Mission Folder: View Mission for 'Phantastic Phage Phinders'

State	Utah
Grade	9th
Mission Challenge	Environment
Method	Scientific Inquiry using Scientific Practices
Students	Kate Watson (microscientist) Rachel Amedee (ScienceStudent) Abigail Atkinson (spacecat03) gavin grose (TheDuck)

Team Collaboration

(1) How was your team formed? Was your team assigned or did you choose to work with each other?

Our Phantastic Phage Phinders team was formed from a unique blend of students with different skills and abilities, and yet a common enthusiasm for STEM. To form our ecybermission team we all completed an application survey from our school Mountain Heights Academy, to determine our common interests in the STEM fields. Each of us were willing to make the efforts (and so were our parents, since none of us can drive) to travel and meet in order to investigate our problem. Our team required a firm commitment in order to be chosen.

Once our advisors looked at our applications and we were accepted on a team, we were assigned to work with each other based on our grade level. Although we were assigned to work with one another, we all work well together and have made some great bonds and friendships. Because we each attend an online high school, we are all from different cities in Utah. This diversity is one of our biggest strengths. Our ability to meet during the day, and through the use of technology, provided a way for each of us to stay in constant contact and work closely together. This project was an incredible opportunity to learn and develop lab skills, critical thinking skills, and problem solve with friends and classmates. It's weird now to think at one point we didn't really know each other. We've become friends with a common goal and purpose. That's what our eCYBERMISSION team did for each of us.

(2) Provide a detailed description of each team member's responsibilities and jobs during your work on the Mission Folder.

Every person in our group had different, yet very critical roles in our project. We learned early on with how our investigation would go, that every member would fulfil an important part. We discovered that working as a team allowed us to accomplish much more than we would've working individually. Here is a brief description of the large division of labor. However, as a team we all pulled together to make this project a success! Each team member consistently attended the lab and worked on the scientific inquiry process,

"ScienceStudent" was responsible for taking notes during our lab meetings and handling all of our data throughout the project. Her role was critical to the overall success of the project. We consistently referred to her amazing notes to pick up where we left off.

"TheDuck" was responsible for frequently going back to the lab to complete tasks. We like to refer to him as the "legs" of the team. While at the lab he would take pictures of our experiment and making sure that it all went according to the plan. His close proximity to the lab allowed the team to rely on him for frequent lab visits.

"Spacecat" handled the creation of our website and designing a domain where our discovery and information could be housed for others to see. Sharing our research was something we wanted to do, and she helped make this part possible.

"Microscientist" was visual presentation for community outreach, as well as team collab expert and survey specialist. She spent countless hours combing through the research to make sure we knew and understood each of the resources.

It's clear to see individually we all had work to do, but together we formed a team with a purpose and mission.

(3) Did your team face any problems working together? If so, how did you solve them? If not, why do you think you were able to work together so well?

The biggest problem our team faced, was the distance each team member had to travel in order to make it to the lab. Team members traveled from 30 minutes by car to 2 hours by train one way to arrive at the lab (see attachment 2 hour train ride). The distance required a certain time commitment, and a resulting difficulty was coordinating work schedules of parents, rides, and time in order to make it into the lab.

Although the time and distance made getting to the lab difficult, we had a team of students committed to their research. Early on as a team we worked out a schedule of what days and times worked for the majority of team members and this is when we met. Although some of the meetings not all of us were able to attend, we worked diligently to make sure most of the team was present. Through the use of a team chat, we would pass on critical information to team members if they were unable to attend. We enjoyed our time in the lab together and learned to be flexible with our time and to work hard and make sacrifices for the good of the whole team. What started out as a problem became a strength as our sacrifice brought greater investment in discovering a solution to our problem.

(4) What were some possible advantages to working together as a team on this project? How would working as individuals have made this project more difficult?

There are numerous advantages from working together. First, we needed all of our minds thinking together to brainstorm ideas for solutions to our problem. We relied heavily on each team member researching components of the information and then coming back and sharing what they learned with the whole team. We also divided up the

workload of the mission folder. With so much to write and so much information to compile, we needed every member of the team. We saw how, although distance was a problem for 3 team members, we could use one team member to help the entire time by going to the lab more often. Our team was in every sense of the word a team. We counseled together, we counseled with our advisors, met together, and we researched, investigated, and worked together. We don't know how this type of project could be done individually.

Together we were much more than we ever could've been as an individual. If this project had to be done individually, it would've been so hard. The amount of work required to contribute a quality mission folder requires more than one individual. The research of one would've been so much less thorough, and the fun we had as a team couldn't of happened alone. In the case of our lab team, if only one individual was working on the project they wouldn't be able to have anyone else fill them in if they couldn't make it back to the lab. Essentially if this was an individual project it never would've happened. "Together" was an important part of our team, and we wouldn't of wanted to do this project any other way.

Uploaded Files:

- [**View**] **Team Intro** (By: spacecat03, 02/14/2019, .pdf)

  Meet our team
- [**View**] **2 Hour Train Ride** (By: ScienceStudent, 02/25/2019, .pdf)

  This is an image from the 2 hour train ride that ScienceStudent took from Layton to the lab at Brigham Young University.
- [**View**] **The Phantastic Phage Phinders** (By: spacecat03, 02/25/2019, .jpg)

  Our team! ScienceStudent (top left), TheDuck (top right), Microscientist (bottom left), Spacecat (bottom right).

Scientific Inquiry

Problem Statement

(1) What problem in your community will your team be investigating through scientific inquiry using scientific practices? Specifically, based on this problem, what question will you be trying to answer?

Our team, the Phantastic Phage Phinders, will be investigating the issue of water contamination through certain bacteria types, specifically E. coli and Cyanobacteria. Contamination in our bodies of water is a severe community problem for our state (Utah) and the country as a whole. Algal blooms of Cyanobacteria in Utah Lake have caused both the animals and people who come in contact with the water to fall ill and E. coli infections of lettuce crops has also caused people to fall ill. The question that our team is most focused on answering is the question of if there are bacteriophages within the habitats of the bacterias themselves that can be used to eliminate Cyanobacteria, and E. coli. Our team is able to investigate this problem and search for the answer to this question through the use of the lab at the Brigham Young University (BYU) and with the assistance of a professor and scientist there, Dr. Julianne Grose.

(2) Research your problem. You must learn more about the problem you are trying to solve and also what testing has already been done. Find AT LEAST 10 different resources and list them here. They should include books, periodicals (magazines, journals, etc.), websites, experts, and any other resources you can think of. Be specific when listing them, and do not list your search engine (Google, etc.) as a resource.

Please see file "Annotated Bibliography" for a list of the resources used in our research. The following is a brief description of our research process.

During the length of our project, we did extensive amounts of research. Some was done online, some with experts, and some with personal experience. When we first heard of algal blooms in Utah Lake, (see annotated bibliography "Utah Lake") we quickly recognized this was a problem in our community. We began researching anything we could on the internet about this harmful phenomenon. We looked at news stories, articles, medical reports, and more. (see annotated bibliography "Algal Blooms") We had to research on Cyanobacteria and which lakes and bodies of water were the most affected. We had to learn about the importance of clean water free of harmful bacteria. This part of research was an incredibly essential aspect to our project. (see annotated bibliography "Utah Lake"/"Algal Blooms")

Then, our research broadened, after hearing of the recent E. coli outbreaks, we decided to include an additional focus on this bacteria to continue our research. It was imperative that we learn all we could about this bacteria, if we were going to study it and look for phages. We read about recent outbreaks all over the country. We researched what types of plants were affected and the damage they were doing to the agricultural industry, businesses, humans, and animals. Through research, we learned just how bad E. coli present in agricultural waters can be. All this research on E. coli is what inspired us to find phages that combat E. coli bacteria. (see annotated bibliography "E. coli") If this research had not been performed we may have never proceeded down the path we are on today.

Although other research was completed, the research that was most critical to our project is the above research on E. coli and Cyanobacteria. Without this research, our project wouldn't be even close to what it is now. We were able to learn, grow, and solve problems through detailed research.

(3) Explain what you learned from your research. What did you find out about your problem that you didn't know before? What kinds of experiments have been done by other people before you? Be sure to put this in your OWN words, do not just copy and paste information. Also, be sure to cite your sources.

Throughout the extent of our project, we learned a great deal about bacteria, phages, water quality and how it all works together. We experienced things we didn't know about, especially when working in the lab, and learned the importance of careful scientific work. We discovered the detrimental effects unclean water, algae blooms, and E. coli had on our water sources, and ways that we can improve it. We learned how to grow bacteria on an agar plate through trial and error, and how to determine what was there. We learned about phages and the way they replicate and destroy bacteria (See annotated bibliography "Phages"). Mostly we learned that science is a process and rarely goes according to plan.

As a team, we originally planned to find some way to combat harmful algal blooms in our lakes and water systems. Algal blooms sometimes produce cyanotoxins that can become a serious health threat to humans and animals. Algal blooms were enveloping Utah Lake, making it unsafe to do almost any recreational or commercial activities (See annotated bibliography "Utah Lake"/"Local Environment"). We found out just how harmful algal blooms can be on our environment, us, our animals, and more. We learned that Cyanobacteria can inflict much harm upon wildlife and animals (See annotated bibliography "Algal Blooms") . Marine animals such as manatees and fish were dying because of algal blooms. A few dogs even died after playing in water that was infected with Cyanobacteria. Farmers were having to change how they watered their livestock, and watered their crops. Bacteria can have some serious effects, if it is not in a balanced ecosystem (See annotated bibliography "Algal Blooms").

We started out growing Cyanobacteria which are prokaryotes also known as blue green algae, and are responsible for algal blooms in bodies of water. Cyanobacteria has very little research done on it, so we had to experiment quite a bit when it came to finding the best way to stimulate growth (See annotated bibliography "Cyanobacteria"). From the article where we learned about what type of growth media would work best we also learned we could help in the discovery. "Therefore, some of the most commonly used growth conditions are described below in the hope that this information may stimulate the interested reader to develop modifications, possibly helpful, for the isolation of those numerous cyanobacteria that so far have escaped culture." (Rippka, "Isolation, Identification, and Culturing", pg 6)

We tried different types of media to grow the Cyanobacteria on, different containers to grow them in, where we grew them, and what is needed for life. We found that the Cyanobacteria will grow best from water samples that were recently collected. We researched and tried to find which type of growth media would work best. Then, we discovered a type of media known as BG11 to be nutrients for the agar, which grew the Cyanobacteria. We also tried using LB growth media, but with no success. It seemed to grow much better when we imbedded the Cyanobacteria into the agar used for growth, instead of just sitting on top. Through trial and error, we were able to grow Cyanobacteria. Although the growth process in the lab was slow we learned a lot about the process. Due to the length of time of just growing successful Cyanobacteria we have yet to isolate a phage for this problem. But our team plans to continue our research and with time and more effort isolate a Cyanobacteria phage.

As we continued to work on Cyanobacteria, we also started looking at E. coli. The nation was racked with another E. coli outbreak our team turned our focus to an additional common water bacteria (See annotated bibliography “E. coli”). We were able to find research that indicated that E coli could be successfully grown on LB growth media. We were able to grow E coli with good success, and turned the attention of our focus to this bacteria. After we had grown E coli well, we were able to move to the next part of the experiment. We wanted to know if there were phages with our E. coli, so we were ready to look at them through an electron microscope. We learned how an electron microscope works and we got to look at our phages through one (See annotated bibliography “Experts”). Electron microscopes send out a stream of electrons onto the object needing magnification, then they bounce back and are able to create a very magnified image of the specimen. Electron microscopes are very delicate so a trained expert controlled the microscope while we watched and looked for phages. We originally found 8 phages within our E. coli, but after handling only a few of them survived. We learned that phages can be fragile and should be handled carefully. We were able to extend our knowledge on phages after we were able to see these microscopic viruses in person. We also learned about the three different types of phages, Podoviridae, Myoviridae, and Siphoviridae. We learned so much about electron microscopy and phages during our project.

Through research on E. coli, we discovered Agriphage. This is a biopesticide that is used commercially as a natural way to combat harmful bacteria on tomato and pepper plants. Created in 2005, scientists have done extensive research on AgriPhage, which is very similar to our project (See annotated bibliography “AgriPhage“. Researchers discovered and isolated phages that will combat harmful bacteria in crops, and made it commercial. Scientists researched, found phages, designed a product, and sold that product to make the agricultural industry more natural and healthy. We were very interested with AgriPhage due to it consisting of natural ingredients and using phage therapy. We chose to focus on AgriPhage because of the impacts is creating in the agricultural industry. Phage therapy is now highly sought after in the agricultural industry, and we want to pursue that (See annotated bibliography “Phages”). AgriPhage research and experimentation is very similar to ours. Researchers found phages that combat bad bacteria in tomato and pepper plants. Our Teams was able to find phages specific to E. coli that may be able to help E. coli outbreaks in water and plants. AgriPhage helped to lead the way and give us ideas on what more we can do with our project to help our community. They helped us learn what our team and findings are capable of.

One of the biggest lessons we learned during our project was that trial and error is necessary, and sometimes science doesn’t work out the way you are expecting. We originally wanted to find a phage that could combat algal blooms in our water. After much experimentation, we realized the time frame to successfully isolate a phage for Cyanobacteria would be years not months. We learned a lot of ways not to successfully grow Cyanobacteria, but discovered a way that worked. Through this process we discovered that E. coli is much easier to work with. It took much less time to go through the process of finding phage for our Utah Lake E. coli. Our project didn’t go in the original direction that we intended, but it sure went down a great path. We learned that science is science and won’t turn out perfectly, but what you discover along the path is almost more important.

Experimental Design

(4) Based on the question you are trying to answer, and your research, what is your team’s hypothesis for this investigation?

If Utah bodies of water are infected with harmful bacteria, then the same bodies of water will contain phages that can combat those bacteria species.

(5) What are the independent and dependent variables in your investigation?

In our experiment, we worked with two different types of bacteria: Cyanobacteria and E. coli. When working with the Cyanobacteria in an attempt to find a phage, the independent variable was the Cyanobacteria and the dependent variable was the existence of a phage (whether or not a phage was present). When working with the E. coli in an attempt to find a phage, the independent variable was the E. coli and the dependent variables were the phages eventually found within the E. coli.

(6) What are the constants in your investigation?

The constants in our experiment include the storing of our samples prior to use, the process we followed when plating samples, the conditions of the lab where our plates were kept, and the safety precautions we took. Prior to use, we stored all of our water samples in a clear, seal-able container, filled about three quarters full with sample water, and kept the container fairly cool. When traveling with the samples, we kept conditions as close to the same as we could. When plating a water sample, we always took a constant amount of sample water/bacteria, agar, and liquid media (bacteria food). After the very first lab, where we used LB growth media for Cyanobacteria and found it did not have the best results, we always plated Cyanobacteria with the liquid media BG-11. All E. coli samples were plated with LB media. After plating, our samples were always stored on a tray in the lab beneath a fluorescent light. They had a consistent light schedule designed to mirror the conditions of the bacteria’s natural habitat. We consistently took safety precautions throughout our experiment, such as always wearing protective latex gloves when coming into contact with our samples and only having Dr. Grose, who assisted us during lab experiments, handle any potentially dangerous substance (such as sewage samples, flame, and neutralized uranium).

(7) Will your investigation have a control group? If so, describe the control group. If not, why not?

Over the course of our experiment, we used two different control groups according to which stage of the experiment we were on at the time. The first control we used was when we were DNA sequencing our bacteria in an attempt to verify if it was Cyanobacteria. For this, we had three test tubes. All three had bacteria, 10 ul of buffer, 3 ul of nucleotides, 3 ul of 16s Forward Primer, 3 ul of Reverse Primer, and 81 ul of water, but only two of them had 1 ul of Taq polymerase (containing DNA from Thermus aquaticus, a heat resistant bacteria found in Yellowstone National Park). The control in this case was the test tube without the Taq polymerase. At a later stage of the experiment, when we put our Cyanobacteria/sample water into tiny test tube wells and tracked the growth, our control group were a few wells that just had distilled water in them.

Experimental Process

(8) List all of the materials you used in your experiment. Be sure to include all physical materials as well as any technology or website used to collect data (not websites you used in your research).

Over the course of our experiment we used several different types of laboratory equipment, including growth medias and all of the supplies used for phage isolation. See file “Materials List” for a full list of the materials and software that we used during this experiment..

(9) Explain your experimental process. Be sure to list all of the steps and safety precautions for your experiment. If no safety precautions are listed it will be assumed none were taken. Remember to write it so someone else could follow the steps and recreate your experiment.

The most important parts of our experiment were done in a lab and so, over the course of our experiment, we learned and followed basic lab procedures for safety (such as wearing gloves, long sleeves, and long pants when working with potentially harmful materials) and sterilization (such as using a flame to sterilize all tools). We also always worked in the lab in the presence of an experienced scientist, Dr. Grose, and let her, or another adult with experience in the lab, handle any expensive or potentially dangerous equipment (such as the Bunsen burner or the electron microscope). See file "Experimental Process" for the exact steps and safety precautions taken during the course of this experiment, as well as pictures of the various steps.

Data Collection and Analysis

(10) Present the data you collected from your experiment. Be sure to include all of the numbers you collected from your observations and measurements. Use of graphs and charts is HIGHLY encouraged.

See file "E. coli Phage Comparison Chart" for phage data. See file "Host Range of E. coli Phage" for our findings on our phages abilities of infecting different bacteria types. See file "PageEMmorphology" for information on phage morphology. See file "Survey Results and Analysis" for the results of a survey sent out to the students in our school in order to find out how much they knew about local water issues.

(11) What are your potential sources of error? Remember, this doesn't mean "Did everything work?", all tests have potential sources of error, so make sure you understand what that means. Explain how these sources of error could have affected your results.

There were several potential sources of error for this experiment. Although we will acknowledge potential sources for error, we will also show how we made every effort to decrease this possibility. The first and largest source of error could have occurred within the ages of the water samples we used. Some of our samples were around 45 days old, while others were collected the same day they were used. This inconsistency could have caused inaccurate results because over time, other things could have grown in the water, or the bacteria could have died. We feel confident although this is a possibility that our fresh water samples were used in our final experimental procedure. Because we had experimented with older samples, and knew from the results that fresh water was better we took measures when isolating phages to gather the water we would use within the hour we would use it.

Another possible source of error could have been the location in the body of water in which we collected our samples. It is possible that collecting the sample on the shore of the body of water (as opposed to the center of the lake or pond) could have picked up other bacteria, along with minerals or pollutants. We took no measures to gather water from the middle of the lake but are interested in comparing the number of bacteria from both locations in the future.

We recognize that due to the lack of experience and skill of the team members there may have been errors in the lab. We feel confident this possibility was decreased as we worked under the strict direction of Dr. Grose. Also, because we were working closely with bacteria, cross-contamination is a common problem and there is a strong possibility that it could have occured somewhere along the way. Again, proper lab procedures and sterilization were consistent, but as students we recognize it is a possibility.

In consideration of all of the possible sources of error we acknowledge that we could have seen errors within the fact that we had to perform a lot of trial and error with our types of media. Different types of bacteria have different nutritional needs, and since we had quite a bit of trouble growing the bacteria (namely Cyanobacteria), we had to try out multiple types of media. Although this is a possible source of error, we felt the information acquired was valuable, and could be shared with others researching Cyanobacteria.

Finally due to the distance the team had to travel to the lab we had longer periods of time between our lab visits. With one team member who lived close making more regular visit to decrease the possibility of error, we still believe that our inconsistency in regular lab dates could have impacted our overall experiment. As a group of ninth grade students we did our best with what we had, and made every effort to decrease the possibility for error. We felt that although we may have had errors along the way, that the knowledge gained through the process far outweighed the potential errors. So do we wish we ran a completely "error-free" experiment, no we do not. We would not trade the learning and knowledge we gained for perfection.

Drawing Conclusions

(12) What conclusions can you draw based on the data you gathered during your experiment(s)?

Our team, the Phantastic Phage Phinders, began our experiment with the idea that we would be able to find phages within the same habitats of the bacteria species that they could combat. This idea was at the center of our experiment and, while we were unable to bring our Cyanobacteria to the phage-finding stage due to a slow growth rate, we were able to find phages for our E. coli within the sample of the bacteria itself. That data goes to support, if not entirely confirm, our hypothesis. Now that we have found our E. coli phages and confirmed that they are E. coli specific, we hope that these phages can one day be a part of a product designed to eliminate E. coli. We hope to continue our research and to be able to educate others on our findings.

As an additional part of our investigation, we did a survey to find out how much about local water contamination issues is known by other students in our school. The results of that survey can be found in the file "Survey Results and Analysis", but it can concluded that while most of our fellow students are somewhat aware of the local contamination issues, many do not know the main causes. As part of our next steps, we will be using a website to educate and inform the public about the issues in our water, our experiment, and our findings. In addition to this, we are also starting the process of deciding on names for the three E. coli phage that we discovered and that survived the electron microscope.

Uploaded Files:

- [**View**] **Materials List** (By: ScienceStudent, 02/14/2019, .pdf)
  This file is a PDF with a list of all materials and software used by the Phantastic Phage Phinders during the course of this experiment.
- [**View**] **PhageEMmorphology** (By: spacecat03, 02/14/2019, .pdf)
  Informational slides on phage morphology created by TheDuck
- [**View**] **E. coli Phage Comparison Chart** (By: ScienceStudent, 02/22/2019, .pdf)
  This is a bar graph showing how many of each type of phage was found when our team looked at E. coli samples using electron microscopy.
- [**View**] **Experimental Process** (By: ScienceStudent, 02/22/2019, .pdf)
  This file is a PDF with explanations of every lab meeting our team had over the course of the experiment and the processes through which our experiment was completed, including pictures of what was done at certain lab meetings.
- [**View**] **Host Range of E. coli Phage** (By: ScienceStudent, 02/22/2019, .pdf)
  This is a table showing our findings when we tested our E. coli phages against other bacteria types.
- [**View**] **Survey Results and Analysis** (By: ScienceStudent, 02/22/2019, .pdf)

This file contains the answers to a survey sent out to students in our school in an attempt to figure out how much they know about local water issues. All answers that could be represented through graphs are shown.

- [**View**] **Annotated Bibliography** (By: microscientist, 02/24/2019, .pdf)

  This is all of our sources, including: Websites, articles, experts, etc.

Community Benefit

(1) Explain how investigating the problem your team chose will help the community. Be sure to include the impacts your research will have on individuals, businesses, organizations, and the environment in your community (if any). Make it very clear why solving this problem would help your community.

Fresh water is crucial to life on earth. As a team we are concerned with the common problems facing our bodies of water including lakes, ponds, and streams (See annotated bibliography "Algal Blooms/Cyanobacteria"). Looking for answers on how to solve the problems of E. coli and Cyanobacteria (harmful blue green algae) in our water systems was critical for our phage phinders team. We wanted to use concrete research and lab practices to discover real answers to real questions. Our team feels that education is a first line of defense about what we can do to help our water systems. Our research will be shared through a public domain website where other scientists, students, teachers, etc. will have access to our scientific inquiry process. But we also want to share what each individual can do to help our water systems and lakes. We want to help our community by making our research and information available. We also have great aspirations to help our nation through our research and inquiry process.

Our team was inspired to make a difference because of research we learned about while investigating E. coli. In 2005, a product called AgriPhage became a commercially available product. It is a biopesticide that uses phage therapy to combat harmful bacteria on tomato and pepper plants (See annotated bibliography "AgriPhage". Additionally, they have biopesticides for citrus fruits. In one milliliter of AgriPhage, over 4 billion phages are present. Each phage can infect an unwanted bacteria and replicate inside of the bacteria, killing it. AgriPhage is completely natural and doesn't contain any harsh chemicals or substances. It is EPA certified and many commercial farmers have found success with it.

Agriphage is interesting to our team because we feel that the phages we have isolated could be used for a product similar to agriphage in the future. Important to this type of product would be a phage that is very narrow spectrum (meaning it is specific to E. coli). With our newly discovered phages and our additional testing of them to make sure they were narrow spectrum phages, we could share research that would lead to the creation of a substance filled with those phages to combat E. coli on plants. This product would be a natural substance that wouldn't inflict harm upon other beneficial bacteria. This could benefit our community in many ways. It could help many farmers, doctors, grocery stores, average people, and much more. Recent E. coli outbreaks on romaine lettuce have spread illness and caused great economic devastation (See annotated bibliography "E. Coli"). Several people got sick, some even hospitalized. Grocery stores had to throw out all of their romaine lettuce, losing money. Farmers had to throw out almost an entire crop. All of this and more had to happen because E. coli infected the water used for agriculture. Using the phages we discovered on agricultural water sources can have tremendous effects on almost everybody in the community. As we consider the difference this could've made to the nation this year, we feel compelled to continue our research and to share it.

See file "Survey Results and Analysis" for the results of a survey sent out to the students in our school as a way to see how much is known by our community about the local water issues.
Visit our informational website at https://phantasticphagephinders.weebly.com/

Uploaded Files:

- [**View**] **Survey Results and Analysis** (By: ScienceStudent, 02/22/2019, .pdf)

  This file contains the answers to a survey sent out to students in our school in an attempt to figure out how much they know about local water issues. All answers that could be represented through graphs are shown.

- [**View**] **Website Page** (By: spacecat03, 02/24/2019, .jpg)

  The "Our Work & Findings" page of our website.

- [**View**] **Google Presentation** (By: microscientist, 02/25/2019, .pdf)

  We created this to present to students at a field trip we all went too that was centered around our research.

Mission Verification

(1) Does your Mission Folder project involve vertebrate testing, defined as animals with backbones and spinal columns (which include humans)? If yes, team must complete and attach an IRB approval form.

No

(2) Did your team use a survey for any part of your project? If yes, team must complete and attach a survey approval form.

Yes

(3) You will need to include an abstract of 250 words or less. As part of the abstract you will need to describe your project and explain how you used STEM (Science, Technology, Engineering and Mathematics) to improve your community

Our team, the Phantastic Phage Phinders, used STEM in an attempt to learn more about water contaminants on both a local and national scale and to attempt to find a method of combating some of the more common contaminants. The two main water contaminants that our team worked with were E. coli and Cyanobacteria, both of which had been recently affecting our state of Utah in hazardous ways. We hoped that, through our use of the lab at Brigham Young University, we would be able to find and isolate bacteriophages within the bacteria that could eventually be developed into two products (one that would target E. coli specifically and one that would target Cyanobacteria specifically) that would serve either as a method of bacteria detection or a method of bacteria elimination. Throughout the course of our project we used various scientific processes (see experimental processes notes) in order to take water samples, and grow our bacteria. Then we continued the experiment to search for, identify, and eventually isolate our phages. Water and crop contamination from both E. coli and Cyanobacteria is a big community problem both locally and nationally. On November 20th, 2018, the infection of a romaine lettuce crop by E. coli caused over 25 people to fall ill in Utah and resulted in major product loss. The goal of our project was that we could use STEM to educate ourselves and others on the specifics of these problems and contribute to finding the solution.

Uploaded Files:

- [**View**] **IRB Form** (By: Advisor, 01/17/2019, .jpg)

Here is the IRB form with approval. We did not think we needed approval since no vertebrate testing was performed. However, we were working in a lab facility during the inquiry process and therefore sought proper approval. All necessary safety forms and permission slips were also filled out and signed prior to work in the lab.

- [**View**] **Survey Form** (By: Advisor, 02/05/2019, .jpg)

  This is the survey form with signed approval to conduct an informational survey for an educational outreach component of the project. Team members conducted the survey of other students general knowledge about cleanliness of lakes in Utah.

Who are the Phantastic Phage Phinders?

We are a team consisting of four freshman all from Mountain Heights Academy. We are planning on entering the eCYBERMISSION science contest. Our team consists of: ScienceStudent, Spacecat, TheDuck, and Microscientist. While our advisors are: Lora Gibbons and Julianne Grose, and a highschool student eCYBERMISSION expert: Kate Larson.

Phantastic Phage Phinders Experimental Materials

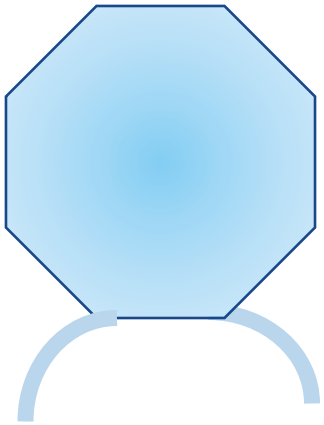
Materials used (see file: “Experimental Process” for exact amounts of materials):

- Sewage samples (for *Cyanobacteria* and *E. coli*)
- Water samples (for *Cyanobacteria* and *E. coli*)
- Plates
- Agar
- LB liquid media (for *E. coli* growth)
- BG-11 liquid media (for *Cyanobacteria* growth)
- Rubber gloves (for safety)
- Test tubes
- Mini-test tubes
- Electron microscope
- Buffer (for DNA sequencing of *Cyanobacteria*)
- Nucleotides (for DNA sequencing of *Cyanobacteria*)
- Forward Primer (for DNA sequencing of *Cyanobacteria*)
- Reverse Primer (for DNA sequencing of *Cyanobacteria*)
- Taq polymerase (for DNA sequencing of *Cyanobacteria*)
- Bacteria suspension
- Sodium carbonate
- Sodium bicarbonate
- PCR machine
- Neutralized uranium stain (for electron microscopy)
- Jars/bottles (for sample transportation)
- Fluorescent light (for sample storage)
- Bunsen burner (for sterilization)

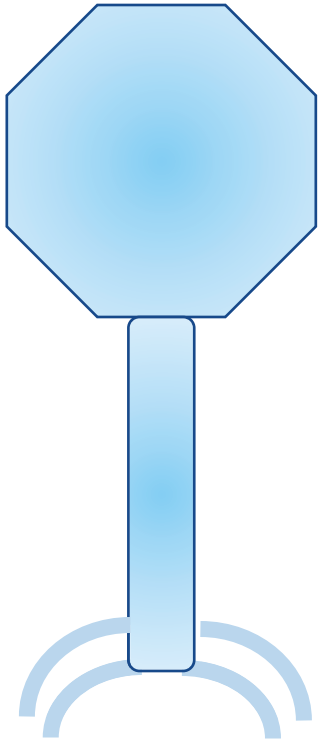
Software and websites used for data collection/display:

- Google Sheets (for graph making)
- Weebly.com (for website making)
- Google Slides (for presentation making)

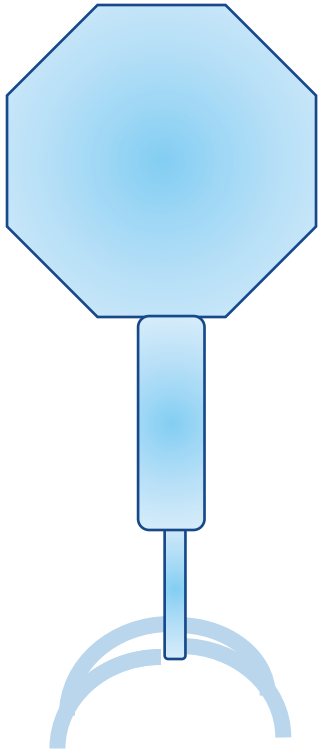
There are Three Common Morphologies for Tailed Bacteriophages



Podoviridae
(short tails)

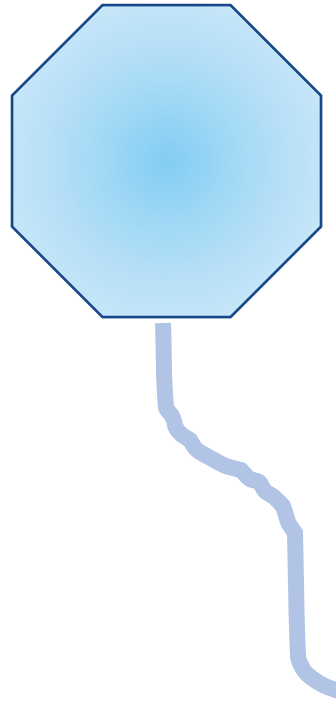


contracted



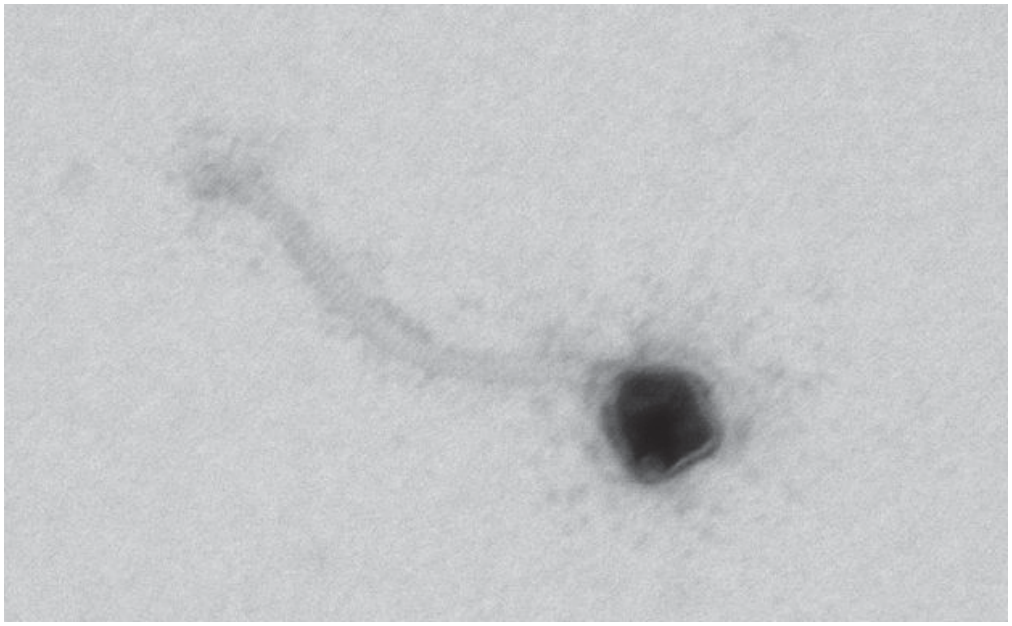
Non-contracted

Myoviridae
(medium/long contractile tails)



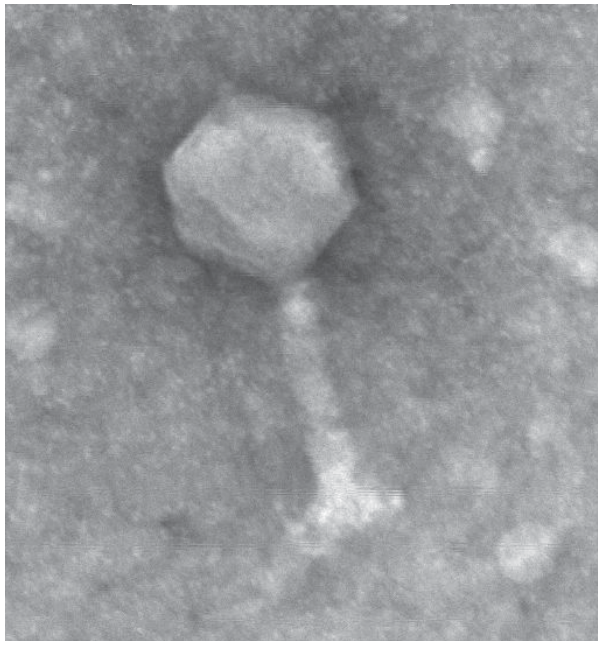
Siphoviridae
(long, flexable, non-contra

Phage (Name)
Siphoviridae

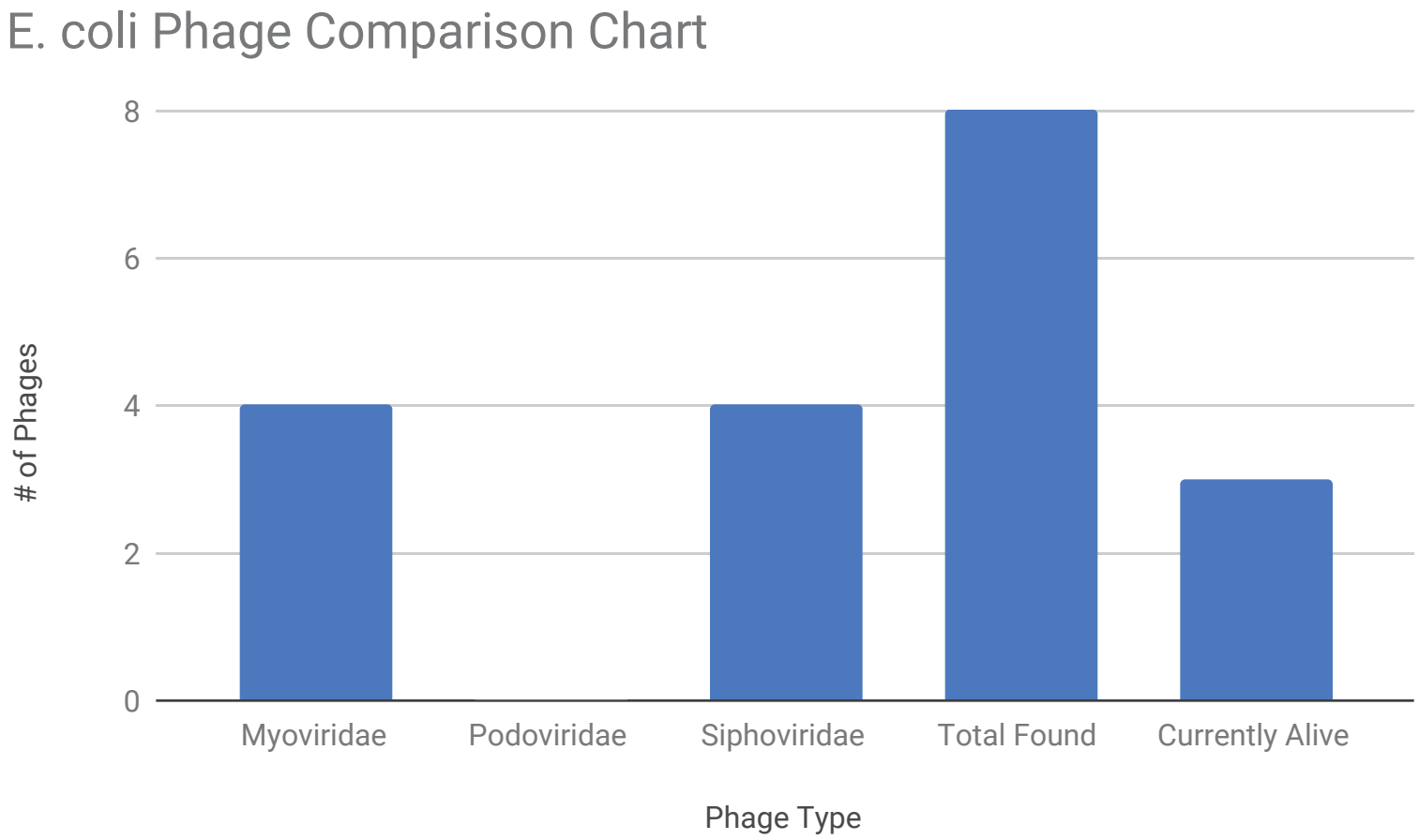


	Length (nm)
Capsid	65.7 +/- 4.0
Tail	305 +/- 19.8
Collar	14.23 +/- 3.0

Phage (Name)
Myoviridae



	Length (nm
Capsid	101.7 +/- 4.6
Tail	99.2 +/- 3.3
Collar	15.4+/- 1.1


E. coli Phage Comparison Chart
8
6
4
2
0
# of Phages
Myoviridae
Podoviridae
Siphoviridae
Total Found
Currently Alive
Phage Type

Phantastic Phage Phinders Experimental Process, Including Lab Dates and Procedures

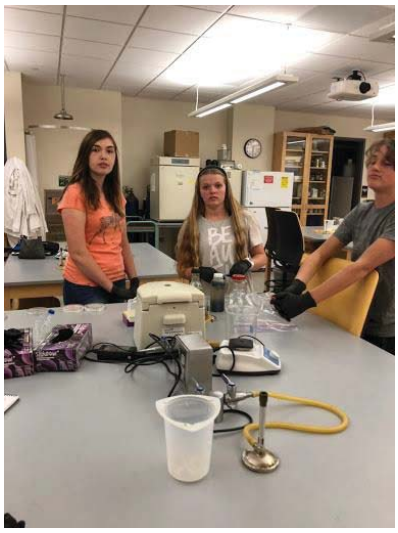
Before meeting at the Brigham Young University (BYU) lab our team had an online meeting and decided to take water samples from several different bodies of water in Utah. After we did so, we met at the Davis Sewer District in order to give our water samples to a scientist from BYU and to take a tour, as well as learn a lot more about the problem of the bacteria in Utah Lake.

June 15
Several team members met at the BYU lab and, with the help of the same BYU scientist, plated the water samples in a media called Luria-Bertani (LB). The goal then was to wait for *Cyanobacteria* to grow.

July 2
We meet again to replate the samples in a different media, as we had not had much growth of *Cyanobacteria*. This time we researched more and and decided to use a media called BG-11.

Pictures of Process and Team Learning How to Plate (siblings of team members and team mentors are in these pictures as well, as we all wanted to learn):





July 10

We met back at the lab and saw that we had *Cyanobacteria* growth. Our next step was to continue growing the *Cyanobacteria* and work on isolating the phage, which we eventually hope to use as a solution to the issue of an excess of toxic *Cyanobacteria* in Utah Lake. To do this we made a solution with 40 ml liquid media (BG-11), 0.5 ml of bacteria suspension, 100 ml of water from a fresh sample, 8 ml of top agar and 0.16 sodium bicarbonate and 2 ml sodium carbonate. We microwaved the mixture for 30 seconds, mixed it, then microwaved it for another 30 seconds. Before using this, we let it sit for 45 minutes. Then we used to solution on each of the plated samples. Our goal then was to wait for the Cyanobacteria to grow enough to cover the entire plate. At that point, we would be able to detect phages by the absence of Cyanobacteria, which is visible to the naked eye.

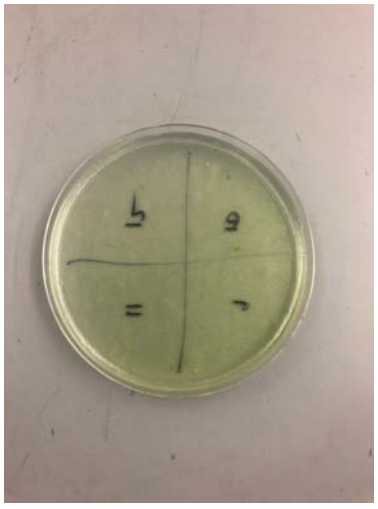
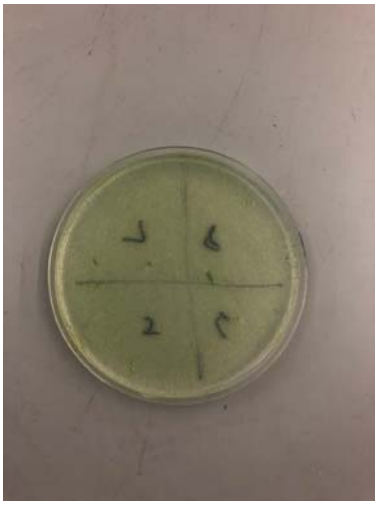
Pictures of Solution Making Process:



July 25

We met again and noted that the *Cyanobacteria* was growing quite slowly, which research proved was normal. We also plated so fresh samples and noted from the older samples that *Cyanobacteria* prefers to be embedded in the agar instead of on top of it, so this time we did embed it in the agar. At that point we just needed to wait for the the *Cyanobacteria* to grow enough for the phage to become visible, since phage is not visible to the naked eye and can only be detected by the absence of bacteria.

Pictures of Plates:


September 21

We met again. The bacteria is slow growing, but Dr. Grose talked to us about DNA replication and sequencing and so we began the process to do so with DNA from our bacteria in order to

verify that the species we had was in fact *Cyanobacteria*. We started by taking a small piece of DNA (the 16s RNA gene that all living organisms have) to use as our primer. Because our bacteria was embedded in the plate, we had to scrape some off with a blade (using water, because the ager had gotten hard) in order to put it in a PCR tube, then we put it in a PCR machine set at 99 degrees Celsius and left it there for 5 minutes. We then assembled the rest of the needed tools. We put the bacteria into three test tubes and used the third test tube as a control (it had everything but the DNA sample), we added 10 ul of buffer to each test tube, added 3 ul of nucleotides to each test tube, added 3 ul of 16s Forward Primer, added 3 ul of Reverse Primer, added 81 ul of water, and we added 1 ul of Taq polymerase (containing DNA from *Thermus aquaticus*, a heat resistant bacteria found in Yellowstone National Park). We then removed the DNA out of the PCR machine and placed in all of the tubes except for the control tube. It was then places in the PCR machine for three hours and would be sequenced at the next lab meeting. Then we plated a fresh sample collected on the 20th of September from Stansbury Lake. We made a plan to meet again and have a team member come in to the lab to spot the plates every other day.

September 27 and October 3
We held online meetings to discuss division of work and our plan to continue the project.

October 11
We held an online meeting so that a team mentor who had done Ecybermission the year before could give us a tutorial on website making. Later that month, more online meetings were held to discuss and plan the making of the website.

October 26th 2018 (In person Lab day)
Notes:

To help with the growth of the *Cyanobacteria*, all available team members met at the lab and replated the older samples into tiny test tubes. Because of the small space, it was hoped that the *Cyanobacteria* would multiply more quickly.

Control: Media with no growth
Control Group: Samples in the wells that have not been treated (clean water)
Independent variable: varying locations of samples
Dependent variable: Any plaques that are present

PHAGE TYPES: 3 different types of Phage

Image created by Adexime.

Myoviridae, Podoviridae and Siphoviridae:

Podoviridae:
- Side tail fibres
- Non-contractile short tail

Myoviridae:
- Contractile tails (long)

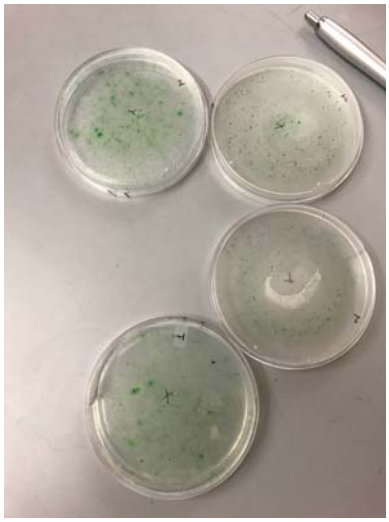
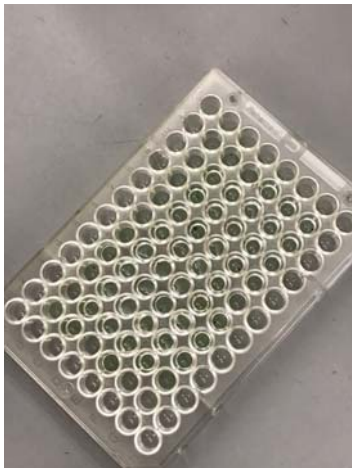
Siphoviridae:
- Long Non-contractile

SAMPLES USED IN WELLS:

Used Wells C,D,E
Older Samples:
#2 Ginger Bottle Utah Lake in spot 6CDE
#3 Utah Lake #2 went into 3CDE
#4 Stansbury sample went into 4CDE
#5 Strawberry Reservoir sample went into 5CDE
#7 Mil Meadow Johnsonville Reservoir 7CDE

New Samples from Today
#8 Utah Lake 8CDE
#9 Utah Lake 9CDE
#10 Utah Lake 10CDE
#11 Utah Lake F345
#12 Distilled water as control F678

Plate Pictures:


Team Pictures from lab day:


November 2

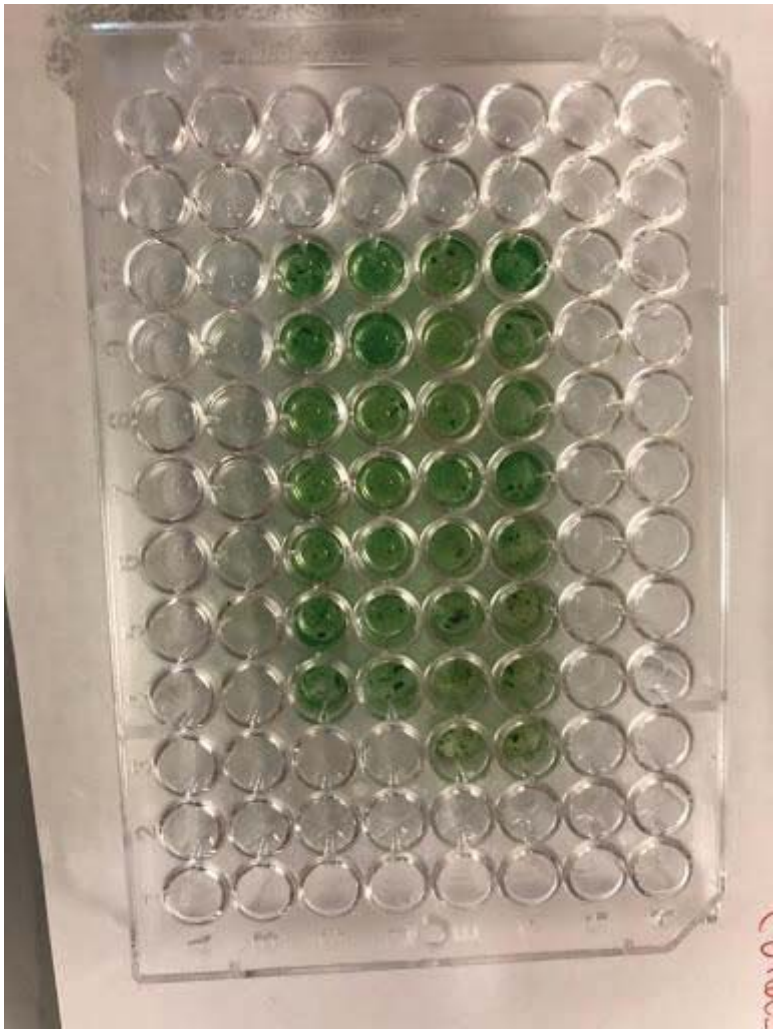
We met at the BYU lab to plate *E. coli* so that we could try to find phages for that bacteria. This process was slightly different than that of the *Cyanobacteria* process (which is still being continued) because we already had *E. coli* in the water samples and had to spend less time growing and isolating the bacteria. We used 9 different water samples that we numbered in the hopes of finding different phage types, with 1 ml of water to 10 ml of *E. coli*.

1. North Davis Sewage District (untreated)
2. North Davis Sewage (Solid Contact)
3. Winterhaven, Florida
4. Utah Lake
5. Stansbury Lake
6. North Davis Davis Sewage (before chlorination)
7. North Davis Sewage (after chlorination)
8. Rancho Cucamonga California

9. South Valley California

Safety measures note: a scientist assisting with our project, Dr. Grose, handled the sewage samples using gloves so that we, as minors, weren't handling potentially harmful substances. We then left that for two days and later had a team member return to the lab to remove from liquid and imbed sample in agar. We then observed the *Cyanobacteria* we had worked with at the previous lab meeting, seeing that the control had no change (which was as hoped for). We also moved samples to different test tube wells and discussed plans for future lab meetings.

Cyanobacteria Well Pictures:

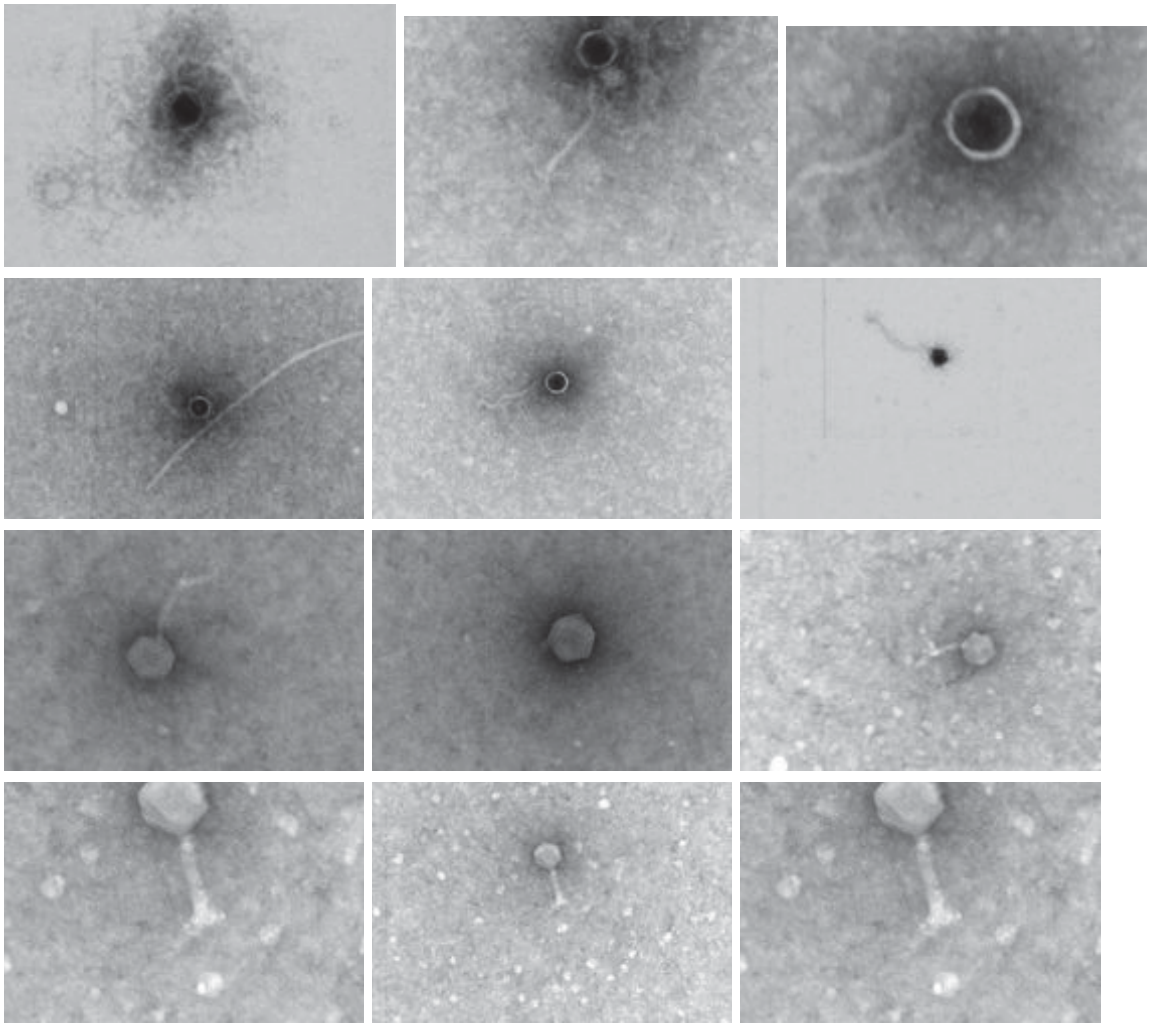


December 10

We met in person at the lab to continue our experiment and do more with the *E. coli* portion. We began by preparing our *E. coli* samples (which by now hopefully had phages) for electron microscopy so that we could see the phages for ourselves. We observed which plates had the best phage plaques and pulled from those (#7,#2, #3(which had a super plaque), and #6), then put in LB media and let sit for a few minutes in wells. We then used uranium, which was in a safe state and handled only by Dr. Grose, as a stain so that we would be able to see the bacteria by

using the electron microscope. On our grid, we had sampes is b6, b7, b8, and b9. We used the electron microscope of 5-6 100,000 times magnification and found a handful of phages: 2 myoviridae phage in b9, 2 siphoviridae phage in b9, 2 siphoviridae phage in b8, and 2 myoviridae phage in b7. We were unable to spot any in b6, but we did find a lot of bacteria in b8 in addition to its phages.

Electron Microscopy Phage Pictures:



January 14 (2019)

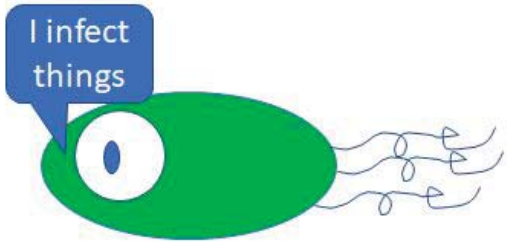
We met at the lab for our final portion of in-lab experimentation for which the entire team would be present. First, we discussed the necessary steps we would need to take that day and our future hopes for the project concerning both types of bacteria (*Cyanobacteria* and *E. coli*). We decided that, because of the slow growth of the *Cyanobacteria* and the recent local infection of *E. coli* in lettuce resulting in removal from stores and the mass loss of crops and profits, that we would end our project focusing primarily on *E. coli* and our *E. coli* phages. Our final step was to test our previously found phages against several other types of bacteria (the samples used were collected

and handled by Dr. Grose, for safety reasons) so that we could see if they fought *E. coli* specifically or if they would be harmful to a wider range of bacteria. We hoped to find that the range of the phages was mostly specific to *E. coli*. This is because the more bacteria the phages are able to combat, the higher the risk that the phages will be harmful to good bacteria that our bodies and the environment need in order to survive. We tested this by first gathering the different types of bacteria we were going to test (all of which are relatives to *E. coli* and cause intestinal issues/diseases):

1. *E. aerogenes*
2. *Citrobacter*
3. *Shigella*
4. *Morganella*
5. *E. cloacae*
6. *Klebsiella pneumonia*
7. *E. coli* (as positive control)
8. *Serratia*

0.5 ml of each bacteria was put into a tube, then 4.5 ml of top agar was added to each tube. In between the additions, Dr. Grose sterilized the tube with fire. Then the bacteria with liquid top agar was added to LB media and plated. We then let it sit for 15 minutes so that the top agar could solidify, then spotted phages on the plate. If our phages were effective against another type of bacteria, we would see this by the absence of the bacteria. If our phages were ineffective to another type of bacteria, there would be just as much bacteria on the plate as before, or more. Over the next several days, the plates would be watched by the team member living closest to the lab, who happened to be Dr. Grose's son. He would then report the results to the rest of the time.

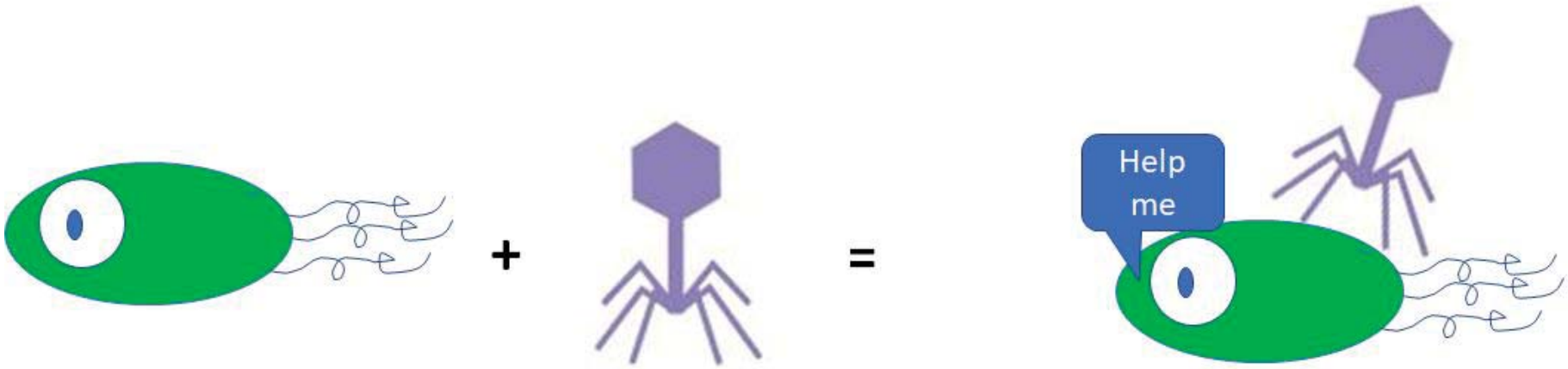
About three weeks later we found out that our phages were *E. coli* specific and did not infect any of the bacteria types that we tested them against, which were *E. aerogenes*, *Citrobacter*, *Shigella*, *Morganella*, *E. cloacae*, *Klebsiella pneumonia*, and *Serratia*. This was what we had hoped for because, since the phage is narrow spectrum, it allows us to further investigate our phage for possibilities in developing a spray to help farmers combat *E. coli* on lettuce plants. That was the end to the in-lab portion of our experiment and the rest of the time we spent working on the Mission Folder and compiling all of our data.



Host Range of our *E. coli* Bacteriophages

	Escherichia coli (E. coli)	*Salmonella typhimurium*	*Klebsiella pneumoniae*	*Serratia marcescens*	*Enterobacter cloecae*	*Enteorbacter aerogenes*	*Citrobacter koseri*	*Pseudomonas aeruginosa*
Phage 1	yes	no	no	no	no	no	no	no
Phage 2	yes	no	no	no	no	no	no	no
Phage 3	yes	no	no	no	no	no	no	no

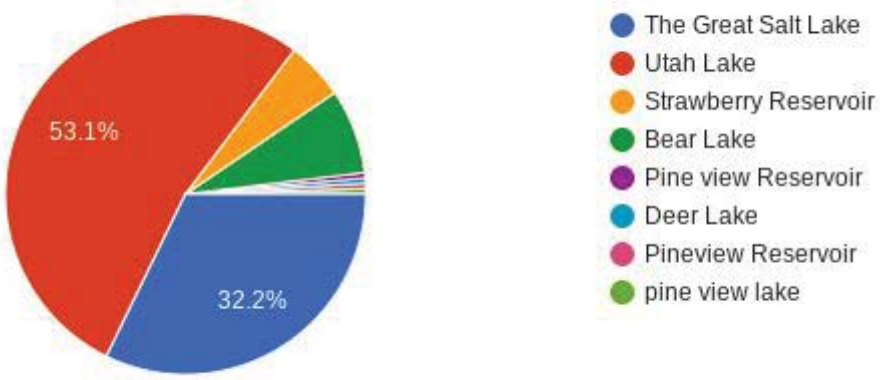
Our phages are specific for *E. coli*



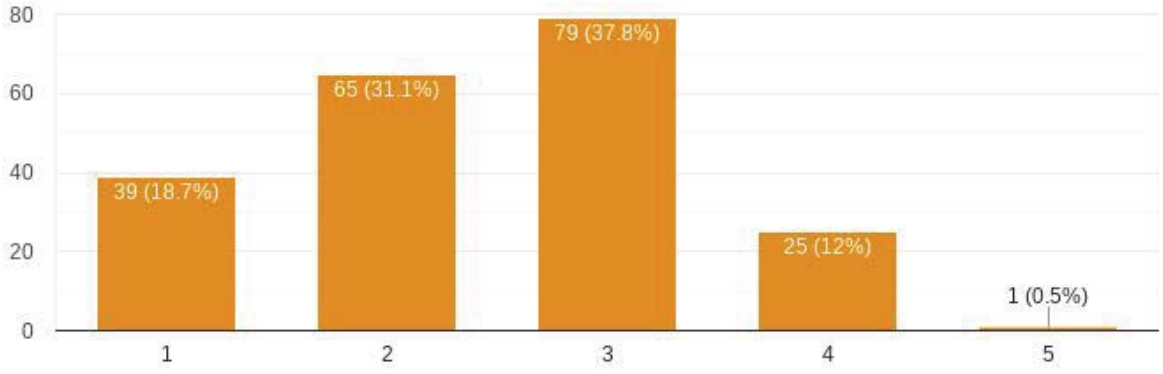
Bacteriophages infect and kill bacteria

Our team, the Phantastic Phage Phinders, made a survey that we gave to some of the other students at our school (Mountain Heights Academy), specifically the students from the Earth Science classes and 8th Grade Science classes, in order to find out how much they knew about the local water contamination issues. A total of 212 students took the survey and results for all questions that could be graphically represented are below.

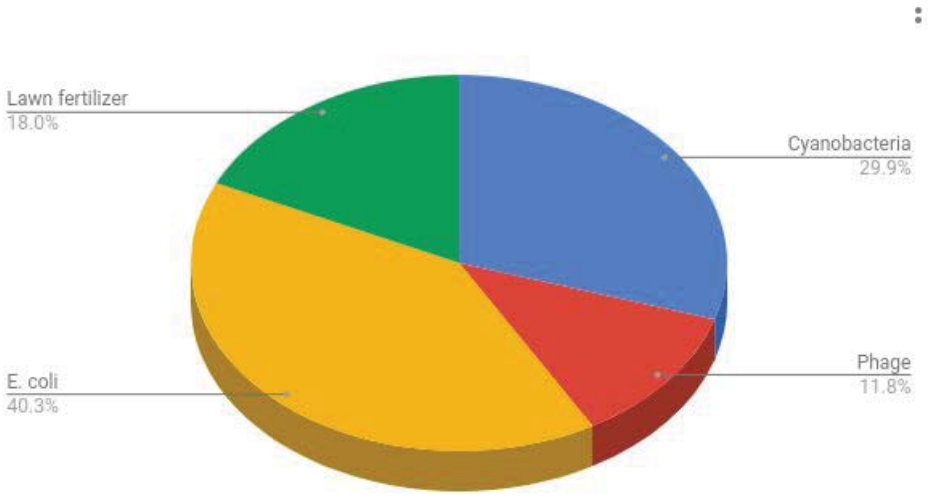
Question 1 (211 responses): Which of these bodies of water in and near Utah do you feel are the most unclean?



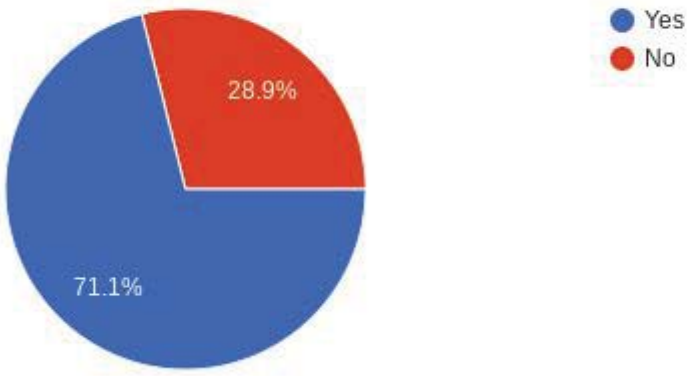
Question 2 (209 responses) : On a scale of 1-5 (with one being very unsafe and five being very safe), how safe do you think Utah Lake is?



Question 3 (211 responses): What do you think is the most harmful substance in our open waters (lakes, rivers, etc.)?



Question 6 (211 responses): Do you ever think about how little things can have big impacts on our water?



Questions were also asked to find out how familiar the other students in our school were with certain terms regarding water contaminants, such as “phage” and “*Cyanobacteria*”. We were unable to graphically represent the responses to those questions but through the survey as a whole we were able to see that while most of the students in our school seem to be aware that our local bodies of water are dirtied by contaminants, many of the students appear to be unsure on the subject of what those main contaminants are. Our goal now is that, through the use of an

informational website, our team will be able to better educate the public on the main dangers of our water and the potential for a solution, as well as possible ways that they can help.

9th Grade Ecybermission Team Sources

Websites/articles:

Algal Blooms/Cyanobacteria

Rippka, Rosmarie. “Isolation and Purification of Cyanobacteria.” *Methods in Enzymology Cyanobacteria*, vol. 167, 1988, pp. 3–27., doi:10.1016/0076-6879(88)67004-2.

This article covers information on culturing and growing Cyanobacteria. We used it when determining how to grow our Cyanobacteria.

Rosenbaum, Leah. “New Tools Aim to Better Predict Blooms of Toxic Algae.” *Science News for Students*, 19 Sept. 2018, 6:45, www.sciencenewsforstudents.org/article/new-tools-aim-better-predict-blooms-toxic-algae

This article showed some ways that people are trying to combat algae blooms and red tides. It also shows the threats of the algae bloom on human health, seafood, wildlife, etc.

Brookshire, Bethany. “Fighting Big Farm Pollution with a Tiny Plant.” *Science News for Students*, 10 May 2018, 7:00 AM , www.sciencenewsforstudents.org/article/fighting-big-farm-pollution-tiny-plant.

This article showed us how other people are fighting off harmful substances and pollution in their water. This person used duckweed to combat pollution in the water.

Mercola, Joseph M. “It's Disgraceful, Scores of Florida Sea Life Killed and Now Puts Human Health at Risk.” *Mercola.com*, Mercola.com, 21 Aug. 2018, https://articles.mercola.com/sites/articles/archive/2018/08/21/toxic-algae-and-red-tide.aspx?utm_source=dnl&utm_medium=email&utm_content=art1&utm_campaign=20180821Z1_UCM&et_cid=DM229611&et_rid=398530468.

This article gives an expert point of view on the effects of harmful algae on humans and wildlife.

“Cyanobacteria (Blue-Green Algae) and Cyanobacterial Blooms.” *LG Sonic*, LG SONIC, 14 Mar. 2017, www.lgsonic.com/blogs/cyanobacteria/.

The above article contains information regarding cyanobacteria, cyanobacteria blooms, the causes of these blooms, and some of the current solutions for these blooms.

“Cyanobacteria/Cyanotoxins.” *EPA*, Environmental Protection Agency, 30 Jan. 2019, https://www.epa.gov/nutrient-policy-data/cyanobacteriacyanotoxins#what2.

This article talks about cyanotoxins, and what types of cyanobacteria create harmful cyanotoxins. It also talks about the most commonly found cyanotoxins in the USA and the risks they pose on us.

Auld, Alison. “Health Officials Suspect Algae in Deaths of Three Dogs Who Played in New Brunswick River .” *Global News*, The Canadian Press, 26 July 2018, https://globalnews.ca/news/4354351/algal-blamed-in-dog-deaths/.

This article helps us understand just how dangerous algal blooms can be. It also shows us that this is even a global problem. It is happening in other countries.

Press, Associated. "Toxic 'Red Tide' Blamed for Rise of Manatee Deaths in Florida." *The Guardian*, Guardian News and Media, 20 Aug. 2018, 19:01, www.theguardian.com/us-news/2018/aug/20/manatee-deaths-toxic-red-tide-algae-bloom-florida.

This article shows us that algal blooms has detrimental effects on wildlife. It also shows us that algal blooms are even an issue nationally.

Utah Lake/Local Environment

Means , Sean P. "Here We Go Again: Algal Bloom Found in Utah Lake Prompts Health Warning." *The Salt Lake Tribune*, The Salt Lake Tribune, 12 June 2018, www.sltrib.com/news/health/2018/06/12/health-warning-issued-after-blue-green-algal-bloom-is-found-in-provo-bay-in-utah-lake/.

This article contains information regarding an algae bloom with Cyanobacteria that took place at the beginning of summer in 2018. This article can help explain what happens when there is an algae bloom and why it is so dangerous.

McDonald, Matt, et al. "Officials Issue Warning as Toxic Algae Spreads in Utah Lake." *fox13now.Com*, Fox 13 News, 25 June 2018, 1:44 PM, https://fox13now.com/2018/06/25/officials-issue-warning-as-toxic-algae-spreads-in-utah-lake/.

The above is a news article reporting on a cyanobacteria bloom earlier in 2018 and discussing why it is harmful.

Ellis, Eric. "Utah Lake: Top 19 Most Interesting Facts." *Utah Lake Official Website*, Utah Lake Commission , 16 July 2011, https://utahlakecommission.org/utah-lake-top-20-most-interesting-facts/.

These Utah Lake facts helped us learn a lot more about the lake we would focus on during the extent of our research.

"Utah Lake Algal Bloom Monitoring 2018." *Utah Department of Environmental Quality*, Utah.gov, 15 May 2018, 5:09 PM , https://deq.utah.gov/health-advisory-panel/harmful-algal-blooms-habs/utah-lake-jordan-river-canals-algal-bloom-monitoring-2018.

This article is an extremely detailed report of algal blooms in Utah Lake, recreational effects, health effects, and more. It has test results and many useful details.

Penrod, Emma. "It's Not Just Utah Lake: Toxic Algae Plagues 20 Waterways, Including Drinking Water Sources." *The Salt Lake Tribune*, The Salt Lake Tribune, 8 Aug. 2015, 10:41 AM, https://archive.sltrib.com/article.php?id=2815139&itype=CMSID.

This article shows that it isn't only Utah Lake that is suffering from harmful algae, but many other bodies of water in Utah as well. It also lists the affected bodies of water and updates on local algae bloom growth.

"Harmful Algal Blooms Home." *Utah Department of Environmental Quality*, Utah.gov, 20 Nov. 2018, https://deq.utah.gov/water-quality/harmful-algal-blooms-home.
This article talks about how cyanobacteria and algae blooms are formed and gives information about them.

"Make a Payment." *Granger Medical Clinic Blue Green Algae In Utah Waters Comments*, Granger Medical , 20 July 2016, https://www.grangermedical.com/blue-green-algae-in-utah-waters/.
This article is the side effects and medical issues surrounding algal bloom. It is from a medical point of view and shows how serious algae blooms can be.

E. coli/AgriPhage

"Outbreak of E. Coli Infections Linked to Romaine Lettuce | E. Coli Infections Linked to Romaine Lettuce | November 2018 | E. Coli | CDC." *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 9 Jan. 2019, www.cdc.gov/ecoli/2018/o157h7-11-18/index.html.
This article told us about the recent E. coli recall, and how E. coli can affect us. This was one of the main reasons we started using E. coli bacteria in our research.

Gray, Sarah. "The Financial Impact of the Romaine Lettuce E. Coli Outbreak Isn't Over." *Fortune.com*, Fortune, 30 May 2018, https://fortune.com/2018/05/30/romaine-lettuce-e-coli-outbreak-impacts/
This was the financial impacts of an E. coli outbreak at such a large scale. It helped us realize how serious these outbreaks are.

"Home » PRODUCT INFO." AgriPhage, AgriPhage, www.agriphage.com/product-info/.
This tells us more about AgriPhage and what it does and the details surrounding the product we were interested in.

Gillham, Felicia. "AgriPhage™-Fire Blight and AgriPhage™-Citrus Canker Approved for Use to Control Bacterial Disease in Citrus and Pome Fruit." *AgriPhage-Fire Blight and AgriPhage™-Citrus Canker Approved for Use to Control Bacterial Disease in Citrus and Pome Fruit*, Gillham & Associates Marketing Communications, 9 Oct. 2018, 6:00, www.businesswire.com/news/home/20181009005359/en/AgriPhage%E2%84%A2-Fire-Blight-AgriPhage%E2%84%A2-Citrus-Canker-Approved-Control-Bacterial.
This article talked about AgriPhage and helped us understand the product more.
It talks about the newly approved natural pesticide for citrus and pome fruits.

Phage

"What Is Phage Therapy?" *Phage Therapy Center*, Phage Therapy Center, www.phagetherapycenter.com/pii/PatientServlet?command=static_phagetherapy&language=0.

This talks about phage therapy and the positive impacts it is having on illnesses. It shows us how others are using phages for good and how they are doing it. It also talks about the pros and cons of phage therapy vs antibiotic another useful tool in our project.

Experts:

Dr. Julianne Grose, Professor Of Microbiology at Brigham Young University - julianne_grose@byu.edu

Dr. Grose helped us extensively throughout our project. She helped us to decide the next step to take after we completed things during our project. She taught us about phage and how fascinating they are. As she is an expert in this field, she always did the things that could be potentially dangerous (such as working with E. coli and sewage). She facilitated our use of the lab at Brigham Young University, and spent countless hours teaching and instructing us.

Ken Burgener, Lab Director, North Davis Sewer District - Email kenburgener@ndsd.org

Mr. Burgener helped us take sewage samples and the NDSD. He talked to us about water quality and cyanobacteria. He showed how important clean water is to our community and gave us lots of information. He helped us continue our project and talked about water conservation and quality.

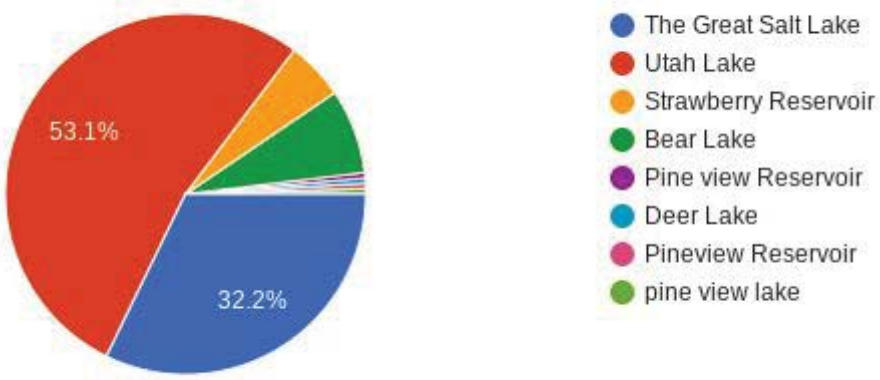
Daniel Thompson

d.william.thompson@gmail.com

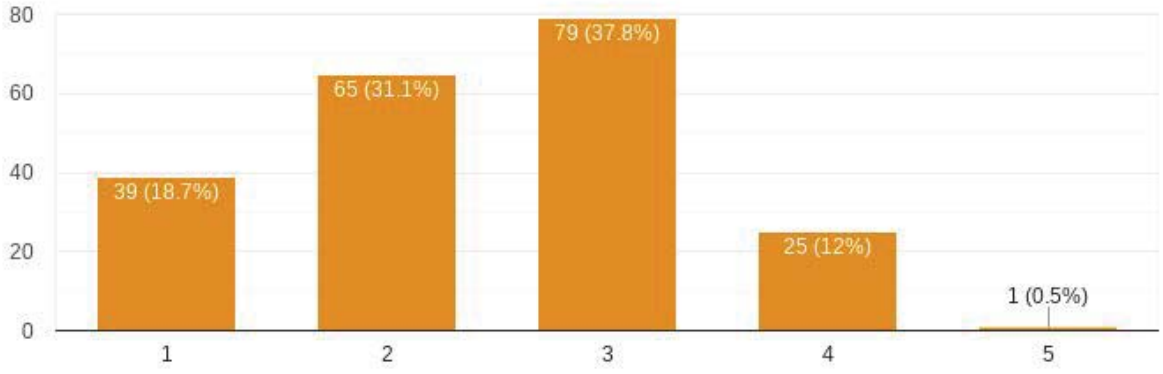
Mr. Thompson helped us look at our electron microscope images. After countless hours of training he was certified and able to use this very expensive piece of equipment. Only trained professionals are allowed to use the electron microscope. He looked at the images and scrolled through our samples, while we watched and looked for phages.

Our team, the Phantastic Phage Phinders, made a survey that we gave to some of the other students at our school (Mountain Heights Academy), specifically the students from the Earth Science classes and 8th Grade Science classes, in order to find out how much they knew about the local water contamination issues. A total of 212 students took the survey and results for all questions that could be graphically represented are below.

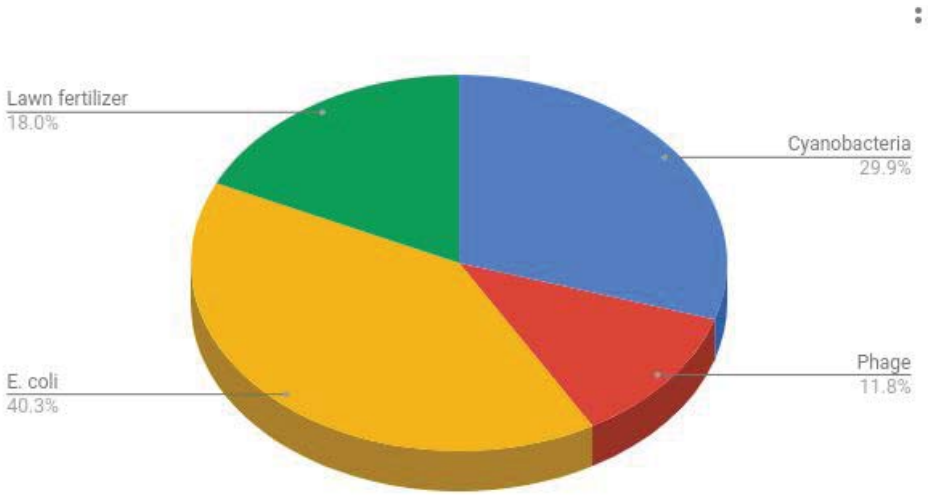
Question 1 (211 responses): Which of these bodies of water in and near Utah do you feel are the most unclean?



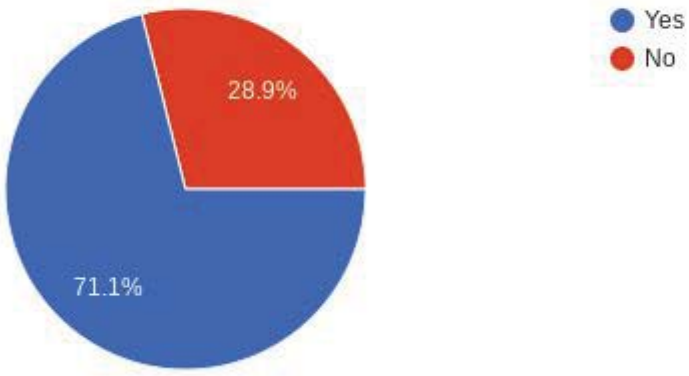
Question 2 (209 responses) : On a scale of 1-5 (with one being very unsafe and five being very safe), how safe do you think Utah Lake is?



Question 3 (211 responses): What do you think is the most harmful substance in our open waters (lakes, rivers, etc.)?



Question 6 (211 responses): Do you ever think about how little things can have big impacts on our water?



Questions were also asked to find out how familiar the other students in our school were with certain terms regarding water contaminants, such as “phage” and “*Cyanobacteria*”. We were unable to graphically represent the responses to those questions but through the survey as a whole we were able to see that while most of the students in our school seem to be aware that our local bodies of water are dirtied by contaminants, many of the students appear to be unsure on the subject of what those main contaminants are. Our goal now is that, through the use of an

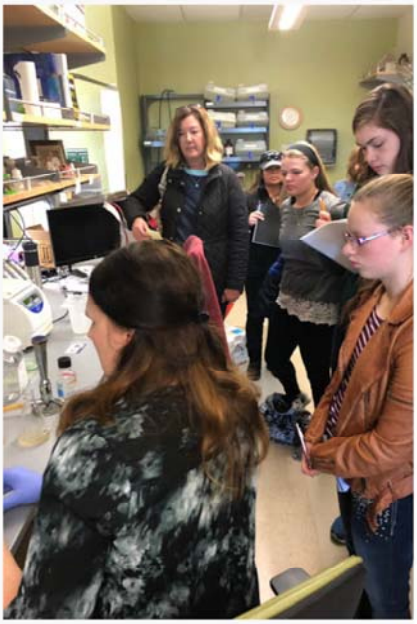
informational website, our team will be able to better educate the public on the main dangers of our water and the potential for a solution, as well as possible ways that they can help.

PHANTASTIC PHAGE PHINDERS

HOME ABOUT US! OUR WORK & FINDINGS HOW CAN I HEL

Our Work & Findings

As a group of high school students, we grew Cyanobacteria and E. coli in a lab in hopes of finding phages inside them. We wanted to use the phages outside the lab to put in our lakes, crops, rivers, and other places, and destroy the bacteria there. We are planning to enter our project and results in the eCYBERMISSION science



Dr. Grose with a few of the team members

Goal: To find phages to kill E. coli (initially it was to kill Cyanobacteria).

Hypothesis: If Utah bodies of water are infected with harmful bacteria, then the same bodies of water will contain phages that can combat those bacteria species.

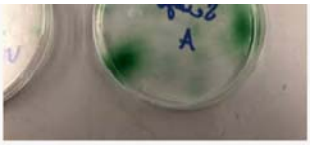
The Experimental Process

Using BG-11

To find these phages, we went through quite a bit of trial and e original intention was to grow Cyanobacteria, so we started wi plated it in a media called LB in hopes of it growing, but with n next step was to see if it would grow in a media called BG-11. Tl get growth, so our next step was to continue growing it to the p would be able to detect if phages were present. The Cyanobacte was slow, so we met as a team and replated the samples into sn tubes in hopes that it would grow faster. (See images below)

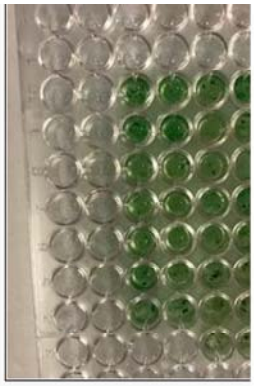
Because the growing process was still slow, we decided to focus our attention to growing E. coli (however, we still intend to keep

working with the Cyanobacteria) because we knew it would grow much faster. About a month later, we met back up and looked at the E. coli samples under the electron microscope to see if we had phages. After looking at 4 different samples, we found 8 phages! (See images below)



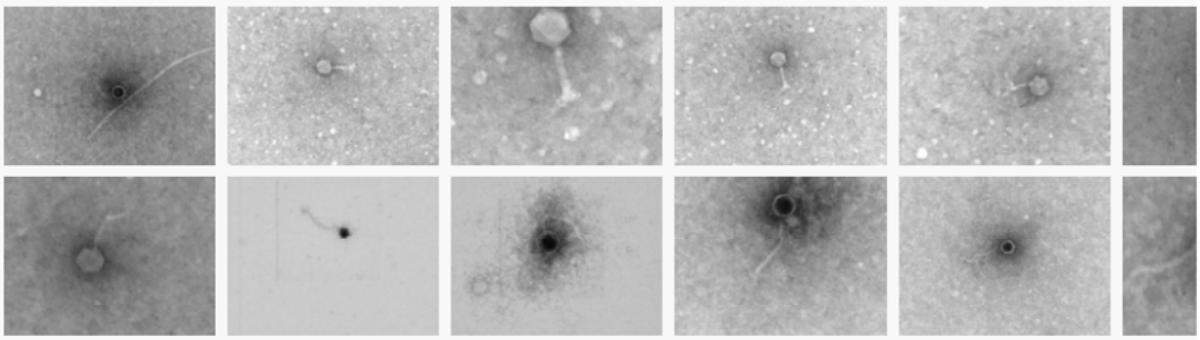
Cyanobacteria plate

Cyanobacteria plate



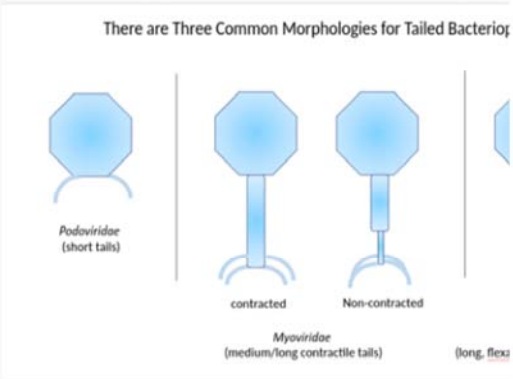
Cyanobacteria we

Electron Microscopy Photos:



We ended up finding eight phages.
Two myoviridae phage in b9, two siphoviridae phage in b9, two siphoviridae phage in b8, and two myoviridae phage in b7.
(b7, b8, and b9 are all types of media.)

We were thrilled by our discovery! Our next step was to find out if these phages targeted and killed E. coli specifically, or if they harmed other bacteria as well. We wanted the phages to be E. coli [illegible] bacteria the phage is able to combat, the higher the risk it is a good bacteria. A few weeks later, we found out that the phages were indeed E. coli specific!



Types of phages. Image created by team member Th

Where will we go next?

As we continue on with this project, we will continue to work closely with both Cyanobacteria and E. coli. Sometime in the n hope to find phages specific to Cyanobacteria as well. Eventually, we would like to develop a product that uses these phages "phagetherapy") to combat bacteria, specifically the two we have been researching.

To learn more about our experimental process, please view the PDF below.

phantastic_phage_phinders_experimental_process.pdf
Download File

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Phantastic Phage Phinders

Our Project

Who are the Phantastic Phage Phinders?

We are a team consisting of 4 freshman all from Mountain Heights Academy. We are planning on entering the eCYBERMISSION science contest. Our team consists of: ScienceStudent, spacecat03, TheDuck, and microscientist. While our advisors are: Lora Gibbons and Julianne Grose, and a highschool student eCYBERMISSION expert: Kate L.

Our Hypothesis

Our hypothesis was:

If Utah bodies of water are infected with harmful bacteria, then the same bodies of water will contain phages that can combat those bacteria species and/or strains.

What is a Phage?

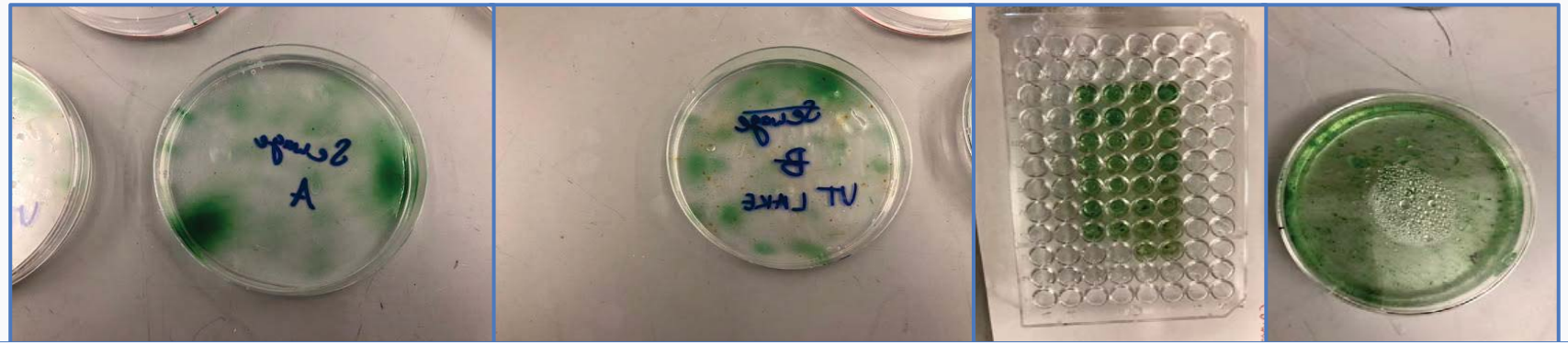
A phage is a type of virus that can infect bacteria by replicating inside of it. A lot of times, phage can be used for beneficial purposes. In one example, a certain phage named Agriphage, went commercial and became a product that people can use to help get rid of harmful bacteria on tomato and pepper plants.

What Types of Bacteria Did We Use to Grow Our Phage?

During the length of our project, we studied two different bacteria types: Cyanobacteria and E. coli, both of which have the potential to inflict harm upon plants, animals, and entire ecosystems. As of now, cyanobacteria is known for being a cause of the harmful algal bloom occurring within Utah Lake, while E. coli often infects plants (sometimes making them harmful to eat). Recently, E. coli infected an immense amount of Romaine lettuce after finding the bacteria in the primary water source. Ultimately, it made over 25 people sick. As a result, we worked with Dr. Grose for a number of weeks in hopes of discovering phage(s) that would infect the E. coli bacteria specifically.

Growing Our Cyanobacteria

After taking water samples from many bodies of water, we then attempted to successfully grow cyanobacteria. Since so little research had been done on this microorganism, it was difficult to find the proper growing conditions. Eventually, there was an immense amount of growth after a number of weeks. However, there was not enough cyanobacteria development to further our research.



Our Experimental Process

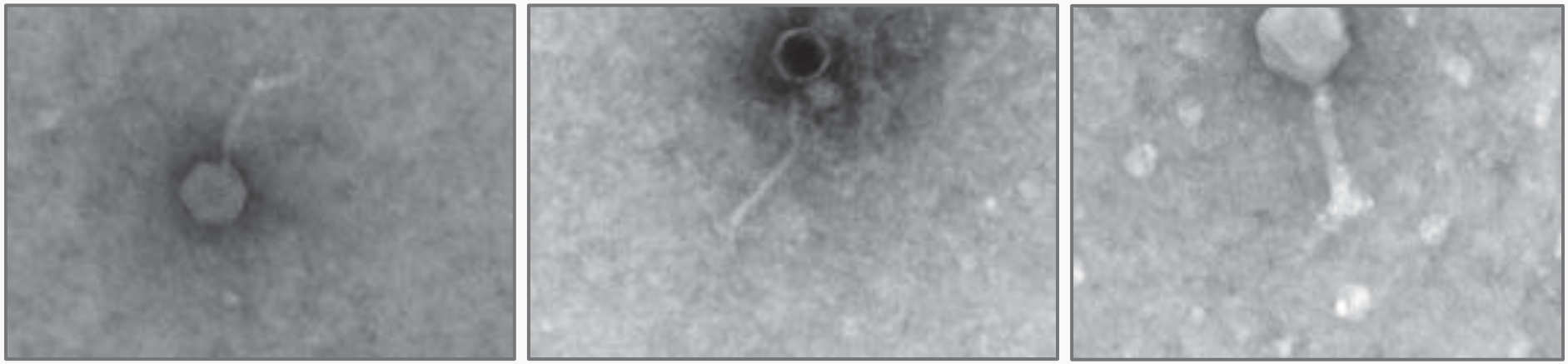
We completed our experiment over the course of several months. Taking time to collect water samples for experimentation with both cyanobacteria and E. coli was our first priority. All of our samples had to be plated and given ample time to grow, especially the cyanobacteria. All of the plates were kept in environments designed to imitate their natural habitats as closely as possible, in order to enhance and advance growth. Despite the fact that our cyanobacteria did grow, it did not develop as much as we needed in order to find a phage. However, our experience with E. coli was different, as we quickly advanced to the phage finding stage.

Phinding our Phages

After about two weeks of being in the lab, our E. coli had grown to the extent that we had the ability to look for phages. We met at the lab, compared the samples that looked most likely to be containing viruses, and took these plates to the electron microscope. The process of electron microscopy gave us the ability to literally see inside of our samples: including being able to see the bacteria cells and a total of eight bacteriophages. Being able to really see our samples and phages for the first time, as well as get true confirmation of what we had found through our experiment, was truly incredible.

Pictures from our Project

Phage Photos



The above pictures are some of the electron microscopy photos from our E. coli phages.

This is a picture from the process of plating the bacteria, with someone holding the plate. Note that gloves were always used when in contact with the bacteria for safety reasons.

This is a picture of the mini test tubes that our samples were eventually plated into.

This is a picture of the bacteria being transferred from the original plate into the little test tubes as part of the growth process.

This is a picture of the agar making process, which was done to prepare the plates for the plating of the bacteria.

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INSTITUTIONAL REVIEW BOARD

APPROVAL FORM

Student(s) User Name(s): TheDuck, spacecat03, ScienceStudent, microscientist

Grade: 9 **Team Advisor:** Lora Gibbons

Team Name: Phantastic Phage Phinders

Brief Description of Project:
Students will be working in a college lab with cyanobacteria and E. Coli isolating phages for research. Students will observe all safety protocol and obtain written parental permission in order to work in the lab environment. Bacteria will be tested, then discarded through an autoclave. Safety is of the highest priority with this group of students.

Team Advisor: Please sign here if the project proposed is a viable eCYBERMISSION Project in which neither animals nor humans will be harmed.

Team Advisor Approval Signature: Lora Gibbons **Date:** June 1, 2018

IRB Waiver of Written Informed Consent for Human or Animal Participation

The IRB may waive the requirement for documentation of written informed consent/assent/parental permission if the research involves **only minimal risk *and* anonymous data collection *and* if it is one of the following**: (NOTE: This statement only applies to providing the written certification mentioned in 1a or 2a above).

- Research involving normal educational practices.
- Research on individual or group behavior or characteristics of individuals where the researcher does not manipulate the subjects' behavior and the study does not involve more than minimal risk.
- Surveys, questionnaires, or activities that are determined by the IRB to involve perception, cognition, or game theory and do NOT involve gathering personal information, invasion of privacy or potential for emotional distress.
- Studies involving physical activity where the IRB determines that no more than minimal risk (Daily Activity) exists and

eCYBERMISSION Survey Approval Form**

eCYBERMISSION team name: Phantastic Phage Phinders

Team Advisor name: Lora Gibbons

Team Advisor email: lgibbons@mountainheightsacademy.org

Team Advisor phone: 801-896-3394

Student usernames: microscientist, ScienceStudent, Spacecate03, TheDuck

School name: Mountain Heights Academy

School address: 9067 1300 W #204, West Jordan, UT 84088

Describe the survey your team will conduct:
The team is seeking answers as to the general level of understanding of students about Utah's water health. The survey is very general and no data will be collected on the individual students. Just their level of understanding.

Describe the participants you plan to distribute your survey to:
This will be administered to various students in grades 7-9.

Project approved by school administration?
☑ Yes ☐ No

Approved by: Emily Andersen

Title: Middle School Principal

Date approved: 1/22/2019

Signature, School Administrator:

*Please have form completed, signed and dated BEFORE surveys are administered.

**As of August 2017, an IRB approval form (below) must be completed for all surveys as well as the information requested above.