Meeting the COVID Challenge - Berg
Supplementary Material. Virtual Experiments

THE NEURO-MUSCULAR BASIS OF EARTHWORM MOVEMENTS: EFFECTS OF PHYSICAL AND CHEMICAL ENVIRONMENTAL AGENTS

Goals. Understanding the neurobehavioral consequences of exposure to natural and manufactured chemicals is the central focus of this module (Weber et al., 2016). In a world of virtual learning, educational goals that apply to classroom experiences are often confronted by potential roadblocks. Using earthworms (*Eisenia fetida*) and their small array of behaviors allows an easy transition from direct to virtual observations. The virtual setting prevents the application of appropriate tools and techniques to conduct controlled experiments using animal models, thus diminishing the students' ability to have a hands-on encounter with behavioral aberrations induced by environmental contaminants. To overcome this deficiency, additional objectives were integrated so that students can discover the impact of potentially toxic environmental agents on specific, easily monitored and analyzed behaviors. These goals included:

- Providing a comprehensive series of training videos that develop students' scientific foundation for understanding the methods used in the module.
- Designing a virtual experiment that allows each student to (i) conduct the experiment at a pace amenable to that student's ability, (ii) observe contaminant-induced behavioral changes under actual experimental conditions, and (iii) collect and analyze scientifically meaningful data.
- Comparing findings with other students who are observing the same videos, thereby gaining insights into biases in behavioral observation, as well as methods to overcome those biases.

Process of Making Videos. The earthworm module is, in itself, modular. The entire set of activities involves five sections that are best done sequentially within a single unit, but can also be conducted individually depending on the specific goals of the teacher. The latter may be particularly attractive to teachers at the beginning of their careers who face the challenge of creating and delivering new courses and are unable to undertake the full module. With this in mind, the videos were generated by a team of two student interns from the UWM Department of Film, Video, Animation, & New Genres, as five separate productions. One student did the videography and the other managed the editing. Their creative insights into how to set up the scenes and assemble the final product were critical to the success of this effort. The staff scientist overseeing these videos was responsible for the final edits.

Video Content. Five videos, ranging in length from approx.15-40 minutes, were completed, each one demonstrating carefully designed and executed experiments as they would be seen by students in a classroom. Before watching a video, students can articulate hypotheses about outcomes they may observe and consider what observations to record. Using their definitions of what constitutes a completed behavior, these data then serve as the basis for analysis of the experiment and determination whether the results support initial hypotheses. Descriptions of the 5 videos follow.

1. Sensorimotor Reactions: How Do Earthworms Sense their Environment? Students begin this module by exploring how sensing the environment is translated into behavior. This is central to developing an understanding of any behavior. Using the simple behavioral repertoire of the earthworm, students discover what environmental stimuli, e.g., light, temperature, or chemical elicit a neuromuscular reaction and how that reaction is dependent upon concentration of the chemical or intensity of the stimulus. The video is designed and produced so that simple household objects like a flashlight and chemicals such as medicinal alcohols (ethyl or isopropyl) can be used in conjunction with the video by students who are learning in a virtual environment. As such, these activities can be done at home.

2. Behavioral Outcomes Related to Chemical Exposures. Having gained an appreciation of how earthworms sense their environment, students have the opportunity to explore how sensorimotor activity becomes translated into behavior. Using the earthworm as a model organism, students discover whether particular chemicals elicit a neuromuscular reaction in a fully controlled experimental setting and how that response relates to the concentration of the chemical (Figure 2). Here, too, household chemicals are included in the video so that these studies can be done either virtually or physically in a home setting if students have access to earthworms.

3. Effect on Behavior of Chemicals in the Earthworm's Environment. Worms, of course, live in soil. Having learned that worms can sense some chemicals but not others in an artificial, controlled environment, students now observe how and why the type of soil in which the worms live can affect their ability to sense chemicals. Viewing this video helps students understand that observed sensory response is dependent on the environmental context of the experiment. Without this realization, conclusions about the impact of chemicals on earthworms living in their natural environment extrapolated from results obtained in the simpler experimental set-up of video 2 may be incorrect.

4. Watching Earthworm Behavior Under the Surface. Now students are ready to "dig" deeper into worm behavior. Since worms usually live below the surface of the soil, it is often difficult to observe how chemicals affect their movement. In this video, students are introduced to an experimental set-up that makes it possible to observe the actions of earthworms below the surface and to use that technique to ask new questions concerning neurobehavioral responses to chemicals.

5. Effect of Direct Exposure on Earthworm Behavior. In the last video, the conceptual and observational complexity of experiments is significantly increased as indicated by the following questions that are addressed in this video. What happens if the worm and not the soil is directly exposed to toxic chemicals? How does the worm's behavior change? What if these questions are investigated using not just one variable, concentration, but two variables, concentration and length of exposure, simultaneously? How do these two variables interact to cause a behavioral effect? What type of data analysis can be employed to derive meaningful information from this complex experiment? In this video, students investigate the effects of chemical **A**mount/concentration, **R**ate/length of exposure, and **T**ype of chemical on the behavior of worms burrowing into soil. These form the basis of the pneumonic used within the video that toxicity is an **ART**.

Each of the above five topics is divided into two videos—a teaching video and a virtual experiment. Producing both sets of videos enhances the modular nature of this unit and provides teachers greater flexibility in implementing the module. The teaching video was developed as a compact version of a longer workshop video that is currently included in our LibGuide. This workshop consists of two, 3-hour sessions that provide hands-on training for teachers and pre-service teachers. The shorter videos were produced so that teachers can use them as a self-guided module overview or as a refresher. They may also provide the videos to students to guide them through the steps and concepts in the module. The advantage is that teachers or students can start and stop the video whenever needed or return to a previous segment of the video to review the information. Close-up views of worm activity allow students and teachers to gain greater appreciation of the effects environmental stimuli and chemicals have on earthworm behavior, specifically neuromuscular activity. Short talks with PowerPoint slides were also produced that show how to construct a data sheet for behavioral observations and how to analyze such data.

The virtual experiment videos comprise the heart of the module and can be used by students either in class, if procurement of animals or materials is difficult, or at home if the student is learning virtually. The videos are of actual experiments conducted by a WInSTEP SEPA program staff scientist. While the teaching videos demonstrate how to set up these experiments, the virtual experiments focus solely on data collection. Use of close-up shots assist students in seeing the behaviors more clearly and, therefore, facilitate data collection. Unlike the training videos, no voice-overs are used that could potentially bias student data collection.

Immersing Students in the Research Experience. Using close-up scenes of earthworm behavior, students observe and collect data under varying environmental conditions. Through this process, the student experience approaches "hands-on", as much as is possible in a virtual experiment. In the last experiment, students gain an appreciation of the complexity of behavioral responses to chemicals by observing the effect and interaction of two variables (concentration and length of exposure) on behavioral outcomes. An additional educational outcome of these videos is that students can easily visualize how even simple experimental designs can yield important scientific insights. The videos allow students to consider how they can conduct their own, at-home experiments using these methods with earthworms to test hypotheses of how chemicals in their own environment may affect living systems, including themselves. Several of these videos were field tested in an in-person middle school setting. Teacher feedback was enthusiastic about the use of the videos to assist students in better understanding the concepts and methods of the module. One teacher noted that use of the videos was a saving grace during a year when in-class experimentation in general and our modules specifically were disrupted.

INTEGRATING PHYSIOLOGY AND BEHAVIOR: USING FATHEAD MINNOWS TO MODEL THE EFFECTS OF ENVIRONMENTAL AGENTS

Goals. Lead (Pb) poisoning remains a critical environmental health issue for many communities, specifically the children in those communities (Hauptman et al, 2017). While it remains high on the list of dangers in older urban locations, where old housing stock containing lead paint and lead water service lines are prevalent, and presents a particular problem for children of color, it is also an issue for rural communities (Carrel et al., 2017). In this module, based upon research conducted by one of the UWM WInSTEP staff scientists, fathead minnows (*Pimephales promelas*) are utilized as model organisms in the investigation of the impact of lead/Pb on reproductive behaviors and outcomes (Weber, 1993). Although the overarching goal of this module is to provide a robust opportunity for research in the context of learning about behavioral biology, it also encourages students to relate their studies to the real-world issue of childhood lead poisoning and become advocates for their own health and the health of their community.

As with the earthworm module, educational goals that apply to classroom experiences may be frustrated when transferred to a virtual experience. Thus, additional objectives were included that ensure the students understand the impact of Pb on specific, easily monitored and analyzed behaviors that relate to physiological and anatomical characteristics affected directly by Pb. These additional goals are similar to those with the earthworm module but include important differences. They include:

- Providing a training video that builds upon those already in our WInSTEP SEPA LibGuide and develops the scientific foundation for the methods used in the module.
- Using digitized tapes of the actual experiment that the module is based upon to create a set of virtual experiment videos (Weber, 1993). The recordings (i) allow the experiment to be carried out at a pace commensurate with each student's ability; (ii) provide flexibility for the teacher to create the best experience for students within the time frame available for the unit; (iii) make it possible for students to observe Pb-induced behavioral and anatomical changes over a 2-week period under actual experimental conditions; and (iv) most importantly, offer students the opportunity to collect and analyze data in a scientifically rigorous manner.
- Encouraging students to compare findings. Because all are observing the same video and identical behaviors, this activity helps students improve their observational skills and may reveal visual biases that affect data collection.
- Combining data to see the value of big data sets.

The teaching video, approx. 30 minutes, was developed as an abbreviated version of a longer workshop experience. The original workshop consisted of 5 days of 3-hour sessions/day that provided hands-on training for teachers. The video was produced so that teachers can use them as a self-guided module overview or as a refresher. If necessary, students can access the video to lead them through the steps and concepts in the module.

Process of Making the Videos. As with the earthworm videos, the virtual experiment videos are central to adapting our program to the pandemic. They can be used by students either in class if procurement of live vertebrates or aquarium supplies is difficult or at home by students who are participating in classes on-line. With this in mind, the videos were produced by a team of two student interns from the UWM Department of Film, Video, Animation, & New Genres. Their creative insights into how to set up the scenes and how to produce the final product were critical to the success of this effort. Unlike the training videos, no voice-overs were used that could potentially bias student data collection (Figure 3).

The training video was produced so that teachers can use it as a self-guided module overview or as a refresher. It may also guide students through the concepts and steps in the module. As with the earthworm videos, teachers or students can start and stop the video whenever needed or return to a previous segment to review the information.

Immersing Students in the Research Experience. Using live animals, especially for behavior studies, is always preferable. Yet, when videos are constructed in a way that allows students to collect data from actual experiments, the experience begins to resemble the real experiment. One facet of this process is that students learn the value of doing behavioral research as a blind study. The set of videos, each one representing one day in a progression, gives students insights as to how behaviors and anatomical structures related to those behaviors develop over time and how toxic chemicals in the environment, even after just a few days of exposure, can alter the intensity of both behaviors and key anatomical features.

Behavioral observation in a classroom setting presents several challenges. Students may have difficulty accurately identifying the various behaviors; data sets are too small for adequate statistical analyses (numbers of aquaria are limited because of animal care and space issues in the classroom); and, due to the need to keep lead/Pb out of the classroom, all fish are pre-exposed. This means that during the 1-3 weeks that teachers keep the fish in the classroom, internal stores of Pb in the fish decrease over time and behaviors in Pb-exposed fish may change during the time frame the students are observing the fish. The use of the videos removes these issues. These new videos (i) help students accurately identify various reproductive behaviors and secondary sex characteristics, (ii) provide the statistical benefits of a large data set (24 pairs of control male-female pairs and 24 Pb-exposed pairs) to track the daily behaviors, and (iii) allow students to observe the effects of constant levels of Pb because the original experiment upon which the video is based involved maintenance of a constant concentration of dissolved Pb in the water. In the future, should teachers decide to use both the virtual experiment and live animals in the classroom, students will be able to compare and contrast the behavioral outcomes under these two experimental regimens.

The minnow virtual experiment videos were field tested in a high school setting. Feedback from students and teachers were helpful in developing a revised version that enhanced the utility of the videos to create a meaningful, virtual research experience. The revised videos were again field tested in two high schools and one middle school. Comments from students and teachers were positive. For example, one teacher wrote, "This year has definitely been a challenge for everybody and having you compile all the videos was a lifesaver. Obviously, having real fish in the classroom is the best, students are constantly looking at screens now and (it) would have been nice to have the real deal." A student team from one of the high schools was chosen to orally present its results at the 2021 SEPA Student Research Conference.

ZEBRAFISH EMBRYO DEVELOPMENT: STUDYING THE EFFECTS OF ENVIRONMENTAL CHEMICALS

Goals. Zebrafish embryo development occurs rapidly over the course of three days (Kimmel et al., 1995). Because embryos are transparent, the details of this process can be observed in great detail. Such features make this process highly attractive to scientists interested in the fundamentals of early development and concerned with abnormal development caused, for example, by exposure to environmental chemicals. In both research contexts, the zebrafish serves as a model organism and surrogate for humans. Taking advantage of expertise within the WInSTEP SEPA staff, students are provided opportunities for experimentation with zebrafish embryos that examine the impact of chemicals on embryo development (Tomasiewicz, H., et al., 2014). The same properties that appeal to researchers make it possible for students in classrooms to observe normal and perturbed development.

There are two objectives of the online portion of the zebrafish module. First is to provide access to key experiences in doing research to those without the means to observe live zebrafish embryos in the context of an experiment. Caring for and manipulating the eggs together with direct observation of development is a great learning experience for students. However, implementation of the module can be difficult in some environments and for newer teachers and especially difficult when instruction is remote.

The second is to provide teachers and students with a much larger pool of animals and experimental conditions from which to collect and analyze data. In the classroom, student observations are generally limited to one chemical at four concentrations (zero, low, medium, high) with three replicates based on classroom logistics and the supply of zebrafish embryos. To enrich the observational experience, we took advantage of an ongoing research project which is studying the impact of a range of chemical exposures on a large number of developmental indices. Several WInSTEP SEPA chemicals were added to the experimental protocol, and their impact on the progress of development visually recorded. These images together with detailed information on experimental conditions have been gathered into an online repository that is available to students. With the repository, students have access to images and videos of zebrafish embryos treated with ethanol, caffeine, or nicotine at nine concentrations, with negative and positive (3,4-Dichloroaniline) controls.

Immersing Students in the Research Experience. We produced short videos outlining the experimental process so students can understand how these images and videos came to be. Essentially, individual zebrafish embryos less than six hours postfertilization (hpf) were placed in the wells of a 96-well plate. Embryo medium was removed and replaced by treatment solution, then plates were moved to a 28^{o} C incubator. The next day at approximately 24 hpf every embryo was photographed, and a short video collected to show spontaneous tail coiling (changes in the tail coiling frequency can be indicative of neurotoxicity or developmental dysfunction) (Figure 4). Media was then removed and replaced with fresh treatment solution, and the plate returned to the incubator. This same image collection process was completed at 48, 72, 96, and 120 hpf. In addition, a short movie of the zebrafish embryo beating heart was captured for data collection opportunities on environmentally-induced heart rate change.

Students decide how to use the repository to follow the development of chemically exposed embryos. The variables include number of organisms, and nature and concentration of the chemical. Perhaps they will want to compare the effects of different chemicals. With the large size of the repository, there are many options for experimental design.

We have created training sets for the students because one must know “normal” to identify “abnormal”. We provide curated examples of the abnormalities so the students can recognize what they are looking for. However, the images taken of experiments have not been evaluated by laboratory personnel, so there are no predetermined answers. Instead, students examine them as new images in the same way they would make observations during their own experiments.

Students create databases of their personal observations. They have the opportunity to evaluate more than 20 developmental and anatomical characteristics of each embryo. Student-collected data are exported in a CSV file for analysis using most database platforms such as Excel. These data can be analyzed alone or in combination with data from others in the class, at the teacher’s discretion, to increase the complexity of the data set and thus to create the challenge of analyzing “big data”. The student experience is greatly enhanced by using the University of Wisconsin-Milwaukee course management system, Canvas™, as the interface for the students to navigate through more than 3500 images and videos.

Process of Constructing the Repository. COVID-19 restrictions imposed by the University (mandated distancing and room occupancy limits) dramatically slowed the process of image and video collection. The biggest challenge was in modifying our existing database program to run within the Canvas™ environment, which provides excellent user authentication and the opportunity for teachers to employ the numerous Canvas™-embedded tools. This is a new use of the Canvas™ platform and it took time to communicate our special needs to the Canvas™ team.