Nematodes:

Model Organisms in High School Biology

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An inquiry-based laboratory involving insecticidal nematodes teaches students about experimental design

Four out of five animals on Earth are nematodes, making them the most ubiquitous multi-cellular organisms on our planet (Bongers and Ferris 1999). Nematodes significantly affect humans ecologically, biologically, and economically, and can be free-living, animal parasitic, plant parasitic, or insecticidal in nature. Model organisms have long been used in research laboratories and in classrooms to advance our understanding of life and human diseases (WORM 2004). Nematode worms, especially insecticidal nematodes (Figure 1), are excellent model organisms for high school biology inquiry-based laboratory activities.

In a collaborative effort between university researchers and high school science teachers, we led an inquiry-based laboratory module using two species of insecticidal nematodes to help high school biology students apply scientific inquiry and elements of thoughtful experimental design. Groups of students investigated the insecticidal properties of two nematode species by "asking questions, planning and conducting investiga-

tions, using appropriate tools and techniques to gather data, thinking critically and logically about relationships between evidence and explanations, constructing and analyzing alternative explanations, and communicating scientific arguments" (NRC 1996, p. 105).

FIGURE 1

The insecticidal nematode *Heterorhabditis bacteriophora*.

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Why nematodes?

Nematodes or roundworms are of inherent biological importance, and although they are popular model organisms in research laboratories and ubiquitous in freshwater, marine, and terrestrial environments, they are often given little significance in most secondary science education curricula. The important ecological niches occupied by these organisms make the study of nematodes in high school classrooms an exciting topic and justify a place in the biology core curriculum (Tylka and Jasalavich 2001).

For instance, free-living nematodes play a major role in carbon cycling and decomposition. One free-living species, *Caenorhabditis elegans*, was the first animal to have its genome sequenced and is currently used as a model organism in biomedicine, genetics, and developmental biology. (**Editor's note:** For an activity investigating *C. elegans*, see "Using Digital Microscopy" in the April/May 2006 Issue of *The Science Teacher*.) Animal parasitic nematodes cause serious diseases—even death—in humans, pets, and stock animals. Plant parasitic species contribute to over 100 billion dollars a year in crop damage worldwide. Conversely, insecticidal nematodes—parasitic roundworms that only infect insects—are increasingly being used as an environmental alternative to chemical

Ecology of insecticidal nematodes.

Entomopathogenic (or insecticidal) nematodes are soil-inhabiting, insect parasites that belong to the phylum Nematoda, commonly called roundworms. The term *entomopathogenic* comes from the Greek word *entomon*, meaning insect, and *pathogenic*, which means causing disease. Although many other parasitic nematodes cause diseases in plants, livestock, and humans, entomopathogenic nematodes, as their name implies, only infect insects. Entomopathogenic nematodes (EPNs) live inside the body of their host, and so they are designated endoparasitic. They infect many different types of soil insects, including the larval forms of butterflies, moths, beetles, and flies, as well as adult crickets and grasshoppers. EPNs are nearly everywhere; they have been found in all inhabited continents and a range of ecologically diverse habitats, from cultivated fields to deserts. The most commonly studied genera are those that are useful in the biological control of insect pests, the *Steinernematidae* and *Heterorhabditidae* (Gaugler 2006). Well-referenced information about EPNs can be found in "On the web" under "Insect parasitic nematodes" at the end of this article.

Experimental design: Francesco Redi and spontaneous generation.

Francesco Redi (February 18/19, 1626–March 1, 1697) was an Italian physician. Born in Arezzo, Tuscany, he is most well-known for his experiment in 1668 which is regarded as one of the first steps in refuting "spontaneous generation"—a theory also known as *Aristotelian abiogenesis.* At the time, prevailing wisdom was that maggots formed naturally and spontaneously from rotting meat. In the experiment, Redi took three jars and put meat in each. He tightly sealed one, left one open, and covered the top of another with gauze. He waited for several days, and saw that maggots appeared on the meat in the open jar, but not in the sealed one, and maggots did not hatch on the gauze-covered jar, even though they did appear (because the flies landed on it). He continued his experiments by capturing the maggots and waiting for them to hatch, which they did, becoming common flies. Also, when dead flies or maggots were put in sealed jars with meat, no maggots appeared, but when the same thing was done with living flies, maggots did appear. For more information, see *www.britannica.com/eb/article-9062979/Francesco-Redi*.

Sample size and statistical theory.

"Class period 3" was on excellent time in the laboratory module to address the issue of sample size and how small versus large sample size can affect empirical conclusions. It was useful to employ real-life examples illustrating the importance of sample size. A simple classroom survey of favorite music genera emphasized the point nicely to students. Two students were surveyed. One of two students said country music was the best. Based on this information, we concluded that one-half of the class likes country music the best. Students normally disagreed with this bold inference, so we asked students to determine the number of students in the classroom we would need to survey in order to positively make a conclusion about the class' music affinities. We continued by discussing necessary sample sizes we would need to make inferences about the students in the whole school, the whole state, and so on. It quickly became apparent to the students that sampling every individual would not always be feasible, leading to a basic discussion on the importance of statistics in making valid conclusions. More information about sample size and basic statistical theory can be found online at *www.robertniles.com/stats/sample.shtml.*

pesticides to *protect* our crops from harmful insects (see "Ecology of insecticidal nematodes").

Due to the significance of insecticidal nematodes, and the advantages of using this model organism at the secondary science level, we developed and integrated a high school-level nemotology module in the 2005 Gains in the Education of Mathematics and Science (GEMS) program at the Walter Reed Army Institute of Research. In GEMS, students in grades 8–12 spend one to four weeks in an Army lab performing experiments that highlight basic scientific principles (*www.usaeop.com/programs/GEMS*).

The initial inquiry-based activity involving insecticidal nematodes, introduced to the 2005 GEMS students, was used as a beta-test of the laboratory procedure. The majority of students cited the module as the favorite part of their internship experience. With the preliminary nematology module revealing such broad student interest in insecticidal nematodes, we set to further develop and field-test the activity in high school biology classrooms. The module, which required three class periods to complete, was integrated in several biology classrooms at Lehi High School in Lehi, Utah with over 300 10th-grade students. The remainder of this article describes the experience and model.

Classroom integration

Class period 1: Introduction to scientific inquiry, experimental design, and nematodes

To give students the tools necessary to carry out scientific experiments involving nematodes, the first day of integrating the module into science classrooms was used to introduce scientific inquiry, experimental design, and general nematology.

To introduce the inquiry methods and elements of thoughtful experimental design, we began the activ-

ity with the example of Francesco Redi's elegant experiment performed to test the theory of spontaneous generation (see "Francesco Redi and spontaneous generation"). This was followed by a short (15–25 minute) introduction to general nematology and insecticidal nematodes using a PowerPoint presentation (see "On the web" for nematology resources). (**Note:** For the original PowerPoint presentation, please see the online extension of this article at *www.nsta.org/highschool/connections.aspx*.) Following the short introduction, we divided students into groups of three or four and supplied each group with a list of basic laboratory materials needed to work with insecticidal nematodes (Figure 2). General lab safety practices were required.

Class period 2: Scientific planning and experimentation

The next step of this activity was scheduled to occur 24–48 hours after "Class period 1." Armed with knowledge and materials (Figure 2) from the first step of the activity, student groups were instructed to plan experiments to answer questions about the insecticidal properties of the two different species of nematodes—*Heterorhabditis bacteriophora* and *Steinernema carpocapsae*. During the planning process, we aimed to give the major responsibility for learning to the students. While some students developed their own questions, other students were provided with questions. Some examples of student- and instructor-derived questions included:

- Which nematode is the more effective insecticide?
- Which environment (wet, dry, light, or dark) is the best for effective parasitism? and
- How many nematodes are needed to effectively parasitize the insects?

To avoid attenuating the critical-thinking component of learning, we were careful to give students only enough information to enable them to design and carry out their own experiments. We found it useful to explain the following few concepts as students discussed their ideas in groups (teachers could also distribute these concepts on a student handout):

- Insecticidal nematodes (entomopathogenic nematodes or EPNs) are aquatic organisms and should always be stored and applied in water or a physiological saline solution.
- Technically, one EPN is sufficient to infect and kill the insect grub *Galleria mellonella*, but to ensure exposure it is best to use upwards of 35–50 nematodes per insect grub.
- EPNs should be applied directly to the filter paper or container using glass or plastic pipettes.
- White filter paper mimics a soil-type environment by retaining water and allowing for natural movement of the EPNs.
- After EPN infection, insect grubs will generally die within 24–48 hours.
- Insect grubs killed by *H. bacteriophora* will appear brick red in color (Figure 3, p. 38). Insect grubs killed by *S. carpocapsae* will appear grayish in color. (**Note:** *Photorhabdus luminescens,* the bacterial symbiont of the nematode *H. bacteriophora,* produce a red pigment that causes the insects they infect to turn red or brownish red. *Xenorhabdus nematophila,* the bacterial symbiont of *S. carpocapsae*, do not produce this red pigment. The function of this red pigment is unknown.)
- Four to five insect grubs will fit comfortably in a medium-sized petri dish or similar container.

Before experimentation, students demonstrated for us how they had incorporated the basic elements of scientific inquiry and experimental design into their procedures. Students then recorded their protocols in a lab notebook

FIGURE 2

Materials needed for laboratory activity.

(**Note:** Nematodes and insect grubs are readily available from various commercial suppliers, easily located through an internet search. The initial cost of less than $100 provides enough organisms for dozens of classes.)

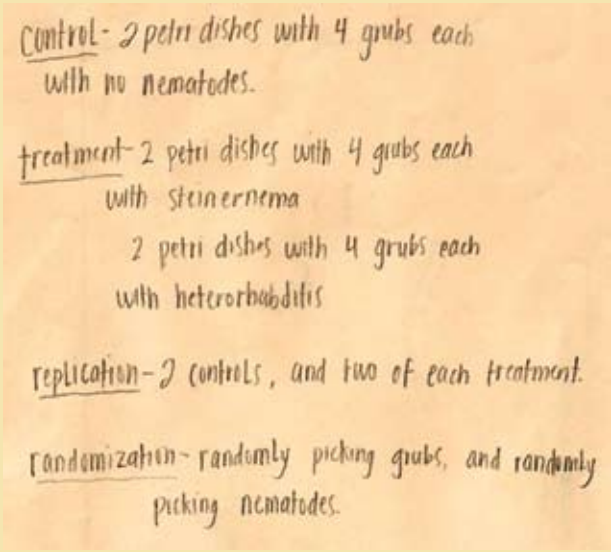
Material	Quantity per group of 3–4 students
The nematode *H.bacteriophora*	1,000 nematodes in 1 mL of water
The nematode *S. carpocapsae*	1,000 nematodes in 1 mL of water
1.7 mL microcentrifuge or similar tube	3 tubes
The wax worm moth grub *Galleria mellonella*	25–30 grubs
Medium size Petri dishes or comparable containers with lids	6–10 containers
Glass or plastic pipettes (with bulbs, if needed)	3–4 pipettes
White filter paper to fit containers	6–10 pieces
Clean tap water	1–2 mL

FIGURE 3

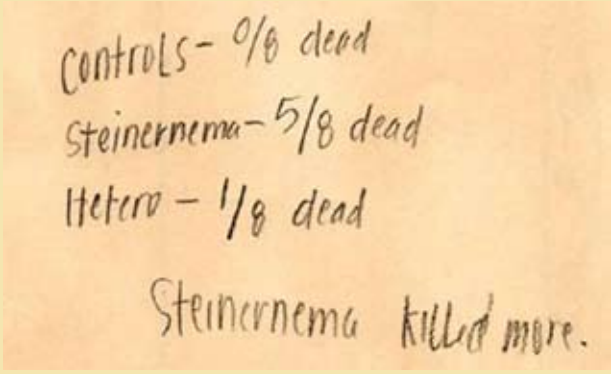
Wax worms exterminated by *H. bacteriophora*.

FIGURE 4

(a) Student group–designed experiment incorporating elements of thoughtful experimental design.



(b) Student results and conclusions based on the experiment outlined in (a).



for assessment and carried out their experiments under instructor supervision.

As each experiment varied from group to group, we cannot provide a step-by-step protocol for conducting the lab portion of this activity. However, an example of a student-designed experiment is given in Figure 4a. The group that produced the lab protocol shown in Figure 4a, for example, asked the question, "Which nematode is the most effective insecticide of grubs?" For a control, these students applied pure water to a total of eight insect grubs in petri dishes. As treatments, students applied each nematode species (*S. carpocapsae* and *H. bacteriophora*) to eight grubs each in other dishes. The students replicated their controls and treatments in additional containers. Grub selection was randomized to reduce investigator bias. Another group of students asked questions about variable environmental conditions on the effectiveness of nematode parasitism. Some of the environmental conditions tested by the students included light, moisture level, and presence or absence of soil.

Class period 3: Results, student-derived conclusions, and research presentation

The third part of the activity was conducted 48–72 hours after "Class period 2." Students monitored their experiments, recorded their results, and discussed findings. We guided student groups as they developed valid conclusions based on their data.

Due to the variance in lab protocols, there was no single expected conclusion. We facilitated students as they determined unique conclusions justified by the data. Students recorded their conclusions in their lab notebooks. The results and conclusions of the student experiment described in Figure 4a are shown in Figure 4b. Of the eight grubs used in the control dishes, zero died over the duration of the experiment. Five of the eight grubs to which *S. carpocapsae* had been applied died within 72 hours, whereas only one of the eight grubs to which *H. bacteriophora* had been applied died in the same time period. The student group concluded that *S. carpocapsae* was a more effective insecticide.

The group of students who tested environmental variables found no apparent effect on nematode parasitism. This outcome, however, provided an excellent opportunity to discuss with the group (and with the

class as a whole) how scientists use all of their data, both expected and unexpected, to come to conclusions. (See "Sample size and statistical theory"on p. 36 for more discussion material.)

Students then presented their findings to the class. Before the presentations, we reviewed scientific methods with the class and explained how methods can be used to organize a scientific presentation, for example: introduction (hypothesis/question), methods and materials (experimental procedure), data, and conclusions. This part of the module was our favorite as students often realized how much they had accomplished on their own through the course of the activity.

Assessment and statistical data

The lab notebooks (described previously) from each student group were used to assess student performance in the module. A lab notebook is easily and readily assessed if it demonstrates an understanding of experimental design, has a written hypothesis, shows recorded results, links results to the hypothesis, makes valid conclusions, and serves as the foundation for the student presentation.

In addition to the notebook assessment, we selected the following four basic learning objectives for statistical assessment:

- Students will improve their ability to think systematically through a problem by using elements of thoughtful experimental design;
- Students will understand and recall the purpose and structure of scientific inquiry;
- Students will be introduced to general facts about nematodes and begin to understand the biological, ecological, and economic importance of nematodes;
- Students will learn and understand some purposes of model organisms and be able to give examples.

To determine if these goals were accomplished, we administered identical pre- and post-assessments to each student. The pre-assessments were administered on the first day of the module before any other activity was performed. The post-assessments were administered after all activities had concluded. The assessments contained five components:

- Solve a given problem by designing an experiment.
- Identify the elements of experimental design used in solving Component 1.
- Describe the characteristics and purpose of scientific inquiry.
- Draw a picture of a nematode and give a description of what nematodes do/are.
- Describe a model organism and cite common examples.

Each component was assigned a point value and rated blindly by two different raters. The average difference on each component from pre-assessment to post-assessment was subjected to a paired-samples student's t-test. The module significantly increased post-assessment scores on all five parts ($p < 0.001$).

Formative data

Students were surveyed three to ten days after the conclusion of the module for formative feedback. Many students indicated they found the module "interesting," "informative," and "useful." Many students said they felt the nematode module was "more interesting than other laboratory activities." A number of students said they talked to people (e.g., family, friends) outside of class about their experience. Examples of student responses to the survey question "What was the most important thing you learned from the module?" included:

- "I learned how fun and interesting science can be."
- "How to conduct an experiment."
- "That even the smallest creatures can be helpful."
- "Nematodes are more important than I thought."
- "That nematodes really do have an affect on our environment."
- "That [nematodes] are essential for life."

Teachers responded to the activity as follows:

- "I liked that it was safe, easy, cheap, and relatively fast."
- "This module 'forced' the students to think and come up with something on their own."
- "There's more to this lab than many other inquiry labs I've done or looked at."
- "I will definitely use this module in my future classes."

Conclusions and extensions

Based on finding statistical significance between our pre- and post-assessments, the module was effective at helping 10th-grade biology students improve critical-thinking skills through application of scientific methods and experimental design. The investigation of nematodes provided an easy and safe opportunity to raise the level of inquiry in laboratory activities, as students designed, experimented, and chose variables to isolate and study. These investigations successfully introduced

students to the study of nematodes and gave them a hands-on experience with unique and important model organisms. Based on formative data, the activity was interesting, useful, and promoted learning for a majority of students. Students' minds were sufficiently engaged by the project to promote discussion with peers and relatives outside of the classroom, which combined with the other formative and statistical information, leads us to conclude that the module positively affected student interest in the subject matter.

Many students suggested the activity be extended beyond three class periods to allow for further investigation and to follow up on results. Some students even returned after school to extend their experiments. Teachers indicated that they would approve an extension of the module timeframe to allow students to explore new avenues of research. Some students suggested using more than two species of insecticidal nematodes. Lastly, for students interested in individual projects or classrooms with longer laboratory time scheduled, we suggest incorporating other types of insects susceptible to these species/genera of nematodes. Mealworms and hornworms are good candidates and are available through various pet stores, bait shops, and online insectaries.

The only notable struggle we encountered was helping some students grasp specific concepts related to nematology or the elements of experimental design. Guiding students to comprehend the concept rather than focus on the "rote answer" is a common struggle faced by teachers in all subject areas, and experimental design can be challenging for students. However, students became excited about generating their data and discussing it, rather than playing a matching game with the answer key, and they enjoyed designing their own experimental procedures, rather than just following the predetermined recipe of a standard laboratory activity. This exercise shifted the focus from passive individual memorization to an active social process of learning.

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References

Bongers, T., and H. Ferris. 1999. Nematode community structure as a bioindicator in environmental monitoring. *Trends in Ecology & Evolution* 14: 224–228.

Gaugler, R. 2006 Nematodes—Rhabditida: Steinernematidae and Heterorhabditidae. *www.nysaes.cornell.edu/ent/biocontrol/pathogens/nematodes.html*

National Research Council (NRC). 1996. *National science education standards.* Washington, DC: National Academy Press.

Tylka, G.L., and C.A. Jasalavich. 2001. Free-living and Plant-parasitic nematodes (roundworms). *The Plant Health Instructor. www.apsnet.org/Education/K-12PlantPathways/TeachersGuide/Activities/Nematode/top.htm*

Wisconsin Outreach Research Modules (WORM). 2004. The use of model organisms in instruction. *www.loci.wisc.edu/outreach/text/model.html*

On the web

General nematology

- *www.nematologists.org*
- *http://plpnemweb.ucdavis.edu/nemaplex/Uppermnus/general.htm*
- *http://nematode.unl.edu/wormgen.htm*
- *www.abo.fi/fak/mnf/biol/nni/lec_bost.htm*
- *www.earthlife.net/inverts/nematoda.html*

Insect parasitic nematodes

- *http://nematode.unl.edu/wormepns.htm*
- *http://kbn.ifas.ufl.edu/kbnstein.htm*
- *www2.oardc.ohio-state.edu/nematodes*

Animal parasitic nematodes

- *http://elegans.swmed.edu/Nematodes/Animal.Parasitic.Nematodes.html*
- *www.intute.ac.uk/healthandlifesciences/veterinary*
- *http://plpnemweb.ucdavis.edu/Nemaplex/General/animal.htm*

Plant parasitic nematodes

- *www.cotton.org/tech/pest/nematode*
- *www.cals.ncsu.edu/pgg/dan_webpage/Nematodes/nema.htm*
- *http://mgd.nacse.org/hyperSQL/squiggles/nematodes1.html*

Free-living nematodes

- *http://creatures.ifas.ufl.edu/nematode/soil_nematode.htm*
- *http://edis.ifas.ufl.edu/pdffiles/IN/IN13800.pdf*
- *www.beorganic.com/nematodes.html*