Environmental Bacteriophage Presence in Drainage Ponds at Coastal Carolina University

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The purpose of this research is to detect naturally occurring, lytic bacteriophages and identify the environmental factors that influence their presence on Coastal Carolina University's campus. Bacteriophages are non-living viruses that only infect bacteria, and these viruses are found in abundance in every environment. Likewise, coliphages are viruses that exclusively infect coliform bacteria. Eleven treated and untreated freshwater sample sites were chosen for weekly sampling on CCU's campus. During water sample collection, ambient environmental conditions were measured. In addition, precipitation was tracked over the collection period as runoff relates to the proliferation of bacteriophages. If plaque assays indicated the presence of lytic bacteriophages or were "non-determinant," then the environmental samples underwent PCR (Polymerase Chain Reaction) identification. Over the collection period, coliphages were detected on CCU's campus and identified based on their genomic material. With the collected precipitation data and observed positive results, the correlation between environmental factors and the presence of bacteriophages on campus was determined, too.

Introduction

Bacteriophages are non-living viruses that infect bacteria, and they are found in abundance in every environment [1]. They outnumber any other entity on the planet, even bacteria. Importantly, they are a naturally created mechanism of bacterial control [1]. Bacteriophages were discovered by Frederick William Twort in 1915 and began to be studied for potential phage therapy by Félix d'Herelle in 1919 [2]. The initial stages of virology incidentally were stimulated through accidental discovery. For example, plated bacteria cultures were contaminated with bacteriophages creating zones of clearing where the viruses had infected the bacteria [2]. This accidental event encouraged research into the lytic behavior of certain bacteriophages [2]. Bacteriophages effectively can be divided into two categories—lytic and temperate [3]. Lytic bacteriophages, as opposed to temperate bacteriophages, are preferred for phage therapy as they effectively lyse and kill their bacterial host [4]. Lysis is initiated by the virus creating enzymes that disintegrate or form holes in their bacterial host's cellular membrane [3]. The evolutionary advantage of lytic activity is that it enables the replicated virions to be released from the host quickly and to be propelled toward neighboring bacterial cells for potential infection [3].

Because of the prevalence of and risks with antibiotic resistance, lytic bacteriophages have been studied for treating bacterial infections, like urinary tract infections (UTIs) caused by *Escherichia coli* and upper respiratory tract infections caused by *Streptococci* recently [5]. Antibiotic resistance refers to antibiotic pharmaceuticals not being able to effectively cure bacterial infections in the human body [6]. This phenomenon is caused directly by the misuse of antibiotics as naturally susceptible bacteria acquire resistance to the antibiotic through either random mutation and/or horizontal gene transfer. Then, the resistant bacteria are selected for while susceptible bacteria are targeted by the antibiotic [6]. Widespread antibiotic resistance poses serious dangers because people can acquire bacterial infections caused by those resistant bacteria. Untreatable infections coupled with the ineffectiveness of antibiotic pharmaceuticals will cause increased mortality rates [6]. For example, recently it was discovered that pathogenic *Chlamydia* can express resistant phenotypes and can acquire genes for resistance when placed under selective pressures [7]. Furthermore, MRSA (Methicillin-resistant *Staphylococcus aureus*) infections are endemic in hospitals and healthcare facilities in many parts of the world [8]. Luckily, bacteriophages are more efficient therapeutically than antibiotics as they are simultaneously very safe clinically and able to adapt to mutated bacteria quickly [5]. Furthermore, it is hypothesized that they could be effective in clearing up bacterial blooms in beaches because of their specificity, effectiveness, and harmlessness.

Coliphages are viruses that only infect coliform bacteria [1]. Coliform bacteria and coliphages originate in the gut, so they both enter the environment by animals, humans, and municipal sewage [9]. The discharge of wastewater treatment plant effluent is the largest source of coliforms and other enteric bacteria in the environment [10]. Additionally, rainfall events can carry and deposit enteric bacteria into water bodies and resuspend bacteria settled in benthic sediments, which encourages fecal contamination [11]. Bacteriophages, including coliphages, will be found in places in which their bacterial host is already present because they are specific to certain species of bacteria and do not pose a threat to bacteria that they are unrelated or dissimilar to [12]. Coliphages and other bacteriophages have been detected successfully on Coastal Carolina's campus and in the surrounding Myrtle Beach community for numerous years [13][14].

Temperature is the most crucial factor in the proliferation of bacteriophages. At low temperatures, fewer bacteriophages can penetrate bacterial host cells; therefore, replication is hindered [15]. Likewise, lower than optimal temperatures can affect their bacterial host negatively, too [9]. Because coliform bacteria, belonging to the *Enterobacteriaceae* family, originates in the gut, it survives best at temperatures like body temperature, ~ 98 °F [16]. Water temperatures greater 35 °F and pH ranges outside of 6-8 better encourage the proliferation of coliphages specifically [17]. So, it is hypothesized that the presence of bacteriophages will be observed in warm water temperatures and in acidic or basic pH ranges. Moreover, rainfall is a key factor in the understanding of bacteriophage presence. For example, during a rainfall event, bacteriophages that are present on land and in benthic sediments become suspended in the water column [18]. Here, they can interact with their bacterial hosts, cause new infections, and proliferate in abundance [18]. So, rainfall will be investigated as it relates to the presence of coliphages and other bacteriophages in this project, too.

The broader, environmental application for this ongoing research project is important to consider in addition to the introduction of bacteriophages. While human health treatment with bacteriophages continues to be developed, they can be used to treat environmental bacterial blooms, too. Beaches are especially important to the city of Myrtle Beach, state of South Carolina, and the southeastern United States in general. There have been countless instances of harmful bacterial blooms following events of rainfall that subsequently close the beaches for days at a time [19]. Obviously, this hurts the economy of cities that depend on tourism. Furthermore, it can put people and animals at risk for harm due to prolonged exposure [20]. Currently, there are no natural ways to address these bacterial blooms, but the purpose of this ongoing research is to identify naturally occurring, lytic bacteriophages that will infect, kill, and clear oceanic bacterial blooms. The descriptor "naturally occurring" is important to highlight in this statement. The objective is to isolate and to introduce lytic bacteriophages that already exist in the environment, so no environmental stress will be created. To better orient the importance of not inducing environmental stress, think of instances where non-native species have been introduced to an ecosystem for a particular purpose. It is possible for non-native species to outcompete native species for resources, and because they have no

predators, they become invasive quickly [21]. That phenomenon is trying to be avoided by locating a mechanism of bacterial control that is already found in abundance in the ecosystem already [22]. As mentioned previously, by determining the environmental conditions that indicate the presence and encourage the proliferation of coliphages, they can be better understood. Through the better understanding of coliphages, a natural solution can be found to suppress environmental bacterial blooms created by coliform bacteria.

Methods and Materials

Sample Sites

Eleven sample sites were chosen on CCU's main campus for weekly collection. These sample sites represent both treated (water has been treated with chemical to alter chemistry and microbial growth by maintenance staff) and untreated water bodies (water has had nothing added by maintenance staff). In Figure 1, an aerial overview of CCU's campus is shown with the labeled sample sites. The significant sample sites to note are the untreated ones. For example, Site 7 is a drainage area in a campus parking lot. It is unknown if the pond represented by Site 8 is being treated or not. Furthermore, Sites 9, 10, and 11 represent a drainage ditch running through the main campus. Aside from the sample sites, it was assumed that the water in the other sites received regular treatment from facilities and the CCU maintenance staff.

Sample Collection

At the eleven sites around the main campus, water samples were collected weekly with the chance of collecting bacteriophages. Approximately 15-50 mL of water were collected from each site using a painter's pole outfitted with a clamp. Also, pH levels and water temperature in °F were measured to establish the ambient, surrounding environmental conditions. Precipitation was tracked over time in inches of rainfall per day.

Sterile Sample Filtering and Viral Amplification

The water samples were filtered with a 0.45 µm filter to remove potential microbes and organic debris. Next, the samples were added to cultures of *E. coli B* or *E. coli K12* to amplify potential bacteriophages to a detectable level. Following the inoculation of *E. coli* bacteria with the experimental environmental samples, the amplified samples remained in a shaking incubator overnight at 37 °C.

Microbial Testing

Two methods were used to detect the presence of naturally occurring lytic bacteriophages. First, a microbial test was conducted to detect the presence of lytic bacteriophage. 2x LB agar plates served as a growth medium for the two different strains of *E. coli*. These plates were divided into four quadrants following the bacterial inoculation. Three quadrants received 5 µL of the amplified site-specific sample while the remaining quadrants received 5 µL of the control. The control was an amplified culture of T4 bacteriophages. Any present bacteriophages will lyse the growing bacteria and leave a zone of lysis, or clearing, that will not have any bacterial growth. This zone of clearing serves as a visual indication that lytic bacteriophages are present. However, if abnormal growth occurred on the plates, then the plaque assay was deemed "non-determinant." Abnormal growth can be due to microorganisms passing through during filtration (0.45 µm filter) and outcompeting any bacteriophages present in the amplified water samples.

Molecular Testing

Secondly, molecular testing was conducted. Samples positive for the presence of lytic bacteriophages or deemed to be "non-determinant" underwent crude DNA extraction to prepare for the molecular PCR (Polymerase Chain Reaction) test. 100 µL of the amplified site-specific sample was added into a microfuge tube and placed in a centrifuge. The samples were spun at the fastest possible speed for five minutes to separate the supernatant from the protein capsid. Approximately 95 µL

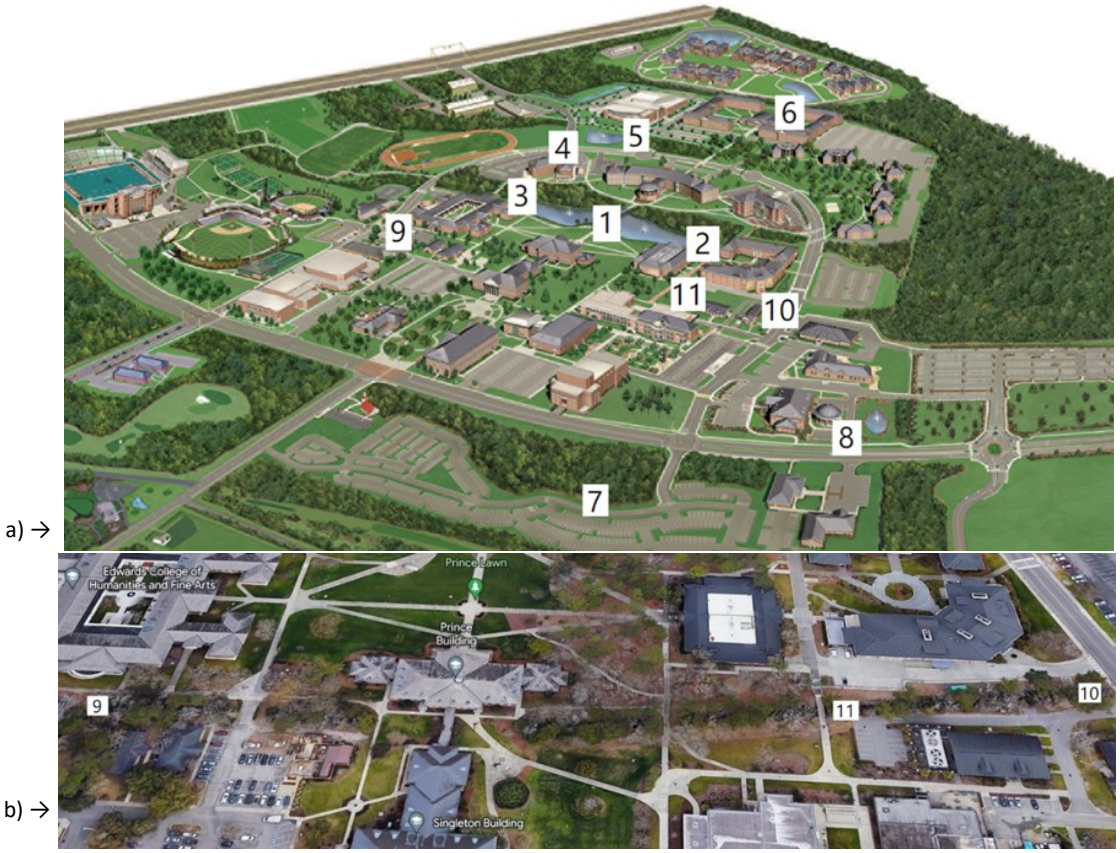

Figure 1: (a) This aerial illustration of CCU's main campus was labelled with the eleven sample site locations. Sites 1-6 were being treated by facilities and the CCU maintenance staff throughout sample collection. However, Sites 7, 9-11 were not treated. It is unknown if Site 8 was being treated actively during sample collection. **(b)** This aerial photograph shows Sites 9-11, an untreated drainage ditch that runs through the main campus.

of supernatant was added to a microcentrifuge tube with 5 µL of proteinase K. Proteinase K is an enzyme that further digests proteins and inactivates nucleases. The samples were put in a shaking incubator for 60 minutes at 37 ºC and then were placed on a heat block for 5 minutes at 95 ºC to deactivate the proteinase K and to encourage further lysing of the viral capsid.

Target viral genetic material was amplified using the three primer cocktails described in Tables 1, 2, and 3. CPA and CPB are primers that have been previously published, and CPO is a primer that Dr. Paul E. Richardson developed using bioinformatics analysis on the genomes of coliphages. The thermocycler used the program is delineated in Table 4.

Gel Electrophoresis

The amplified samples were run using gel electrophoresis on a 2% agarose gel with 3-5 µL of ethidium bromide added at 60 volts for 1.5 hours. A one hundred base pair (bp) DNA ladder (Promega) served as the reference for band sizes.

Statistical Analysis

To evaluate the variance among the observed environmental factors, mathematical computations were done. Specifically, linear regressions and single factor analyses of variance (ANOVA) were conducted to determine the variance in means and the presence or absence of correlative relationships. For the linear regressions, a regression coefficient, r^2, will describe the presence or absence of a correlative relationship. Moreover, for the single factor ANOVA, the variance of means will be reflected in a p-value with its alpha level, α, equaling 0.05.

Results and Discussion

First, the observed environmental factors were evaluated. There was no statistically significant difference between the average pH or average water temperature (°F) across the experimental sample sites (p-values > α). So, on any day of collection, the water temperatures and pH levels across each sample site were similar. From a linear regression, it was determined that precipitation had no correlation with the frequency of positive bacteriophage results ($r^2 \approx 0$). Also, water temperature (°F) had no correlation with the frequency of positive bacteriophage results ($r^2 \approx 0$). Similarly, pH did not have a correlation with the frequency of positive bacteriophage results neither ($r^2 \approx 0$). Although, water temperature (°F) and pH did vary significantly across the collection dates (p-values < α) as reflected in Figure 2.

In the combination graph comparing the water temperature (°F) with the presence of coliphages, there is a sharp peak in early November 2022. This spike in water temperature (°F) was due to abnormally warm air temperatures late October through early November. It is easy to recognize that water temperatures decreased as the seasons transitioned from late Summer to Fall, and to Winter. Also, by looking at Figure 2, it can be recognized that no bacteriophage presence was found during the Winter months. However, once water temperatures increased as Winter transitioned into Springtime, bacteriophages were detected again on campus. Furthermore, bacteriophages were detected in both neutral pH and acidic conditions as illustrated in Figure 2.

Secondly, this project sought to detect bacteriophages on campus successfully with microbial and molecular tests. A recurring presence of coliphages and other bacteriophages were seen at Sites 9, 10, and 11, which comprise an untreated drainage pond that runs through the main campus, as illustrated in Figure 3. As shown in Figure 1, these sample sites are different sites on the same drainage ditch, so they experience similar environmental influences, too. As mentioned earlier, it is unknown if Site 8 has treated or untreated water (campus maintenance was not certain when asked about this issue on treatment of the water at this site); however, it is important to recognize that Figure 3 shows that noticeably more bacteriophage positives were observed there. Coliphages were detected at Site 7, which is an untreated drainage area in a parking lot off the main campus. Bacteriophages were found at Sites 1, 5, and 6, which are all treated water bodies interestingly. In the history of this ongoing project, no coliphages or other bacteriophages had been detected in the treated ponds on campus. So, it is important to recognize

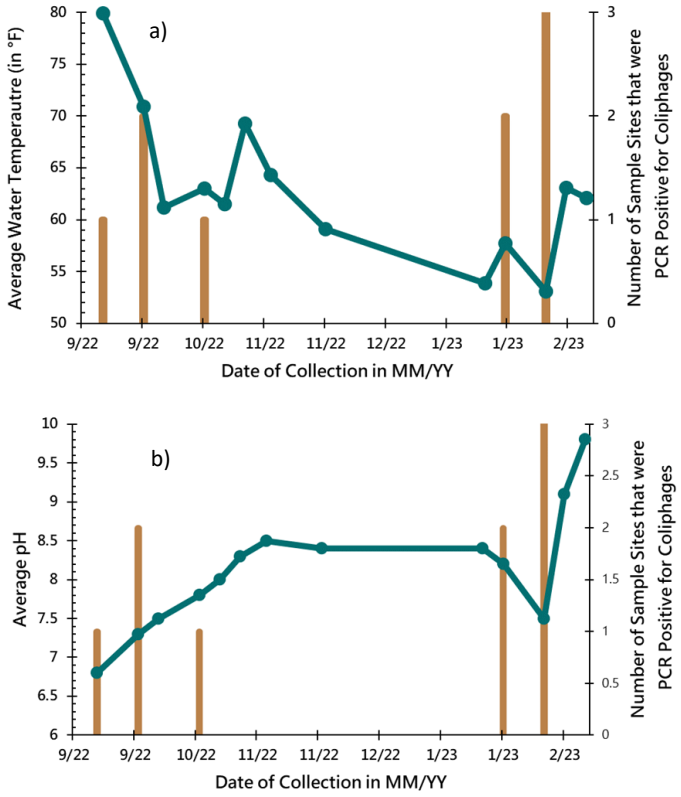

Figure 2: (a) This combination graph illustrates the change in water temperature, in °F, compared to the presence of coliphages over the collection period extending from September 2022 to February 2023. Water temperatures were at their highest in September and decreased as the seasons transitioned to Wintertime. In late October, there was a spike in water temperature, which was precipitated by abnormally warm weather. As expected, water temperatures increased in late January and climbed until the end of the collection period. As illustrated, coliphages were detected consistently in September and October 2022. However, during the Winter months, no coliphages were able to be detected at any sample site. Once the water temperatures began to increase, coliphage presence reemerged at the sites. Visually, this figure indicates a potential seasonality of coliphage presence on CCU's campus. This seasonality will be investigated further as this project continues this Summer and next school year. **(b)** This combination graph depicts the change in pH levels compared to the presence of coliphages over the collection period. Coliphages were detected on campus at neutral and slightly acidic pH levels.

that this is the first time that bacteriophages have successfully been detected both microbially and molecularly in treated water sources on the CCU campus.

On those tests that confirmed bacteriophage presence, the most notable result was the successful identification of one viral family, *Siphoviridae*, in the environmental samples. Sample sites positive for this viral family had varying visual indications on the plaque assays. For example, in Figures 5-7, the coliphages were outcompeted on the plaque assays by bacteria that were small enough to pass through the filter and resulted in "non-determinant" determination on these samples. However, in Figures 4 and 8, the plaque assays clearly have distinguishable zones of lysis. Reflected in Figures 9-12, sites PCR positive for *Siphoviridae* contained amplified DNA strands indicative of belonging to that family, which Primers CPA and/ or CPB and CPO detected. Also, it is important to recognize samples positive for *Siphoviridae* were collected at various points in the year but under similar environmental conditions. The *Siphoviridae* family was first detected on campus at Site 9 on October 18, 2022, as shown in Figure 9. Then, over the Winter, nobacteriophages were detected on CCU's campus. However, on January 30, 2023, the

Table 1: This table describes the CPA primer used for PCR identification. The targeted genomic sequence and viral family are included. For replication purposes, the commercial primer name is stated, too. Following gel electrophoresis, the PCR fragment size was referred to in order to determine the viral family the detected coliphage belonged to.

Viral Family	Sequence	Group	Primer Name	Primer Size	PCR Fragment Size
Siphoviridae	CACAGCGAGAAATTGATCGC CTAATCGGACTGATGTCTG	HK	HKsetFor HKsetRev	20 bp 19 bp	177
Podoviridae	GCAATACATCAAACGCCG GCGAATGCCAGCGGCG	933	933For 933Rev	18 bp 16 bp	488
Myoviridae	GATATTTGTGGCGTTCAGCC GTCAAATACACCAGCTTTAGAACC	T4	T4setFor T4setRev	20 bp 24 bp	704
Microviridae	GCTGCCGTCATTGCTTATTATGTT GYTAYCGBMMCATYAAYTAHTCACG	Micro	MicroFor MicroRev	25 bp 25 bp	1039
Podoviridae	TGGAAGCCCGTGAGAC GCAGCGTCAATCGCTCGG	K1F	K1FFor K1FRev	16 bp 18 bp	2110

Table 2: This table describes the CPB primer used for PCR identification. The targeted genomic sequence and viral family are included. For replication purposes, the commercial primer name is stated, as well. Following gel electrophoresis, the PCR fragment size was referred to in order to determine the viral family the detected coliphage belonged to.

Viral Family	Sequence	Group	Primer Name	Primer Size	PCR Fragment Size
Myoviridae	GAAAACGACTCAATCCTTGCC TCATCAGGTCTTTTGTTGTGG	Mu	MusetFor MusetRev	21 bp 20 bp	171
Siphoviridae	TGGGCGTACTTTATGGGGCG CGGACCTGCTGGGCAAAAAT	1	lambdaFor lambdaRev	20 bp 20 bp	307
Siphoviridae	GYGAYCAGATGGTTCC CAATRTCYTCYTARTTG	JK	JKsetFor JKsetRev	16 bp 16 bp	878
Podoviridae	GCACATGCAGAATAAGGTTG CCATTAGTAACACCATCTGC	N4	N4For N4Rev	20 bp 20 bp	2285

Table 3: This table describes the CPO primer used for PCR identification. The targeted genomic sequence and which virus it was derived from are included. The CPO primer detects two genes that are present in coliphages—the genes coding for either DNA polymerase or the major head protein, respectively. This primer was developed by Dr. Paul E. Richardson using bioinformatics in coliphage DNA. The base pair size was referred to following gel electrophoresis to determine if the detected bacteriophage was a coliphage.

Virus	Sequence	Gene Target	Primer Name	Primer Size	Base Pair Size
Coliphage T2/ T4	CCCTGCGCCTTTCATAATAA ATCGCAGGAACAGCTCCTAA	DNA Polymerase Orf 43	Orf43 For Orf43 Rev	20 bp 20 bp	198
Coliphage T2/ T4	TGGCGCAGTAACTCAGATTG GCACCAGCTTCCATTTGTTT	Major Head Protein Orf 23	Orf23 For Orf23 Rev	20 bp 20 bp	405

Table 4: This table describes the T4 program used during PCR amplification. Following the initial denaturation, the subsequent denaturing, annealing, and extension processes were repeated forty times to replicate the targeted genomic sequences sufficiently.

	Temperature	Time
Initial Denaturation	95 °C	4 minutes
Denaturation	94 °C	30 seconds
Annealing	55 °C	1 minute
Extension	72 °C	2 minutes

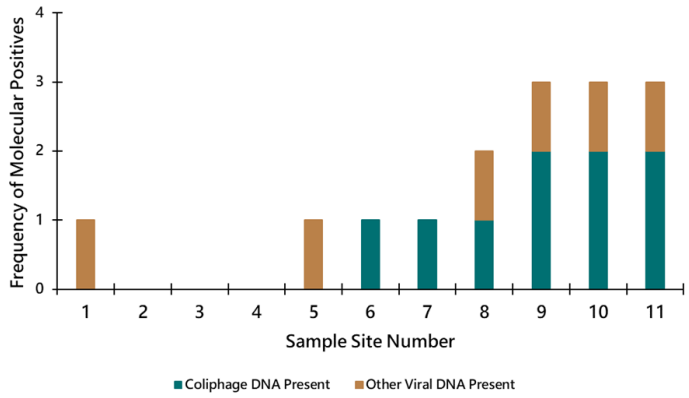

Figure 3: This bar graph illustrates the cumulative coliphage presence at each sample site across the collection period beginning in September 2022 and ending February 2023. The greatest frequency of coliphage presence was observed at Sites 9, 10, and 11, which constitute the untreated drainage ditch on campus. In the history of this research, no bacteriophages have been detected in the treated water bodies on campus. However, as pictured in this bar graph, coliphages and other bacteriophages were observed in Sites 1, 5, and 6.

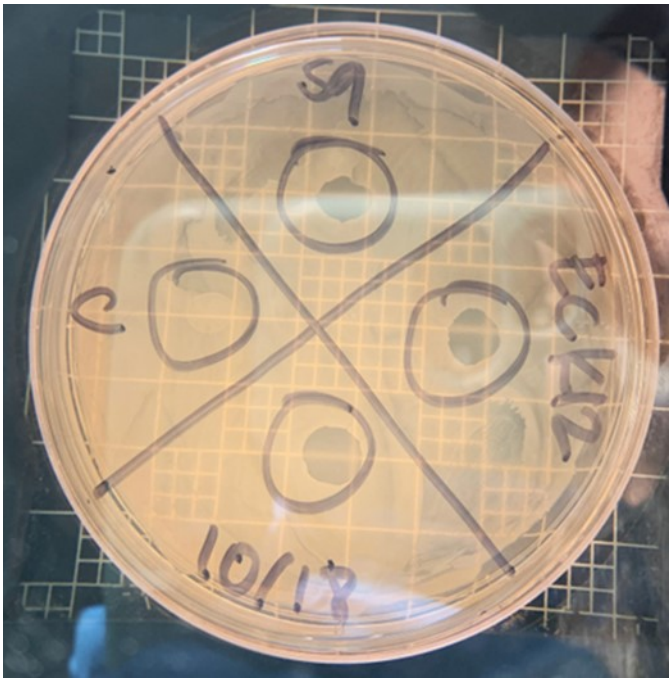

Figure 4: This plaque assay was conducted for a sample collected on October 18, 2022, from Site 9. There are zones of lysis—clearing caused by the lytic infection by bacteriophage in the cells of the plated *E. coli* culture—in the three experimental quadrants. The experimental quadrants are those containing information on the site-specific sample used. After being filtered, it was amplified with *E. coli K12*. The control quadrant noted by "C" appears as expected with no abnormal growth nor lysis. Because the control quadrant appears uniform and as expected, the plaque assay had been conducted correctly.

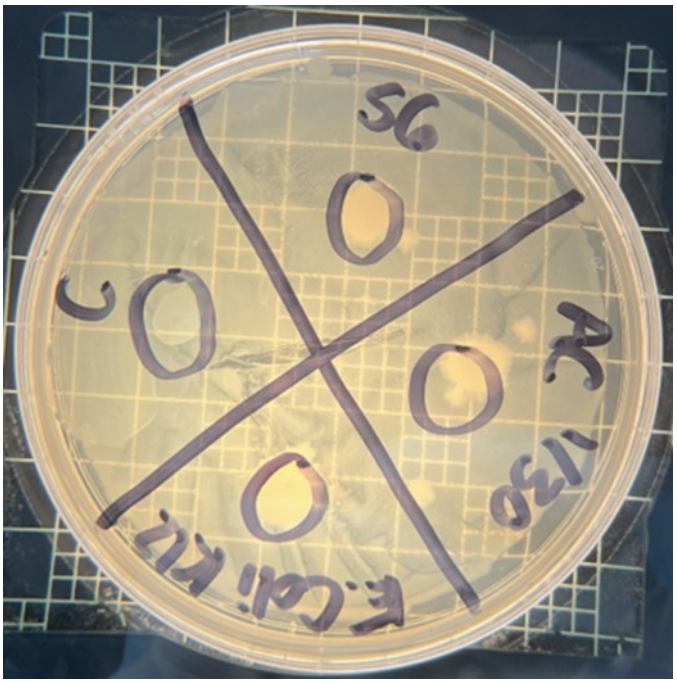

Figure 5: This plaque assay was from an environmental sample that was collected on January 30, 2023, from Site 6, and it was amplified using *E. coli K12*. In the experimental quadrants, there appears to be abnormal growth, so it was deemed to be "non-determinant." Perhaps, fungi or another organism outcompeted the present bacteriophage in the sample. However, the control quadrant appears uniform, so the plaque assay was completed correctly even though the experimental quadrants appear abnormal.

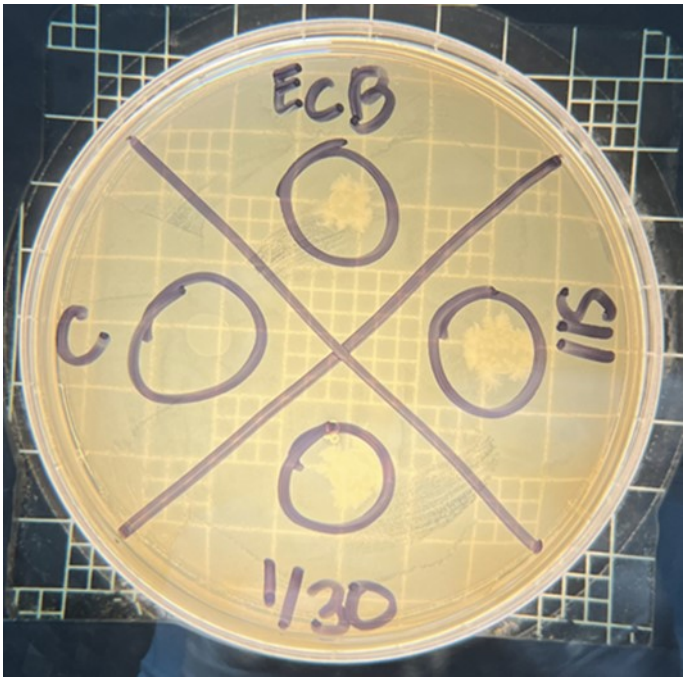

Figure 6: This is the plaque assay from a water sample taken on January 30, 2023, from Site 11 and amplified with *E. coli B*. There is unexpected growth in the control quadrants illustrating that the present bacteriophages were outcompeted on the plate. So, this plaque assay was deemed to be "non-determinant."

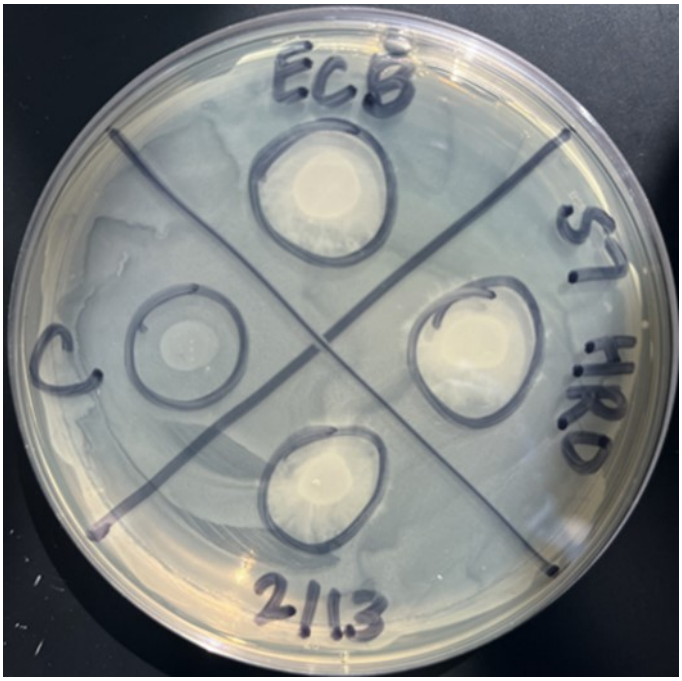

Figure 7: This plaque assay was conducted for a water sample collected on February 13, 2023, at Site 7 and amplified with *E. coli B*. There is unexpected growth in the control quadrants illustrating that the present bacteriophages were outcompeted on the plate. So, this plaque assay was deemed to be "non-determinant."

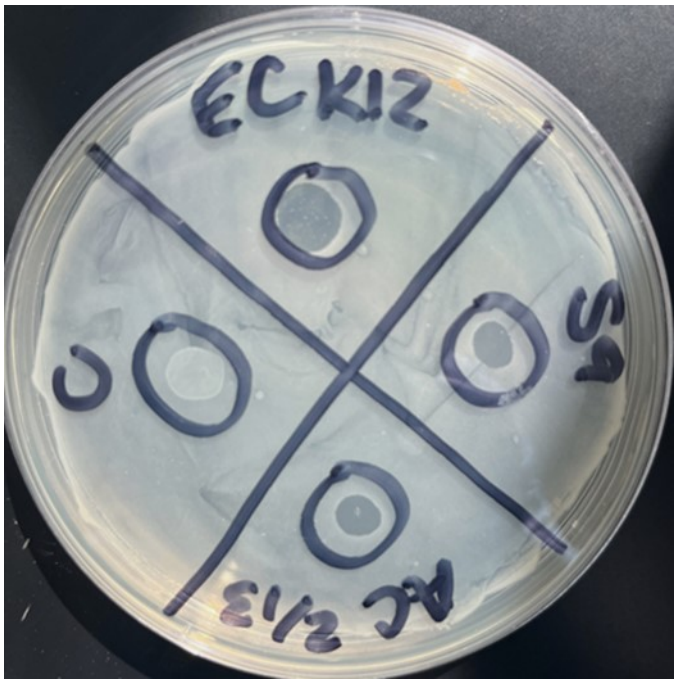

Figure 8: This plaque assay was done for a water sample taken on February 13, 2023, from Site 9 and amplified using *E. coli K12*. This plate has a clear zone of lysis in each of the three experimental quadrants. The bacteriophages present in the amplified environmental sample infected and lysed the growing coliform bacteria inoculated on the plate.

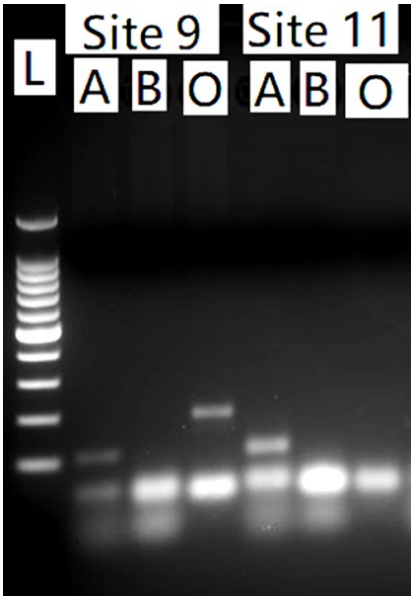

Figure 9: This is an image of the results from PCR testing run using Gel Electrophoresis. The "L" represents the one hundred bp ladder that was used for reference on the gels. The sample sites in which the samples were collected are noted at the top of the figures. Also, in this case "A," "B," and "O," represent the primer—CPA, CPB, or CPO—that was added to the PCR. In this gel, there are bands present in the second and fourth wells. From the CPA primer, there is a band present between the one hundred to two hundred bp range. Also, from the CPO primer, there is a band present in the two hundred bp size range. These bands match the bp sizes of coliphages from the *Siphoviridae* viral family shown in Table 1 and Table 3. Because of this, it is concluded that there is coliphage present in the sample from October 18, 2022, at Site 9 belonging to the *Siphoviridae* family.

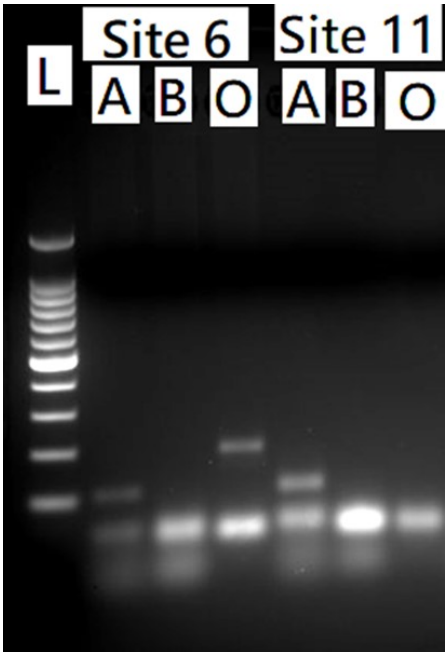

Figure 10: There are bands present in the second, fourth, fifth, and seventh wells. For Site 6, there is a band present by the CPA primer between the one hundred to two hundred bp range and a band present by the CPO primer at the two hundred bp size range. Identically, for Site 11, there is a band present by the CPA primer between the one hundred to two hundred bp range and a band present by the CPO primer at the two hundred bp size range, too. The bands indicate the presence of coliphages from the *Siphoviridae* family at those sites on January 30, 2023.

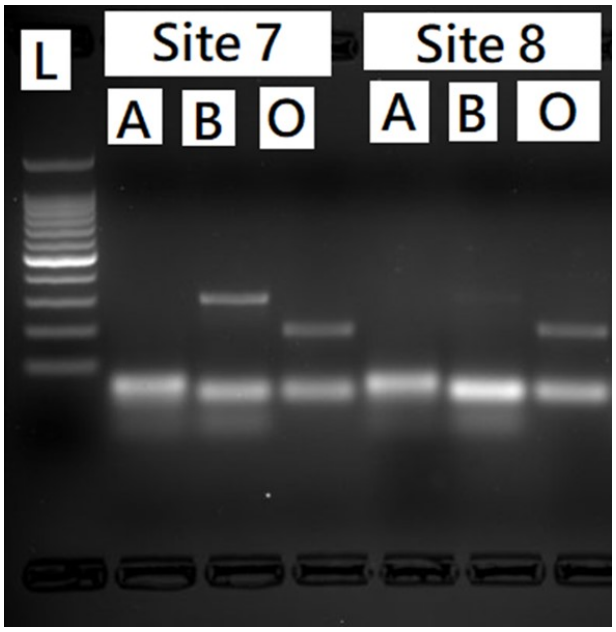

Figure 11: There are bands apparent in the third and fourth wells. By the CPB primer, there is a band present in the three hundred bp size range. Furthermore, with the CPO primer there is a band present at the two hundred bp size. Even though the CPA primer did not pick up viral sequences like those in Figure 9 and Figure 10, it can still be concluded that the viral family *Siphoviridae* is present again in the sample from Site 7 taken on February 13, 2023. Reflected in Table 2, the CPB primer indicates that viral family at a longer bp length.

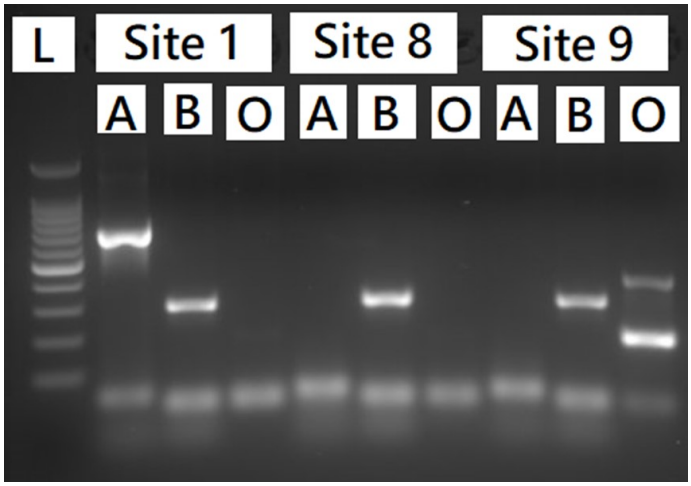

Figure 12: Figure 12 illustrates bands present in the ninth and tenth wells indicative of the *Siphoviridae* family. First, the CPB primer identified a viral sequence consisting of approximately three hundred bp. Second, the CPO primer found viral sequences of approximately two hundred bp and four hundred bp in length. These bp sizes represent the viral sequences present in coliphages and are represented in Table 2 and Table 3 again. This is a clear indication that this same viral family was also present at Site 9 on February 13, 2023.

Siphoviridae family was detected again at Sites 6 and 11, which is reflected in Figure 10. Again, this same viral family was detected on February 13, 2023, at Sites 7 and 9, as illustrated in Figure 11 and Figure 12, respectively.

This study's purpose was to detect naturally occurring coliphages and identify the environmental influences that encourage their presence. Coliphages and other viruses were successfully found and identified in many of the eleven sample sites. Of the detected coliphages, the viral family *Siphoviridae* was identified successfully by the molecular test repeatedly. This viral family was found at four sample sites across three separate collection dates across different seasons. Three of those four sample sites represent untreated drainage areas on CCU's campus—either the drainage ditch running through main campus or the drainage area in parking lot KK. Additionally, the plaque assays completed for sites visually differed. While a couple plates displayed appropriate results indicating lytic activity, most appeared to be "non-determinant," meaning something outcompeted the *E. coli* and bacteriophages and could not be detected by the plaque assay. Because this viral family was found in environmental samples across several collection dates, there must be a profuse source of its bacterial host present in proximity to the sample sites (I.e., all campus).

The ambient environmental conditions, measured by water temperature and pH, significantly fluctuated across the collection period. As pictured in Figure 2, the presence of bacteriophages is seasonal. In November through early January, no coliphages were identified in the sample sites by both a microbial and a molecular test. This supports the hypothesis that coliphages proliferate in warmer water temperatures [16]. However, the hypothesis that coliphages exist better outside of the 6-8 pH range was not supported by the results [17]. In both neutral and acidic environments, coliphages were found. It is important to recognize that most coliphages and other viruses existed in the untreated drainage ponds on CCU's campus. These environments are more likely to experience long-term, persistent pollution, so bacteria can grow uninterrupted, which naturally warrants the presence of bacteriophages.

Conclusion

In conclusion, the methodology for detecting bacteriophages outlined in this research project was found to identify the viral families of the environmental bacteriophages quickly and accurately. As this project is ongoing, it is crucial to continue to illustrate proof of concept. Furthermore, the results supported the background research described in the introduction. For example, the potential seasonality of bacteriophage presence substantiates their proliferation in warmer water temperatures rather than cooler. To further investigate this potential trend, long-term data collection and subsequent analysis will need to be conducted overtime throughout multiple seasons.

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Notes and References

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