

Measuring Changes in Blood  
Vessel Diameter in the Tail  
of the Goldfish

RECOMMENDATION

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**ABSTRACT**

This laboratory exercise enables students to measure changes in blood vessel diameter in the tail of the goldfish (*Carassius auratus*) using ImageJ software. The lab can be adapted for different course levels and equipment. Students can also perform statistical analyses on the results.

**Key Words:** Vasodilation; vasoconstriction; goldfish; blood vessel; measurement; ImageJ software; temperature; graphing.

**○ Introduction**

Vasoconstriction (decreased diameter of vessels) and vasodilation (increased diameter of vessels) are affected by multiple factors, both external and internal (Shier et al., 2012). This lab exercise focuses on the effects of hot and cold temperatures on blood vessel diameter, including techniques for observing and measuring these changes. It can also be used as a model for the role of cutaneous vessels in thermoregulation in endotherms. Goldfish are excellent lab specimens because they are inexpensive, hardy, easy to house, and easy to acquire. Aquarium equipment is readily available, and since goldfish can survive in lower-oxygen situations, supplemental aeration and heaters are usually not required, although a filtration system is recommended.

This experiment has three main objectives:

1. Identify arterioles, capillaries, and venules, using the size of the vessel and the direction of blood flow in relation to the heart.
2. Measure the changes in vessel diameters (vasoconstriction and vasodilation) using ImageJ software. Options for observation of these changes without measurements are also described.

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3. Use Excel to analyze and graph data and calculate  $R^2$  values.

This lab exercise is appropriate for high school and college students. It can be used in Human Anatomy and Physiology or Animal Physiology courses. It can be modified for different academic levels by adjusting the level of discussion about blood vessel physiology. The discussion could include more details about vessel diameter changes for higher-level classes. Vasoconstriction and vasodilation are caused by contraction and relaxation of small fibers of smooth muscle found in and around the vessel walls. There are also precapillary sphincters (also small fibers of smooth muscle) that regulate vessel diameter at the junction of the arteriole and capillary (Shier et al., 2012). Graphing of data points can be done by both high school and college students; statistical analyses may be more applicable for college students.

Pre-lab assignments requiring students to research arterioles, capillaries, and venules may be helpful. How do these vessels compare in size, and in what direction does blood flow through these vessels? Arterioles carry blood away from the heart, venules carry blood toward the heart, and capillaries connect the arterioles to the venules (Shier et al., 2012). Oxygen, carbon dioxide, waste, and nutrients are exchanged between the semipermeable walls of the capillaries and the tissues. The direction of blood flow can be observed under the microscope – but remind students that the direction of flow is reversed when looking through a microscope. Blood flow is usually slower through capillaries, and this can be seen through the microscope (Shier et al., 2012). Ask students to predict how temperature might affect vessel diameter. You might pose the question, “Given different water temperatures, propose several hypotheses based on what you know about vasodilation and vasoconstriction.” You might also ask them to design a way to identify arterioles, capillaries, and venules under the microscope.

## ○ Materials List

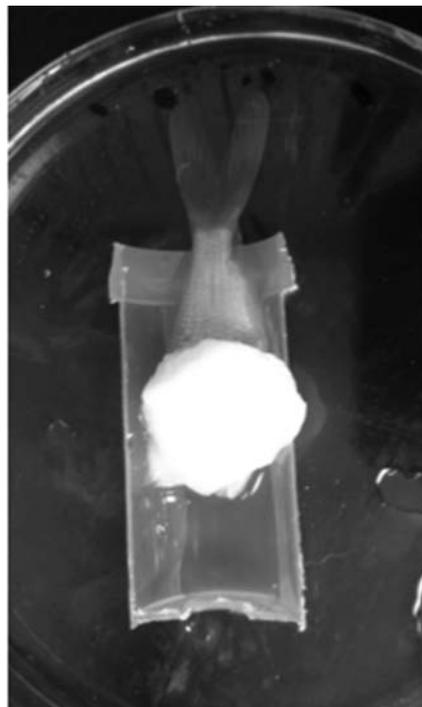
- Goldfish (fish should have light-colored or clear tails)
- Equipment for housing (aquarium with filtration)
- Prescription bottles (7 × 3 cm) without lids, cut in half lengthwise (can be purchased from a pharmacy)
- Cotton balls
- Petri dish: 150 × 20 mm
- 5-mL disposable droppers
- Thermometers
- Microscope with video camera interfaced to computer (the microscope used for this experiment was an inverted compound microscope, but portions of this experiment can be performed using a conventional compound microscope with 40× lens)
- ImageJ software – free download from National Institutes of Health website (<http://imagej.nih.gov/ij/download.html>)
- Ice water: several samples between 1°C and 10°C
- Hot water: several samples between 40°C and 50°C

## ○ Procedures

The apparatus to contain the goldfish is made by cutting a medium-sized prescription bottle in half longitudinally. Smooth the cut edges with a metal file, and place the apparatus inside an open Petri dish.

Students should be reminded to wash their hands before and after the lab. Thoroughly wet one cotton ball, using water from the goldfish tank or distilled water; do not use chlorinated tap water. Stretch the wet cotton ball until it is flat and place it inside the prescription bottle. (*Note:* Students should wet their hands or gloves – gloves are recommended – before touching the goldfish, to avoid removing the mucous layer protecting the fish's skin.) Place the fish into the pill bottle on top of the cotton so that the entire tail is hanging out of the open end of the container. Lay a wet, stretched cotton ball gently over the head of the goldfish, covering the gills and eyes completely (Figure 1). Some fish are more cooperative than others, and covering the eyes with the wet cotton ball helps calm the fish and keep it still. Flush the cotton and gills with 2–3 mL of tank or distilled water. A small amount of water will accumulate in the area of the bottle under the gills and will serve as a reservoir of water for the fish to breathe. Continually flush the cotton and gills with tank or distilled water (about three times each minute) to renew the water passing over the gills. The Petri dish will catch any water draining out of the container, preventing the water from leaking onto the microscope. Using this procedure, the goldfish can be kept out of water for 4–5 minutes without adverse effects.

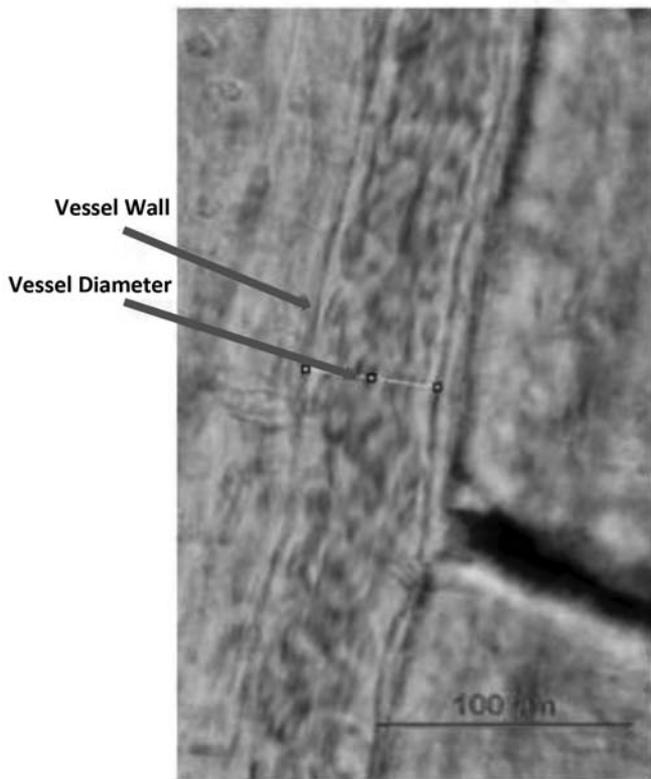
Move the extended tail under the microscope lens; we use an Olympus IX71 microscope (40× lens) connected to a camera and computer. Focus on an arteriole or venule, but do not use capillaries because capillary walls are thin and difficult to measure under the microscope. You will see blood flowing through the vessel, and the direction of blood flow will determine whether it is an arteriole (flows away from heart), a venule (flows toward the heart), or a capillary (flows between arteriole and venule). Set the camera to



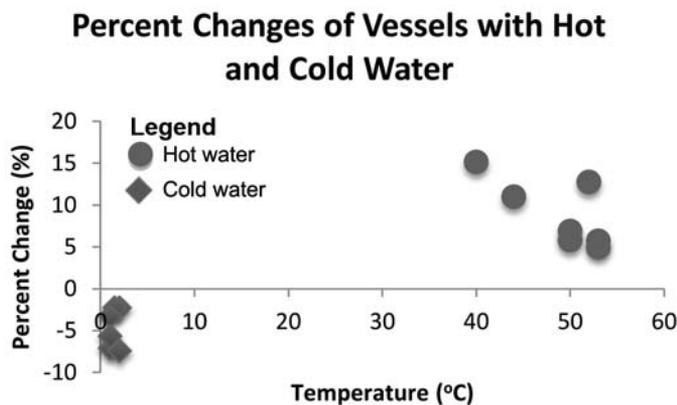
**Figure 1.** Apparatus to hold goldfish.

record a video of a specific blood vessel, about 30 seconds long. This will allow visualization of the maximum change in blood vessel diameter. (*Note:* If the fish moves, reposition the tail so that the two measurements [treated and untreated] can be made on the same area of the vessel to ensure accuracy.) Use a thermometer to measure the temperature of the hot or cold water (1–10°C for cold water and 40–50°C for hot water; Ford & Beitinger, 2005), draw the water into the dropper, and immediately flush the tail with several milliliters of water. Record the temperature of the water placed on the tail. The vessel diameter will change very quickly and can be observed on the computer screen, so continue the video for about 10 seconds after the change occurs. When the video has finished recording, return the fish to the tank.

Select two still photos from the video, one before treatment and one after treatment. (The videos and/or pictures are stored on a thumb drive for later use as needed.) Using ImageJ software, measure the vessel diameter in both the “before” and “after” pictures (Figure 2) and record the diameters in an Excel spreadsheet. Calculate the percentage change in vessel diameter to allow for comparisons among vessels of different sizes. Use negative percentages for vasoconstriction and positive percentages for vasodilation. The data are graphed as percentage change of vessel diameter on the y-axis vs. temperature on the x-axis (Figure 3).  $R^2$  values can also be calculated using Excel. Once the values have been entered into the Excel spreadsheet, make a chart using the temperature values for the x-axis and the percent change values for the y-axis. Right-click one of the data points, and select Add Trendline. This will automatically add the Best Fit line to the data, and the options to show the line equation and the  $R^2$  value will become available in the menu that appears. The  $R^2$  values determine the correlation between two dependent variables; a higher  $R^2$  value means greater correlation. Results will vary, depending on the range of temperatures used and the number of measurements made, but a larger range



**Figure 2.** Untreated vessel measurement using ImageJ software.



**Figure 3.** Excel graph of hot and cold water temperatures versus percent change in vessel diameter.

of temperatures with multiple data points within that range (i.e. >10–15) showed the best statistical results.

## ○ ImageJ Software Operations

ImageJ is a free Internet download available from the NIH website (see link under “Materials”). Once downloaded, the software can be used to open the pictures of the blood vessels, making them available for editing. On the toolbar, select the option to draw a line (the default option is to draw a square) on the picture. The first step in measuring the diameter of the blood vessel is to set the standard

measurements with which to compare the results. Measure the length of the scale (100 μm; see Figure 2) as shown on the picture obtained from the microscope’s camera. Select the Analyze drop-down menu, and select Set Scale. Enter the known distance of the scale and the length in pixels. Once this has been set, the vessel diameter can be measured. Using the same line-drawing tool, draw a line that extends from one side of the blood vessel to the other, the length of its diameter (see Figure 2). Select the Analyze drop-down menu again, and this time select Measure. Your results, and the length of the vessel diameter in the units set by the scale, will appear in a separate box that can then be saved to a computer or flash drive. When performing this procedure on multiple pictures, use the same scale length as the standard for each picture to provide fewer points of error among the pictures.

## ○ Procedure Options

If a computer-interfaced video camera is not available, this experiment can be done using observations instead of measurements of vasoconstriction and vasodilation. A conventional compound microscope with a 40× lens can be used. Students will measure the temperature of the water before flushing the tail and observe the changes in vessel diameter. Vasoconstriction can be recorded as “+” or “++” for a greater increase in diameter, and vasoconstriction can be recorded as “–” or “– –” for a greater decrease in diameter. Students can also identify arterioles, capillaries, and venules by the direction of blood flow in relation to the heart; and they can draw the blood flow they observe through these vessels. The specific measurements needed for calculating percentage change, graphing data, and calculating  $R^2$  values cannot be obtained using this option.

Additional variables, such as drugs or chemicals, can have a measurable systemic effect on blood vessel diameter when added to the gills. Acetylcholine, epinephrine, or caffeine can be used to cause changes in heart rate and vessel diameter. Students using ImageJ software can measure these changes, and students not using the software can observe the changes.

This lab exercise has been successful in the classroom because it is easy to prepare, it is easy to implement, and data can be collected in a 2- or 3-hour period. Students are enthusiastic about seeing blood flowing through living vessels, and the direction of blood flow between arterioles, capillaries, and venules illustrates blood’s circular path from the arterial system back through the venous system.

## ○ Institutional Animal Care & Use Committee

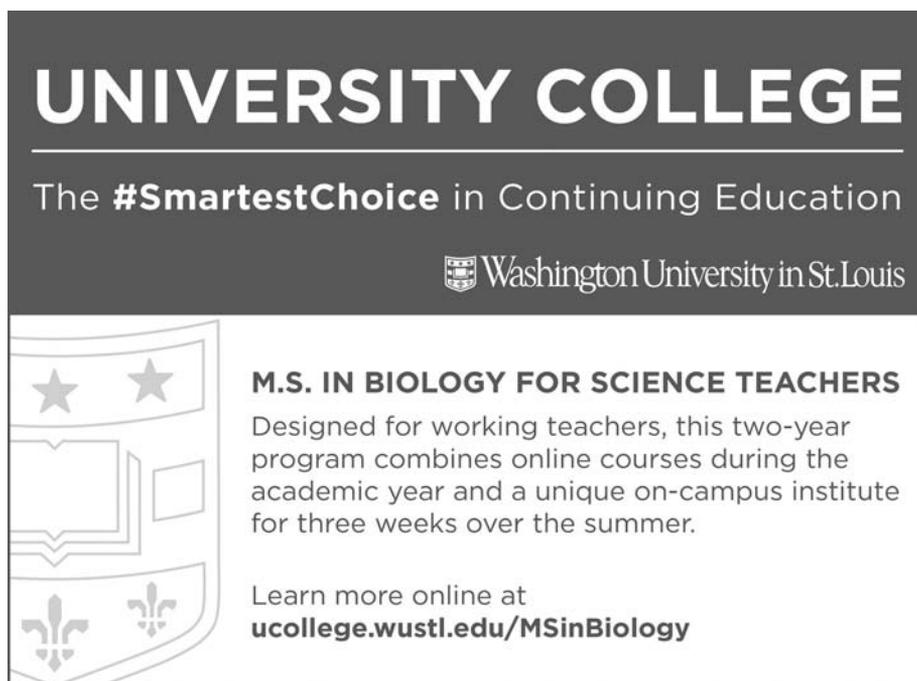
Some institutions require approval from the Institutional Animal Care and Use Committee (IACUC) for the use of vertebrates in research. An IACUC may not be available at all levels, and it is generally required for research involving grants and publications. This approval may not be necessary at all institutions for animals used for teaching laboratories, so the first step is to find out whether you need approval. If approval is necessary, it is important to know the scheduled meeting times of your IACUC so that you can apply for what may be an extended approval time. If you need approval,

the following process may be necessary. For application at our institution, the committee meets twice each year, and the approval process begins by completing a form, Protocol for Laboratory Use of Animals. The information requested includes a description of animals used, a description of the procedures of the experiment and the handling and restraint of the animals, justification for the number of animals used, and details on housing and care for the animals. Documentation for any training courses completed on caring for or handling animals is also required. The approval process also includes announced and unannounced inspections of animal housing (in this case, the aquarium setup). Our IACUC prefers that the animals are kept in a room with limited access (a door that can be locked). The IACUC outcomes may include approval of the project, approval with revisions, or not approving the project.

## References

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- Shier, D., Butler, J. & Lewis, R. (2012). *Hole's Essentials of Human Anatomy & Physiology*. New York, NY: McGraw-Hill.

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