When Harry Met Gabby: A Hypoxia Story

by
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"Ours is a watery world, and we, its dominant species, are walking sacks of sea water. The presence of large amounts of liquid water on Earth makes our planet unique in the solar system." *

Homework: Watch the video titled *When Harry Met Gabby, Part I* <https://youtu.be/pJ2NMZYIWWY> and then read the background information on the scientific method and hypoxia below. We will then demonstrate impacts of aquatic hypoxia in an experiment following the scientific method of investigation.

Background

Water is the solution of life. The early biosphere was not conducive to life because it contained low levels of atmospheric oxygen. As photosynthetic bacteria evolved, they consumed carbon dioxide and produced simple sugars and oxygen, creating sufficient atmospheric oxygen for more advanced life forms to develop (Brown, 1994).

Breathing oxygen is crucial for survival not only for land dwellers but for most fish and other aquatic organisms as well. The oxygen present in the atmosphere must be dissolved into water for use by aquatic organisms (dissolved oxygen, DO) (Mitchell and Stapp, 1992). When dissolved oxygen (DO) levels become unsustainable for life, a condition known as a dead zone occurs and amplifies the life-killing forces that depleted the oxygen, resulting in mass fish kills.

The inability to escape low oxygen areas makes immobile species such as crustaceans (e.g., shrimp, oysters, and mussels) particularly vulnerable to oxygen deficiency (i.e., hypoxia) (JSOST, 2010). These organisms can become stressed and may die due to hypoxia resulting in significant impacts on marine food webs and the economy. Mobile organisms can flee the affected area when dissolved oxygen becomes too low. Nevertheless, fish kills can result from hypoxia, especially when the concentration of dissolved oxygen drops rapidly. New studies are better at illustrating how habitat loss associated with hypoxia avoidance can impose ecological and economic costs, such as reduced growth in commercially harvested species and loss of biodiversity, habitat, and biomass.

There are two main sources of dissolved oxygen: (1) from the atmosphere, including turbulence from waves and rapidly moving rivers which help mix oxygen from the atmosphere with water; and (2) from photosynthetic aquatic organisms (algae and benthic, bottom-rooted plants), which consume carbon and release oxygen as a waste product into the waters. When disturbances occur in one or both of these important processes, impaired DO can have detrimental effects to aquatic life.

Using aquatic freshwater tanks in the laboratory, we will learn about the scientific method by scrutinizing causal relationships between fluctuating availability of an abiotic factor, abundant dissolved oxygen vs. hypoxia, which can impact the quality of aquatic life. An indicator species, the viper shrimp *(Atya gabonensis*, aka vampire shrimp), will be used to monitor water quality. A photograph of the viper shrimp is shown in Figure 1.

Many factors can be detrimental to aquatic life, and a low level of oxygen (hypoxia) is one of the most severe. To understand how water can lose its oxygen content, we need to understand the role of temperature on dissolution of oxygen into water, as well as atmospheric pressures, salinity, and a process called eutrophication. These are some of the most critical factors affecting the ability of oxygen gas to dissolve into water.

*Hill, 1992, p. 477.

Figure 1. Atya gabonensis, viper shrimp, adapts to changing salinity, not low levels of DO. The back appendages on the abdomen are swimmerets, or pleopods, which function in swimming. The front appendages on the thoracic segment are for walking, called pereopods. The claw adaptation used for grasping rocks is significantly larger in the adult male and is used for sex identification.
Credit: Wikipedia Commens user Ray eye, CC BY-SA 3.0, <https://commons.wikimedia.org/wiki/File:Blaue_Monsterfächergarnele.jpg>.

Important Factors That Impact Dissolved Oxygen Levels

- *Temperature, Atmospheric Pressure, and Salinity*
  The solubility of all gases, including oxygen, is inversely proportional to temperature, which means that the solubility of gases goes down as the temperature goes up, and vice versa (Figure 2). The concentration of dissolved oxygen also varies directly with atmospheric pressure and atmospheric oxygen concentration. When the atmospheric pressure or atmospheric oxygen concentration goes up, the level of dissolved oxygen goes up (Roskowski and Marshall, 1993). Salinity also affects chemical conditions within waterways, particularly DO. The amount of oxygen that can dissolve in water, or solubility, decreases as salinity increases. The solubility of oxygen in seawater is about 20% less than it is in fresh water at the same temperature (NOAA NERRS, *n.d.*).

- *Degradation of Organic Matter*
  Bacteria play a role in decreasing DO by enriching the water with plant nutrients. A reasonable amount of breakdown is good, so the water won't become oligotrophic or nutrient poor. But too much organic breakdown will decrease DO and leave an excess of nutrients. Eutrophication is a term used to describe a body of water in which the organic nutrients reduce the level of dissolved oxygen to such a point

Figure 2. Dissolved oxygen levels are impacted by increasing temperatures. Adapted from Lomborg, 2001.

that plant life is favored over animal life. Algae blooms cause excessive organic material also. When algae die, they become a part of the organic wastes (Nebel, 1990). Once DO in water is decreased by excessive organic matter and ongoing degradation, the process shifts to anaerobic processes. Animal life can be permanently suppressed in this environment and the kinds of aquatic organisms shift (Hill, 1992). Mayfly nymphs, stonefly nymphs, and caddis fly nymphs are replaced by pollution tolerant worms, fly larvae, nuisance algae, and other anaerobic organisms (Mitchell and Stapp, 1992).
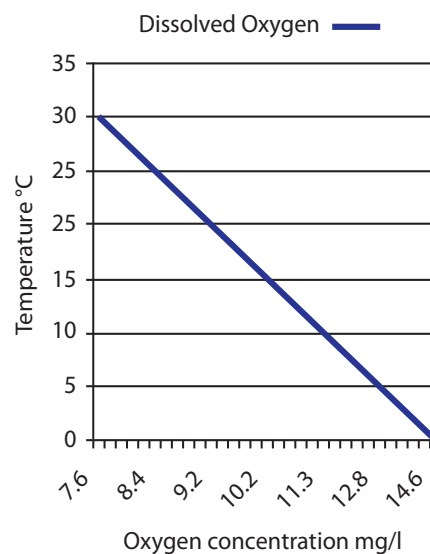
So what is a good level of dissolved oxygen? Less than 4 ppm DO is *not* good (Hidaka, et al., 1991). But what about too much dissolved oxygen? Scientists determine optimal and sustainable levels of oxygen by posing these types of questions. Experiments are then designed using the scientific method to analyze factors that are essential to healthy aquatic life.

The Scientific Method

The scientific method is used to answer questions and provide explanations about natural phenomena. The scientific method follows a logical process based on careful observation and experimentation. It is not based on any belief system, dogmatic assertions, or even a consensus when it comes to creating or accepting hypotheses. Since scientists assume natural events follow predictable patterns, these patterns are discernable by observation and established methods of scientific analysis.

In the first step, an observation gives rise to a question or questions about the observed phenomena. Based on these observations, scientists generate a hypothesis, which is a tentative explanation for what was observed. Hypotheses are written statements that explain the phenomena and are testable. The hypothesis should provide only one explanation and predictions (this is where we can use the "if-then" statement) that are tested using experimentation.

Not every question can be answered using the scientific method. Certain questions may be answered instead by using other "ways of knowing," including personal opinion, consensus, faith, tradition, and moral/ethical considerations, which have no basis in science (Alexander and Steffen, 2015). Science asks questions of the natural, observable world, not of the supernatural.

Scientific hypotheses are not only testable, they are also predictable, falsifiable, repeatable, and verifiable. Experiments involve two or more hypotheses. The null hypothesis (H_0) typically states the observed phenomenon is due to random causes or factors. The alternative hypothesis (H_1) covers other outcomes, which cannot be explained by H_0.

If the experiment has been designed properly, the data will support or reject your original hypothesis. (If you can neither support nor reject the hypothesis, then the experiment has been poorly designed.) If the experimental analysis leads to rejection of your hypothesis, then a new or revised hypothesis can be considered and the testing process repeated. In fact, if the hypothesis is supported, experimentation continues. This is because repetition supports and strengthens mounting evidence for the hypothesis. Scientific inquiry is an ongoing process. A given hypothesis is never considered to be proven, but rather is supported by the evidence. It is always possible that new data may lead one to modify or or even reject a previously supported scientific explanation.

During examination of a hypothesis, the object of study is placed into different treatment groups. Treatment types include at least one experimental group (examined by some kind of change or manipulation) and a control group (no change, kept at initial values). Well-designed scientific experiments test only one factor (variable) at a time, with all other conditions of the experiment kept constant. In some observable manner, an independent variable is manipulated or changed and causes an effect to a responding dependent variable. The dependent variable is not under direct control of the scientist; it is observed and measured in response to changes in the independent variable(s). The values of the dependent variables are thought to be caused by changes in one or more independent variable.

As an example expressing the relationship between independent and dependent variables, we can pose the question: What is the effect of density of different mediums (air, glass, etc.) on the speed at which light travels? The independent variable is the density of different mediums (isn't affected by speed of light travel), whereas the speed of light travel is a dependent variable (affected by density of different mediums). There are also variables that should be controlled (not to be confused with the control treatment) in order for the experiment to give a clear understanding of each variable's effect. If you are testing the impact of sunlight on plant growth, you would not also change air temperature at the same time.

In our laboratory experiment, we will need to understand the relationship between dissolved oxygen levels and shrimp mortality. Spend a moment to see if you can determine some dependent variables and independent variables based on the above description.

Based on the experimental data, you will need to make some conclusions. The data collected will be analyzed to give meaningful results from which to draw conclusions. Scientists do not follow the approach of trying to prove their hypothesis because both true and false hypotheses can lead to true predictions. Einstein said "A thousand scientists can't prove me right, but one can prove me wrong." It is easier to disprove a hypothesis, where just one observation can refute the hypothesis, than it is to prove a hypothesis. Since it is impossible to test every possible outcome, science advances only through disproof.

Questions

1. Give an explanation of H_0 and H_1.
2. What are dependent variables and independent variables?
3. Why is it important to execute clearly defined experiments?
4. What causes hypoxia in crustaceans like the viper shrimp?
5. What do you expect adding an excess of photosynthetic organisms will do to DO levels in a water system?

First Lab Period

The goal of this lab period is to formulate and examine a scientific hypothesis based on decreased oxygen solubility vs. mortality of freshwater shrimp *(Atya gabonensis)*. Two exercises will be conducted, a "black box" practice experiment and setting up an actual shrimp hypoxia study.

Preparation

- Review your lecture notes on water chemistry and factors impacting DO in water, including temperature, atmospheric pressure, turbulence, salinity, and pH.

- Read the "Background" section of this case study.

- Watch the video *When Harry Met Gabby, Part II* <https://youtu.be/WuVYLyqIfxk>

Thinking Inside the Box

First we will practice hypothesis-based experimentation using an exercise called "What's inside the black box?" Your task is to determine the contents within a mystery box provided by your instructor using the scientific method. Keep in mind that good experiments should only test one variable at a time, so you may need to conduct several experiments in order to come up with a complete list of the box's contents. Also keep in mind that you may be able to test the same hypothesis in different ways. Record your hypotheses, experimental design, data, and conclusions.

Black Box Questions

1. If your predictions were not correct, what may have led to your erroneous conclusions?

2. How could you have conducted the experiments differently to come up with the correct conclusions?

3. How else might you test this hypothesis?

4. What were the dependent and independent variables?

Shrimp Study

Your instructor will describe which treatment conditions are used in each of four aerated 10-gallon dechlorinated, freshwater tanks and how to introduce viper shrimp into the tanks for a one-week observation period. The instructor will then perform a small demonstration for the class on physiological effects to viper shrimp under unsustainable DO conditions (<0.5 mg O_2/L). While waiting for observable changes to the shrimp, lab rules for working with invertebrate animals will be reviewed. The class will then give predictions, considering the relationship between oxygen levels and crustaceans and the varying conditions in each tank. Students should follow a process similar to the black box experiment as they seek to understand this more complex situation involving both biotic and abiotic factors.

Tank Parameters: The instructor will set up two tanks with separate independent variables, another with both independent variables, and, finally, a control (no variable).

The experiments will occur under aerated, 12:12 light:dark photoperiod with strong light intensities avoided by use of black plastic sheeting or brown paper on three sides. Using the laboratory's water meter probe, pH and salinity (if using brackish water) will be monitored daily to standardized pH 7.5 and 20 ppm (<1.005 specific gravity), respectively, as well as monitoring and adjusting DO concentrations. Water temperatures of the four tanks will be recorded daily.

Experimental Setup: Once the tanks are ready, students will add laboratory-acclimatized viper shrimp directly into the tanks. These tanks are set up under varying conditions in order to measure shrimp response to each variable. Experimental observations will be conducted at time zero, 60 minutes (end of lab period), and in seven days (during next week's lab), and will be recorded in your lab journal as data collection. While waiting for the first time point,

the class will divide into teams to generate hypotheses, determine the dependent and independent variables in this experiment, and come up with predictions. Toward the end of the period, students will observe and report impacts to small invertebrate viper shrimp in freshwater tanks and compare data to the other time points. Groups will each collect a shrimp from the experimental tanks to view specimens, obtain an average wet weight, and biopsy any deceased shrimp under the dissecting microscope to identify anatomical structures and make drawings.

Students should discuss together and make end point predictions using the following table prior to completing the experiment. "End Point" is defined as a measure that allows us to decide whether the null hypothesis should be accepted. After observing results, students should record actual findings for all time periods in their lab journals and draw diagrams depicting what has been observed.

Observations and End Point Markers (norms vs. abnormal)		
Mortality	Alive	Dead
Discoloration/Spotting		
Pre-Molt State		
Wet Weight (gm)		
Length (cm)		
Behavior		
Location in Tank		
Rotifer Density		
Temperature		
Oxygen Levels		
Salinity		

Note: On the first day, two sets of data should be collected, Time Point Zero (0) and Time Point 60 minutes. The final Time Point, 7 days, will be collected at the beginning of next week's lab period.

Post-Lab Questions

1. Make predictions for each parameter and end point.

2. What are your dependent and independent variables?

Second Lab Period

Preparation

- Review your ecture and/or lab notes on use of model organisms in the study of water quality, osmosis, and diffusion.
- Watch the video:
  "Sick Fish, Eutrophication and Dead Zones" <https://youtu.be/0JnKkit5ocI>
- Answer the following questions.

Pre-Lab Questions

1. What is the definition of a bioindicator? Can you think of another good bioindicator in nature?

2. Is it possible for algae to function as a photosynthetic organism, providing oxygen to aquatic life, or does it function as a destroyer of aquatic life?

3. Define eutrophication and dead zones. Which comes first? And, if they cause fish to be sick, why would crustaceans like the viper shrimp be useful as a bioindicator species for fish?

In Class

Observation Period (5 minutes). This time should be devoted to observing the shrimp populations in each tank, and noting any visible end point markers. Weigh shrimp for final growth calculations (see instructor for formula). Discuss findings with your team members.

Post-Lab Questions

1. Was the hypothesis supported?

2. How do you know that the findings you report are not due to another parameter not factored into your experiment design, or whether the results were simply due to chance?

3. Can you think of ways to change the experiment and still predict an outcome that supports your hypothesis?

4. What is the effect of adding metabolically active aerobic bacteria to the tank? What would happen if you increased the density of rotifers? Would the addition of algae change your results?

5. Why do biologists collect this type of data rather than study fish populations?

6. Did either the rotifer or shrimp thrive during low oxygen levels? If so, give an explanation why one or both were impacted.

7. How might aquatic life benefit as scientists learn more about the relationship between viper shrimp and varying oxygenating events in streams and brackish water?

Final Report on "Hypoxia in Viper Shrimp" is due by next laboratory period.

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