INQUIRY & INVESTIGATION

Lights, Chemicals, Action: Studying Red Worms' Responses to Environmental Contaminants

NABT BioClub RECOMMENDATION

DANIEL N. WEBER, RENEE A. HESSELBACH, DAVID H. PETERING, LOUISE P. PETERING, CRAIG A. BERG

Abstract

We have developed an experimental module that introduces high school students to guided scientific inquiry. It is designed to incorporate environmental health and ecological concepts into the basic biology or environmental-science content of the high school curriculum. Using the red worm, a familiar live species that is amenable to classroom experimentation, students learn how environmental agents affect the animal's locomotion by altering sensory neuron–muscle interactions and, as a result, influence its distribution in nature. In turn, the results of these experiments have direct application to human-caused environmental disruptions that cause changes in species distribution and indirectly increase the recognition that environmental chemicals affect human health. Students undertake a series of explorations to identify how red worms sense their environment and then apply that knowledge to understand the effects of chemical exposure on locomotor behavior. The activities are designed to generate critical thinking about neuromuscular processes and environmental pollutants that affect them.

Key Words: *Animal distribution; behavior; biology; red worm, environmental health; inquiry-based learning; model; neuromuscular.*

Introduction

What cues do animals use to know where to live? How do they translate that information into behaviors that determine species distribution? Are the processes of sensing the environment and transferring that sensing into purposeful movement common to all animals, including humans? If so, can an animal as simple as a red worm be a model for our own sensorimotor pathways? Such fundamental questions of ecology and physiology are the framework for an inquiry-based experience in which students study live animals' behavioral responses to their chemical and physical surroundings.

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Andrewartha and Birch's classic book *The Distribution and Abundance of Animals* (1954) asked, "How does the environment influence survival?" This approach has yielded a rich literature not only in ecology but in other scientific disciplines such as physiology and molecular biology. Yet answering that question in a high school setting, using live animals, and applying that knowledge to modern-day issues of human and ecosystem health have proved challenging. Using a simple animal model, the red worm, we have developed a module that successfully answers that need.

The red worm serves as an effective classroom model because its survival depends, as does ours, on the ability to respond to what happens in its environment. While red worms and humans are able to react physically to a number of changes in the environment, their responses are limited to the stimuli each is able to sense. Harmful stimuli are usually avoided, but only if an organism can sense deleterious changes in the environment. For students to understand the underlying biological basis of such behavior, they must first develop an appreciation for the sensorimotor pathway. Red worms are an excellent, inexpensive, easy-to-obtain model to examine this pathway by studying repeatable, stereotypic behavioral responses to a range of environmental stimuli. Use of red worms as a model of ecotoxicity for classroom experiments has been proposed previously by Paradise (2001) – but in that paper, mortality was the endpoint. Use of this metric, however, does not lend itself well to an inquiry approach that allows students to investigate more subtle measures of environmental toxicity (e.g., how soil contaminants alter species distribution without lethality). Furthermore, it has been our experience that the death of an animal, even a red worm, can be upsetting to students and can cause them to have a negative attitude toward science.

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Historically, environmental scientists have used the lowest observed effective concentration (LOEC) to define the lowest concentration of a specific substance, under defined conditions of exposure, that causes an adverse change to the exposed population. Ecologists have often employed the LOEC to understand the effects of chemicals on species distribution, as did Marques et al. (2009) with earthworms exposed to an herbicide. While not in vogue today, LOEC remains a useful educational tool for students to gain an appreciation of how animals respond to sublethal concentrations of environmental contaminants that may have relevance to their own lives – for example, the behavioral effects of specific metals are especially important for students who live near heavy industries, and pH levels can be used as a model for acid rain and acid mine drainage. Lastly, students are provided an opportunity to explore how multiple variables simultaneously influence toxic responses and to correlate those data with human sensitivity to chemical exposures. Using more than one variable is a departure from many standard experiments conducted in high schools and will encourage students to expand their ideas on how species, including humans, function in disturbed ecosystems.

Table 1. ***Next Generation Science Standards*** **(NGSS Lead States, 2013) applicable to this module.**

Life Science • Interdependence of organisms • Behavior of organisms	**Scientific and Engineering Practices** • Asking questions and defining problems • Developing and using models • Planning and carrying out investigations • Analyzing and interpreting data • Using mathematics and computational thinking • Constructing explanations and designing solutions • Engaging in argument from evidence • Obtaining, evaluating, and communicating information
Disciplinary Core Ideas – Life Sciences • From Molecules to Organisms: Structures and Processes (LS1) • Ecosystems: Interactions, Energy, and Dynamics (LS2) • Biological Evolution: Unity and Diversity (LS4)	**Nature of Science** • Scientific investigations use a variety of methods • Scientific knowledge is based on empirical evidence • Scientific knowledge is open to revision in light of new evidence • Science models, laws, mechanisms, and theories explain natural phenomena • Science is a way of knowing • Science addresses questions about the natural and material world • Science is a human endeavor
Crosscutting Concepts • Patterns • Cause and effect • Systems and system models • Structure and function • Stability and change	**Science and Society** • Connections between science research and societal concerns and issues
Science as Inquiry • Abilities necessary to do scientific inquiry • Understandings about scientific inquiry	

○ Objectives

Our goal is to lead students to an understanding that chemicals in natural and human-influenced environments affect animal behavior and distribution. To achieve this goal, the module engages students with three aspects of science learning: (1) knowledge and application of basic concepts and principles (the sensorimotor circuit), (2) skills in scientific inquiry and processes (using a series of questions that mirror how scientists approach their studies), and (3) understanding science as a human endeavor designed to address problems and issues (red worms as a model for ecosystem health).

In this module, students

- articulate and refine questions about animal behaviors, their structural and functional basis, and their susceptibility to environmental agents;
- use appropriate tools and techniques to conduct controlled experiments;
- gather and analyze data regarding behavior of control and experimental organisms;
- generate explanations and predictions, interpret data to draw conclusions, and propose new hypotheses; and
- apply results and conclusions to guide personal and social decisions about ecosystem health.

Through this process, many *Next Generation Science Standards* for high school students are addressed (Table 1).

○ Methods

Overview

The experiments in this module present a progression of experiences that integrate physiology, behavior, and ecology. Red worms (*Eisenia fetida*) are used because their small size allows for the small-scale experiments students will be conducting. The first set of lessons provides the background and basic skills for the remaining instructional days. Student background knowledge of red worms is explored. Red-worm locomotion (function) and red-worm anatomy (structure) are modeled by squeezing a water balloon to approximate the actions of circular and longitudinal muscles. Student-generated inquiries (e.g., "How do red worms know what the temperature is?") build on this by observing how red worms sense their environment and the roles that sensory stimuli (touch, light, temperature) play in producing locomotor behaviors. With that understanding, they are in a position to comprehend how red worms react to chemicals in their environment. These two experiences are the basis for the next level of experimentation, in which they first see how red worms' behavior changes when they are exposed to chemicals and then how they change their burrowing behavior when they come in contact with chemicals in the soil. Students are now equipped to develop their own experiments to determine how chemical contaminants that are of interest or importance to them alter the movements of the red worm and thereby affect its health, survival, and distribution. Inherent in any behavioral experiment is accounting for the effect of individual variability through statistical analysis (e.g., means and standard errors). Therefore, experiments employ at least four red worms for each exposure regimen, a number chosen to balance the need for sufficient replicates with the time constraints found in most high school class periods. All materials needed for this module are listed in the Appendix.

Basic Sensorimotor Responses

Students examine basic red-worm behavioral responses to stimuli that are critical to defining where the animal lives: light, temperature, and chemical presence. Before creating hypotheses and experiments to test them, students ask themselves questions, just as scientists do (Table 2). Using a concept map, students identify "What do I already know?" about the ability of red worms to sense and react to their environment. At the end of the module, students return to this concept map and add to it with knowledge gained through their experiments. Assessing what they already know allows students to identify the gaps in their knowledge as a basis to formulate the next question, "What do I need to know?" And rather than being told how to proceed, the students discuss "What do I do to learn what I need to know?" This invests students in the scientific process and teaches them how to create a viable experiment including control and treatment groups. Once the data are collected, students ask, "What do I now know?" The answer follows from data analysis, interpretation, and communication. The process, of course, is not over. Instead, it has just moved into the next phase, "What do I now need to know?" Asking questions permeates class activities before and during each investigation. Note how each experiment leads to more student-generated questions for additional investigations.

Response to Light

If you ask students whether red worms can see, most will hypothesize that, because red worms do not have eyes, they cannot see. This can be tested by using a small flashlight. Students shine a light near the red worm and record its reactions in qualitative terms (strong, mild, or no response) as it crawls toward the light. Observing that the red worms react to the light by immediately withdrawing from it challenges their original hypothesis. After reading relevant resources, they learn that red worms do indeed have a simple eye. Then they can ask, "What do I now need to know?" For example, students might ask if the color of light matters (using color transparencies) or can the red worm sense light from any part of its body. When followed up with more experiments, students learn that red worms are differentially sensitive to different colors of light. Through critical thinking, they now confront why the color of light matters – that is, why do red worms not retract from red light but do retract from blue or white light? (Hint: What time of day are red worms active, and what does that have to do with wavelength of light?)

Table 2. Prediction/Reflection Log. Science as a process is taught to students through a series of engaging questions that lead them to develop experimental procedures, analyze and interpret data, and communicate their findings to others.

Question	What Do I Already Know?	What Do I Need to Know? (Hypothesis Development)	What Do I Do to Learn What I Need to Know? (Methods)	What Do I Now Know? (Data Collection)	What Do I Now Know? (Data Analysis)	What Do I Now Need to Know? (Next Experiments)
How do red worms know what time of day it is?		What will a red worm do when light is shone in its path?				What will a red worm do when light is shone behind it? On top of it? What if a different color of light is used?
How do red worms know what is bad for them?		What will a red worm do when alcohol is placed on or near its head?				What will a red worm do when alcohol is placed on or near its tail?
How do red worms know what the temperature is outside them?		How will a red worm respond when a heated object is placed near its head?				How will a red worm respond when a heated object is placed near its tail?

Response to Temperature

We have found that some students hypothesize that red worms only sense the flashlight because it gives off heat. To investigate the red worm's ability to sense different temperatures, students use a heated glass rod to observe the red worm's sensitivity to heat. The heated rod is placed only *near* the head or other body sections. This finding stimulates another round of questions. For example, students might now ask if similar behaviors occur with cold (place rod in an ethanol-ice bath) or room temperatures. Now they are able to puzzle over how soil temperature determines red-worm distribution. It is now appropriate for the students to discuss how they sense temperature in their own bodies and if the sensory neurons we have for detecting temperature are similar to those in red worms.

Response to Chemicals

Investigating the red worm's response to ethanol forms the basis for the next series of inquiries involving how animals react to the presence of potentially toxic chemicals. After applying a single drop near the red worm's head or tail, students record their observations as the red worm comes in contact with the solution. They discover that the body region most sensitive to a chemical stimulus is the anterior region. They are now in a position to reflect on why such differential sensitivities along the body axis might be important for both them and red worms, and how red-worm chemosensory structures are similar to those of humans.

The first three investigations have demonstrated to students not only that red worms have many ways to sense their environment, but that they are useful models for human sensorimotor responses. The students are now prepared to use this knowledge and style of investigation to examine how exposure to chemical contaminants alters behavior.

First, students discuss why detecting changes in water or soil chemistry might be important to red worms and how sensitive these organisms are to the presence of different chemicals. By extension, students consider how this models human responses to toxic chemicals. In each of the investigations below, students should ask each of the questions in Table 2 to guide them through the process of discovery. During these experiments, students should wear appropriate laboratory clothing to shield them from chemical exposure.

Making test chambers. Students create an experimental apparatus by transforming an ant farm purchased from any pet store into a worm behavioral test chamber. The ant farm is dismantled so that the clear plastic panels can be used for the experiments described below and can be reassembled into a chamber for examining burrowing behavior in control vs. contaminated soil. While any plastic sheet or container will suffice for these experiments, the advantage of the ant farm is that, once reassambled, it can be used to observe the worm's movement as it burrows through the soil.

Effect of pH. How do red worms know if the pH of their environment has changed? This is important, given the anthropogenic sources of acid rain and acid mine drainage. Students are encouraged to develop an experimental protocol to examine whether and how red worms react to different pH levels. One method is illustrated in Figure 1. Discuss with the students why a circle of drops rather than a single drop is used. This will actively engage the students in the process of creating their experiment. On their data sheet, students record the red worm's withdrawal reaction (strong, mild, or no reaction) when it comes in contact with pH 4, 7, or 9. After finding that red worms react strongly to pH 4 but not to pH 7 or 9, they can now discuss how to discover a pH value closer to the actual LOEC. Is this correlated with where red worms are found in nature? Allow students to research whether red worms disappear if soil becomes acidic as a result of acid rain and whether they return when the original soil pH is reestablished (e.g., Cácamo et al., 1998; Ohno, 2001; http://northernwoodlands.org/discoveries/digging-for-worms).

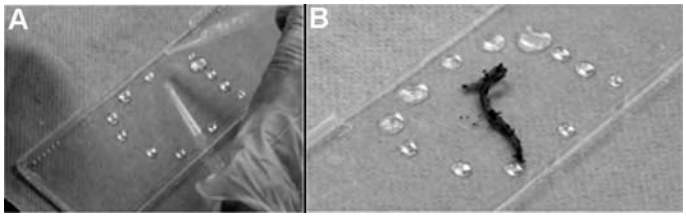

Figure 1. Circle of droplets to test reaction to pH or metals. (**A**) Placing drops of pH or metal solution in a circle. (**B**) Placing a red worm in the circle.

Effect of metal ions. Soil can become contaminated with industrial metals, and this can be a major issue for students living in urban regions. How do red worms react to such contamination, and what do these animals tell us about human responses to metal ion exposures? Because students are identifying the LOEC, they will test the highest concentration first and then examine decreasing concentrations of metal ion until the red worms no longer react. As with pH, students can create experiments to more accurately identify the LOEC for a specific metal. For "next-step experiments," students could develop other environmentally relevant questions, for example investigating differential effects of metals with mutiple valences (Cu^{+} vs. Cu^{2+}) or whether long-term exposure to a low concentration changes the level of sensitivity. These questions also are relevant to human health.

Can red worms choose? What if a red worm is presented with a choice between two different chemical environments? Will it seek the "safer" side? What does that behavior mean ecologically? Are there human parallels to this situation? Using the ant farm, students investigate these questions. With one row of drops being the control side (pH 7, or 0 ppm metal ion) and the other row the treatment side (see Figure 2), students record the red worm's preference (i.e., to which side the worm crawls) on their data sheets.

Effect of contaminated soil on sensorimotor response. Red worms, of course, do not live on plastic sheets. Students now are ready to investigate the effect of potting soil on toxicity. Specifically, is the red worm's sensitivity to chemicals the same when exposed in the soil as it was when exposed directly to a solution? The preference experiment is repeated, except this time students add enough potting soil to fully cover the plastic surface to a depth at least equal to the thickness of the ant farm's wall but with space for placing the worm (Figure 3). One side will be made a control by adding ≤30 mL of distilled water to the soil so that it is damp but not soaking. The soil on the other side will contain an equal aliquot of pH or metal ion solutions.

Developing a model to explain observations. Students will find that soil mitigates the effect of pH or metal exposure on red-worm behavior. After developing hypotheses to explain this observation, students develop a model in which a drop of a positively

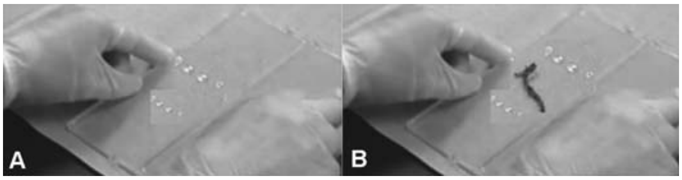

Figure 2. Using an ant farm to discover whether red worms can choose. (**A**) Placing drops of either pH or metal solution in rows. (**B**) Placing a red worm between the two rows.

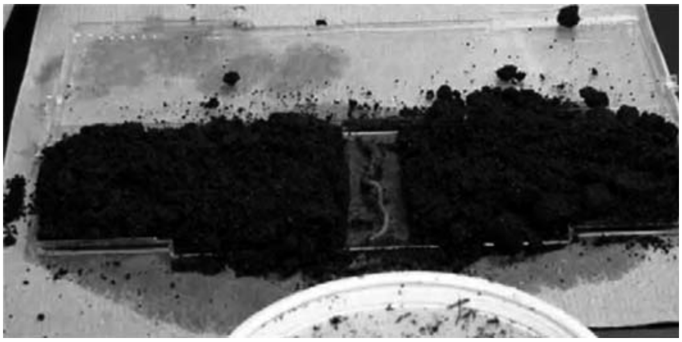

Figure 3. Identifying the response of a red worm to potting soil. Photograph demonstrates how to determine whether potting soil can alter the toxicity of pH levels or metal. Red worm is placed between the two sections of potting soil, one dampened with dechlorinated water and the other chemically treated as described in the text.

charged chemical, methylene blue, is placed on a small amount of potting soil on a slide and viewed under a microscope. Being positively charged, methylene blue models the attraction of the positively charged metal and H^+ ions to the negatively charged soil particles. The time it takes for the blue color to visibly begin disappearing and the time at which all the blue color is gone are recorded. Now the students are in a position to develop a hypothesis to explain the behavioral observations (HINT: potting soil is negatively charged). With this knowledge, students might ask whether different soil types, such as sand or loam, provoke different behavioral responses. Challenge them to create and conduct an experiment to test their new hypothesis. How does the methylene blue react in those soils? That the blue color does not disappear in sand indicates that sand has no or little negative charge on the surface of each particle. What does that mean for chemical reactivity in sand vs. potting soil? Discuss with the students how this new knowledge might be important to the health of humans as well as plants and wildlife.

Using multiple variables to explore toxic effects. Most high school science experiments include just one variable. That, however, is not how nature operates. Exposure to a contaminant, for example, means more than just the amount or concentration of that chemical. Among the many other factors that are important to understanding the effects of toxic chemicals is how long that exposure occurred. In this exercise, students are challenged to analyze the simultaneous effects of *two* variables on a behavioral outcome: concentration and length of exposure. Student-generated hypotheses and experiments

Figure 4. Simple burrowing-time test chamber. An 8-ounce plastic cup provides an ideal and inexpensive behavioral test chamber for examining the dual effect on red worms' burrowing activity of concentration and time of exposure to a chemical. Photo credit: Sofi Garcia-Velez, Sarah Brennan, and Riordan Ryan, students at Divine Savior Holy Angels High School (Milwaukee, WI). Parental permission provided.

are developed by asking the questions outlined in the scientific process (Table 2). Importantly, this exercise has direct relevance to human environmental health. While any behavior is a coordinated series of muscle contractions triggered by motor neurons that are controlled by excitatory and inhibitory neurons, specific muscle structures and neurotransmitters that control muscle contraction differ between red worms and humans (Hooper et al., 2008; Robinson, 2013). Still, these experiments provide students with insights into how chemicals in their environment may affect them.

A simple, inexpensive exposure chamber is created using an 8-ounce plastic cup with a piece of paper towel at the bottom that is wetted with the test solution. The red worm is placed on the paper towel and covered with a second piece that is also wetted with the test solution. A second plastic cup is then inserted into the first cup to prevent the red worm from crawling out. The red worm is removed with a blunt-edge forceps after the designated exposure time (we recommend 0, 2, 5, 10, and 20 minutes) and placed into another 8-ounce plastic cup filled with damp potting soil (Figure 4). If the students want to be able to observe the red worm as it actually burrows through the potting soil, they can reassemble the ant farm and, after the red worm is exposed, place it inside. Time to completely burrow into the potting soil and observations of any behavioral abnormalities are recorded. Given classroom time constraints, if the red worm has not completely

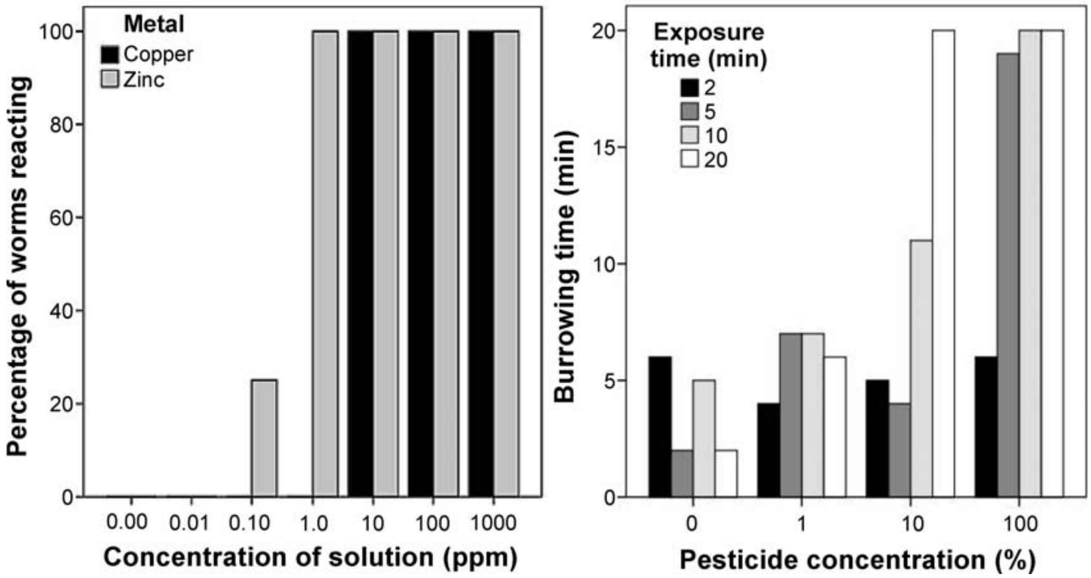

Figure 5. Example of student-generated data. (**A**) Comparing red worms' responses to different concentrations of either copper or zinc. Red worms placed in a ring of either copper or zinc solutions demonstrate the value of using the lowest observed effective concentration (LOEC) to understand animal distribution in nature. Data collected and graphed by Abby Bruders and Thomas Sievert, students at Eisenhower High School (New Berlin, WI). (**B**) Effect of two variables, concentration and time of exposure, on red worms' burrowing behavior. Red worms were exposed to the commercial pyrethroid pesticide Bonide at different concentrations paired with different lengths of time. Results demonstrate to students that both variables can be important in explaining toxicity. Data were collected and analyzed by Sofi Garcia-Velez, Sarah Brennan, and Riordan Ryan, students at Divine Savior Holy Angels High School (Milwaukee, WI). Parental permission for both graphs provided.

burrowed into the potting soil by 20 minutes, the trial is ended and the burrowing time is recorded as 20 minutes (this explains the truncated bars in Figure 5B).

Videoing this behavior can be a dramatic method of communicating to other students the effects of the chemical exposures. Students will learn that both concentration and time of exposure are important components that drive a red worm's response to chemical exposure. To enhance interest in and ownership of the module, students are free to test a chemical that is of interest to them. In past years, choices have included a wide range of chemicals, such as sugar water, caffeine, nicotine, energy drinks, and household pesticides.

○ Data Analysis

Student-Generated Data

While students with knowledge of basic statistics can more formally analyze their data, students without such background can still gain insights from their results. This represents the "What do I now know?" column in Table 2. Examples of student-generated data are shown in Figure 5. Note how red worms exposed to either Cu^{2+} or Zn^{2+} react differently at the lower concentrations. This now becomes yet another discovery about sensitivity to chemical contaminants and can form a basis for further inquiries, such as whether metals in the same column of the periodic table induce similar reactions in red-worm behavior. Questions such as these help students comprehend the importance of chemistry in understanding biological processes. Students with knowledge of statistics can generate a multiple regression equation to more accurately reflect the LOEC value and one-way (concentrations) or two-way (concentration × exposure time) analysis of variance to identify significant differences between concentrations.

Understanding that multiple variables affect behavioral responses to toxic chemicals (Figure 5B) is important to student appreciation of the complexities of human and ecological health issues. It also opens up greater possibilities for individual explorations that have relevance to real-world situations. For example, what if the exposure was done as a series of short exposure times but the total exposure time was the same as the single-exposure regimen? Advanced analyses may include identifying the LOEC for the two variables together and asking how that differs from the LOEC for either variable alone. That investigation introduces students to the important concept of additive vs. synergistic effects. Such additional student-generated studies represent the "What do I now need to know?" column in Table 2.

Module Evaluation

Each year we conduct a rigorous evaluation procedure. The professional evaluation of the module's ability to achieve the goals described in this paper was based on pre- and post-module tests of student knowledge and perceptions, as well as interviews with teachers. All the teachers indicated that the module improved their students' scientific and analytical skills and their students' environmental science literacy (Goldberg & Associates, 2013).

The data indicate that the red-worm module shifted student opinions significantly toward preferred responses for three statements included in the survey from the time the pretest was administered to the time of the posttest. At the time of the posttest, significantly more students agreed that (1) environmental agents acting upon red worms also act upon humans, (2) experiments with red worms demonstrate how the environment affects humans, and (3) red worms are adversely affected if there is too much pollution in their environment. Students who participated in this module demonstrated increased knowledge about the biological, physical, and chemical factors that influence red-worm burrowing behavior. Student responses about the red-worm module also indicated a mostly positive affective experience. For example, approximately two-thirds (66%) strongly or moderately agreed that "Learning about the red worm taught me things about the environment that I didn't know before." More than half of respondents agreed that they enjoyed doing the red-worm module (57%) and that they would like to do similar experiments in future science classes (55%).

○ Conclusion

Using the ability of high school students to reason deductively and to recognize connections between explanations and evidence, we designed, tested, and implemented a classroom module to investigate the effects of environmental stimuli on the spatial distribution of animals and the effects of neurotoxic chemicals on animal behavior. The module is flexible enough to accommodate a wide range of learning styles and abilities and to meet a variety of curriculum needs. Using their data, students use critical-thinking skills to gain insights into fundamental sensorimotor mechanisms of behavioral control, conduct further experimentation, and correlate their data to real-world environmental issues. Using an inquiry approach, students comprehend how the scientific process creates opportunities to view science as an endeavor that can provide answers to issues confronting society today. This module allows students to comprehend the value of using live animal models to understand the effects of environmental contaminants on their own bodies.

○ Acknowledgments

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DANIEL N. WEBER (dweber@uwm.edu), RENEE A. HESSELBACH, DAVID H. PETERING, and LOUISE P. PETERING are on the staff of the Children's Environmental Health Sciences Core Center, University of Wisconsin–Milwaukee, 600 E. Greenfield Ave., Milwaukee, WI 53204. CRAIG A. BERG is a Professor in the School of Education, University of Wisconsin–Milwaukee, Milwaukee, WI 53201.

APPENDIX

Materials List

Class:

- pH 4, 7, and 9 (Use the strong acids H_2SO_4 or HNO_3 as models of acid rain – HCl works too. Optional: vinegar or lemon juice – use 10% and 1% solutions)
- 1000 parts per million (ppm) of Copper, Manganese, Nickel, and/or Zinc
- Distilled water
- Garden or potting soil (no sand or ash content)

Group:

Paper towel

- Squeeze bottle of dechlorinated tap water
- Glass rod
- Matches
- Small dropper bottle of 70% ethyl alcohol
- Alcohol lamp or butane lighter for a cool flame
- Small flashlight
- One small ant farm
- Red worms (purchased from bait shop)
- Test tube rack
- Six test tubes (10 mL) per solution tested to create serial dilutions of 1000, 100, 10, 1, 0.1, 0.01 ppm + a separate tube with dechlorinated water (0 ppm)
- Transfer pipets
- Forceps
- One 10 mL or 25 mL graduated cylinder
- Precut paper towels (5 × 5 cm)
- Red, yellow, and blue acetate transparencies cut into 2-inch squares to cover flashlights
- Stopwatches

Student:

- Goggles
- Nitrile gloves (one pair per student per class period)