## **Using Regression Models to Examine The Effect of Carbon Dioxide (CO2) on Fluid pH Instructor Guide**

* BIOLOGY GOALS/LEARNING OUTCOMES
	+ Explain why carbon dioxide levels in the extracellular fluid affect the pH of blood
	+ Measure pH changes as air is exhaled into water.
	+ Compare the rate of pH changes in someone at rest and the same person after exercising.
* MATH GOALS/LEARNING OUTCOMES
	+ Determine and graph the regression equations for linear and logarithmic models for the data to determine which is a better fit for the data.
	+ Interpret the slope and y-intercept of the linear equation.
	+ Use a regression model to make predictions, calculate and interpret residuals.
* Prior learning
	+ This activity needs no prior skills and can be used to introduce students to the concept of pH at the most basic level such as in an Introductory Biology course or Introductory Chemistry course. Instructors in these courses could modify which assessment questions are used, removing or replacing questions more suitable to the knowledge level of their students.
* How can the instructor help students who are weak in their prior knowledge?
	+ The instructor could give a very brief (15 minute) lecture reviewing cellular respiration and ATP production (information provided in the background), the relationship between hydrogen ion concentration and pH values (pH measures the concentration of hydrogen ions in a solution with lower pH values indicating higher concentration of hydrogen ions due to the negative logarithm), and carbon dioxide diffusion into alveoli to be exhaled and removed from the body during breathing.
* TIME: How long will the module take to complete?
	+ If both at rest and after exercise data is collected, the collection of data should take students approximately 45-50 minutes. If the instructor is pressed for time the data collection could be shortened by having students only collect “at rest” data points and then forming a hypothesis about how the readings may change if someone exercises based on the background material.
	+ If students complete the data collection, lab clean up, and post lab assessment questions the module will most likely take around 2 hours to complete in its entirety.
* Are there parts that THE INSTRUCTOR should do BEFORE class?
	+ The instructor should obtain pH meters, pH indicator strips or cabbage water before class and make sure that there are enough beakers and distilled water for each group. It is also important to ensure that there is a squirt bottle of distilled water and container or sink to rinse the pH probes between readings.
* Are there parts that THE STUDENTS should do BEFORE class? How long will it take?
	+ Students should read the background information that is given or any sections of their textbook or lab manuals discussing cellular respiration, ATP production, lung function, and skeletal muscle contraction before class. Students should also complete the pre-lab questions before class.
* What parts really need to be done in class (and how long will it take)?
	+ The data collection has to be done in class (estimated time: 45-50 minutes). Post lab assessments can be done as homework assignments if needed.
* How can the instructor facilitate the activity in class?
	+ If possible, it would help to give students a brief, very short review of what pH measures, cellular respiration, how ATP is used in muscle contractions, and factors affecting breathing rate changes.
	+ The instructor should demonstrate exhaling into the water as directed to avoid “puffing” cheek air into the water.
	+ The instructor should circulate among lab groups during the activity monitoring the rinsing of probes, correct pH reading techniques, and answering any questions students may have.
* Are there parts that should be done AFTER class?
	+ If the instructor is short on time the post lab assessments could be done after class.
* Assessment:
	+ There are questions to assess understanding in my original “Student Instructions and Assessment” that seem to have been removed from the current documents. These questions combined with the pre-lab questions and Questions to Assess Specific Knowledge should be used to assess the learning outcomes.
* Resources:
	+ Any human anatomy and physiology textbook contains all of the biology information.
	+ Any elementary statistics textbook contains all of the mathematics information.

**Set Up for Lab Instructors**

1. This lab is intended to be straightforward with minimal material requirements and also to be easily customizable. Please make it your own.
	1. **Materials needed:**
		1. **Small glass beakers or plastic cups and straws.**
			1. Preferred: Clear beakers or cups.
			2. Adaptation: Any small vessel that holds water.
		2. **Distilled water.**
			1. Preferred: It is very important to use distilled water if at all possible, since this will ensure more accurate pH measurements.
			2. Adaptation: If you do not have access to distilled water, purified water in a jug from the grocery store or even tap water will work fine.
		3. **pH meters**.
			1. Preferred: It is best to use pH meters for this experiment in order to detect the small pH changes and create better graphs if you are including graphing in this exercise.
			2. Adaptation 1: If you are forced to use pH strips, it is preferrable that you use strips that detect incremental pH changes (the values between the whole numbers such as 4.5, 5.2, 6.8, etc.). pH strips that only detect whole number values (4.0, 5.0, 6.0, etc.) should only be used as a last resort since they will not be sensitive enough to record the readings needed to do the graphing exercise.
			3. Adaptation 2: This experiment can also be done with colored fluid such as purple cabbage water or liquid aquarium pH indicator that measures pH increments such as this one:



1. **Troubleshooting:**
	1. **Problem:** The pH value of the first breath is significantly lower than pH value of distilled water (the water should be at or close to pH 7.0) and changes very little in subsequent readings.
		1. **This occurs when students exhale forcefully or for too long and the water rapidly becomes saturated with carbon dioxide.** Ask the students to repeat the experiment and carefully monitor the amount of bubbles produced during their exhalations. When exhaling, “less is more” applies to this experiment.
	2. **Problem**:There is little or no difference between the rate of change of pH values at rest and after exercise.
		1. Students must elevate their heart rate for 5 minutes with fairly vigorous exercise in order to see changes occur. Encourage them to exercise until they are at least slightly out of breath and feel like their heart rate is elevated. The more vigorous the exercise, the more dramatic the changes in pH.
	3. **Problem**: The pH values remain close to 7.0 or barely change while using a pH meter.
		1. This may occur if students do not carefully rinse off their pH probes between measurements and fluid from previous measurements remains on the tip of the probe.
2. **Ideas for Discussion:**
	1. **Chemistry**
		1. The formula for the reaction that is occurring is:

  **CO2 + H2O + carbonic anhydrase ↔ H2CO3 (carbonic acid) ↔ H+ + HCO3-**

* + 1. Carbon dioxide combines with water to form carbonic acid, even in the absence of the enzyme carbonic anhydrase. However, in organic life forms ranging from bacteria to humans contain carbonic anhydrase to speed up the process.
		2. Carbonic acid is very unstable and quickly dissociates into protons (H+) and bicarbonate ions (HCO3).
		3. **pH is a measurement of the H+ concentration of a solution**. It is a logarithmic equation and as the concentration of H+ increases in a solution the pH number decreases. pH values of less than 7.0 represent acidic solutions and pH values of more than 7.0 represent basic solutions.
		4. Increases in CO2 concentrations drive the above reaction to the right, resulting in the production of more carbonic acid, and subsequently, more H+ protons and a decrease in pH (acidification).
	1. **Ecology/Geology/Environmental Studies**
		1. Carbon dioxide immediately reacts with water to form carbonic acid (see equation under “Chemistry” above and introduction).
		2. How does acid rain form?
			1. There is water in Earth’s atmosphere in the form of water vapor and clouds.
			2. This atmospheric water vapor combines with carbon dioxide, making acidic water droplets that eventually fall to Earth as rain.
				1. What are some causes of increasing carbon dioxide levels in the atmosphere?
				2. What organisms REMOVE carbon dioxide and lower atmospheric carbon dioxide levels? (cyanobacteria, plants, any organism utilizing photosynthesis).
			3. Carbonic acid dissolves rock such as limestone and marble.
				1. **Question:** What happens to statues carved from limestone or marble when they are left outside in urban areas?
			4. Acidic rain also lowers the pH of bodies of water such as ponds, lakes, and streams.
				1. **Question:** Would lowering the pH of bodies of water have any effect on the organisms living in the water? If so, discuss some specific problems that acidification of water might cause the living organisms.
				2. **Question:** Have you learned about any organisms that thrive and live happily in acidic water? \*This is a good jumping off question to discuss acidophilic or acidotolerant organisms.

*Helicobacter pylori* is an acidophilic bacteria that can live in the stomach, where the pH is close to 2.0. *H. pylori* cause ulcers.

*Eschericia coli* is an acidotolerant organism that can tolerate the low pH of the stomach long enough to survive and travel into the intestines and cause disease.