INQUIRY & INVESTIGATION

Phage Hunting in the High School Classroom: Phage Isolation and Characterization

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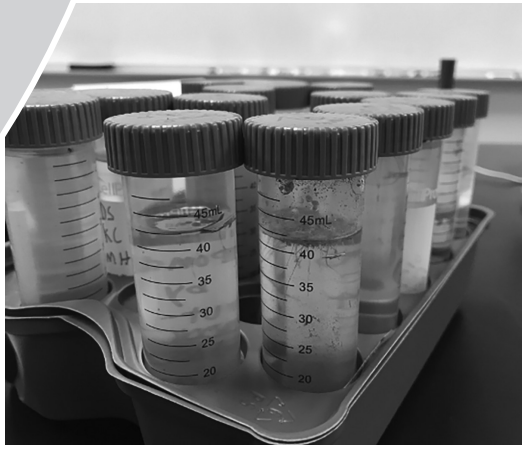

Abstract

National science, technology, engineering, and mathematics education emphasizes science practices, such as hands-on learning. We describe a weeklong activity where students participate in real-world scientific discovery, including "hunting" for bacteriophage in a variety of environmental samples. First, the students collect samples, then look for evidence of phage on "bait" bacteria, and finally amplify/purify the phages for further study.

Key Words: *bacteriophage; K12.*

○ Introduction

Easy access to information has shifted science education from fact-based to inquiry-based learning. New education practices such as Next Generation Science Standards (NGSS) emphasize critical thinking and effective communication skills (Helen et al., 2012). However, most hands-on learning still relies on low-risk "cookbook"-style laboratories that focus on expected results with little innovation. Therefore, we are still failing to expose students to the entire scientific process. Notably missing are the skills of troubleshooting, optimization, and reworking hypotheses.

Bacteria and their viruses ("bacteriophages" or "phages") grow quickly, are easy to work with, and are relatively inexpensive. Due to these low resource requirements, laboratory exercises where students isolate phages are already becoming more prevalent in undergraduate biology programs, which led the way for our modified high school module (Jordan et al., 2014; Williamson et al., 2014). Here we present a weeklong, in-class activity where students at Lincoln Southwest High School (LSW) work with instructors from Michigan State University (MSU) to utilize scientific practices to "hunt" for bacteriophages. Working in small groups, LSW students collect environmental samples, filter out any contaminating particulates and microbes such as bacteria, and then apply the filtrate to various nonpathogenic bacterial hosts to look for novel phage types (*isolation*). The students then pick plaques and transfer them to new plates to ensure (1) it really was a phage and (2) there is only one phage strain per sample (*purification*). Any positive samples are then shipped to MSU for follow-up studies including producing larger qualities of the samples for advanced characterization (*amplification*) and identifying unique qualities such as morphology and host range (*characterization*). Follow-up interactions between MSU and the LSW students occur over Zoom where we show the students the results of their research by live streaming imaging of their samples using electron microscopy. Lastly, the students have the opportunity during the Zoom session for "Q&A" where they can ask any questions related to the activity, to careers in science, or what to expect in college. The intention of this exercise is multifaceted. High school students become exposed to real-world science experiences and have the opportunity to learn from experts in the field. Hopefully, this engenders a general interest in science and also can inspire some students to pursue college education. Additionally, it provides professional training in pedagogy for the undergraduates, graduate students, and postdocs. Finally, it integrates into federally funded original scientific research conducted at MSU.

○ Class Profile

To date we have implemented this activity in over seven classrooms of students ranging a variety of skill levels from sophomore general Biology and honors Biology and up to the highest level of senior students in Advanced Placement biology with classroom sizes generally between 28 and 30 students. These classes meet daily for 90-minute block classes. Each classroom had a teaching team made up of one instructor from LSW and one representative from MSU: The MSU representative was either the lead professor, a postdoctoral researcher, a graduate student, or an undergraduate student.

○ Learning Objectives

The content goals of this activity are for students to:

- Use their knowledge of phage biology to hypothesize or predict where phages might be naturally occurring.

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- Interpret scientific data and communicate their results to their classmates.
- Explain where phages are and are not observed in the local environment.

The science practices goals of this activity are for students to:

- Learn basic microbiology techniques:
  - Pipetting and micropipetting.
  - Plating bacteria and phage in top agar overlays.
  - Plaque purification methods.
- Communicate what they have learned:
  - Interpret their plating results and communicate their findings to their peers.
  - Compare and contrast their data to those data presented by other groups.
- Apply what they have learned:
  - Predict other environmental samples where phages might or might not be present.

○ Who, Where, and When?

This activity started as a partnership between the Parent Lab and LSW as a result of a father–son relationship between Jason Schrad (earned his PhD at MSU) and Kevin Schrad (an instructor at LSW). In order to jump-start this project, this work was supported by the National Science Foundation which provided funds needed for the initial infrastructure (incubators, hot blocks, micropipettors, etc.) as well as consumables (petri plates, etc.) and travel costs. In order to maintain this project past the five-year funding cycle, costs are very minimal for renewing basics such as the media, as most of the other materials can be re-sterilized with an Instant Pot and reused. The largest expense after the initial period is related to travel. Virtual classroom visits via Zoom can reduce travel expenses considerably.

This activity can be implemented at any time during the school year, although it is best to avoid severe cold (frozen samples are less likely to contain infectious phage) and avoid collecting samples after several days of intense rainfall. Various environmental situations can also impact the outcomes. For example, in spring of 2019, there was a high level of *Escherichia coli* (*E. coli*) in Lincoln waterways due to a very wet spring and we isolated almost exclusively *E. coli* phages. In general, the phage host range we observed tended to match our expectations given the environments sampled. We typically found *Salmonella* and *Shigella* phages in agricultural settings with animals present, either livestock or wild. For example, chicken coops, duck ponds, and water from cow troughs historically had diverse phages present. If this activity is to be adopted in other high school settings, care should be given to match "bait" bacteria to species that would be reasonable to find in that local environment.

○ Materials

***For a complete protocol follow the steps in the "Phage Hunting Worksheet," see Supplemental Material available with the online version of this article.

An abbreviated flowchart of the procedure is shown in Figure 1.

See Supplemental Material available with the online version of this article for short instructional videos on the basic techniques used.

Communal equipment/supplies/consumables (we set up a station that each group of students can come up and use):

- Incubator for plates (37°C).
- Small shaking incubator (37°C).
- Hot block (42–45°C) and glass tubes for top agar.
- Vortexers.
- Micropipettors and tips.
- Bleach bucket for decontaminating tubes (5–10% bleach).
- Bacterial "seed" stocks and transfer pipettes.
- Positive control phage solution.
- LB media* for growing overnight bacterial stocks.
- Instant Pot.
- Microwave.

Items per group of four students:

- 2 × 50 mL conical tubes*.
- 1 × 60 mL Sterile Syringe*.
- Sterile toothpicks*.
- Keurig disposable coffee filters (for groups that have solid samples or liquid samples with a lot of particulate matter).
- 1 × 0.2 µm filter.
- One Sharpie.
- One to two colorimetric plates (we have used both HardyChrom SS NoPRO and HUrBi from Hardy Diagnostics).
- 8 × Agar plates* (both 35 mm and 10 mm plates work well—this activity is described for the 10 mm plate size).
- 8 × 1 mL 0.7% top agar* (melted and located in the hot block).
- Instructions and data tables (one photocopy packet per student).

*To sterilize plastics for reuse, and to make sterile media from powder, we use an Instant Pot based on work from Swenson et al. (2018).

○ Procedure Overview

- *Pre-class:* Students need to have an understanding of cell morphology, especially the difference between eukaryotic and prokaryotic cell types. This can be accomplished through traditional lecture, exploratory learning, or a combination of the two. Due to the dynamic nature of the phage hunting lesson, students are able to expand their understanding of bacterial cell types and functions. Ecological concepts are also woven into the overall lesson thereby creating an integrated science teaching method.
- *Sample Collection* can occur pre-class, or in-class. Each group of four students is given a 50 mL sterile tube and asked to collect a specimen. See Figure 1 for a graphical overview of the procedure.
- *Day One of Sample Processing:* Introduction (5–10 min by MSU team), students plate unfiltered samples on the HardyChrom/HUrBi plates to search for bacteria, and then filter their samples (40 min).
- *Day Two of Sample Processing:* Students plate the filtered samples on bacteria test lawns, inspect their HardyChrom

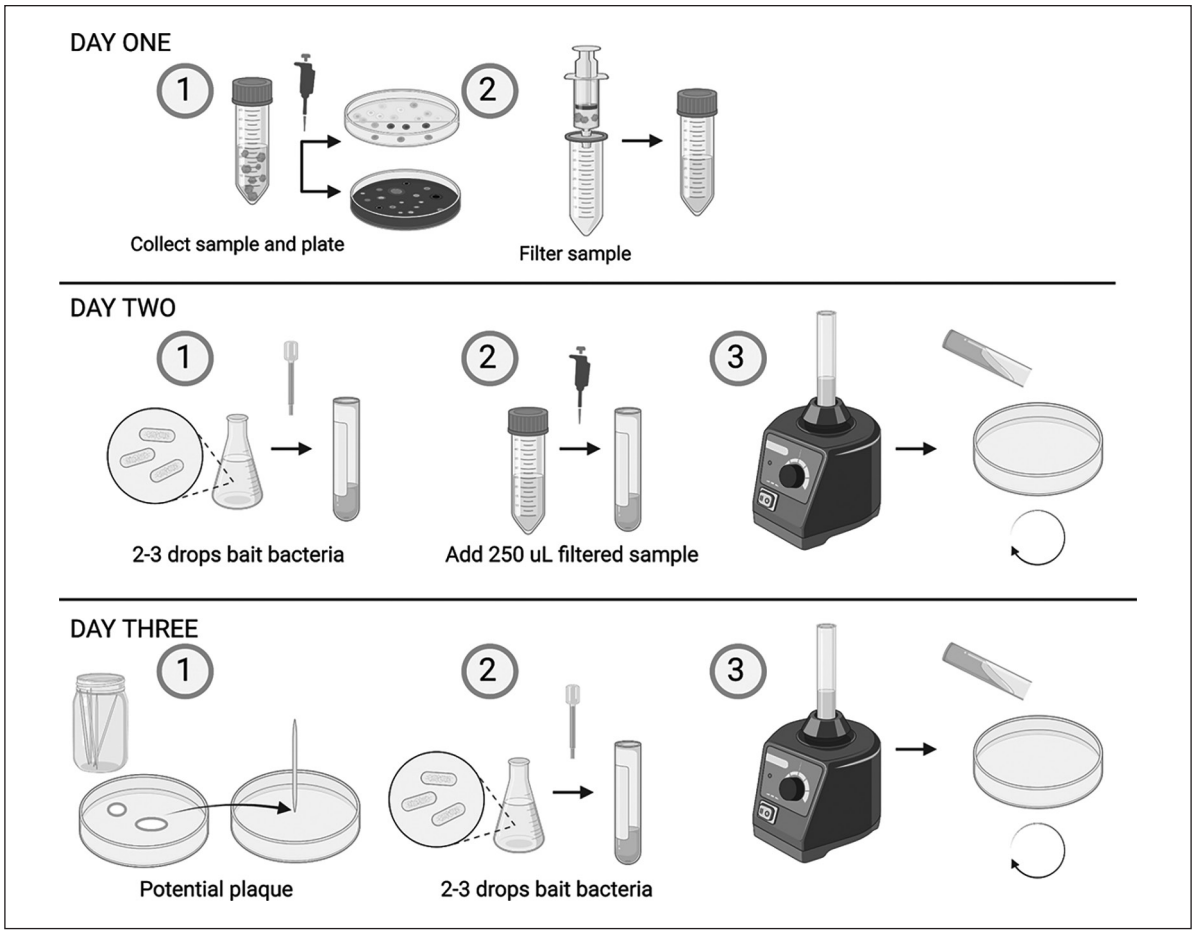

Figure 1. Typical workflow of the procedure. Day One, students collect a sample and plate a portion of the sample on the colorimetric plates (approximately 250 uL – 1 mL). Next, they filter out any bacteria and transfer the filtrate into a new sterile tube. The filtrate is stored at room temperature until the next class period. On the second day, the students seed molten top agar with 2–3 drops of a desired host bacteria to "bait" the phage to infect, and then add approximately 250 µL of their filtrate to the tube, mix by vortexing, transfer to a sterile agar plate, and spread by "swirling" the plate. The sample is incubated overnight. On the next day, the students inspect their plates for the presence of potential phage plaques. If a plaque is observed, the students use a sterile toothpick to "stab" into the plaque and transfer this to a new, sterile agar plate. They then add 2–3 drops of the same host bacteria to the molten top agar, pour over the "stabbed" area, and spread by "swirling." The sample is incubated overnight. In parallel, the students repeat the process on both Day Two and Day Three using a known phage and host as a positive control. See Supplemental Videos available with the online version of this article for tutorials showing these techniques.

or HUrBi plates, and record the results of HardyChrom/HUrBi plates.

- *Day Three of Sample Processing:* Students check for phage and then perform purification of potential plaques by toothpicking. Each group should have a positive control plate (known phage and bacteria host), so even if no environmental isolates are observed in a group, the students can perform this step using the control plate.
- *Day Four of Sample Processing:* Students check for phage, record and discuss classroom data as a group, and wrap up the activity.

○ Preparation

At LSW, the instructors prepare for this unit by starting with a phenomenon. We have used many different phenomena to kick off our environmental unit or cells unit. For example, LSW instructors have used their own storyline, "Sargassum," that they developed a few years ago. It begins with a video of the sargassum problem in the Caribbean and currently parts of the United States. Students participate in a Diving Questions Board in which students propose various questions about the problem they just viewed. During the course of answering questions created by students, they end up investigating local aquatic systems that model the larger ocean system (the original phenomenon). The local aquatic system is a supporting phenomenon indicating specific times of year in which eutrophic conditions exist as well as increased *E. coli* outbreaks. This allows for the class to discuss solutions to excess nutrients due to animal wastes, a common problem in Nebraska. Bacteriophages become one of the solutions to the *E. coli* problem in lakes, typically indicative of excess animal wastes. The Bacteriophage Hunting Lab is "real-world solution" to a local problem; a problem that is modeled all over the world where agriculture and water meet. The goal of the introduction is to allow students to observe, question, then investigate. All aspects of the scientific process are integrated into

the pre-lab lessons and topics, allowing for a seamless transition into the phage hunting lesson. Each teacher has the agency to create the appropriate on-level work that leads into the phage hunting work. This autonomy is only possible due to the collaboration among participating teachers and MSU collaborators. Each participating teacher works toward the same outcomes for all students, the implementation of the virus work.

- *Prior to Day One of Sample Processing:* Workstations are set up with Sharpies, plates (agar and colorimetric plates; HardyChrom and/or HUrBi), 0.2 µm filters and syringes, sterile 50 mL tubes, micropipettors, and tips. See Figure 2 for what a typical workstation looks like.
- *Prior to Day Two of Sample Processing:*

  The night before: Instructors set up overnight cultures of the test bacteria in the shaking incubator in liquid LB. For this, we found making several copies of each test strain in 15 mL conical tubes works well so that if students spill or contaminate the test bacteria, there are plenty of backups.

  The morning of: Workstations are set up with Sharpies, plates (HardyChrom and/or HUrBi), 0.2 µm filters and syringes, sterile 50 mL tubes. The main classroom station is set up with the hot blocks, top agar in glass tubes, vortexers, test bacteria with transfer pipettes, and bleach buckets.
- *Prior to Day Three of Sample Processing:*

  The night before: Instructors set up overnight cultures of the test bacteria in the shaking incubator in liquid LB as before.

  The morning of: Workstations are set up with Sharpies and agar plates. The main classroom station is set up with the hot blocks, top agar in glass tubes, vortexers, test bacteria with transfer pipettes, and bleach buckets.
- *Prior to Day Four of Sample Processing:* No set up required—This period is for examining the plates and having a class discussion on the entire module.

○ Procedure

Prior to the first day of the visit from MSU scientists as well as Day One of the lab work, students are given instruction on how to collect water samples in the field. This "fieldwork" is an essential part of any scientific study involving the outside environment. Students learn how to collect and maintain sterile samples, store them, and document the location of those samples. Students do know that they will be using their water samples to search for viruses, but they do not yet know the methodology for extracting bacteriophages from their samples. Day One of the actual lab work highlights the reasonings and skills needed to extract the viruses. Parent notification is important prior to students bringing home a collection vial. Due to the parent communication about the project, many students get parent interest in the science happening at LSW. This provides a greater impact in the community due to parental discussions, positive student encouragement to collect samples (also known as parental motivation) and dispels myths about viruses and their function in the larger ecosystem.

Figure 2. Typical work station for students to plate phage.

Sample collection can occur pre-class, or in-class (students think about what they want to collect and then go get samples). Year one we collected samples around the LSW campus, however we discovered that students wanted to have a larger sample area by sampling lakes, ponds, or other bodies of water they are more familiar with in their community. Both have worked well. Pre-class collection tends to bring about more creativity and more unusual samples (e.g., dog's water bowl, chicken coop samples, kiddie pool left out all summer, etc.), but comes with the caveat that some students forget to bring a sample. Therefore, it is important to have extra samples on hand so each group can complete the activity. In-class collection works great at LSW where the school grounds have an "outdoor classroom" rich with different areas (e.g., pond, marsh areas, woods, etc.). Of course in-class sample collection is weather permitting. In addition, at LSW students have access to a "living classroom" rich with plants, and a variety of animals (e.g., turtles, fish tanks, snakes, etc.). The water from these terrariums is often a good sample last minute if a group forgot to collect a sample at home, or if the weather is not suitable for sample collection outside.

Following sample collection, on *Day One of Sample Processing* we have a brief discussion of the sample contents—there are likely to be bacteria and/or phages in the samples and we need different methods to detect the presence of each one. In groups of four, students use micropipettes to add a portion of their samples to colorimetric agar plates to detect the presence of different types of bacteria (see Figure 1 for a flowchart of the process). These plates (either HardyChrom or HUrBi) have a dye indicator that can identify different species. For example, on the HardyChrom SS NoPRO plates *Escherichia* strains appear pink, *Salmonella* strains appear black, and *Shigella* strains appear light green). Other types of bacterial species appear beige. The HUrBi plates have a divider in the middle and one side of the plate selects for Gram positive strains and one side selects for Gram negative strains. We have used either individually or both plates in combination depending on the goal of the particular experiment (some years we were hoping to isolate phages on particular species of bacteria and we chose the plates that best matched the test strains we used). This is also a good time in the exercise to explain the importance of laboratory safety—best practices and the importance of handwashing after handling the samples.

A major research focus in the Parent Lab at MSU is on enteric bacteria and their phages. Therefore, we have focused on bacterial species that are relevant to our broader research goals (mainly *Shigella*, *E. coli*, and *Salmonella*), using nonpathogenic and safe to handle versions of these strains which are suitable for the high school classroom. In principle, this activity could be done with any

type of bacteria, provided the bacteria forms a robust and confluent lawn on a top agar overlay plate. One caveat is that the bacteria should reflect those likely to be found in the natural environment to increase likelihood of capturing phage (e.g., deep sea microbes might not make good "bait" bacteria for samples in Nebraska farmlands).

Students next filter out the samples to remove the bacteria and retain any potential phages. If the students brought a clear liquid specimen, they simply pass this through a 0.2 µm filter into a new, sterile 50 mL conical tube. If the students brought in a solid sample, they first add some water or liquid LB media to the solid sample to resuspend it, then use a coffee filter to remove large particulates. The cleared liquid then can be passed through a 0.2 µm filter into a new, sterile 50 mL conical tube. During this time, the instructional team walked around from group to group facilitating discussion and answering any questions students may have had. As one example of a discussion point, we asked the students where they collected their specimens from, and why they hypothesized there may be phage present. Once the samples have been filtered, we collect them in a rack and set them aside for additional processing the next day. The HardyChrom/HUrBi plates are placed in the incubator overnight. At the end of the day, the instructors decontaminate the bench-top surfaces and any glassware using a dilute solution of bleach (approximately 5–10%).

On *Day Two of Sample Processing*, the students prepare their plates for the samples. Each group typically has four different plates, one for each student. One student chooses the control phage/host pair, and the other three students will test the sample against three different hosts. To do this each group labels their plates and then as a group they come to the central workstation that is instructor-led. Each student will receive a tube of top agar (approximately 1 mL solution) and they are instructed to add 2–3 drops of test bacteria using a transfer pipette. The suspension is vortexed and then added to the top of the agar plate. Once the plate has dried (the small approximately 10 mm plates dry very quickly), the students pipette approximately 250 µL of their filtered sample on top of the plate. The plates are allowed to dry and then are placed in a 37°C incubator overnight to allow the phage to grow.

While the students are doing this in groups, the rest of the class is inspecting their colorimetric plates from the day before (see Figure 4 for representative plate images). We have them record their data on paper (one copy for the student and one copy for the MSU team to take back to East Lansing with them—see Phage Hunting Worksheet available in the Supplemental Material online). We also create a giant table on the classroom whiteboard and have the students list their results there (see Table 1 and Figure 3). Once the table is complete, the instructional team prompts discussion about the bacterial diversity in the community and we pass the plates around so students can see how their samples compare to other environmental samples.

On *Day Three of Sample Processing*, the students inspect their bacterial test lawns for the presence of phages. The instructional team talks with the groups and helps to interpret the plates. It is important that the instructional team inspects each of the plates to make sure the students didn't overlook any difficult-to-see plaques. Potential plaques are highlighted by circling the plate and will be used for the next step: "toothpicking." Groups that did not find any potential plaques on their plates from environmental samples should have plaques on their positive control plate, and all group members can use that to participate in the next step. For this activity, both lytic phages (that form clear plaques) or lysogenic/temperate

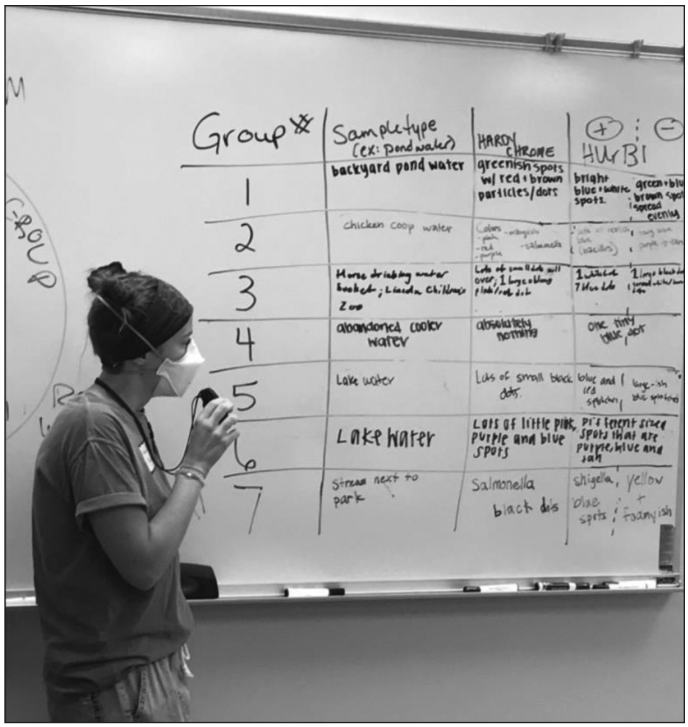

Figure 3. Kendal Tinney explaining the results of the colorimetric plates.

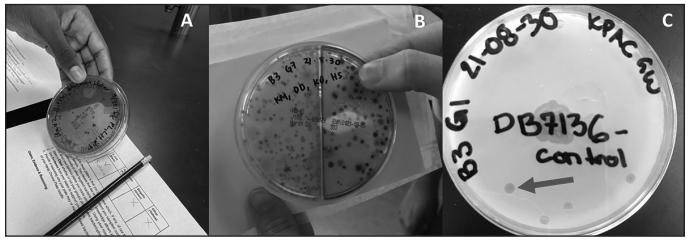

Figure 4. Students inspecting and recording data from the colorimetric plates, both HardyChrom (A) and HUrBi (B). Examples of plaques formed on bacterial lawns (arrow) (C).

phages (that form "halo" or turbulent plaques) can be isolated. In our experience, the lytic phages are much easier for the students to identify and have been much more commonly found. Any zone on the plate that we think could be a plaque is subjected to replica plating in order to identify any false positives and to confirm actual phages (see next paragraph). However, based on the research interests of the Parent Lab at MSU, lysogenic/temperature phages are generally not considered for further assessment.

Toothpicking is a process where a potential plaque is poked with a clean, sterile toothpick, and then this toothpick is inserted into a fresh sterile LB plate. Students then add top agar seeded with bacteria (the same host that showed a potential plaque), the plates are gently swirled and then left still to harden (approximately 2–5 min). Plates are then placed in the incubator at 37°C overnight.

On *Day Four*, the students inspect their plates for the presence of phages (Figure 4). If the potential plaque was real, the students should see amplification, or multiple plaques originating from the toothpick site. If there are no plaques, the putative plaque was a

Table 1. Example of results from colorimetric plates from LSW

Group #	Sample Type/Location	HardyChrom	HUrBi (+)	HUrBi (–)
Group 1	Creek water	No black or light blue; lots of pink spots, dark blue and purple	Different shades of blue spots and different sizes	White, purple, and dark blue spots
Group 2	Pond water	No black, blue, or pink; lots of yellow	Light blue (different sizes and textures)	Light purple and blue mixed together
Group 3	Boat ramp on Yankee Hill Lake	Purple, black, red, and pink	Lots of green and blue	Blues and light purples
Group 4	Pond on S 21st street	Blue, black, purple	Dark blue, light blue, white and tan, some green and yellow	Purple (dark and light) and white
Group 5	Chicken coop water	Purple and tan; no black or blue	Blue, tan	Blue, pink, red
Group 6	Abandoned cooler water	Pinks, blues, and yellow/ cream color	Purple, dark blue, white/ yellow	Light blue, some darker blues and yellows

false positive (likely a bubble arising in the soft agar, or an area on the plate that bacteria did not cover).

Once the plates are inspected, groups again record their data on paper (one copy for the student and one copy for the MSU team to take back to East Lansing). We also create another giant table on the classroom whiteboard and have the students list their results there. At this point the instructional team leads a group discussion. We ask the students: "Are you surprised by the results, why or why not?" and "If you were to repeat the experiment what type of sample would you collect next time, and why?" We also discuss how "negative" results are also important in science: We want to know where phages both are and are not found in the environment. Therefore, a lack of plaques is not a "failure."

○ Post Visit Work and Follow-Up

After the visit to LSW, the MSU team ships plates with confirmed plaques to MSU for additional amplification and purification procedures. We then do a variety of characterizations such as host range studies, basic characterization using standard biochemical approaches and for phages with interesting behaviors, we do whole genome sequencing. Examples of the results we have obtained have been published (Doore et al., 2018; Doore et al., 2019). We have also made the activity available to the public through a *Scientia* publication and corresponding YouTube video (Parent, 2022).

We also set up a virtual Zoom session with the students where we show them the electron microscope facility at MSU and we image the purified phages by negative staining the samples together. One discussion we have with the students ahead of time has to do with phage morphology. For example, phages with long, noncontractive tails like a siphon belong to the family *Siphoviridae*. Phages with long, contractive tails like a big muscle belong to the family *Myoviridae*. Lastly, phages with short tails like little feet belong to the family *Podoviridae*. We then ask the students to look at the phage images from the microscope and ask them to classify the individual isolates. We also take suggestions for the phage names from the groups and if appropriate, we adopt their names or help them come up with newer ideas. For example, one year we had phage "Silverhawkium" which is based on LSW's mascot (Doore et al., 2019). The last part of the activity is the open the floor for Q&A to allow the students the opportunity to ask any questions about science, or college, and so on.

Examples of student questions that arose from the activity:

- "What is your favorite thing about being a scientist?"
- "Were there ever times in college where you second guessed whether you actually wanted to study viruses and bacteria?"
- "If phages didn't exist, would we all survive?"
- "Do you like bacteria or bacteriophages more?"
- "Why do so little samples have phage?"
- "Would the sample being salt water effect the experiment?"
- "How many viruses did you discover in total?"

○ Reflections

Overall, students responded positively to this in-class exercise. This reaction was largely monitored by open-ended questions to both students and parents.

Below are some examples of student answers (from question prompts):

1. *Question*: Describe how the Bacteriophage investigation made a difference in your understanding of Biology and science in general.

Student Responses:

1. *The Bacteriophage investigation made me understand biology and science better because when I do things first hand I can understand what is happening and why easier. By being able to do this I could ask more questions and was involved in participating instead of just reading a book that I would surely forget soon. This investigation however is not likely to escape my memory seeing as it actually interested me and was a fun way to learn about science in what usually would have been a boring lecture.*
2. *The Bacteriophage investigation made a difference in my understanding of Biology and science in general by showing me another field that I may be interested in. I*

thought it was really cool all of the different tools were introduced to and how things worked. Not only are the bacteria and viruses living (depending on who you ask), but we also learned how they affect other living things even though they are on completely different scales. I think it just helps my understanding in science in general because if you're going to study any living organism, this is a factor. Anything can be affected by certain bacteria or viruses so I think it just really helped me understand the big picture.

3. *This investigation made me appreciate the little things that live everywhere in the world around us, and the diversity in them. Before this, I didn't realize that bacteriophages even existed, I was under the impression that viruses were something that infected multicellular organisms only. It also caused me to come to a greater understanding on the makeup of both bacteria and bacteriophages, and the strategies they use to reproduce.*

2. *Question*: What was the most impactful part of the Bacteriophage experience?
   1. *The most impactful part of working with the phages was definitely the hands-on nature of the experiment. It made the whole experience better in every aspect; it was more interesting, more enjoyable, and caused me to absorb the information much better than reading out of a textbook.*
   2. *The most impactful part of this experience for me was learning about how bacteriophages could be used to target and kill specific, harmful strains of bacteria. It was very interesting that bacteriophages could even be used as a better alternative to antibiotics. The fact that the phages could continue to be effective even when bacteria evolved to become resistant to them is very interesting because this would be a huge breakthrough in modern medical science.*
   3. *The most impactful part about this experience is the fact how we actually found a good one and that it will go to the university for investigation. This is impactful because it's cool to know that something that could help people came out of some high schoolers from Nebraska.*

- Parent quotes (after the exercise):
  - *"We want to thank you and the other staff for having this research with scientists from MSU."*
  - *"This project sounds amazing!! Wow!! Thanks for providing this amazing opportunity to your students!"*
  - *"Super cool! What an awesome opportunity!" "Thank you so much for the updates and for the upcoming hands-on work. That sounds very exciting."*
  - *"Wow, this sounds like a really cool lab experiment."*
  - *"Just a quick note that Lane is loving your class! Biology is right up his alley, so I'm glad that you are continuing to fuel that enthusiasm."*

○ Conclusions

Our activity allows students to use a hands-on method to isolate bacteriophage from environmental samples. This activity employs active learning and allows students to participate in scientific practice and to communicate their ideas and results. We aim to combine skill development and scientific content to generate enthusiasm for science in general and to help train the next generation of students to be scientifically literate and to think critically. Over the past few years, the phage hunting lesson and collaboration between LSW and MSU has increased student interest in the sciences. The broad nature of the activity has allowed all ability levels and cultural backgrounds to benefit. Throughout the lesson, students had multiple ways to present their findings beyond the qualitative and quantitative data gathered. By the end of the week, students became comfortable speaking scientifically about lab skills, scientific equipment, and the role of viruses in the environment.

NGSS are found throughout the lesson, including disciplinary core ideas, science and engineering practices (SEPs), and crosscutting concepts (CCCs). Seven out of eight SEPs are explicitly addressed in this lesson as well as five out of seven of the CCCs are directly addressed by students. The following SEP was the only one NOT directly addressed: using mathematics and computational thinking. All other SEPs were directly addressed. The following CCCs were directly addressed: patterns, similarity and diversity, cause and effect, scale, proportion and quantity, structure and function, and stability and change. All educators in this project aligned the lesson with the DCI in Life Science, which met multiple standards among all four main high school topics: HS-LS1, HS-LS2, HS-LS3, and HS-LS4. Overall, we believe that this activity is a great addition to high school biology courses.

○ Acknowledgments

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