Through A LOOking Glass

An Invertebrate Inquiry

by

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[NOTE: The procedures in this write-up use flat-tipped plastic culture tubes to view worms. An alternative procedure, which uses glass capillary tubes to view worms, is more difficult to do but offers additional opportunities for student inquiry. The write-up for the alternative procedure was originally presented by C. Drewes and B. Grosz at the 1999 NABT meeting and is available upon request.]

"Through a Looking Glass! - An Invertebrate Inquiry"

INTRODUCTION

This simple, inquiry-based exercise emphasizes students' powers of observation, attention to detail, problem-solving ability, and creative thinking about the biology of a user-friendly freshwater invertebrate -- *Lumbriculus variegatus* (Phylum: Annelida; Class: Oligochaeta; common name: blackworms). The 'looking glass' used to view worms is a clear tube with a narrow space at the tip. Generally, worms prefer to be in narrow, tight spaces because such spaces offer protection. This behavioral orientation is termed "thigmotaxis." As worms occupy the tips of tubes, their movements are reduced and viewing of internal and external features may be done with enhanced clarity.

MATERIALS LIST

1) *Lumbriculus* worms (medium-sized; 3-5 cm in length). Three commercial sources include:
(A) www.holidayjunction.com/aro/ (B) www.novalek.com/korgde1.htm (C) www.carolina.com
[Contact C. Drewes for worm culture instructions, background information, and wormy lab exercises.]
2) Flat-tip Demoslide culture tube with cap (Connecticut Biological, Cat #: LW2250; $43/120 tubes]
3) [Optional: LW2256 Demoslide stage adapter; $4.50/ea]
4) 100 x 15 mm plastic Petri dish
5) Disposable polyethylene transfer pipet; standard bulb (example: Fisher Cat # 13-711-7)
6) Spring water (Alternative: tap water that is aerated for several days, or "aged" for about 2 wks)
7) Clear plastic mm ruler [NOTE: I suggest photocopying the grid lines of a couple transparent mm rulers onto a transparency sheet (100% copy size). Then, cut up the photocopied images to much smaller sizes (about 1 cm each), thus making numerous "mini-ruler" transparency strips. For measurements, each strip can be submerged along side a tube. Strips can be re-used.]
8) Binocular dissection microscope (side-illumination is preferred). An alternative is a compound microscope with a low-power or scanning objective
9) Separate light source, if not part of microscope set-up
10) Clock, watch, or stop-watch for timing to nearest whole second

PROCEDURES

1) Obtain a whole *Lumbriculus* that is isolated in a petri dish of spring water.
2) Obtain a flat-tipped Demoslide culture tube and fill it with ~ 2 ml of spring water.
3) Obtain a plastic pipet with a stretched tip (see Part II of "Pencil Pipet" write-up).
4) Insert the pencil pipet tip into the tube and suck up some water from the tube.
5) While the tip of the pipet is fully inserted into the tube, forcefully expel water from the pipet into the very tip of the tube. The idea is to try to fill the entire flattened tip of the culture tube with water, leaving no visible air spaces in the tip of the tube. It may take several attempts to do this.
6) Use a regular plastic pipet to transfer a worm from the petri dish into the culture tube.
7) Hold the tube vertically and cap it. The worm should sink to the bottom and slowly attempt to crawl into the thin, water-filled space at the tip of the tube. [NOTE: The tendency of an organism to maximize contact between its body and surrounding surfaces or substrates is called "thigmotaxis."]
8) Using a stereo-dissecting microscope, view the worm as it is positioned in tip of the tube. Turn the tube over, as needed, to view the worm from dorsal and ventral sides.
9) Read and answer the questions below.

TWENTY QUESTIONS FOR: *"Through a Looking Glass!"*

1) What is the classification of this animal? (Phylum? Class?) Is it a vertebrate or invertebrate? How do you know?

2) Is the animal segmented? About how many segments does it have?

3) Look closely for tiny structures projecting from the surface of the body. How many do you see in each segment? Describe these structures and their arrangement in each segment? Use references to find the names of these structures? To what advantages are these structures used by the animal?

4) Which is its head end and which is its tail end? Which is the dorsal and ventral surface? How can you tell?

5) Is the skin pigmented? If so, describe the location, color, and pattern of pigments?

6) What features do you see *inside* the body? Look at different locations along the body length. List, describe, and explain internal features that you see.

7) Does the animal have blood? Blood vessels? Describe the location of the main longitudinal vessels along the body, especially in relation to *dorsal* and *ventral* body surfaces. To make this comparison you will need to rotate the tube to observe the worm from different sides and angles.

8) What causes the blood to move or circulate? Carefully observe and describe blood vessel contractions. Do the pulsations have a regular rhythm?

9) At any given instant of time, do you see more than one pulsation occurring along the body length? Use a ruler to measure/estimate the distance between two on-going pulsations?

10) Try to minimize the worm's movements by reducing the light intensity and then try to determine how fast a blood pulsation wave moves over a 1 mm distance along the worm. Use a mm ruler and a clock with a second hand to determine this velocity. Express wave velocity in units of mm/second.

11) What is the frequency of blood vessel pulsations? [Suggestion: Focus on one segmental location and count the pulses that occur over a 15 or 20 second interval. Express the pulse frequency in units of pulsations/minute

12) Does the pulsation frequency differ in different parts of the body? Especially compare pulsation frequency near the tail end to the frequency in mid-body segments?

13) Using your computed pulse velocity (#10), estimate the <u>volume of blood that moves through the vessel in one day</u>. In doing this, you should assume that the pulse velocity equals the velocity of blood flow in the vessel, and that this value is a constant throughout the day. First, estimate the cross-sectional area of the dorsal blood vessel. To do this, you will need to use a microscope and a small microruler to try to measure the diameter of the vessel (remember that the radius, r = ½ D). Then, use the formula ($A = \pi \cdot r^2$) to calculate the cross-sectional area of the vessel. Knowing the cross-sectional area and the velocity of blood flow, use the formula for the volume of a cylinder (V = cross-sectional area $\cdot$ length) to calculate the volume of blood that flows past a given point per second. What are the units of volume? Convert these units to cubic centimeters (= ml) of blood per day.

14) As the worm moves in the tube, do segments in the body change diameter and length? What muscle group causes segments to shorten and increase in diameter? What muscle group causes segments to lengthen and decrease in diameter?

15) Find the chaetae, or bristles, that are present in each segment. Does it appear that chaetae are under muscle control? Describe the position of the chaetae when segments are increasing in diameter. Describe chaetae position when segments are constricting in diameter. Explain why it is advantageous for these animals to control their chaetae in this way.

16) Where is the worm's mouth? What types of food do you think this animal eats in nature?

17) Can you see food in the worm's digestive tract? Look especially near the posterior end. Is the food moving? In which direction?

18) How do you think the animal obtains oxygen and performs gas exchange?

19) Where do you think the animal lives in nature? Find information about its habitat and ecology.

20) Develop some additional questions about the biology or behavior of this animal that you would like to know answers to. Describe experiments you might do to discover answers to these questions?

OTHER *"LOOKING GLASS"* INQUIRIES

Track and quantitate the progress of body segment regeneration in worm fragments

→ obtain worm fragments of known size and origin, as described in the "Head or Tails" write-up for studies of *Lumbriculus* regeneration

→ place fragments in tubes with 1-2 ml of spring water per tube (one fragment per tube)

→ sketch the appearance and measure the size of fragments at 2 day intervals during the regeneration process. After about one week, it should be possible to count the number of newly regenerated segments on the anterior and posterior end of the fragment.

Effects of ambient temperature on circulatory function

→ transfer tubes with worms to dishes containing spring water at different temperatures

→ while tubes are being held at one temperature, determine the pulsation frequency; compare frequencies at various temperatures

→ try using worm fragments, instead of whole worms, to determine temperature effects