

**Exercise 2: Factors Affecting Respiration in Goldfish**  
**Developed by Dr. Michael Dorcas**

**INTRODUCTION**

To understand the physiological problems animals face, it is first necessary to understand the abiotic properties of the environment in which they live. This is especially true when trying to understand the problems animals face in gas exchange and how those problems vary with the environments in which they live. Aquatic animals live in environments in which oxygen is found in relatively low concentrations (compared to air, at least). Additionally, the high viscosity and density of water makes it difficult to move the fluid back and forth into respiratory organs like we do with air in our lungs. Consequently, most aquatic animals have special adaptations that help acquire oxygen.

First, most aquatic animals have one-way or flow-through respiratory systems that pump water over their respiratory organs, usually some sort of evagination called a gill. Instead of pumping water into and out of a chamber containing the gills, the animals pump water in one opening and out another, producing a one-way flow of water across the organs. Consequently, the animals don't expend energy stopping the flow of water and starting it in the opposite direction, which would be much more difficult than performing the same task with air. Second, to increase the concentration gradient of gasses between the blood and water, most aquatic animals have some sort of counter-current exchange system that allows for maximal gas exchange. In this system, the blood flows in the opposite direction as the water, thus maintaining a substantial gradient across the entire gill surface. Finally, because oxygen is often limiting in aquatic environments, the nervous systems of aquatic animals are usually most sensitive to low oxygen levels in their blood, in contrast to terrestrial animals that usually are more sensitive to CO<sub>2</sub> levels.

In this laboratory, you will design and conduct an experiment examining a factor or factors that potentially affect(s) respiration in an aquatic animal, the goldfish (*Carassius auratus*).

**General Objective:** To develop and conduct original experiments investigating factors affecting respiration in an aquatic animal.

**Specific Objectives or Hypotheses: To be determined by you and your partners.** We will spend part of the week before this laboratory planning and designing your experiment.

**MATERIALS AVAILABLE:**

goldfish acclimated to room temp.	air pumps with air stones	pH meter
goldfish acclimated to 10°C	low dissolved oxygen water	hydrochloric acid
many beakers of different sizes	magnifying glasses	small nets
dechlorinated water at 10°C	stopwatches	thermometers
dechlorinator or dechlorinated water	sodium bicarbonate	dissolved oxygen meter
pipets	balance	ice

(If there is something else you need, ask. We may be able to get it for you).

**EXPERIMENTAL DESIGN**

Goldfish have been chosen for this laboratory because they are small, easily handled, tough, and inexpensive (about 8 cents each) aquatic animal. The primary dependent variable we will measure is respiratory rate, which can be measured easily by carefully observing the fish and counting gill ventilations (using a magnifying glass will help and sometimes it is easier to count mouth openings).

However, you can use other dependent variables if desired or if we have the ability. Respiratory rate calculations should be recorded as ventilations/minute.

I will give you some ideas for treatments during our introductory discussion, but I will let you decide on the specific treatments (independent variables) you will examine. You can subject goldfish to different treatments in any number of ways. Some treatments to consider are listed below. But you can come up with other factors that are of interest. **We encourage creativity!!** After you decide on the independent variables you would like to examine, write out your hypotheses (research and null). Consider and think about why these factors might affect respiration rates – they may increase stress or the need to metabolize at a higher rate, therefore causing a larger oxygen requirement.

You will need to plan, in detail, your experimental design. Remember to consider such factors as sample size, number of treatments, and control of extraneous variables. Sample size **is important**, since the fish are different sizes and ages, and randomly selecting only two for a particular treatment will cause high variance in your estimate of respiration rate. For instance, you might randomly select a stressed fish and a healthy fish, or a large fish and a small fish, and in both scenarios the different individuals will respond differently to the treatment. This is why we always try to have larger sample sizes, at least within the confines of our other constraints, such as total number of fish available and time to perform the experiment. One way to increase sample size without using more total fish is to **expose each individual fish to every treatment** (with a recovery time period in between each treatment exposure), which is called a repeated-measures design. Using each fish in each treatment or randomly selecting fish within the same size category will both help to reduce error variation, and are considered part of controlling extraneous variables (i.e., you might control for fish size, which will reduce variation caused by size). The treatments will be dependent on the question or hypothesis being addressed.

Other considerations you should make before proceeding include such things as how you will measure respirations. You may count gill operculum openings or mouth openings – both will be measurements of respirations, and one may be easier for you to observe than another, depending on your treatment. Count only for 15 second intervals instead of a full minute. This decreases your chances of losing count and allows you to take repeated counts for each individual to calculate an average +/- standard deviation. Also, you should consider dividing up the workload so that there is no bias in treatments caused by individual experimenters. How can you reduce this bias?

Finally, before you begin you also need to decide how you will analyze your data, based on your experience with various statistical tests (e.g., ANOVA, Regression, Chi-square). Before you begin your experiment, you must run through your hypotheses, proposed design, and proposed analysis with me.

**Potential independent variables to consider are:**

temperature	effects of acclimation	exercise
pH	crowding	oxygen concentration
aeration	individual size	

**Assignment**

Prepare a 10-15 minute oral presentation, with your group, on your experiment. Format it like a laboratory report – that is, incorporate all the components of a full report. Use the guidelines and suggestions below to assist you. **The presentations will be given during the last lab session of the semester, so plan accordingly. Email a PPT document to me prior to class. The file should be named using the following convention: lastnames\_fish.ppt.**

### Guidelines/Suggestions for Laboratory Symposium Presentation

In addition to using the chapter on oral reports in Pechenik, use these guidelines to help prepare your presentation. Prepare it as you would a report, in an organizational sense, with Introduction, Methods, Results, Discussion, and References. The presentation should run about 10-15 minutes, with all group members participating equally in preparation and presentation. Consider the following points as you prepare your presentation.

1. Avoid large amounts of text. Use bullets with short points, and then discuss those at greater length.
2. You may want to think about adding pictures of your organisms or setups. You can search the internet for pictures. If you include downloaded pictures, you must include the URL where the picture was obtained, or some other acknowledgement of the source of the picture.
3. Your introduction should address the following questions. What is the general phenomenon under consideration and why is it important? What is your purpose? What is your hypothesis?
4. For methods and materials, avoid long discussion of detailed methods. However, because each group designed their own study, you must go into some detail about your design, how it addresses your hypothesis, and how you analyzed the data.
5. **Results** should not have much text, but should focus more on high quality figures and tables that are readable by someone at the back of the room and back up the points you make about the data. You may want to include one slide of text with major points, which you describe, and then show with your visual aids. Include averages and standard deviations where appropriate.
6. **Figures and tables** can be made in Excel, tables can be made in Word, or both can be made in PowerPoint.
7. **Discussion** slides should emphasize two or three major conclusions. Be sure to address why the research is significant and how you might be able to generalize your conclusions to more than just one species. Do results support research hypotheses, how will your future experiments (that follow up questions from this one) be designed to reduce error or bias, and what new questions arise from this experiment?
8. Presentations often have acknowledgements thanking people that assisted with the project. For instance, Dr. Peroni supplied all the seeds for the LAP lab, and other groups often supplied data for certain populations.
9. Use large fonts and high contrast for ease of viewing by the audience. Apply the principles of preparing professional-quality figures discussed in Exercise 1.
10. Each person in the group should present equal portions of the presentation; you can divide it up by section, or each person can do part of each section.
11. Each person will receive a grade based on the overall presentation, and your individual performance. Grades will be based on: 1) organization and delivery, 2) visual aids (e.g., PowerPoint), 3) ability to convey how your study contributes to the science of biology, 4) ability to focus on 2-3 three major points, 5) ability to stay within the time limit, 6) ability to answer questions at the end, and 7) how well your Discussion addresses the broader significance of the research and relates back to issues raised in the Introduction.