LAB #1: DATA GRAPHING AND DILUTIONS

This lab is the first of two labs focused on laboratory techniques. This week we'll work on computer skills and dilutions, and next week we'll practice wet-bench techniques.

I. Graphing Exercise

The goal of this exercise is to provide an introduction to using a computer for data manipulation and graphing. We will be using Microsoft Excel, a common spreadsheet program. This software is excellent for data entry and manipulation, and passable for graphing (there are much better graphing packages around, but they require learning another set of software). Your lab instructor will lead you through the exercise in one of the computer labs on campus.

We will use the dataset below for our exercise.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Photoperiod (hours)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>O2 consumption (µl/hr)</td>
<td>Caterpillar mass (mg)</td>
<td>O2 consumption (µl/hr)</td>
<td>Caterpillar mass (mg)</td>
</tr>
<tr>
<td>15</td>
<td>0.078</td>
<td>0.23</td>
<td>0.59</td>
</tr>
<tr>
<td>18</td>
<td>0.18</td>
<td>0.42</td>
<td>0.43</td>
</tr>
<tr>
<td>21</td>
<td>0.21</td>
<td>0.32</td>
<td>0.46</td>
</tr>
<tr>
<td>24</td>
<td>0.22</td>
<td>0.22</td>
<td>0.52</td>
</tr>
</tbody>
</table>

These data are from an experiment measuring metabolic rate of *Helicoverpa zea* larvae under different conditions. *Helicoverpa zea* is a caterpillar that is a major pest on cotton, corn, and tomatoes. The adults are non-descript moths. Being an insect, this species is poikilothermic (i.e., cold-blooded) which means that the internal temperature of the animal is determined by the environment. Furthermore, metabolic rate of the animal (that is, the rate of the cellular respiration chemical reactions) will be affected by temperature. This is to be expected, since in general, rates of reactions increase as temperature increases. However, it is unclear to what extent photoperiod (i.e., how many hours of daylight per day) will affect this temperature/metabolic rate relationship. Photoperiod is one environmental variable that changes between seasons – for example, there are more daylight hours during the summer than in the fall. This experiment was designed to explore this relationship.

Experimental design: The researchers conducted this experiment using 8 caterpillars. Four of the caterpillars were grown in a chamber with a 10-hour photoperiod, whereas the other four caterpillars were grown in a chamber with a 14-hour photoperiod. Among the caterpillars in the 10-hour photoperiod, each was grown at one of four different temperatures.

Data Measurement: The metabolic rate of each caterpillar was measured when the caterpillar was approximately 1 week old. The metabolic rate was estimated as the amount of oxygen consumed (in microliters) by the caterpillar in one hour. The weight of each caterpillar was also measured.
**Data Entry & Data Manipulation:**
1. First, enter the data into an Excel spreadsheet.
2. Generate a standardized measure of O₂ consumption for each caterpillar – amount of oxygen consumed per mg of caterpillar body mass per hour. Do this by dividing O₂ consumption rate by caterpillar mass.

**Data Analysis and Graphing**
1. The researchers wanted first to see if the caterpillars in the two photoperiods were the same size. For example, if the short-photoperiod caterpillars were all much larger, then any differences in perceived metabolic rate could be due to caterpillar size differences, not photoperiod.
   ➔ Check this by calculating the average caterpillar mass for each photoperiod group on your spreadsheet.
2. Plot caterpillar metabolic rate versus temperature for each photoperiod group. What we want here is both sets of photoperiod data on the same graph.
3. Fit a line to each set of photoperiod data. Have the computer draw the line a little past the data points in both directions.
4. Show the equation for each line on the graph. Each equation should be an equation for a line of the form:
   \[ y = mx + b \]
   where \( x \) = temperature, \( y \) = oxygen consumption rate, \( m \) = slope of the line, and \( b \) = y intercept.
5. Make sure that your axes are clearly labeled – what the data are and the measurement units.
6. Put a caption below the figure – see pages 35-37 in Knisely's "A Student Handbook for Writing in Biology."

**II. Dilutions**

Conducting successful experiments depends on attention to detail, and the correct preparation of the chemicals and tools to do and analyze the experiment. As such, being able to mix chemicals of the appropriate concentration is a necessary skill of all biologists. However, many students have trouble calculating and making dilutions. This need not be the case – with a few concepts and a simple equation, you’ll be on your way to mixing any solution needed.

**Dilutions in General**

To understand dilutions, it is convenient to think about making juice from concentrate, such as orange juice. When you make orange juice from frozen concentrate, the directions say to use 3 cans of water added to the can of concentrate. This represents a 1:4 dilution: you end up with 1 part of frozen concentrate and three parts of water, for 4 parts total. You’ve made the concentrate 4 times as dilute, and a result much more drinkable.

**Molarity**

A mole can be defined in a number of ways. For instance, it can be a pigmented spot or mark on the human body. Or a burrowing insectivorous mammal. OR even a massive wall of stones laid in the ocean as a breakwater. For this course, however, a mole is the amount of substance that has a weight equal numerically to the molecular weight of the substance.
Example Calculation:

Suppose you have a 0.5M stock solution of glucose and you want to make 75 ml of 0.15M glucose solution. How much of the stock solution should you use, and how much water should you add?

Substituting into our dilution equation:

\[ v_1 c_1 = v_2 c_2 \]

\[ v_1 (0.5 \text{ M Glucose}) = (75 \text{ ml})(0.15 \text{ M Glucose}) \]

Solving for \( v_1 \), we get

\[ v_1 = \frac{75 \text{ ml}(0.15 \text{ M Glucose})}{0.50 \text{ M Glucose}} = 22.5 \text{ ml} \]

Answer: We should use 22.5 ml of the 0.5M stock solution. Since the final volume should be 75 ml, we should add 52.5 ml water.

For you visual learners:

```
0.5M Glucose

Water

22.5 ml

52.5 ml

Final Solution: 75 ml of 0.15M Glucose
```
III. Hand-in Assignment – Due at the beginning of lab next week

A. Graphing Assignment
   1. Graph of caterpillar data as described above.
   2. Copy of your spreadsheet used for graphing.
   3. Show the average mass of the caterpillars in each photoperiod. Did the average seem to differ?
   4. Statement of the slope of the line for each photoperiod. How did photoperiod affect the relationship between temperature and metabolic rate?

B. Answer the following dilution problems:
   1. In a 0.05 M solution of NaOH, how many mmoles are there per ml?
   2. In a 16 mM solution of anything, how many µmoles are there per ml?
   2. You have just plotted a standard curve for a protein concentration determination (see lab 2 if you want to know what a standard curve is). One of the standards had 24 µg protein in a 60 µl sample. **What is the concentration of the standard in terms of mg/ml?**
   3. Using a protein assay (an assay is a quantitative analysis of something), you have found that 0.1 ml of a 1:10 dilution of your unknown substance contains 320 µg of protein. **What is the concentration of the undiluted sample in mg/ml?**
   
   **Note:** A 1:10 dilution means a "10-fold" or a "10x" dilution. When people write "1:10 dilution," they really mean 1 part of the stuff you're diluting in 10 parts TOTAL solution. You're making something ten times weaker than it started out.

   4. You have a stock solution of 0.8 M Tris buffer (a buffer is a solution that does not change pH readily), and you want to make a 250 ml working solution with a final concentration of 10 mM Tris buffer. **How much of the stock solution and how much water do you need to add to get this?**

   5. The protocol for an enzyme assay requires that you add 20 µl of a 100 mM solution of p-nitrophenol phosphate to a total volume of 3.03 ml (this final volume includes the 20 µl that you added). **What is the final concentration of the p-nitrophenol phosphate?**

   6. Using the following stock solutions, describe how you would make 100 ml of a working solution containing all of the components at the indicated concentrations.

   **Stock solutions you have:**
   1.5 M Tris buffer
   50 mM EDTA
   0.1 M DTT

   **Working solution you want:**
   60 mM Tris
   50 µM EDTA
   2 mM DTT

   **Hint:** Use the dilution equation to figure out how much of each stock solution to use, and then add the appropriate amount of water at the end to make the final total volume of 100 ml.