INQUIRY & INVESTIGATION

Minnows as a Classroom Model for Human Environmental Health

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Abstract

Understanding human environmental health is difficult for high school students, as is the process of scientific investigation. This module provides a framework to address both concerns through an inquiry-based approach using a hypothesis-driven set of experiments that draws upon a real-life concern, environmental exposures to lead (Pb^{2+}). Students learn how scientists use model organisms to understand basic biological concepts, and how these models relate to human and environmental health. Students observe how Pb^{2+} alters fish behaviors. Because many levels of biological organization are involved, this module has application for multiple units within general and advanced biology classes. Beginning with what is known about Pb^{2+} toxicity, students develop testable hypotheses about how it may affect behavior, apply this knowledge to human populations, and identify the "next experiment."

Key Words: *Behavior; environmental health; fish; inquiry-based learning; lead; minnow; model.*

Perhaps more than any other poison, lead (Pb^{2+}, the ionic form most commonly used in research) from residential, commercial, and industrial sources (Silbergeld & Patrick, 2005; Del Bene Davis, 2007; Chen et al., 2011; Mielke et al., 2011) conjures up images of lower IQs, behavioral disorders, and sensory-motor deficits – and with good reason. This pollutant has been associated with a range of childhood behavioral problems at ever lower levels of exposure (Bellinger, 2004, 2008; Olympio et al., 2009; Winneke, 2011; http://www.atsdr.cdc.gov/csem/csem.asp?csem=7&po=8). For most students, these dangers remain largely abstract because they are outside their normal set of experiences. The challenge is to get students to internalize issues of environmental health through real-world, engaging, and interactive science experiences. After 15 years of experience using this module with middle and high school students, including some who were lead-poisoned themselves, followed by a rigorous, professional evaluation protocol, we have found that students are fully engaged in conducting the experiment while gaining empowerment over issues surrounding their own health.

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○ Why Study Behavior?

Behavior results from integrated neurological and hormonal processes responding to external environmental stimuli (Weber & Spieler, 1987). Because behavior is observable and measurable, students are actively engaged in hypothesis testing, data collection, and assessing cause and effect. First, students use animal activities to model their own behavior. If Pb^{2+} changes fish behavior, students have a strong, visual example of what that might mean for them. Second, chemical-induced behavioral disorders have a physiological context, thus demonstrating the integration of different levels of biological organization. Third, behavior is sensitive to contaminants. Observing and analyzing these changes can be accomplished with inexpensive, reusable laboratory equipment. Fourth, students develop the concept that pollutant-induced changes in the development and expression of behavior can be just as harmful as physical damage. Fifth, it is fun and engaging. Live animals in the classroom provide a means by which students learn affective skills of patience and teamwork, as well as cognitive skills of observation, data collection, and data analysis.

○ Why Study Fathead Minnows?

Fathead minnows (*Pimephales promelas*) provide information on fundamental principles of biology while simultaneously allowing students to identify effects of environmental contaminants within the time and space constraints of classrooms. Minnows are an excellent model for classroom studies of environmental health because they are easy to maintain under classroom conditions. Of importance to this module, fathead minnows are sexually dimorphic: males exhibit a suite of secondary sexual

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characteristics and a repertoire of easily observed and recorded reproductive behaviors.

○ Goals & Objectives

High school students are generally able to use deductive reasoning and recognize connections between explanations and evidence. Therefore, we designed, tested, and implemented a classroom module to investigate substances harmful to human health using a hypothesis-driven experiment with a live animal model and well-defined set of variables centered around the impact of Pb^{2+} on reproductive behaviors. Using an inquiry approach, students learn basic concepts and principles of living organisms (e.g., behavior) while applying this knowledge to a real-world problem.

Table 1. A guide to fathead minnow breeding behaviors (modified from McMillan & Smith, 1974).

Activity	Description
Hovering	Occupying upper space under spawning structure, regardless of activity.
Nest Preparation	Contacting spawning structure ceiling with head or physically removing debris or dead eggs.
Spawning	Side-by-side interaction between male and female under spawning structure associated with egg deposition. Male will flip female upside-down to allow her to deposit eggs while he fertilizes them.
Chase	Male swims rapidly after female; sometimes female chases male.
Patrol	Male swims outside spawning structure to feed, chase, or display behavior not directed to reproductive activity.

Choosing observable and measurable variables. When studying behavior, it is crucial to identify and clearly define the variables of interest. To accomplish this, students begin with a training video provided by the authors that allows them to define and practice observing specific behaviors (Table 1) that are requisite for minnows to attract a mate and guard the developing eggs. An alternative method is to use the fish in the control tank to help students, with guidance from the teacher, generate their own behavioral categories and definitions.

If you build it (correctly), they will breed. Before assembling the tank, students should think about what a minnow needs to survive, grow, and reproduce and what variables should be recorded. By dividing the list into constants (e.g., temperature, date, and time) and variables (e.g., treatment regimen), students learn the complexities of, and multiple interactions between, the physical and biological world and how different species balance those interactions. Only then can they begin to appreciate the changes induced by Pb^{2+}.

To create an artificial environment that will be conducive to eliciting breeding behaviors, students identify relevant characteristics about minnows. Female minnows are fractional spawners that sequentially oviposit a fraction of their eggs in more than one nest over many days or weeks. During breeding activity, males display secondary sex characteristics of dorso-ventral color bands, dorsal epithelial pads, and rows of tubercles on the snout (Figure 1). Females are smaller, lack the darker banding coloration, possess no tubercles or epithelial pads, and, when, sexually mature, display an ovipositor (tube through which eggs are deposited) between the pectoral and anal fins.

Males use the underside of any object in slow-moving water as a nesting site. After cleaning it with their tubercles, mouth, and fins, they spend time attracting females to their nest. If a female enters the nest, the male will attempt to flip her so that she can lay her eggs (200–500 day^{-1}; Figure 2) on the structure's ceiling. Sperm is then released from the testes (Figure 2) by the male to fertilize the eggs, after which females leave. Males remain to tend to the eggs (e.g., egg fanning to increase oxygen flow and removing dead embryos) until they hatch (female students like the idea that male minnows are responsible for ALL parental care!). All behaviors and structures are quantifiable and easily observed, making recording of data easy for students.

To ensure that minnows will enjoy their home, students consider what physical components of that home are needed (e.g., oxygen, clean dechlorinated water – chlorine will kill fish – and places to lay eggs). To stimulate specific reproductive behaviors, photoperiod, temperature, and food must be considered. For minnows, a 16 hours light:8 hours dark photoperiod with water temperatures of 19–25°C provides optimum conditions to stimulate breeding. Connecting these physical necessities with specific aquarium structures enhances students' comprehension of life's complex interactions.

Each 10-gallon tank (1 control with no Pb^{2+}, 1 treated with Pb^{2+}/16 students) requires an aquarium light (standard fluorescent bulbs are sufficient) attached to a timer. Because minnows begin breeding activity at lights on and continue for about 2 hours, the teacher can manipulate peak activity depending on the time of day that class(es) will be doing observations. For example, if class period begins at 9:15 AM, lights should go on at 9 AM and off 16 hours later. Water is maintained at 20°C with a submersible aquarium heater. Air is pumped in to ensure sufficient oxygen and to distribute heat evenly throughout the tank. Feeding with frozen brine shrimp is done daily at the beginning of the light period.

Because aquarium filters contain activated charcoal that removes Pb^{2+} from the water, no filters are used in the tanks. Therefore, every other day, 50% of the water is siphoned to reduce ammonia levels and remove uneaten food and feces. Dechlorinated water is used to reestablish the 20-L volume and, by adding the appropriate amount of stock solution, to reestablish Pb^{2+} concentrations (1 mg/L). Because minnows remove almost all Pb^{2+} within 2 days (Weber, 1991), waste water can be poured directly down the drain. Blood lead levels of laboratory fish exposed to this concentration for the 2 weeks of this module are similar to that found in lead-poisoned children (Weber et al., 1997). All water is dechlorinated before fish are placed into the aquarium by letting the water stand in clean plastic gallon jugs uncovered for 48 hours.

Breeding substrates require a structure with a ceiling to which the female will attach her eggs. A 4-inch-diameter PVC pipe cut lengthwise and then into 4-inch sections works well, is easy to clean and store, and allows viewing of breeding behavior from both sides

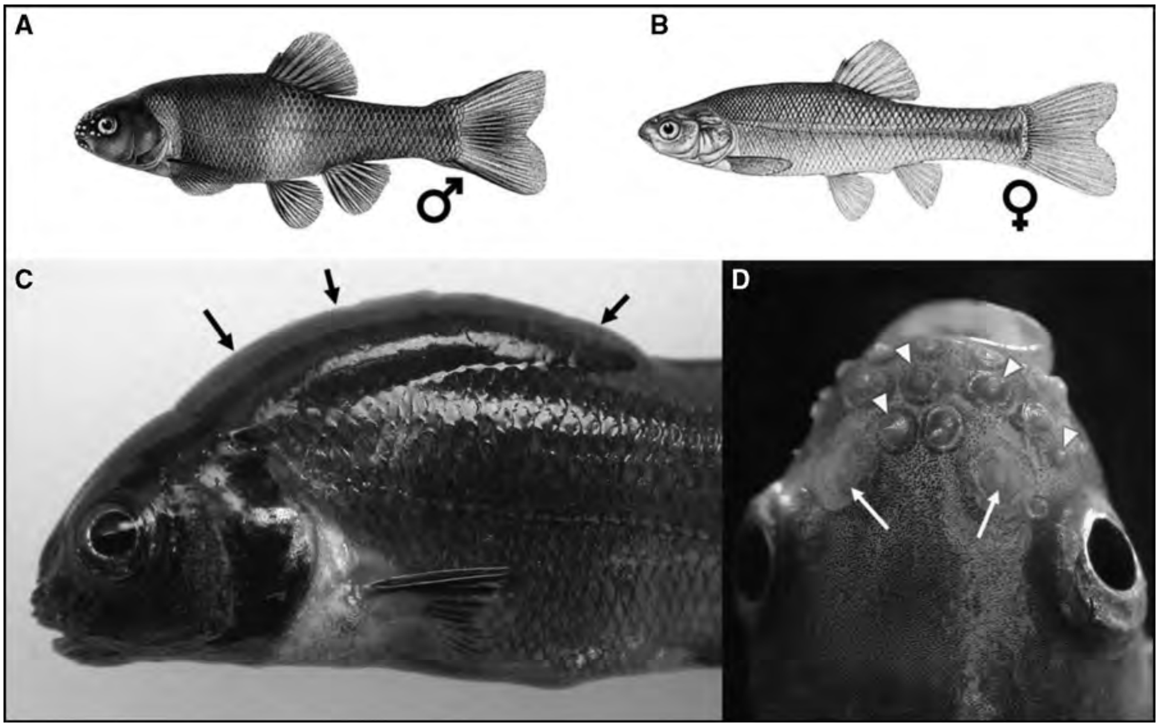

Figure 1. Fathead minnow sexual dimorphism. (A) Adult male fathead minnow in breeding condition, showing tubercles, epithelial "fat" pad, and vertical banding pattern along the body. (B) Adult female fathead minnow, typically smaller than mature males, and lacking tubercles, epithelial pad, and banding coloration. Drawings by Joseph Tomelleri, with permission. (C) Photograph of mature male fathead minnow in breeding condition. Note epithelial pad (arrows) and tubercles. (D) Dorsal view of male fathead minnow, showing tubercles of various sizes (arrowheads) and nares (arrows). Photos by Andrew Kane.

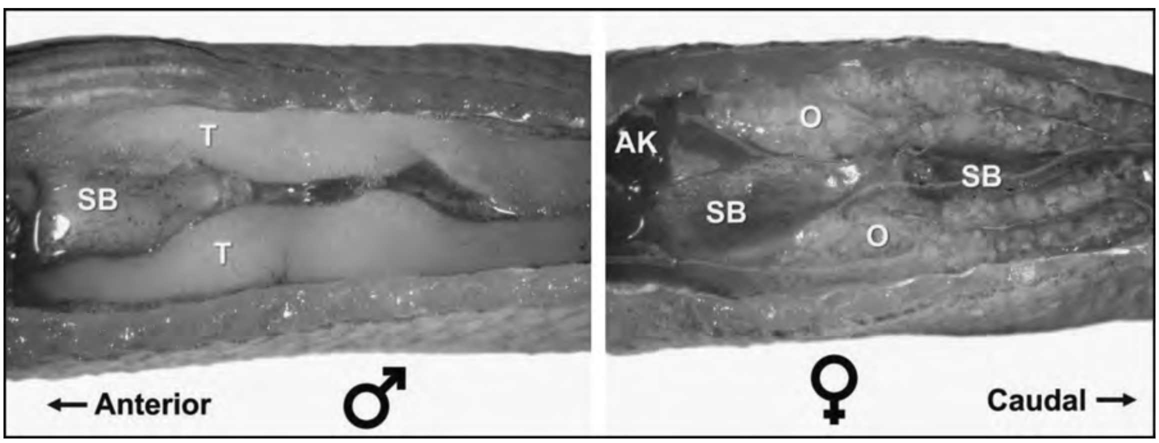

Figure 2. Ventral view of dissected male (left) and female (right) adult fathead minnows revealing testes (T), swim bladder (SB), ovaries (O), and anterior kidney (AK). Photos by Andrew Kane.

of the tank. Perforated, transparent aquarium dividers are inserted into the tank to create three sections, each with one male: female breeding pair and one breeding substrate (Figure 3). This makes it easier for students to follow each fish and collect their data.

A more complete description of fathead minnow husbandry techniques can be found in Denny (1987) and can be obtained at no cost through the U.S. Environmental Protection Agency (http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=2000HU6Q.txt).

Planning timetable. Minnows (≥120 days old) are obtained from our laboratory or scientific supply vendors to ensure that the fish have not been exposed previously to any toxicant. If there is a private or government bioassay laboratory near the school that uses minnows for testing, they are often willing to donate their unused fish. A genetic variation of fathead minnows commonly sold by vendors or pet stores that would be a suitable substitute is called Rosy Red. Although teachers who participate in our workshop receive all supplies at no cost, our experience is that some teachers have been able to take advantage of donations from their school's PTA to purchase these items or have received items directly as donations to the school.

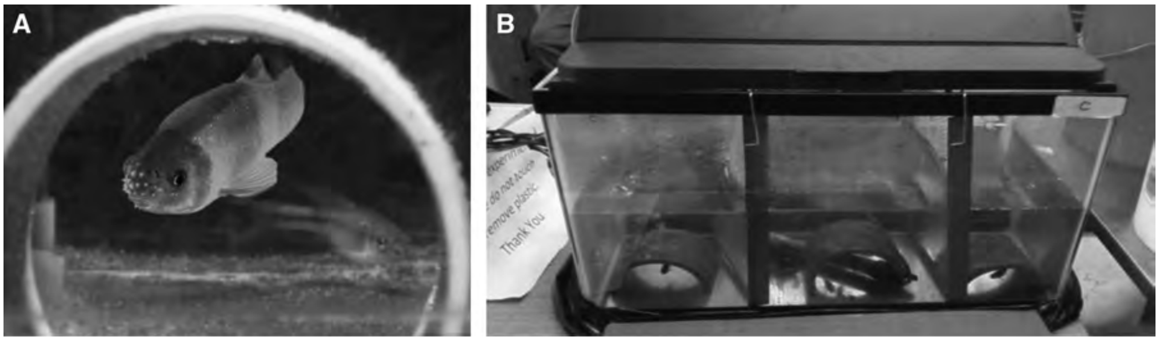

Figure 3. (A) Male fathead minnow under breeding substrate. Male is "hovering," with female in background. Photo by Daniel Weber. (B) Complete aquarium setup. Photo by Michael Mullen, Menomonee Falls High School, Menomonee Falls, WI.

Advanced teacher preparation of aquaria is required, including Pb^{2+} exposures and successively longer light regimens necessary to support sexual maturation and associated breeding behaviors. Teachers can request that fish be sent that are pre-acclimated to a 14 hours light:10 hours dark photoperiod (14L:10D) to reduce class acclimation time. Once the fish have arrived, teachers should place all the fish into a single large tank set up and maintained as described above. Each week, the timers should be shifted to lengthen the photoperiod by 1 hour until a photoperiod of 15L:9D is achieved. At that time, males and females can be differentiated (males show their dorsal fin spot; Figure 1), and the test tanks can be set up with one male:female pair placed into each section per tank (Figure 3). Lead treatment begins at that time. Student enthusiasm is generated through observations of week-to-week changes in fish body coloration and size as the photoperiod increases. Knowing that the hormonal basis of sexual behavior is similar for fishes and humans solidifies, in the students' minds, the value of using minnows to model human environmental health as they gain an appreciation for the many commonalities between living organisms.

Students will observe effects of Pb^{2+} within days after exposure, which become more pronounced over time. Therefore, observations should continue for at least 2 weeks to clearly document the effects of Pb^{2+} exposure. Comparing these data to those reported in human studies will enhance student appreciation of the dangers of pollution.

Safe handling of Pb^{2+}. Teachers should handle lead salts with utmost caution, wearing gloves (latex or nitrile), lab coat, and protective eyewear. If any solution should splash on the skin, wash with water immediately and thoroughly. For more safety information (e.g., what to do in case of a spill), check http://www.sciencelab.com/msds.php?msdsId=9927204.

Lead (as $PbCl_2$, $PbNO_2$, or Pb acetate; from Fisher Scientific, VWR, etc.) stock solutions (100 mg Pb^{2+}/L distilled water = 100 parts per million [ppm]) acidified with HCl to allow the Pb^{2+} to dissolve are made **ONLY BY THE TEACHERS** and stored in labeled plastic bottles at pH 2. The final concentration of Pb^{2+} in the aquaria is 1 ppm (i.e., 1 mg Pb^{2+}/L of dechlorinated tap water). This will amount to 200 mL of the stock solution going into the 20 L of water in the aquarium. For a 2-week experiment, 2 L of stock solution is needed for each treated aquarium. Although lower concentrations of Pb^{2+} can produce similar results over longer time frames (Weber, 1993), the tight schedules of many science curricula require teachers to use the higher concentration to produce quicker results.

Caution: *Students Should Never Handle The* Pb^{2+} *Solutions!*

Students should calculate how much Pb^{2+} is needed to make the solution. It is important to have students understand what "parts per million" means and that these small amounts of toxic exposures are very dangerous. Proportional thinking and use of ratios is a difficult concept for many high school students. Therefore, equivalents such as 1 second in 11 days (not much from your spring break!) are useful.

○ Methods

Each group has an observer and recorder. During each 3- to 5-minute observation session (length related to size of class and length of class period), the observer calls out each behavior when noticed and the recorder marks it on the data sheet (Figure 4). Because there are three pairs of breeding fish per tank, each separated by the perforated tank divider, students can view multiple breeding pairs within a class period or there can be multiple groups observing each tank simultaneously to maximize the number of students able to record data during each class period.

Observations can be made up to 2 hours after lights go on. Thus, consecutive classes can use similar sets of tanks for collecting data. Using this observation method allows all students to view the fish, even within a 40-minute class period.

At the end of the daily observation period, check the underside of each breeding substrate for eggs that may have been deposited. The eggs can be removed by one student (wearing nitrile gloves for protection against lead-contaminated tank water or bacteria from fecal material) gently rubbing them off the substrate while another student uses a squirt bottle with dechlorinated tap water to wash loosened eggs into a beaker. Using a transfer pipet, eggs can be collected and counted as an assessment of reproductive success.

○ Data Analysis

Graphing is important for visualizing patterns and communicating data to others. Many high school students have difficulties constructing or interpreting graphs (e.g., unscaled axes and randomly choosing which axis to use for the dependent and independent

Day/ Date/ Temp/ Time	Recorder/ Observer/ Timer/ Duration	Nest Prep	Hover	Spawn	Chase
Day ____ Date ____ Temp ____ Time ____	Recorder ________ Observer ________ Timer ________ Duration ________	### ### ### //	### /	///	### ////
Total		17	6	3	9

Figure 4. Sample data-collecting table.

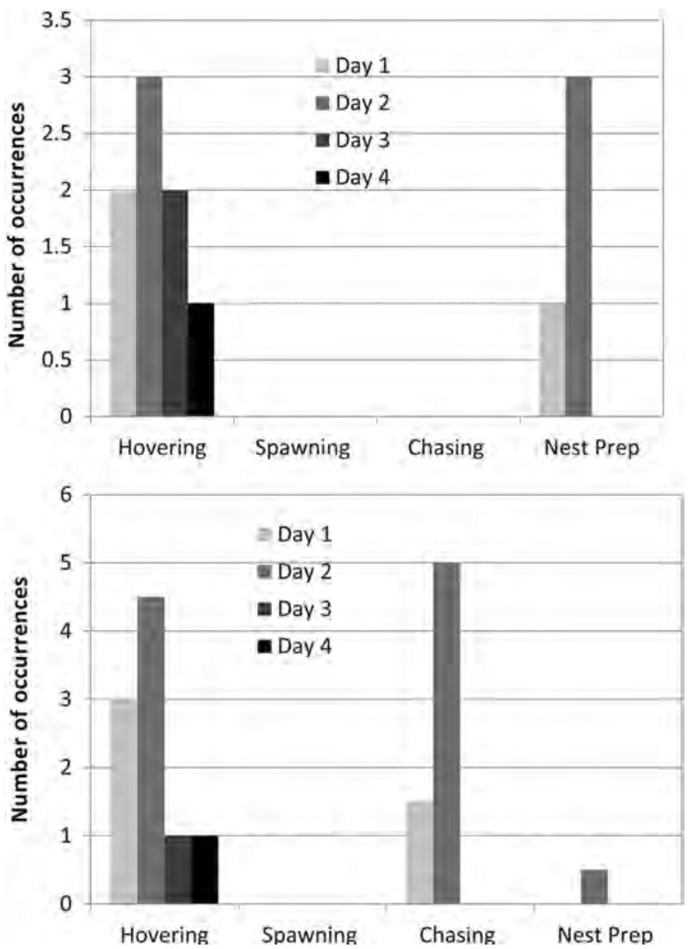

Figure 5. Example of a student-generated graph. (Mikey Pflughoeft, Menomonee Falls [WI] High School.)

variables; Berg & Phillips, 1994; Berg & Smith, 1994). The module allows students to generate data, express results graphically, and understand the context of what they are graphing.

Students draw their graphs (Figure 5), depending on the questions they want to answer – for example, do behaviors change over time? The students are now ready to interpret their data, identify trends, compare their data to published scientific findings, make a new set of hypotheses based on their data, and suggest possible new experiments for their new set of questions. These last two steps are critical to the module's inquiry approach (i.e., applying the process of science in which the student identifies new gaps in our knowledge and the next steps of scientific investigation).

○ Conclusion

The module introduces environmental health issues by engaging students in three aspects of science learning: (1) skills in scientific inquiry and processes, (2) knowledge and application of biological and environmental concepts and principles, and (3) understanding science as an endeavor that addresses human problems and issues. Scientific inquiry is embedded into the module. Students articulate and refine their own broad questions about the effects of environmental agents; become acquainted with appropriate tools and techniques to conduct a controlled experiment; gather and analyze data regarding reproductive behaviors of control and experimental organisms; interpret data to draw conclusions and generate explanations and predictions; gain and use skills in scientific processes and reasoning as they generate and clarify questions, conduct investigations, and gather and analyze data; and make critical connections between evidence and explanation.

Use of minnows as a model allows students to learn the negative consequences associated with chemical exposure (e.g., lowered reproductive success due to decreased breeding behaviors; for a more complete discussion, refer to Weber, 1993) as they gain deeper insights into science as a human endeavor that seeks to characterize health impacts of human action on environments. Using their appreciation of the role of a model system in biology, students are now in a position to link changes in fish behavior to those observed among children their own age who have been exposed to Pb^{2+} and how the scientific process is able to address the environmental health issues that affect them. Students can go to http://www.epa.gov/lead/ for additional information.

Module activities foster thinking, understanding, and growth toward goals for students as defined in both the *National Science Education Standards* and the emerging Next Generation Science Standards (Pratt, 2012), which serve as primary guides for key and essential elements of a K–12 science curriculum (Table 2). When studying impacts of environmental agents on minnow reproductive behaviors, students learn basic concepts and principles of living organisms, including structure and function, regulation and behavior,

Table 2. Comparing a science education framework (Pratt, 2011) to module activities.

Practices Essential to K–12 Science	Module Activities
Asking Questions, Defining Problems: Scientists formulate answerable questions to establish what is already known and to determine what questions are not yet satisfactorily answered.	Students progress through defining a research question, methods and data collection, results, conclusions, and next series of scientific questions.
Developing, Using Models: Science often involves the construction and use of models and simulations to help develop explanations about natural phenomena.	Minnows are a model to represent how Pb^{2+} affects behavior and reproduction. Students apply knowledge gained to other chemical agents.
Planning, Doing Investigations: Planning and conducting systematic investigations requires identifying variables and clarifying what counts as data.	Controlling variables and filtering out non-important factors is critical to reaching conclusions about effects of Pb^{2+} on minnows.
Analyzing, Interpreting Data: Scientific investigations produce data that are analyzed to identify significant patterns.	Students identify and compare normal minnow behavior versus environmental agent-exposed reproductive behaviors of fathead minnows using data tables and graphs to determine effects.
Using Math, Information/Computer Technology, Computational Thinking: Math and computation are fundamental tools for representing variables and their relationships.	Quantitative comparisons serve as evidence of the presence or absence of effects of Pb^{2+} on minnows.
Constructing Explanations, Designing Solutions: Develop hypotheses that provide explanatory accounts of the natural world.	Students are immersed in activities and discussions regarding data, how science regards a hypothesis, and weighing solutions to problems caused by environmental contaminants.
Arguing From Evidence: Reasoning and argument clarify strengths and weaknesses of evidence and identify best explanations for natural phenomena.	Students present their research, which is reviewed by students in order to receive feedback on strength of method, data collection and analysis, results, and conclusions based on critical thinking skills.
Obtaining, Evaluating, Communicating Information: Science advances when findings are shared.	Students communicate findings using papers, posters, and presentations to peers.

and populations and ecosystems. By this process, students meet specific standards for high school science education.

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