaled?

What was the mass of C you exhaled and how does that compare to the mass of carbon you ate?

What percentage of glucose is composed of C?

What was the mass of water formed in addition to the CO_2?

How many moles of water were formed?

Actual yield from a C_6 fatty acid via beta-oxidation (twice):

- 2 rounds of beta-oxidation = 3 molecules Acetyl-CoA (=10 ATP per Acetyl-CoA = 30 ATP total)
- 1 ATP per round to prime (-2 ATP)
- 1 NADH per round (=2.5 ATP * 2 = 5)
- 1 FADH2 per round (=1.5 ATP * 2 = 3)
- Total = 30 - 2 + 5 + 3 = 36 mol ATP mol^{-1} fatty acid
- Fatty Acids yield > 20% than Glucose

6. For you overachievers (*this means you don't have to answer this if you don't want to! – it's a bonus question*) - of course this candy is not pure glucose. The "Nutritional Facts" of the candy tells me there are 42g of fat! If you can correctly calculate the energy available (in kcal, and mols ATP) assuming the Fat is a six carbon fatty acid that is broken down by beta-oxidation (from the lecture notes) and the remainder of the mass is glucose you can have the candy bar (or at least enter a raffle for it if more than one of you gets the answer right). You'll need to assume the fatty acid is 120g mol^{-1}. Of course if no one tries this, your TA's get to eat the chocolate!

II. Enzymes

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:

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

Rate or velocity (v) of the reaction can now be calculated as:

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

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. For those of you who are numerically inclined, we've included a paper describing the derivation of the Michaelis-Menten equation.

Lets start by calculating **Michaelis-Menten** values for a hypothetical reaction. This is just a sample exercise to get used to analyzing these types of graphs. This is actually very simple to do, just plot these data in excel (just as you see below). Highlight all of the numbers you entered, click insert, chart, XY (scatter), fill in the X and Y labels, insert as an object in the sheet you are working in. 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 by using the information on the graph).

Substrate *(S)*	Reaction rate *(v)*
0	0.0
1	33.3
2	50.0
3	60.0
4	66.7
5	71.4
6	75.0
7	77.8
8	80.0
9	81.8
10	83.3
12	85.7
14	87.5
16	88.9
18	90.0
20	90.9

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 during your consumption of the Dove milk chocolate you took your blood glucose level from 5 to 12 mM.

Use the **Michaelis-Menten equation** to make a plot of hexokinase reaction rate vs. glucose concentration 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}) effect the rate of the reaction.

Before you begin, ***start by forming a hypothesis*** about how the reaction rate will be affected by increasing and decreasing both K_m and V_{max}. (Question 1 on the Answer Sheet)

You will test your hypothesis by analyzing three graphs. All three graphs will plot blood glucose levels from 0-29 mM. The reaction velocity will need to be calculated based on the provided information and the **Michaelis-Menten** equation. (Question 2 on the Answer Sheet)

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

Remember to include X and Y labels and units on all of your graphs.

From graph one, what was the rate of the hexokinase reaction before you ate the chocolate (when your blood glucose was 5 mM) and after (when your blood glucose was 12 mM)? (Question 3 on the Answer Sheet)

III. Forest Tree respiration and climate change

Now lets change our focus to tree and canopy respiration, and how it might respond 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 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 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 by my colleagues and I here in NY at **Black Rock Forest** (http://blackrockforest.org/). 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 below), 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 temperatures responses (*E_o* above).

1. **Use the Leaf-level section in the Tree Respiration tool** (http://blackrock.ccnmtl.columbia.edu/respiration/) 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 – $R_{15} = 0.86$ mmol m^{-2} s^{-1}, $E_o = 40000$ J mol^{-1}

b. Maple – $R_{15} = 0.84$ mmol m^{-2} s^{-1}, $E_o = 27000$ J mol^{-1}

Be sure you understand and are comfortable with the equation and the effect each variable has on the temperature response. To do this:

a. Experiment with changing variables and observing the effects.

b. What is the effect of a doubled R_{15} vs. a halved E_o?

c. Is the rate of change constant across the entire temperature range?

d. How do Oaks compare to Maples?

e. 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).

f. 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?

g. At what temperature is the respiration of the two species equal and how does this relate the ambient air temperature in NY?

Use the background information provided from the graph produced in section 1 to formulate a hypothesis for the next section (2.)

2. From this plot speculate (make some hypotheses) on the general effect of:

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

3. 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 section 1, 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 section of the Tree Respiration tool** (http://blackrock.ccnmtl.columbia.edu/respiration/). 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.

For each scenario plot and report the total amount (moles) of carbon respired from a m^{-2} of forest.

a. assume the forest is all oak

b. assume the forest is all maple

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.

Use the data from the Open Lowland Station in 2005 – an "average precip" year. 2005 temperature plots from the Open Lowland station follow below:

Figure 1a. Seasonal trend in air temperature measured at the open lowland site in Black Rock Forest during 2005.

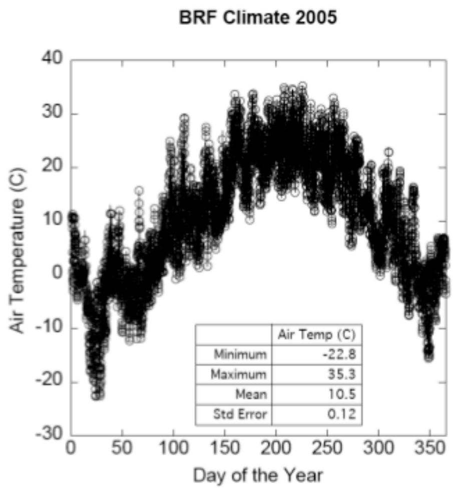
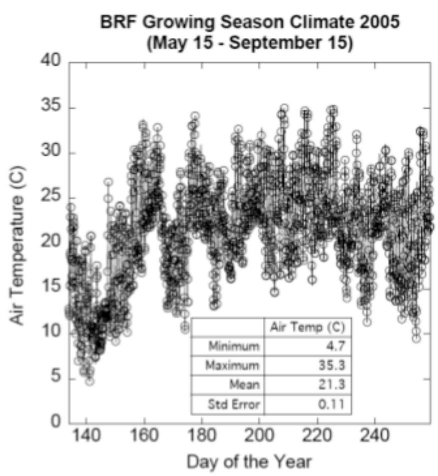


Figure 1b. Growing season air temperature (May 15 to September 15) measured at the open lowland site in Black Rock Forest during 2005.



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

4. 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).

5. In the final section of this lab, I want you to use the virtual forest tool to explore the relationships 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 hypothesis

b. test this hypothesis 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.

For example:

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 (you have data available from 1996 – a wet year - and 2002/2005 - average rainfall years.)

The climate has warmed by 1ºC in the last 100 years, has this had an effect on the amount of respiration?

Gypsy moths can dramatically defoliate the forest, what would happen if half the leaf area were consumed by these moths?

What sort of leaf level characteristics might favor the invasion of the forest by an introduced species and what effect would this have on the total carbon respired during a season?

We have climate data from two different stations in the forest, does it matter which data set we use – what does this tell us about stand of trees in different areas of the forest?

The combinations and scenarios are nearly endless, so be creative, have fun and try to learn something. Remember using the Tree Respiration 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)