EFFECT OF ENVIRONMENTAL FACTORS ON COLIPHAGE CONCENTRATIONS IN SURFACE WATERS

Brianna A. Young

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Approved by:

Jill R. Stewart

John F. Griffith

Rachel T. Noble

ABSTRACT

Brianna A. Young: Effect of Environmental Factors on Coliphage Concentrations In Surface Waters
(Under the direction of Jill R. Stewart)

Coliphages have been suggested as water quality indicators. The objective of this research was to evaluate effects of environmental factors on coliphage concentrations in San Diego surface waters using both field sampling of recreational waters and controlled mesocosm experiments. Water samples were collected from beach sites during rainfall and tidal events and analyzed for F+ and somatic coliphages, and from a controlled freshwater system in summer and winter. Regression models determined significance of coliphage concentration with different environmental factors. Coliphage concentrations were significantly affected by sample location, rainfall, water temperature, and season, but not by surf height, sea state, salinity, kelp coverage, tide height, wind speed, and turbidity ($\alpha = 0.05$). Potential coliphage die-off was observed along the San Diego River. This research informs how environmental factors affect coliphage concentrations and demonstrates timing and conditions for viral contamination of surface waters.

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CHAPTER 1. INTRODUCTION

Waters polluted with fecal contamination pose a health risk to humans. There are multiple sources of fecal contamination entering waterways, such as municipal sewage, domestic and wildlife feces, and soils (Hilton and Stotzky 1973, Santo Domingo and Edge 2010). Exposure occurs through recreational activities as well as consuming fish and shellfish. Fecally-contaminated waters have been seen to cause gastrointestinal illness, respiratory health effects, and other health effects at higher rates in swimmers than non-swimmers of polluted marine and freshwater environments (Cabelli 1983, Cabelli et al. 1979, Cabelli et al. 1982, Calderon et al. 1991, Marion et al. 2010, Prüss 1998, Soller et al. 2010, Wade et al. 2006, Wade et al. 2003, Wade et al. 2010). In addition, associations have been observed between illness and increasing densities of *Enterococci* and *Eschericha coli* in swimmers (Dufour 1984, Marion et al. 2010, Wade et al. 2003).

Water quality regulation in the United States began in 1948 with the passage of the Water Pollution Control Act by Congress (EPA 2014). Over the next several decades, additional acts were passed mandating changes to water quality, until the passage of the Clean Water Act in 1972 established measures to control water pollution and required states to create standards that regulated pollutant limits (EPA 2014). In 1974, public drinking water supply regulations were first mandated by Congress with the Safe Drinking Water Act (EPA 2014), and in 1986, ambient water quality criteria standards were set for coastal recreational waters (EPA 1986). Both of these measures reduced the general population's risk of becoming sick due to pathogens in their drinking water and in recreational waters the public bathes in, such as beaches. In 2006, the Ground Water Rule was passed to reduce disease incidence caused by microorganisms in drinking water from groundwater supplies (EPA 2015b). The GWR was the first rule to incorporate coliphages as a microbial indicator of fecal contamination in drinking water, thereby using them as a measure of water quality.

Fecal indicator bacteria (FIB), such as coliform and *Enterococcus* bacteria, are indicators traditionally used to determine water quality. However, FIB may not be adequately protective for all pathogens that may be in recreational waters, especially viruses. This issue proves problematic for public health officials who use only FIB when trying to determine when water is safe for human consumption and contact. Recent research has suggested that the majority of waterborne illnesses are likely caused by viruses (Santo Domingo and Edge 2010, Soller et al. 2010). Human enteric viruses may be transmitted through water and can cause a variety of health effects, such as gastroenteritis, meningitis, enterovirus, and hepatitis (Bosch 1998, EPA 2013). Coliphages are viruses that infect *E. coli* and have been proposed as an alternative indicator of viral pathogens in recreational water, but most municipalities do not to test for coliphages in recreational waters. Recent studies have documented associations between coliphages and health outcomes, such as nausea, gastrointestinal illnesses, fever, and cough (Abdelzaher et al. 2011, Colford Jr et al. 2007, Griffith et al. 2016).

Although coliphages are a promising alternative water quality indicator, it is currently not well studied how certain environmental factors may affect coliphage occurrence. Recent research has suggested that parameters such as temperature, pH, salinity, and sunlight can affect the incidence and survival of coliphages. Feng et al. (2003) observed that higher temperatures had higher inactivation rates and that a pH of 6 to 9 had lower inactivation rates. Jonczyk et al. (2011) found that temperature determines the occurrence, viability, and storage of bacteriophages, and that salinity affects bacteriophages as well. Sinton et al. (1999) concluded that somatic coliphages were more persistent in sunlit seawater than fecal coliforms, enterococci, and F-RNA phages. Sinton et al. (2002) observed that with higher salinity, sunlight inactivation increased of sewage F-RNA phages.

Coliphages need more study to understand their frequency of detection and potential as water quality indicators. The objective of this research is to determine the presence and persistence of coliphages in surface water. First we examined how environmental factors affect coliphage concentrations in beach water over a three month study period. Six sites were chosen in San Diego, California and tested at different tidal stages and during rainfall events. We hypothesized that rainfall

events and low tides would increase the presence of coliphages in recreational waters. Then we sampled further upstream in the same San Diego watershed to determine potential locations where coliphages may enter the San Diego River. Finally, we conducted a controlled experiment of coliphage die-off in surface water mesocosms, and compared coliphage inactivation with coliforms. This research will provide more information on how environmental parameters affect coliphage concentrations in surface waters. This research may also help inform local, state, and federal governments on whether coliphages would be an appropriate water quality indicator in addition to traditional FIB.

CHAPTER 2. OBJECTIVES

1. Determine how environmental factors, specifically rainfall and tidal events, affect coliphage concentrations in marine environments over a three month study period.
2. Determine potential locations of where coliphages may enter the San Diego River to determine potential sources of pollution.
3. Determine the occurrence and persistence of coliphages and traditional fecal indicator bacteria in mesocosms under controlled conditions.

CHAPTER 3. REVIEW OF THE LITERATURE

i. What is Water Quality?

The assessment of water quality requires the ability to detect microbial pollutants. Water should not be an infection risk or have unsafe levels of hazardous chemicals, and should be aesthetically acceptable to consumers (Payment et al. 2003). Presenting water to consumers that is free of microbial pollutants is important as many microbes can make a population ill. Worldwide it is estimated that 3.4 million people die due to water-related diseases each year (WHO 2001). According to the CDC, the incidence of outbreaks associated with treated and untreated recreational waters has been increasing over the last several decades, and in the United States from 2011 to 2012, there were 90 recreational water-associated outbreaks reported by 32 states and one territory (Hlavsa et al. 2015). The highest number of outbreaks occurred from June through August. This resulted in at least 1,788 cases, 95 hospitalizations, and one death. In 77% of these outbreaks, the etiology was confirmed to be infectious pathogens.

Throughout history, fecally-contaminated waters have caused illness in the human population. Gastrointestinal illness, respiratory health effects, and disabling health effects have been seen at higher rates in swimmers than non-swimmers of polluted marine and freshwater environments (Cabelli 1983, Cabelli et al. 1979, Calderon et al. 1991, Marion et al. 2010). In addition, associations have been observed between illness and increasing densities of *Enterococci* and *Eschericha coli* (*E. coli*) in swimmers (Dufour 1984, Marion et al. 2010). There are multiple sources of fecal contamination entering waterways, such as municipal sewage, domestic and wildlife feces, and soils (Hilton and Stotzky 1973, Santo Domingo and Edge 2010). Municipal sewage is the primary source of coliforms entering rivers and estuaries, but can also come from animal feces, soil runoff, and kitchen and laundry wastes (Hilton and Stotzky 1973). Human enteric viruses may be transmitted

through water, and can cause a variety of health effects, such as gastroenteritis, meningitis, enterovirus, and hepatitis (Bosch 1998, EPA 2013).

According to the CDC, the top 10 causes of outbreaks in public water systems are *Giardia*, *Legionella*, Norovirus, *Shigella*, *Campylobacter*, copper, *Salmonella*, Hepatitis A, *Cryptosporidium*, and a tie between *E. coli* and excess fluoride (CDC 2015b), and the top 10 causes of outbreaks in untreated recreational water systems are *Cryptosporidium*, norovirus, *E. coli*, algal bloom, *Giardia*, *Shigella*, *Campylobacter*, Avian schistosomes, *Leptospira*, and *Plesiomonas* (CDC 2015a).

There is a documented history of associations between fecally-contaminated waters and illness, particularly gastrointestinal distress. These illnesses have been seen in both coastal marine (saltwater) environments as well as freshwater environments. One area of concern for exposure is through contact in marine and fresh recreational waters (also known as bathing waters). In marine environments, Ferley et al. (1989) found a relationship between morbidity and microbiological contamination of bathing water, as well as a predominance of gastrointestinal morbidity, whereas other illnesses, such as respiratory tract, skin and ear infections were less frequent. Cabelli et al. (1979) found higher rates of gastrointestinal, respiratory, and disabling health effects in swimmers compared to non-swimmers in sewage-contaminated water. In addition, it has been observed that swimmers in municipal wastewater-impacted marine waters and polluted beaches had an increased risk of gastrointestinal illness related to the water quality indexed by the mean enterococcus density than non-swimmers (Cabelli 1983, Cabelli et al. 1982). In freshwater recreational waters, Dufour (1984) found a direct linear relationship between swimming-associated gastrointestinal illness and bacterial densities of enterococci and *Eschericha coli* (*E. coli*) in the bathing water. In addition, Dufour (1984) stated that the criteria developed for marine water cannot be applied to fresh water, as it was observed that at equivalent indicator densities, marine swimmers had an illness rate about three times greater compared to that in freshwater swimmers. Marion et al. (2010) found an increased risk of gastrointestinal illness in swimmers compared to non-swimmers, as well as an increased risk of gastrointestinal illness when swimmers were exposed to increasing densities of *E. coli*. Calderon et al.

(1991) looked at non-point source pollution (fecal pollution came from animals) and found that on dry days, the density of the fecal bacteria was significantly less than after rain events. In addition, Calderon et al. (1991) found that the symptomatic gastrointestinal illness rate in swimmers was significantly higher than non-swimmers. In general, inland waters contain more rural areas and have more agricultural land use, therefore they are more likely to be affected by wildlife and livestock (Dorevitch et al. 2010).

Due to the wide range of types of enteric viruses, they can cause a variety of health effects, such as gastroenteritis and meningitis (EPA 2013). A wide range of human enteric viruses may be waterborne transmitted, such as enterovirus, rotavirus, parvovirus (Bosch 1998).

ii. The History of Water Quality Regulations

The American Public Health Association's Committee on Bathing Beaches released a report in 1924 stating that there was not enough evidence to develop bathing water standards for natural waters (Simons Jr. et al. 1924). In 1933, the APHA again did not set any microbial standards due to a lack of epidemiological information (Ferguson et al. 1933), and in 1957, they found that there was little reliable data to implicate bathing places as a source of disease spread (Dufour and Schaub (2007), referencing American Public Health Association (1957) *Recommended Practice for Design, Equipment and Operation of Swimming Pools and Other Public Bathing Places*. American Public Health Association, Washington D.C.)

However, in 1948, Congress passed the Water Pollution Control Act, which was the first comprehensive legislation for water pollution control (EPA 2014). Congress passed additional acts in the following decades: the Federal Water Pollution Control Act of 1956 and the Water Quality Act of 1965. In 1972, the Federal Water Pollution Control Act Amendments, or better known as the Clean Water Act, was passed by Congress, and established water quality and technology based approaches to controlling water pollution, established measures to control water pollution and required states to create standards that regulated pollutant limits (EPA 2014). In 1974, public drinking water supply regulations were mandated by Congress with the Safe Drinking Water Act (EPA 2004, 2015a) to

reduce the general population's risk of becoming sick due to pathogens in their drinking water. The SDWA was amended in 1986, and again in 1996. The 1996 SDWA amendments required EPA to consider detailed risk assessment and cost assessment when developing the standards, in addition to enhancing source water protection, and recognizing the importance of funding for water system improvements, operator training, and public information (EPA 2004). This law allows the EPA to protect against naturally-occurring, as well as man-made, contaminants found in drinking water by setting health-based standards. In addition, actions are required to protect drinking water and its sources (rivers, springs, reservoirs, lakes, and groundwater wells) (EPA 2004). In 1987, Congress passed the Water Quality Act of 1987 after states had not adopted toxics standards.

In 1986, the EPA set ambient water quality criteria standards for coastal recreational waters (EPA 1986). These standards were meant to protect the public from the risk of waterborne illness in recreational waters due to pathogens. However, many states had not adopted the EPA's suggested bacteria regulations for recreational coastal waters by 2000, or regulations that were as protective as the EPA's. Based on this lack of action, and to ensure the entire U.S. population had protection from pathogens in recreational waters, Congress passed the Beaches Environmental Assessment and Coastal Health (BEACH) Act in 2000, which was meant to encourage states to either adopt the regulations recommended by the EPA, adopt regulations that are as protective as the recommendation, or modify the EPA's recommended criteria to reflect site-specific conditions (Congress 2000). If states did not create their own regulations by 2004, the EPA would then enforce the federal standards in those states. In 2012, the EPA updated the recreational water quality criteria (EPA 2012a, b), which again focused on coastal waters, but were applied to inland recreational waters as well. When indicator bacteria concentrations exceed regulatory standards, the recreational water body is either closed or the public is warned of the potential for illness if they choose to enter the water.

In 2006, the Ground Water Rule was signed, and was applied to all systems that use ground water as a source of drinking water (EPA 2015b). It was meant to reduce disease incidence caused by

microorganisms in drinking water from groundwater supplies (EPA 2015b). The Ground Water Rule established a risk-based approach to identify ground water systems vulnerable to fecal contamination, and require them to take corrective action before illness occurred from exposure to microbial pathogens. The Ground Water Rule was the first rule to incorporate coliphages as a microbial indicator of fecal contamination in drinking water, thereby using them as a measure of water quality.

iii. How Water Quality is Measured

Water quality is measured using indicators, as it is not feasible to test for all potential pathogens that could be present in a water sample. An indicator should be present when the contamination source is present, absent when the water is unpolluted, be easily isolated and enumerated, respond to treatment processes in a similar manner as pathogens, and be inexpensive (Medema et al. 2003). Additionally, an indicator should be applicable to all types of water, have constant characteristics, have a dose-response relationship (numbers present are associated with the risk of enteric illness or the amount of contamination), and should be in a greater quantity than the number of pathogens (Goyal 1983).

An indicator should not be pathogenic (i.e. it should be harmless to humans and other animals), present in a smaller number or persist for a lesser time than the pathogen, or able to multiply in the environment (no aftergrowth or regrowth) (Goyal 1983, Medema et al. 2003).

iv. Current Techniques to Measure Water Quality

Currently, traditional fecal indicator bacteria are used to measure water quality. Membrane filtration methods are approved by the U.S. EPA for *Escherichia coli* and Enterococcus. The membrane filtration method for *E. coli* filters a water sample through a membrane, the membrane is placed on a selective and differential medium, incubated for 2 hours at 35°C to allow injured or stressed bacteria to be resuscitated, then incubated for 22 hours at 44.5°C. After incubation, the number of red and magenta colonies are recorded as *E. coli* colonies. However, any other equivalent method may be used as well that measures culturable *E. coli* (EPA 2009b). The membrane filtration method for *Enterococci* filters a water sample through a membrane, the membrane is placed on a

selective medium, then incubated for 24 hours at 41°C. Colonies with a diameter greater than or equal to 0.5 mm and a blue halo are recorded as enterococci colonies. However, any other equivalent method may be used that measures culturable enterococci (EPA 2009a). Other common methods include multiple fermentation tubes and biochemical assays.

There are two current U.S. EPA recommendations for standards for *E. coli* and Enterococci using the membrane filtration methods (EPA 2012a). The first recommendation has an estimated illness rate of 36 per 1,000 people, given a geometric mean (GM) of 35 cfu/100 mL and a statistical threshold value (STV) of 130 cfu/100 mL of Enterococci in marine and freshwaters, or a GM of 126 cfu/100 mL and a STV of 410 cfu/100 mL of *E. coli* in freshwater. The second recommendation has an illness rate of 32 per 1,000 people, given a GM of 30 cfu/100mL and STV of 110 cfu/100 mL of Enterococci in marine and freshwater, or a GM of 100 cfu/100 mL and a STV of 320 cfu/100 mL for *E. coli* in freshwater.

However, it has been indicated that the general public is still getting sick when the bacterial levels are below the set standards. This could be indicative that there are pathogens other than bacteria present in the water, such as viruses, and that the current bacterial indicators cannot account for viruses in the water, or viruses persist differently than bacteria in waters (Medema et al. 2003). One option to address this issue is to incorporate viral indicators, such as coliphages, into water quality testing parameters, in addition to the current bacterial indicators.

v. Viral Indicators

Viral indicators currently utilized include bacteriophages, which are viruses that infect bacteria. Bacteriophages are metabolically inert in the virion form and reproduce using the host's metabolism (ASM 2015). This reproduction occurs when the bacteriophages genome is inserted into the bacteria's cytoplasm, and the bacteria's cellular machinery is used to produce more virions. The host cell is then either lysed or a coexistence occurs in which harmful genes are not expressed but a small set of beneficial genes to the host are expressed (ASM 2015).

A specific group of these bacteriophages include coliphages, which are viruses that infect *E. coli*. Two types of coliphages are male-specific coliphages (F-specific RNA bacteriophages) and somatic coliphages, both of which are found in human and animal fecal waste. The coliphage structure consists of a head made of a protein coat surrounding the DNA or RNA, a tail, and tail fibers. There are seven classes of coliphages. Two specific classes are Class II and Class IV. Class II coliphages have a single-stranded DNA genome, and an example is ΦX174, a viral strain used in this research. Class IV coliphages have a single-stranded RNA genome, and an example is MS2, a viral strain used in this research. Male-specific coliphages infect a host *E. coli* through the F- or sex pili, and somatic coliphages infect a host *E. coli* cell through cell wall receptors (Payment et al. 2003). Male-specific coliphages can contain either RNA or DNA, are similar to human enteric viruses since they can be single-stranded RNA surrounded by a protein coat, and there are four major subgroups (Payment et al. 2003).

Male-specific coliphages are useful as in indicator of sewage contamination and treatment efficiency for groundwater since they are found in large amounts in sewage, they have a relatively high persistence like enteric viruses, and they are similar to enteric viruses in size, shape, and composition (Payment et al. 2003).

Coliphages have been suggested as an alternative indicator of water quality. However, there is conflicting evidence in the scientific literature surrounding whether there is an association between coliphages and the presence of enteric viruses. Some studies have reported an association between the presence of coliphages and human viruses (Ballester et al. 2005, Havelaar et al. 1993), while other studies have found no association between the presence of coliphages and human viruses (Jiang et al. 2007, Viau et al. 2011).

Havelaar et al. (1993) found that enteric virus concentrations may be predicted from FRNA phage data as the phages were significantly correlated with enteric virus in five of the eight water types and with enterovirus in four of six water types. Havelaar et al. (1993) concluded that FRNA phages are model organisms for human viruses, making them a suitable alternative for detection of

viruses in recreational waters. Ballester et al. (2005) found that enteric viruses were significantly correlated with male-specific coliphages and somatic coliphages, but not indicator bacteria. In addition, they found that coliphages were significantly correlated with one another and the presence of adenovirus, and male-specific coliphages were significantly correlated with the presence of rotavirus and enterovirus.

However, Jiang et al. (2007) found that although F+ coliphages had temporal and spatial distribution similar to FIB, the occurrence of viral genome detection was not correlated with F+ coliphage or FIB. They suggested that the detection of human viruses depended on distribution patterns that were opposite of coliphages and FIB. Viau et al. (2011) did not find an association of F+ coliphages and virus occurrence for adenovirus and norovirus, and salmonella and campylobacter.

Additionally, there is inconsistent evidence in the literature regarding whether coliphages could be used as water quality indicators. Lee et al. (1997) found that there was a relationship between illness incidence and the count of F-specific bacteriophages after the use of recreational freshwater. The authors concluded that F-specific RNA bacteriophages could be an indicator of risk. However, van Asperen et al. (1998) did not observe an exposure response relationship with enteroviruses, faecal streptococci, and F-specific RNA bacteriophages.

CHAPTER 4. MATERIALS AND METHODS

i. Beach Water Study

IV.i.1. Sampling Sites

Water samples were collected in southern California by SCCWRP and shipped to the University of North Carolina for coliphage analysis. In the beach water study, water samples were collected from six beach sites during tidal events and eight sites during rainfall events. Figures 1 and 2 depict the sampling locations of the beach water study conducted in San Diego.

Figure 1. Sampling sites in Tourmaline Surfing Park in San Diego. Courtesy of SCCWRP.

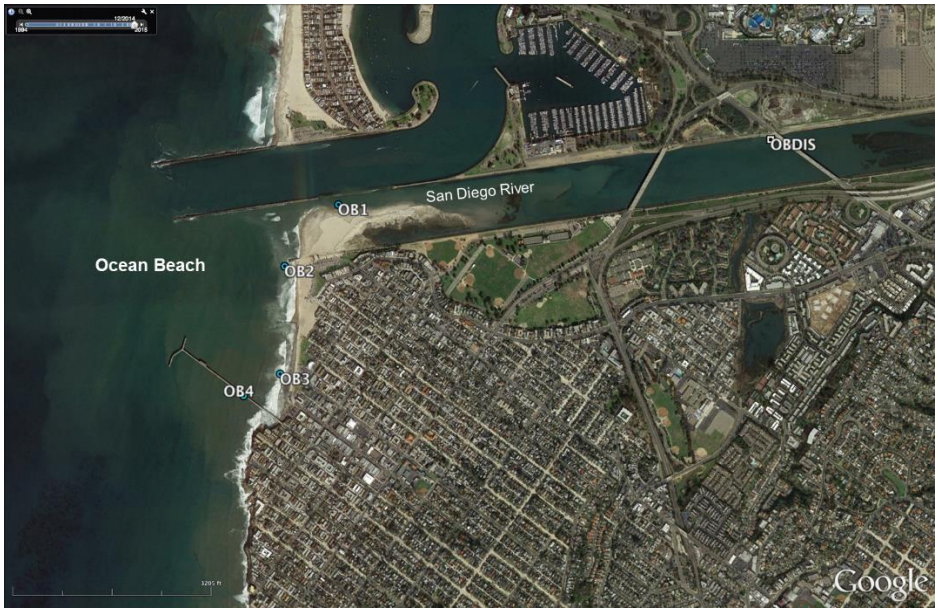

Figure 2. Sampling sites in Ocean Beach in San Diego. Courtesy of SCCWRP.

IV.i.2. *Field sampling*

Water samples were taken from two locations in San Diego, California: Ocean Beach (adjacent to the San Diego River) and Tourmaline Surfing Park (adjacent to Tourmaline Creek). Samples were collected during the wet season (January through March 2015). From these two locations, six beach sites were sampled during tidal events, and two additional discharge sites were sampled during rainfall events.

Samples were collected between 7:00am and 9:00am. During extreme tidal events, samples were collected the day before, the day of, and the day after the tidal event. During rainfall events, samples were collected the initial day of rainfall and the three following days, or as long as the discharge site had water flow. Samples were collected at ankle depth at Tourmaline Surfing Park (sites FM030 and Tourmaline South) and Ocean Beach (sites FM010, PL110, and PL100). An additional sample was taken at the Ocean Beach pier near the surfer lineup. Following wet weather, additional samples were collected from discharge sites at Tourmaline Creek and San Diego River. Wet weather was determined using rainfall and channel flow data from rain gauges and flow meters placed in

Tourmaline Creek and San Diego River, as well as information from the USGS flow gauges on the San Diego River.

Rainfall and flow sensors, and programmable peristaltic pumps were used during rainfall events to collect composite samples from Tourmaline Creek and San Diego River. Sampling occurred when either the water flow increased by 10% or the pump intake was covered. Flow-weighted 20 L composite samples were collected from 6 hours after the first sample was collected. If the storm surge continued for more than 6 hours, a second 20 L composite sample was collected until either the storm surge returned to within 10% of baseline flow or 12 hours after sampling began.

From these composite samples, 1 L samples of water were shipped to UNC-Chapel Hill overnight for coliphage analysis in coolers containing ice packets for proper storage conditions. Over weekends, samples were kept refrigerated at 4 ^{0}C until the samples could be shipped. We analyzed 480 samples for F+ and somatic coliphage analysis. This included two storm events, six extreme high tide events, and six extreme low tide events from January 5, 2015 through March 30, 2015. Surf height, sea state, wind, current, water color, water temperature (^{0}C), salinity (ppt), kelp coverage (%), turbidity, and the number of surfers, dogs, birds, and sea lions, were recorded as well.

IV.i.3. EPA Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure

Somatic and F+ analysis was performed based on the EPA Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure (EPA 2001a). This method was chosen because previous research has suggested a potential association between coliphages measured by this method and health outcomes at marine beaches (Griffith et al. 2016).

IV.i.3.1. 1X Tryptic Soy Broth (TSB)

A 1X TSB concentration was made when needed throughout the study for overnight *E. coli* host and log-phase *E. coli* host. This was prepared by adding 30g of TSB powder per 1000mL deionized (DI) water. The bottle was then autoclaved for 15-25 minutes and cooled to room temperature or colder before use.

IV.i.3.2. Antibiotics

When needed throughout the study, antibiotics were made. The 100X nalidixic acid antibiotics were made by adding 1g of nalidixic acid per 100 mL of sterile DI water, vortexed or mixed by hand until the power completely dissolved, and filter sterilized using a sterile 60mL syringe and 0.22 µm filter. The 100X streptomycin/ampicillin (strep/amp) antibiotics were made by adding 0.15 g of streptomycin sodium salt and 0.15 g of ampicillin sodium salt per 100 mL of sterile DI water, vortexed or mixed by hand until the powder completely dissolved, and filter sterilized using a sterile 60mL syringe and 0.22 µm filter.

IV.i.3.3. Phosphate Buffer Solution (PBS)

Phosphate buffer solution was made by adding 1 phosphate buffer solution tablet per 200mL of DI water, mixing thoroughly, and autoclaving for 15-25 minutes.

IV.i.3.4. 4M Magnesium Chloride ($MgCl_2$)

When needed, 4M $MgCl_2$ was made by adding 814g of magnesium chloride hexahydrate to 1000mL of deionized (DI) water. This was then placed in the autoclave for 15-25 minutes.

IV.i.3.5. Overnight E. coli *F_{amp} and CN-13 hosts*

Fresh overnight *E. coli* F_{amp} and CN-13 hosts were made each night before sample analysis. One flask was made for each host. The overnight F_{amp} host was made by adding 25mL of 1X TSB into a sterile 100mL flask, 0.25mL strep/amp antibiotic, and a scraping of frozen F_{amp} E. coli. The overnight CN-13 host was made by adding 25mL of 1X TSB into a sterile 100mL flask, 0.25mL nalidixic acid antibiotic, and a scraping of frozen CN-13 E. coli. The flasks were placed on a shaker plate in a walk-in incubator overnight for 18-24 hours, and were loose-capped to allow oxygen to enter bottles.

IV.i.3.6. E. coli *log-phase host*

The F_{amp} log-phase host was made by using 10mL of 1X TSB per 100mL of sample, as 10 mL of log-phase *E. coli* host was needed per 100mL sample. Based on the volume used, 1% of the strep/amp antibiotic was added to the total volume, as well as 1% of the overnight F_{amp} *E. coli* host. The CN-13 log-phase host was made by using 10mL of 1X TSB per 100mL of sample, as 10 mL of log-phase *E.*

coli host was needed per 100mL sample. Based on the volume used, 1% of the nalidixic acid antibiotic was added to the total volume, as well as 1% of the overnight CN-13 *E. coli* host.

Once the log-phase hosts were prepared, the bottles or flasks were placed loose-capped (so as to allow oxygen to enter the bottles) on a shaker plate in a walk-in incubator and incubated for 1.5 to 3 hours. The cultures were determined to be in log-phase growth using the spectrophotometer. An absorbance value of 0.2 – 0.8 was considered log-phase growth for the *E. coli*. The spectrophotometer was set to read at a wavelength of 520, and blanked using 1mL of sterile TSB in a cuvette. After the spectrophotometer was blanked, the F_{amp} and CN-13 cultures were checked in the same manner, using 1mL of each culture in a cuvette. If the cultures were close to the absorbance cutoff of 0.8, they were placed in the refrigerator to slow bacterial growth until they were ready to be used.

IV.i.3.7. 2X Tryptic Soy Agar (TSA)

For each batch of samples, 2X TSA was made by adding 60 g of Tryptic Soy Broth powder and 20 g of agar powder per 1000 mL of DI water. For every 100 mL of sample water to be tested, 100 mL of TSA was made. The bottles were mixed thoroughly using a stir bar and stir plate while being heated, then placed in the autoclave for 15-25 minutes. After the bottles of 2X TSA were removed from the autoclave, they were placed on a stir plate and kept on low heat until ready for use.

IV.i.3.8. 40% Glycerol Solution

Autoclave 60mL of DI water, add 40mL of glycerol into 60mL of DI water about 10 minutes after removing the DI water from the autoclave so that the water is still warm to mix the sugars, and filter syringe the solution through a 0.22μm filter to filter out any viruses. This glycerol solution is meant to act as a preservative.

IV.i.3.9. Coliphage Stock

To make the coliphage and E. coli stocks for this study, we followed the dual agar layer (DAL) method. On the first day we made overnight *E. coli* host (described above). The second day involved making the log-phase host (described above), 40% glycerol solution (described above), and 0.5X TSB by adding 3g of TSB power per 200mL of DI and autoclaving. We then aliquot out the *E. coli* hosts

into 1.5mL screwcap tubes. This was done by mixing 15mL of the log phase and 15mL of glycerol solution into a beaker and aliquot 1mL of host into 1.5mL screwcap tubes, therefore the tubes consisted of half log phase and half 40% glycerol solution, resulting in a net 20% glycerol solution in each tube. These were then stored in a - 80^0C freezer for the duration of the study. Overnight phages were then made by mixing 25mL of 0.5X TSB, 75µL of 4M $MgCL_2$, 0.3mL of appropriate antibiotic (strep/amp for MS2 or nalidixic acid for ΦX174), 20µL of appropriate phage (making sure that it was completely defrosted and vortexed before adding), 5mL of log-phase, and incubating overnight in a shaker. On the third day we extracted the MS2 and ΦX174 by centrifuging the overnight phages. This was done by putting the overnight phages into centrifuge tubes, putting them in the centrifuge at 3000 rpm for 30 minutes at 4^0C, adding 5mL of chloroform and vortexing the centrifuge tube, then centrifuging again at 3000 rpm for 30 minutes. The phages were then extracted by taking 20mL of the centrifuged solution, mixing with 20mL of 40% glycerol solution in an empty container, mixing well by swirling, and then aliquoting 0.3mL into pop top tubes and storing in a box in a -80^0C freezer for the duration of the experiment.

The phages were then titered. The first day of titering involved making the overnight *E. coli* hosts (described above). Day 2 of titering involved making the log-phase hosts (described above) and diluting the MS2 and ΦX174 stocks. Three replicates of dilutions of 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} (method described below) were made, as well as three negative controls (only log-phase *E. coli* hosts were added). The dilutions were made by filling each microcentrifuge tubes with 900µL of sterile PBS, 100µL of the appropriate phage stock to the 10^{-1} microcentrifuge tube, which was then capped and vortexed. After changing tips, 100µL of the 10^{-1} dilution was taken out and added to the 10^{-2} microcentrifuge tube, then capped and vortexed. This process was repeated until the 10^{-10} dilution was made. The bottom agar layer was made by making 150mL of 1.5X TSA (4.5g TSB powder + 2.25g agar poweder), mixing, autoclaving, and adding 1.5mL of the appropriate antibiotic and 0.375mL of 4M $MgCl_2$. Twelve mL of the bottom layer TSA solution was pipetted into each plate and allowed to dry. The top agar layer was made by making 100mL of 0.75X TSA (3g TSB powder + 0.75g agar

powder), mixing, autoclaving, then adding 1mL of the appropriate antibiotic and 0.25mL of 4M $MgCl_2$. Using sterile glass tubes and caps, place the tubes in a water bath, and put 5mL of the top agar into each glass tube. We then added 100µL of each phage dilution (vortexing prior to the sample),and 100µL the appropriate log-phase *E. coli* host into each glass tube, gently rolled each glass tube in our hands to mix, and pouring the top agar solution onto the bottom agar plate. The negative control plates were made in the same manner above, except only *E. coli* log phase was added to each glass tube. The top agar on all plates was then allowed to dry. Once the top layer was dry, the plates were inverted and placed in an incubator overnight. The third day of the titer involved counting the plaques and calculating the titer. The titer was calculated by dividing the total number of plaques by the sum of the dilutions used and reported as PFU/100µL.

IV.i.3.10. Dilutions

Frozen coliphage stocks were used to make 10-fold dilutions of MS2 and ΦX174. New dilutions were made for each day that samples were tested. The MS2 dilution was made by filling seven microcentrifuge tubes with 900µL of sterile PBS and one 15mL falcon tube with 4.5mL sterile PBS. A micropipeter was used to add 100µL of MS2 stock to the 10^{-1} microcentrifuge tube, which was then capped and vortexed. After changing tips, 100µL of the 10^{-1} dilution was taken out and added to the 10^{-2} microcentrifuge tube, then capped and vortexed. This process was repeated until the 10^{-7} dilution was made. Once the 10^{-7} dilution had been prepared, 500µL were taken and added to the 10^{-8} dilution in the 15 mL falcon tube. This process was repeated to make the ΦX174 stock, except a 10^{-7} dilution was the final dilution made in the 15mL falcon tube.

IV.i.3.11. Plates

Five 150-mm petri plates were used per sample. Each plate was labeled with the sample ID, which was the collection site and the date the sample was collected, and the type of coliphage type being tested (F+ or somatic). Control plates were labeled with the processing date, whether the plate was the positive or negative control, and the type of coliphage being tested for (F+ or somatic).

IV.i.3.12. Controls

The F+ positive controls were made by combining 20mL of sterile DI water, 0.1mL $MgCl_2$, 0.4mL strep/amp, 2mL of log-phase F_{amp} *E. coli* host, 0.1mL of a 10^{-8} dilution of MS2 virus, and 20mL of 2X TSA into a 50mL falcon tube. The somatic positive controls were made by combining 20mL of sterile DI water, 0.1mL $MgCl_2$, 0.4mL nalidixic acid, 2mL of log-phase CN-13 *E. coli* host, 0.1mL of a 10^{-7} dilution of PhiX174 virus, and 20mL of 2X TSA TSA into a 50mL falcon tube. The F+ negative controls were made by combining 20mL of sterile DI water, 0.1mL MgCl2, 0.4mL strep/amp, 2mL of log-phase F_{amp} *E. coli* host, and 20mL of 2X TSA into a 50mL falcon tube. The somatic negative controls were made by combining 20mL of sterile DI water, 0.1mL MgCl2, 0.4mL nalidixic acid, 2mL of log-phase CN-13 *E. coli* host, and 20mL of 2X TSA into a 50mL falcon tube. After adding the agar in, the tube was capped and inverted to ensure a good mixture and immediately poured into single 150mm petri dishes.

IV.i.3.13. 10X TSB

A 10X TSB concentration was made by mixing 300g of TSB powder per 1L of DI water. This was then heated and mixed on a stir plate, and then autoclaved for 15 minutes. The 10X TSB concentration was stored in a refrigerator until ready for use.

IV.i.3.14. Procedure

For each sample, 100mL of sample water was added to each of two 250mL screw cap bottle: one bottle for F+ and one bottle for somatic, using a total of 200 mL of the sample water. To each bottle, 0.5 mL of 4M MgCl2 was added, 2mL of appropriate antibiotic (100X strep/amp to F+ bottles and 100X nalidixic acid to somatic bottles), and 10mL of appropriate log-phase E. coli host (Famp to F+ bottles and CN-13 to somatic bottles). The lids were tightly screwed onto the sample bottles and place in a water bath for several minutes. Once the TSA had cooled to an appropriate temperature (the bottle could be grasped comfortably for a few seconds), 100 mL of the TSA was added to each sample flask using a sterile graduated cylinder. Each bottle was then immediately poured across five 150mm petri dishes that corresponded with the sample being poured. The plates were then left

uncovered until the agar dried, then capped, inverted, and placed in a 36^0C incubator. After incubating the plates for 16-24 hours (this differs from the procedures 18-24 hours due to time constraints in sample processing), the plates were read by counting the total number of plaques on each group of five plates, summing the total and recoding as PFU/100mL.

IV.i.4. EPA Method 1601: Male-Specific (F+) and Somatic Coliphage in Water by Two-Step Enrichment Procedure

In addition to the EPA Method 1602, somatic and F+ enrichments on high tide samples was performed based on the EPA Method 1601: Male-Specific (F+) and Somatic Coliphage in Water by Two-Step Enrichment Procedure (EPA 2001b). We used 1 L samples to determine if the water contained the coliphages of interest. This procedure was undertaken due to the large number of negative samples using EPA Method 1602, as the EPA Method 1601 analyzes a higher volume of water (1 L).

IV.i.5. Spot Plates

Separate spot plates were made to test for F+ and somatic coliphages. Spot plates were made by adding 3g of TSB power and 0.75g of agar to two separate bottles each containing 100mL of DI water, the bottles heated and mixed on a stir plate, then autoclaved for 15 minutes. Five 150mm petri dishes for each coliphage were gridded out to contain nine spots, and each spot was labeled for each sample: negative control, positive control 1, positive control 2, PL110, PL100, Tourmaline South, FM030, FM010, and OB Pier. Once the agar had cooled to a temperature where the bottles could be grasped comfortably, 1.25mL of $MgCl_2$ was added to each bottle, 2mL of F_{amp} log-phase *E. coli* host and 1mL of strep/amp antibiotic was added to one bottle and 2mL of CN-13 log-phase *E. coli* host and 1mL of nalidixic acid antibiotic was added to the second bottle. The TSA was then distributed across the five petri dishes using a pipette to put 20mL into each plate. The plates were then left uncovered until they dried.

IV.i.6. Positive Control

Positive controls were made by autoclaving 1L of DI for the F+ positive control and 1L of sterile DI for the somatic positive control for 15 minutes. When ready for use, the water was added into 2L bottles and 1mL of appropriate virus stock (MS2 or ΦX174) was added to the appropriate 2L bottle. A secondary positive control of undiluted virus was also used.

IV.i.7. Method Blank

A method blank (or negative control) was made by autoclaving 1L of DI water for 15 minutes.

IV.i.8. Procedure

On the first day, twelve 2L sterile bottles were labeled with the coliphage being tested for (F+ or somatic) and the sample name. To each bottle a 1L sample aliquot was dispensed, 12.5mL of $MgCL_2$, 50mL 10X TSB, 5mL of the appropriate E. coli log-phase host (F_{amp} or CN-13), 10mL of the appropriate antibiotic (strep/amp or nalidixic acid). The positive control and method blank were made as well. All bottles were then capped, inverted seven times, and placed in a 36^0C incubator for 16-24 hours. On the second day, the spot plates were made. The 2L bottles were removed from the incubator and inverted 10 times. An aliquot of 10µL was taken from each of the bottles and spotted to their corresponding place on the plate. For each aliquot a new sterile micropipette tip was used. Each somatic sample was spotted onto the corresponding spot on the somatic plate and each F+ sample was spotted onto the corresponding spot on the F+ plate. The method blank (negative control) and each positive control were also spotted. The spots were allowed to absorb into the medium for between 30 and 60 minutes, then the plates were capped, inverted and placed in a 36^0C incubator for 16 to 24 hours. On the third day, the plates were removed and read to determine if the samples were positive or negative.

IV.i.9. Confirmation Procedure

A confirmation procedure was ran on the F+ samples. Lysis zones from each sample on the F+ plate were picked with a sterile micropipette and transferred into a centrifuge tube with 0.5mL of 1X TSB. The inoculated broth stood at room temperature for 5 minutes, then the tube was capped and

vortexed on a medium-high setting until well mixed. A 10μL aliquot was taken from the tube spotted onto a spot plate. The plates were left for 30-60 minutes to allow the spots to absorb into the medium. The plates were then covered, inverted, and placed in a 36^0C incubator for 16 to 24 hours. The plates were removed and read to determine if the samples were positive or negative.

IV.i.10. Data analysis

Plaque counts were used to determine the occurrence of coliphages in the water over time under various environmental conditions and reported as plaque forming units (PFU) per 100mL. General descriptive statistics, such as mean values, standard errors, and ranges, were calculated using Microsoft Excel. Coliphage concentrations were transformed to log10 values (Sobsey et al. 2005). Coliphage occurrence was then compared to the occurrence of bacterial indicators (data obtained from SCCWRP). The occurrence of both coliphages to environmental parameters, as well as against bacterial indicators, were compared using regression models. Environmental parameters tested included site location, rainfall, tidal stage, tidal height, turbidity, water temperature, wind speed, current, water color, salinity, surf height, sea state, kelp coverage and numbers of surfers, dogs, birds, and sea lions at the beach. An α-criterion of 0.05 was used to determine significance of statistical tests.

ii. Tributary Study

IV.ii.1. Sampling Sites

A tributary study was conducted to evaluated potential sources of coliphages and other viruses detected during the beach study. Samples were collected from 12 sites upstream of the San Diego River discharge site during a single rainfall event. Figures 3 depicts the sampling locations of the tributary study conducted in San Diego.

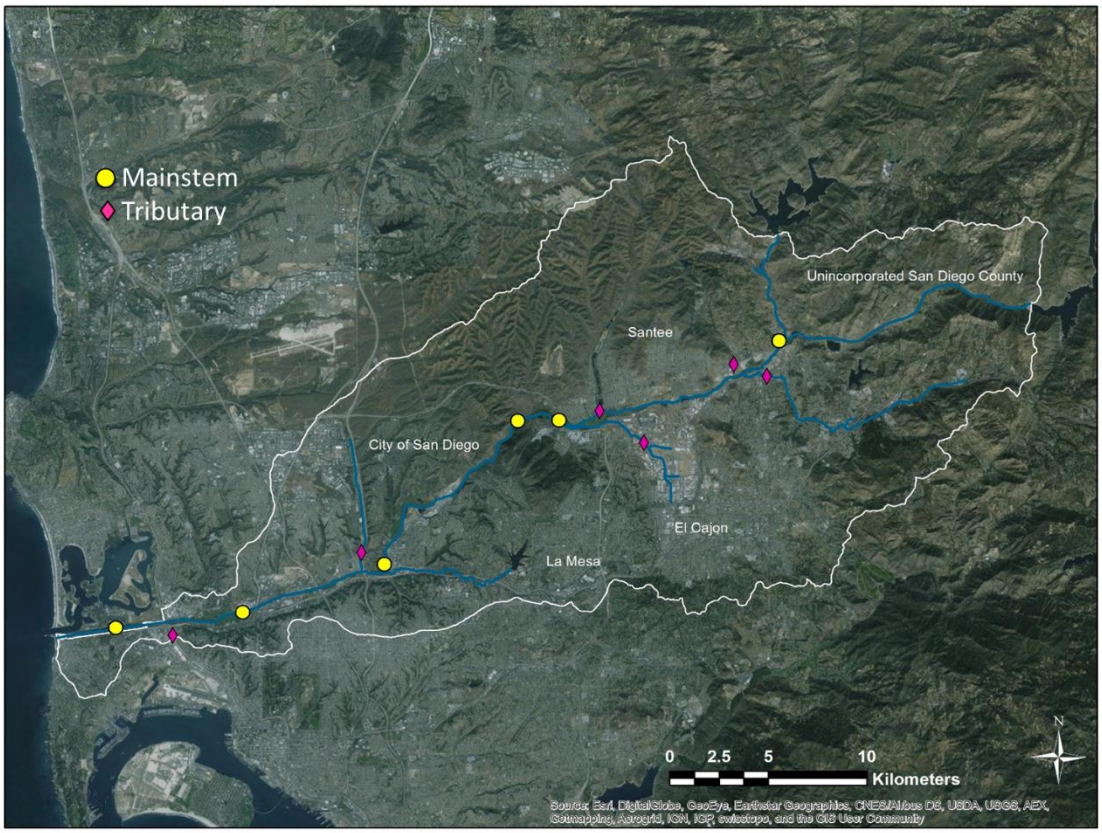

Figure 3. Sampling sites upstream of the San Diego River discharge site in the tributary study. Courtesy of SCCWRP.

IV.ii.2. Field sampling

Water samples were taken from 12 mainstream and tributary locations upstream of the San Diego River discharge site. Samples were collected in January 2016 during a single day rainfall event. Composite samples were collected at all 12 locations. From these composite samples, 1 L samples of water were shipped to UNC-Chapel Hill overnight for coliphage analysis in coolers containing ice packets for proper storage conditions. During analysis, samples were kept refrigerated at 4 ^{0}C until analysis was completed.

IV.ii.3. EPA Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure

Coliphage concentrations were quantified using the single agar layer method as described above for the beach water study.

IV.ii.4. Data analysis

Plaque counts were used to determine the occurrence of coliphages in the water reported as plaque forming units (PFU) per 100mL. Coliphage occurrence was compared to the occurrence of bacterial indicators (data obtained from SCCWRP). The occurrence of coliphages and bacterial indicators were compared using Spearman's Rank correlation. General descriptive statistics, such as mean values, standard errors, and ranges, were calculated using Microsoft Excel.

iii. Decay Study

IV.iii.1. Sampling Site

In the decay study, water samples were collected daily by SCCWRP from a baseflow freshwater creek site located in Southern California. The sampling site was located in the San Joaquin Marsh (33° 39' 57.9" N, 117° 50' 46.8" W), and receives water directly from the San Diego Creek, a freshwater creek.

IV.iii.2. Field Sampling

Surface water in Southern California was seeded with human sewage using *in situ* field diffusion devices for 10 days by SCCWRP (summer: August 8th, 2015 through August 18th, 2015; winter: January 9th, 2015 through January 19th, 2015), allowing the bacterial community to be affected by ambient environmental fluctuations. A control diffusion device was not seeded to observe amplification of background contamination under environmental conditions. The experiment was run during the summer, with samples in shade and without shade, and repeated in the winter under ambient sunlight.

After the seeding period was completed, water samples of 1 L were collected daily. These samples were collected by diffusion bags being removed from the creek experimental site, transported in a cooler to the SCCWRP laboratory, and transferred to 1 L sampling bottles. An approximate 200 mL portion of these samples was shipped to UNC-Chapel Hill for analysis of relative decay rates of F+ and somatic coliphages. Throughout the study, additional aspects of the water matrix were measured, including turbidity, water temperature, specific conductance, salinity, chlorophyll-a, dissolved oxygen saturation, and dissolved oxygen concentration.

IV.iii.3. EPA Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure

Coliphage concentrations were quantified using the single agar layer method as described above.

IV.iii.4. Data analysis

Plaque counts were used to determine the decay of coliphages in the water over time and reported as plaque forming units (PFU) per 100mL. Decay profiles were made by plotting $\ln(C_t/C_0)$ versus time *t*, where C_t and C_0 denote coliphage concentration at time *t* and the beginning of the sampling period, respectively (adapted from (Sobsey et al. 2005)). The coliphage decay rates for each of the conditions in warm water (data obtained from Stewart Lab) were then compared to the relative decay rates in cold water for each experimental condition, as well as to the decay rates of bacterial indicators (data obtained from SCCWRP). The decay rates were compared using regression analysis. General descriptive statistics, such as mean values, standard errors, and ranges, were found using Microsoft Excel. An α-criterion of 0.05 was used for statistical significance.

CHAPTER 5. RESULTS

i. Beach Water Study

During January 2015 through March 2015, measurements were taken to determine the effect of environmental conditions on coliphage concentrations.

V.i.1. Physical and chemical parameters

Physical and chemical parameters at each sampling site recorded for the beach water study included water temperature, salinity, antecedent rainfall, type of tide (high or low), surf height, sea state, turbidity, kelp coverage, tide height, and wind speed and wind direction (summarized in Appendix I). The mean (SD) water temperatures ranged from 16.7^{0}C (1.3 ^{0}C) to 17.2 ^{0}C (1.8 ^{0}C) across all of the beach sites during entire study period. The mean (SD) salinities across all of the beach sampling sites ranged from 31.5 ppt (3.0 ppt) to 33.3 ppt (0.4 ppt).

V.i.2. Independent comparison of F+ and somatic coliphages to environmental parameters

Two regression models were run to compare how environmental parameters affected F+ and somatic coliphage occurrence. Each regression model compared a different number of environmental parameters. The first regression model included all eight sampling sites and the parameters that had information available for all sites. The second regression model included only the six beach sites as information on water temperature and turbidity were not available for the discharge sites.

Coliphage concentrations in marine environments were significantly affected by sample location, rainfall, and water temperature. The environmental factors initially included in each model, and then omitted from the final models as they were found to not explain coliphage variability were surf height, sea state, salinity, kelp coverage, tide height, wind speed, and turbidity.

In the first regression model, which compared F+ concentrations to the environmental parameters sample location, antecedent rainfall, and type of tide, the only environmental parameter

found to significantly affect F+ concentrations was sample location ($p < 0.05$). Rainfall events were found to affect F+ concentrations, though not significantly (Tables 1 and 2). Tide type had no observed effect on F+ concentrations (Tables 1 and 2). The second regression model, which compared F+ concentrations to the parameters sample location, antecedent rainfall, tide stage, water temperature, and turbidity, the environmental parameters found to significantly affect F+ concentrations were water temperature and tide stage ($p < 0.05$; Tables 3 and 4). Sample location, rainfall, and turbidity had no observable effect on F+ concentrations in this study (Tables 3 and 4).

Table 1. Associations between coliphages and other parameters during the beach water study at the beach sampling sites.

F+ coliphage association in Model 1 (*p*-value)		**Somatic coliphage association in Model 1 (*p*-value)**	
Sample Location	0.023*	Sample Location	0.000*
Tide stage	0.928	Tide stage	0.361
Rain event	0.060	Rain event	0.018*

*$p < 0.05$

Table 2. Regression analysis of F+ coliphages during winter beach water study. (N = 240 in each model)

	Model 1	**Model 2**
	Coefficient (SE)	Coefficient (SE)
Sample location*		
FM010	-	-
FM030	-0.0557 (0.0542)	-0.1038 (0.0506) ‡
OB Pier	-0.0419 (0.0537)	-0.0888 (0.0503)
PL100	-0.0688 (0.0537)	-0.1046 (0.0501) ‡
PL110	-0.0511 (0.05337)	-0.0860 (0.0496)
Tourmaline South	-0.0741 (0.0542)	-0.1406 (0.0523) ‡
Tourmaline Creek	0.138 (0.128)	-
San Diego River	0.402 (0.128)*	-
Rainfall		
No	-	-
Yes	0.0986 (0.0542)	0.0709 (0.0503)
Tide type		
High	-	-
Low	-0.0030 (0.0331)	0.1309 (0.0422)
Turbidity		
Clear	-	-0.0242 (0.0701)
Clear/slightly turbid	-	0.028 (0.187)
Slightly turbid	-	-0.0316 (0.0702)
Turbid	-	-0.0764 (0.0945)
Water temperature‡	-	-0.0650 (0.0173) ‡

* $p < 0.05$ in Model 1

‡ $p < 0.05$ in Model 2

When comparing somatic coliphages, the first regression model compared somatic concentrations to the environmental parameters sample location, rainfall, and type of tide. Sample location and rainfall were found to significantly affect somatic concentrations (Table 1). The second regression model, which compared somatic concentrations to the parameters sample location, rainfall, tidal stage, water temperature, and turbidity, the only environmental parameters found to significantly affect somatic concentrations was rainfall ($p < 0.05$; Tables 3 and 4). Sample location, tidal stage, water temperature, and turbidity had no observed effect on somatic concentrations in this study (Tables 3 and 4).

Table 3. Associations between coliphages and other parameters during the beach water study at the beach sampling sites.

F+ coliphage association in Model 2 (*p*-value)		**Somatic coliphage association in Model 2 (*p*-value)**	
Sample Location	0.157	Sample Location	0.112
Water temp (^{0}C)	0.000*	Water temp (^{0}C)	0.765
Tide type	0.015*	Tide type	0.742
Rain event	0.161	Rain event	0.014*
Turbidity	0.932	Turbidity	0.621

*p < 0.05

Table 4. Regression analysis of somatic coliphages during winter beach water study. (N = 240 in each model)

	Model 1	**Model 2**
Sample location*		
FM010	-	-
FM030	-0.1310 (0.0568)*	-0.1538 (0.0570) ‡
OB Pier	-0.1254 (0.0562)*	-0.1403 (0.0567) ‡
PL100	-0.0696 (0.0562)	-0.0848 (0.0565)
PL110	-0.0753 (0.0562)	-0.0814 (0.0559)
Tourmaline South	-0.1002 (0.0568)	-0.1040 (0.0590)
Tourmaline Creek	1.033 (0.134)*	-
San Diego River	2.037 (0.134)*	-
Rainfall*‡		
No	-	-
Yes	0.1302 (0.0544)*	0.1415 (0.0567) ‡
Tide type		
High	-	-
Low	0.0318 (0.0347)	0.0157 (0.0475)
Turbidity		
Clear	-	-0.0443 (0.0790)
Clear/slightly turbid	-	-0.131 (0.210)
Slightly turbid	-	-0.0874 (0.0791)
Turbid	-	-0.106 (0.107)
Water temperature	-	0.0059 (0.0195)

* p < 0.05 in Model 1

‡ p < 0.05 in Model 2

The significance of each sampling location with coliphage occurrence can be seen in Table 5. The FM010 sampling site was used as the dummy variable in all models, and the coefficient indicates the difference in log(number of coliphages) compared to the FM010 site. The F+ coliphage

concentrations were found to be significantly different from the FM010 site for only the San Diego River sampling site in the first model, but were found to be significantly different from FM010 for the sampling sites FM030, PL100, and Tourmaline South in the second model. Somatic coliphage concentrations were found to be significantly different from FM010 for all sampling sites in the both models except PL100, PL110, and Tourmaline South.

The environmental parameters surf height, sea state, wind, current, water color, salinity, kelp coverage, and the number of surfers, dogs, birds, and sea lions were not found to be significantly associated with F+ or somatic coliphage concentrations in marine environments in both regression models (results not shown).

Table 5. Associations between coliphages and sampling location during the beach water quality study.

Sample location	**F+ coliphage association in Model 1 (*p*-value)**	**Coefficient**	**Somatic coliphage association in Model 1 (*p*-value)**	**Coefficient**	**F+ coliphage association in Model 2 (*p*-value)**	**Coefficient**	**Somatic coliphage association in Model 2 (*p*-value)**	**Coefficient**
FM010	-	-	-	-	-	-	-	-
FM030	0.306	-0.0577	0.022	-0.1310	0.042	-0.1038	0.008	-0.1538
OB Pier	0.437	-0.0419	0.027	-0.1254	0.079	-0.0888	0.014	-0.1403
PL100	0.202	-0.0688	0.217	-0.0696	0.039	-0.1046	0.136	-0.0848
PL110	0.343	-0.0511	0.182	-0.0753	0.085	-0.0860	0.147	-0.0814
Tourmaline South	0.174	-0.0741	0.079	-0.1002	0.008	-0.1406	0.080	-0.1040
San Diego River	0.002	0.402	0.000	2.037	-	-	-	-
Tourmaline Creek	0.281	0.138	0.000	1.033	-	-	-	-

V.i.3. Coliphage association with other indicators

Coliphage persistence and decay was also evaluated using regression modeling against that of several other microbial measures during rain events at the two discharge sampling sites (San Diego River and Tourmaline Creek). These microbial measures, provided by collaborators, included *adenovirus, C. coli, C, jejuni, campylobacter, enterovirus, norovirus G1, norovirus G2, salmonella invA*, and *salmonella ttr*. Somatic and F+ coliphage presence at the two discharge sampling sites was not significantly associated with any of the other measured indicators (data not shown).

V.i.4. Coliphage association with regulatory FIB

Descriptive statistics of enterococci, fecal coliform, F+ coliphage and somatic coliphage concentrations can be seen in Table 6. The geometric mean (SE) for enterococci is 32 (203) CFU/100mL, with a median of 23 CFU/100 mL and a range of 2 CFU/100 mL to 30,000 CFU/100 mL. The geometric mean (SE) for fecal coliforms is 15 (32) CFU/100mL, with a median of 10 CFU/100 mL and a range of 2 CFU/100 mL to 4800 CFU/100 mL. The geometric mean (SE) for F+ coliphages is 1 (0) PFU/100mL, with a median of 0 PFU/100 mL and a range of 0 PFU/100 mL to 16 PFU/100 mL. The geometric mean (SE) for somatic coliphages is 2 (3) PFU/100mL, with a median of 0 PFU/100 mL and a range of 0 PFU/100 mL to 549 PFU/100 mL. The number of enterococci samples and fecal coliform samples above and below regulatory threshold values can be seen in Figures 4 and 5. A majority of the enterococcus samples fell below the 35 CFU/100mL regulatory threshold, and a majority of the fecal coliform samples fell below the STV regulatory threshold of 200 CFU/100 mL for California.

Table 6. Descriptive statistics for bacterial and viral indicators.

Statistical measure	Enterococcus (CFU/100 mL)	Fecal coliforms (CFU/100 mL)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)
Arithmetic Mean	706	161	1	12
Geometric Mean	32	15	1*	2*
Standard Error	203	32	0	3
Median	23	10	0	0
Mode	2	2	0	0
Standard Deviation	3165	495	1.78	48
Range	29998	4798	16	549
Minimum	2	2	0	0
Maximum	30000	4800	16	549

*Geometric mean found using n+1 for each data point; error returned otherwise due to 0's

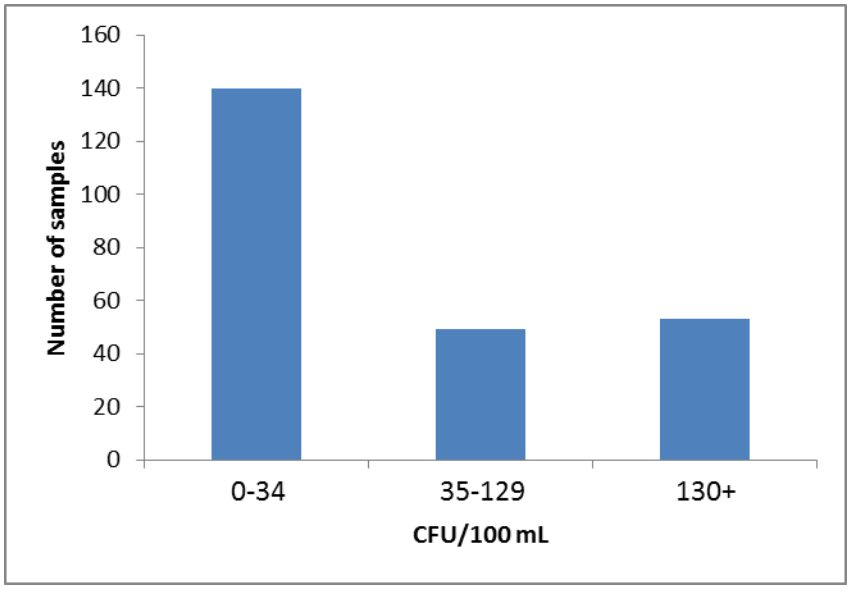

Figure 4. Number of enterococci samples 0-34 CFU/100 mL, 35-129 CFU/100 mL, and more than 130 CFU/100 mL.

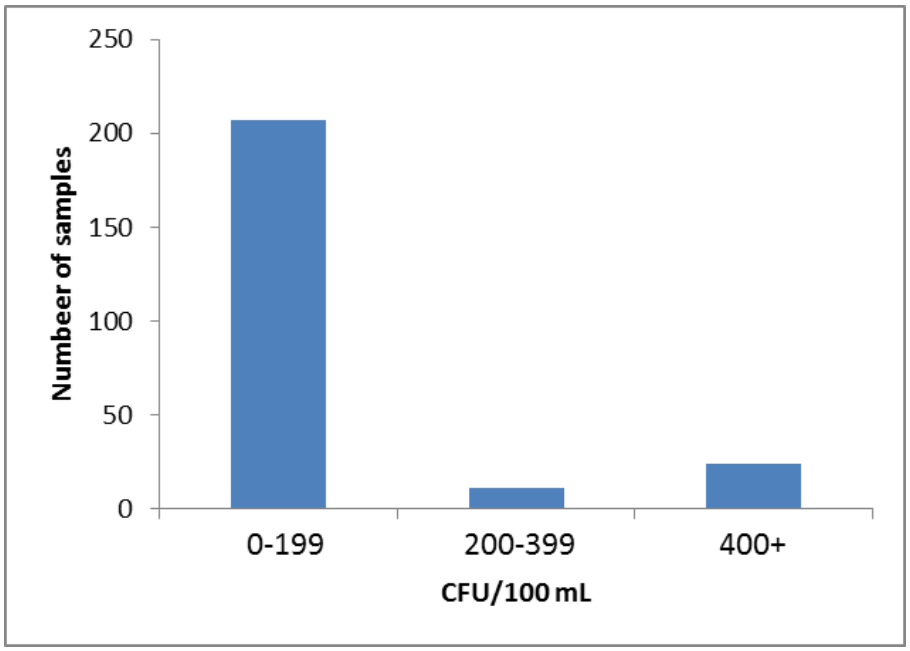

Figure 5. Number of fecal coliform samples 0-199 CFU/100 mL, 200-399 CFU/100 mL, and more than 400 CFU/100 mL.

The geometric mean of F+ coliphage and somatic coliphage concentrations for each bacterial regulatory standard category can be seen in Table 7. A majority of the F+ samples fell below the enterococcus GM 35 CFU/100mL regulatory threshold, and a majority of the somatic samples fell below the fecal coliform STV regulatory threshold of 200 CFU/100 mL for California.

Table 7. Geometric means of coliphages for each bacterial regulatory standard category.

	F+ (PFU/100 mL)	SE	Number of samples within category	Somatic (PFU/100 mL)	SE	Number of samples within category
Enterococcus 0-34 CFU/100 mL	1	0	140	1	1	140
Enterococcus 35-129 CFU/100 mL	1	0	49	2	4	49
Enterococcus 130+ CFU/100 mL	2	0	53	6	13	53
Fecal coliforms 0-199 CFU/100 mL	1	1	207	2	3	207
Fecal coliforms 200-399 CFU/100 mL	1	0	11	3	6	11
Fecal coliforms 400+ CFU/100 mL	2	1	24	3	11	24

All six relationships of Spearman's rank correlation indicate that overall as one measure increases, the other measure increases as well (i.e. as bacterial indicator concentrations increased, coliphage indicator concentrations increased; Table 8). The strongest relationship was seen between enterococcus and somatic coliphages (Spearman's rank correlation = 0.53), and the weakest relationship was seen between enterococcus and F+ coliphages and between F+ and somatic coliphages (Spearman's rank correlation = 0.29 for each). All six Spearman rank correlations were not significant (Table 8), indicating that all six relationships are not significantly related.

Table 8. Spearman's rank correlation comparing coliphages to bacterial indicators

	Fecal coliforms to F+ coliphages	Fecal coliforms to somatic coliphages	Enterococcus to F+ coliphages	Enterococcus to somatic coliphages	Enterococcus to fecal coliforms	F+ to somatic coliphages
Correlation (ρ)	0.19	0.13	0.29	0.53	0.48	0.29
p-value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

ii. Tributary Study

In order to determine potential sources of the observed coliphages in the beach water study, 12 locations upstream of the San Diego River discharge site were tested for coliphages. These sites were tributaries and mainstems of the river.

V.ii.1. Observations at each sampling location

Countable plaques were observed at 7 of the 12 sites for F+ coliphages and 11 of the 12 sites for somatic coliphages. Forrester had the highest concentration of F+ coliphages (582 PFU/100 mL), and was the ninth furthest site upstream from the San Diego River discharge site (Table 9). Murphy Canyon had the highest concentration of somatic coliphages (389 PFU/100 mL), and was the fourth furthest site upstream from the San Diego River discharge site (Table 10). Additional sites with over 100 PFU/100 mL included Mission Trails (289 PFU/100mL; seventh furthest upstream), Carlton Hills (152 PFU/100 mL; tenth furthest upstream), and Los Coches (105 PFU/100 mL, eleventh furthest upstream).

Table 9. Locations with F+ coliphage plaques upstream of San Diego River discharge site.

Sample name	**Sample Date**	**Total PFU/100mL**
01-Ingraham (22:53)	1/31/2016	<1 PFU/100mL
01-Ingraham	1/31/2016	1
02-Morena	1/31/2016	14
03-Fashion Valley	1/31/2016	<1
04-Murphy Canyon	1/31/2016	<1
05-SD Mission Rd	1/31/2016	<1
06-Alvarado	1/31/2016	<1
07-Mission Trails	1/31/2016	12
08-Sycamore Canyon	1/31/2016	3
09-Forrester	1/31/2016	582
10-Carlton Hills	1/31/2016	5
11-Los Coches	1/31/2016	8
12-Upper Euchlid Hills (13:35)	1/31/2016	<1
12-Upper Euchlid Hills	1/31/2016	<1

Table 10. Locations with somatic coliphage plaques upstream of San Diego River discharge site.

Sample name	Sample Date	Total PFU/100mL
01-Ingraham (22:53)	1/31/2016	39
01-Ingraham	1/31/2016	21
02-Morena	1/31/2016	19
03-Fashion Valley	1/31/2016	55
04-Murphy Canyon	1/31/2016	389
05-SD Mission Rd	1/31/2016	5
06-Alvarado	1/31/2016	- *
07-Mission Trails	1/31/2016	289
08-Sycamore Canyon	1/31/2016	12
09-Forrester	1/31/2016	1
10-Carlton Hills	1/31/2016	152
11-Los Coches	1/31/2016	105
12-Upper Euchlid Hills (13:35)	1/31/2016	3
12-Upper Euchlid Hills	1/31/2016	2

*Sample not tested due to lab error

iii. Decay Study

Coliphage and traditional fecal indicator bacteria concentrations were measured, which included F+ and somatic coliphage, as well as total coliforms and *E. coli*. The initial day (D0) concentrations of F+ and somatic coliphages in summer were 943 PFU/100 mL and 5.53×10^4 PFU/100 mL, respectively, and in winter were 70 PFU/100 mL and 1.81×10^4 PFU/100 mL, respectively. The initial day (D0) concentrations of total coliforms and *E. coli* in in winter were 6.4 log10MPN/100 mL and 5.8 log10MPN/100 mL.

V.iii.1. Physical and chemical parameters

Physical and chemical parameters for the decay study included turbidity, water temperature, specific conductance, salinity, chlorophyll-a, dissolved oxygen saturation, and dissolved oxygen concentration (summarized in Appendix III). The mean (standard deviation [SD]) water temperature in summer was 25.3 ^{0}C (0.5 ^{0}C) and 13.4 ^{0}C (0.8 ^{0}C) in winter. The mean (SD) turbidity, specific conductance, and salinity in winter were 16.75 (6.40) NTU, 2.471 (0.090) mS/cm, and 1.282 (0.049) ppt, respectively. These values were not reported during the summer sampling period. The mean (SD)

dissolved oxygen and chlorophyll-a concentrations in summer were 6.06 (0.35) mg/L and 16.84 (4.20) µg/L, respectively, and 4.05 (1.19) mg/L and 6.06 (1.18) µg/L, respectively, in winter.

V.iii.2. Effect of season on coliphage decay in independent replicates

Field replicates were collected simultaneously in the form of two dialysis bags being retrieved at the same time point (R1 and R2). Table 11 displays the decay rates observed for F+ and somatic coliphages in summer and winter. Figures 6 through 9 depict the decay of F+ and somatic coliphages for R1 and R2 samples under sunlight and shade conditions for summer and R1 and R2 samples in winter.

Table 11. Decay rates of F+ and somatic coliphages in summer and winter.

Replicate	**F+ Decay Rate**	**Somatic Decay Rate**
R1 Sun Summer	0.2928	0.4633
R1 Shade Summer	0.3466	0.3987
R2 Sun Summer	0.3182	0.4685
R2 Shade Summer	0.3766	0.4134
R1 Winter	0.07529	0.04183
R2 Shade	0.08861	0.02440

There was no significant difference observed between R1 and R2 samples ($p = 0.710$) or between F+ and somatic coliphages ($p = 0.129$) for any of the test conditions. A significant difference in coliphage decay was observed between summer and winter ($p = 0.000$).

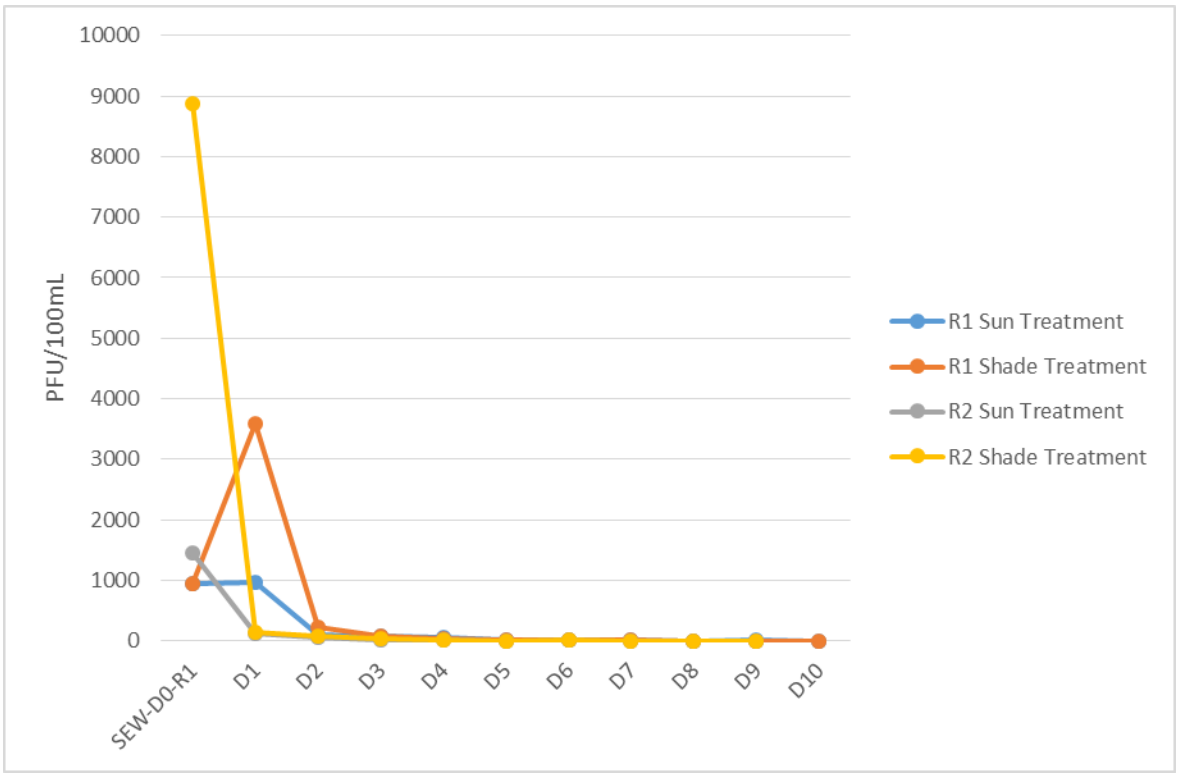

Figure 6. Concentration of F+ coliphage R1 and R2 samples under sunlight and shade treatment.

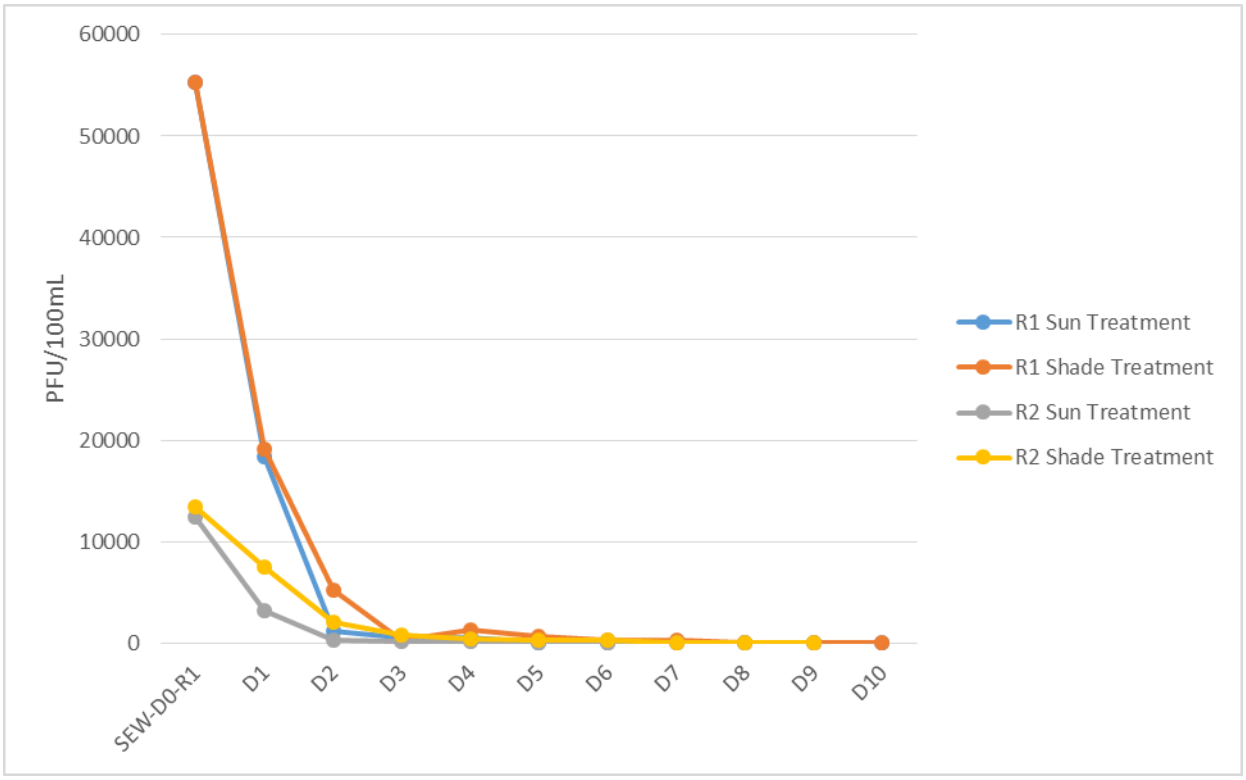

Figure 7. Concentration of somatic coliphages R1 and R2 samples under sunlight and shade treatment.

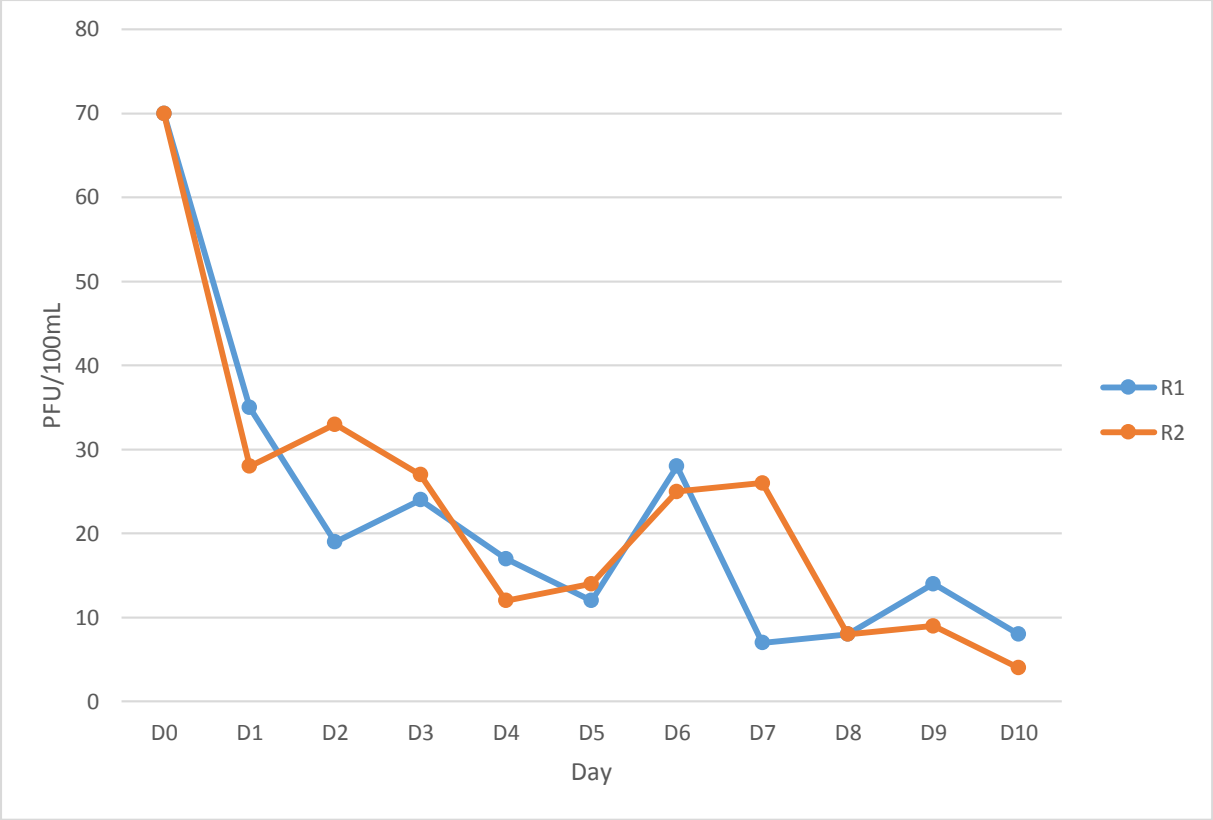

Figure 8. Concentration of F+ coliphages R1 and R2 during the winter experiment.

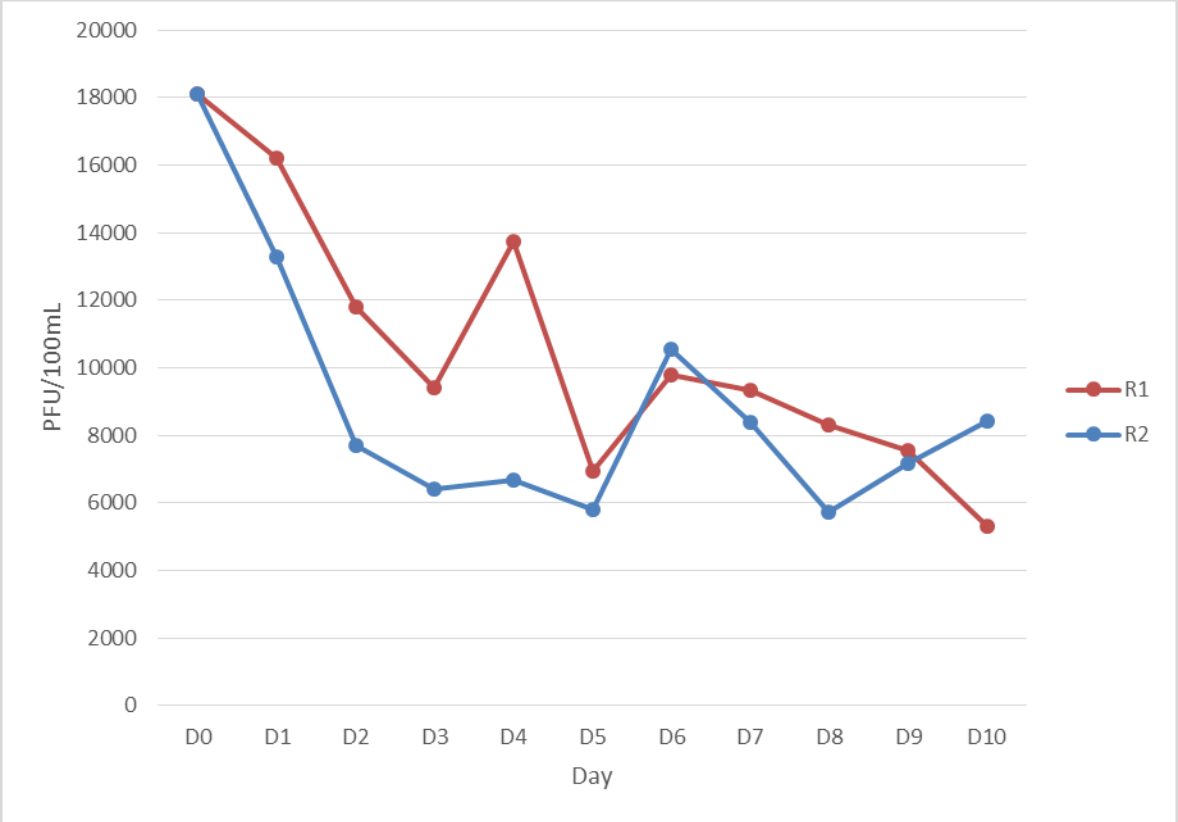

Figure 9. Concentration of somatic coliphages R1 and R2 during the winter experiment.

V.iii.3. Effect of sunlight on coliphage decay in summer: Examining R1 and R2 averaged

In summer, coliphage decay was observed under sun conditions and shade conditions to determine if sunlight affected the rate of coliphage decay. A visual representation of the data indicates that sunlight had a greater effect on somatic decay than on F+ decay (Figures 10 and 11). The F+ sun and shade treatment decay rates appear to closely overlap, while the somatic sun treatment appears to increase coliphage decay greater than the shaded treatment. The F+ decay rate under the sun treatment was found to be 0.28 per day, and under the shaded treatment the F+ decay rate was 0.36 $\log_{10}$PFU/100mL per day. The somatic decay rate under the sunlight treatment was 0.45 $\log_{10}$PFU/100mL per day, and under shaded treatment the decay rate was 0.41 $\log_{10}$PFU/100mL per day.

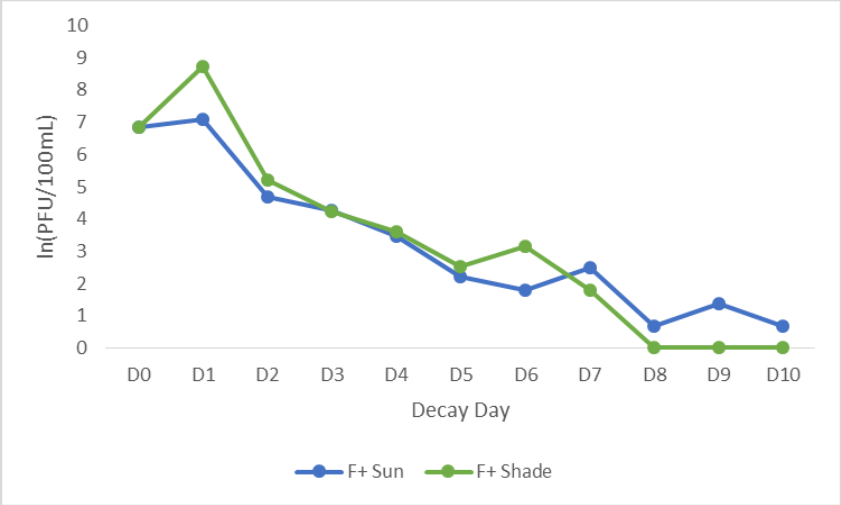

Figure 10. Decay of F+ coliphages under sun and shade treatments.

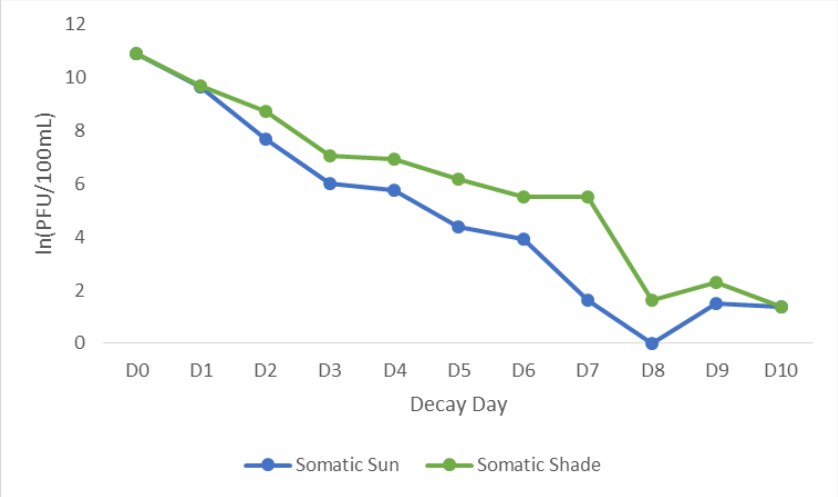

Figure 11. Decay of somatic coliphages under sun and shade treatments.

V.iii.4. Effect of season on coliphage decay: Examining R1 and R2 averaged

Significant differences were observed in coliphage decay rates between summer and winter. Coliphages were found to persist significantly longer during winter for both F+ coliphages and somatic coliphages than in summer under both shade and sun conditions (winter vs. summer: 0.016) (Figures 12 and 13). The F+ decay rate in winter was 0.075 per day compared to 0.28 per day in the summer under sun conditions and 0.36 per day under summer shaded conditions. The somatic decay rate in winter was 0.033 per day compared to 0.45 per day in the summer under sun conditions and 0.41 per day under summer shaded conditions. However, in summer, the F+ and somatic coliphage

decay rates were not significantly different from one another. This association held true for the winter experiments as well.

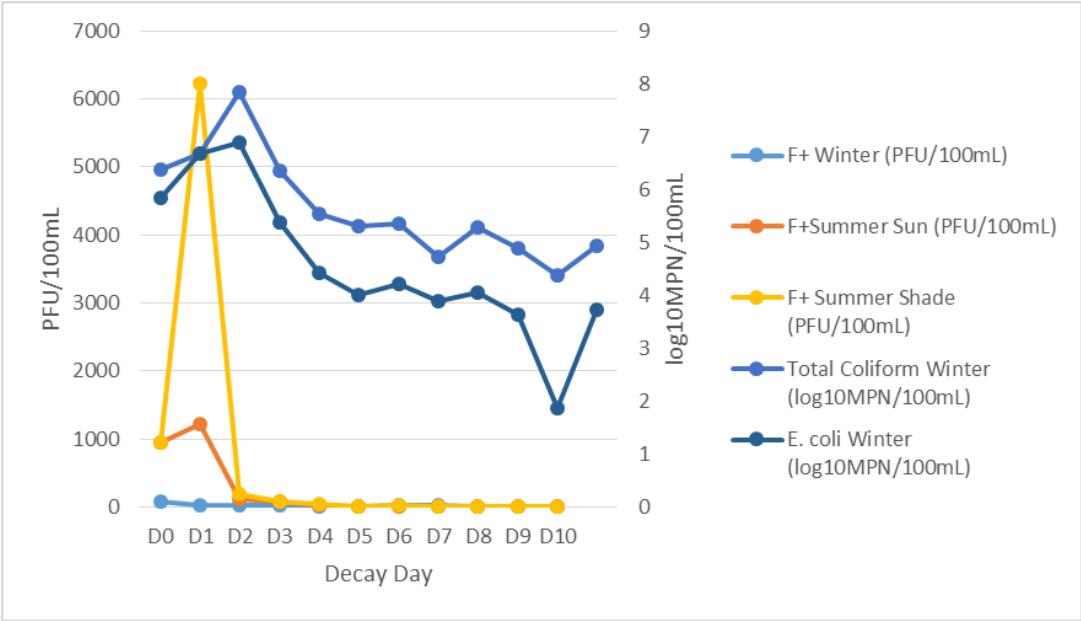

Figure 12. Summer versus winter F+ coliphage and bacterial persistence.

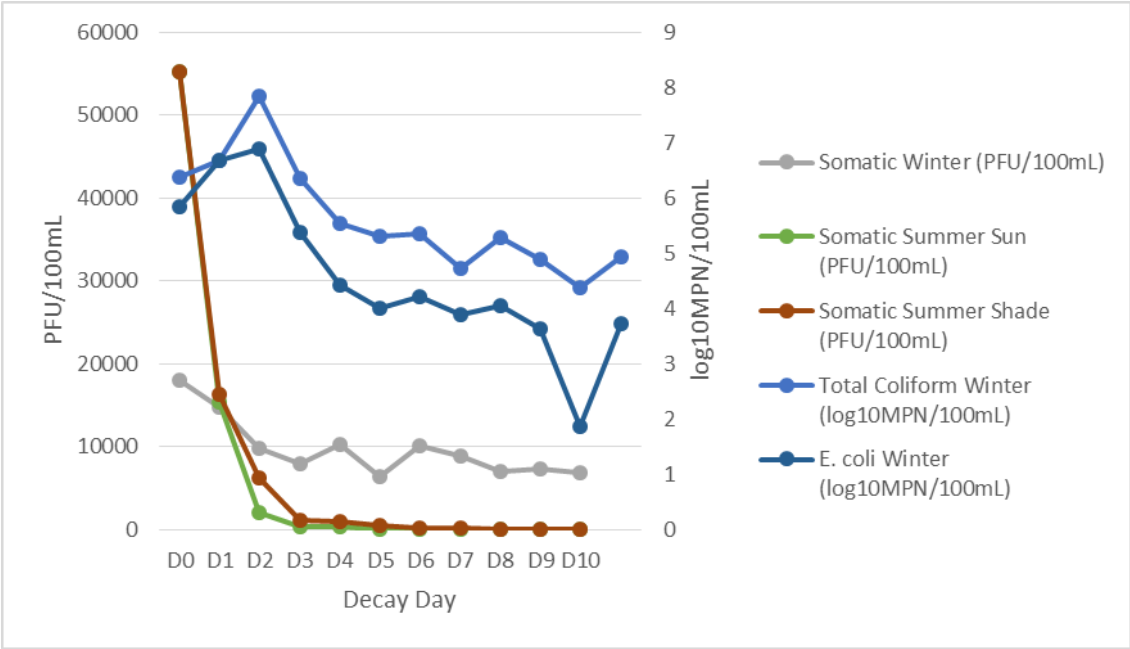

Figure 13. Summer versus winter somatic coliphage and bacterial persistence.

V.iii.5. Effect of water parameters on coliphage decay in winter: Examining R1 and R2 averaged

Several of the measured water parameters during the winter decay experiment were found to affect the decay of coliphages. As salinity concentrations decreased, the decay of coliphages increased (Figure 14). This was also seen in reverse, in which as salinity concentrations increased, the decay rate of coliphages decreased (Figure 14). These associations were present in both F+ and somatic coliphage decay during the winter decay study.

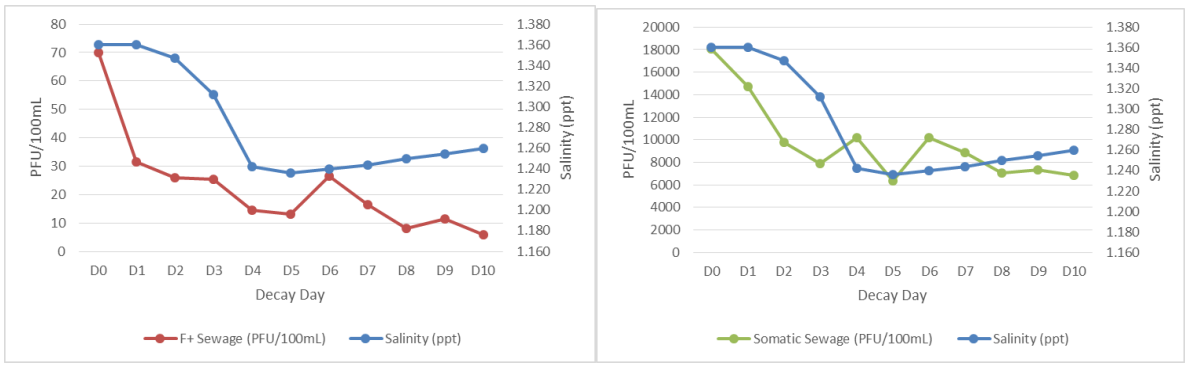

Figure 14. Salinity versus F+ coliphage (left) and somatic coliphage (right) during the winter decay study.

Similar associations were seen with specific conductance. As specific conductance decreased, the decay rate of F+ and somatic coliphages increased, and as specific conductance increased, the decay rate of F+ and somatic coliphages decreased (Figure 15).

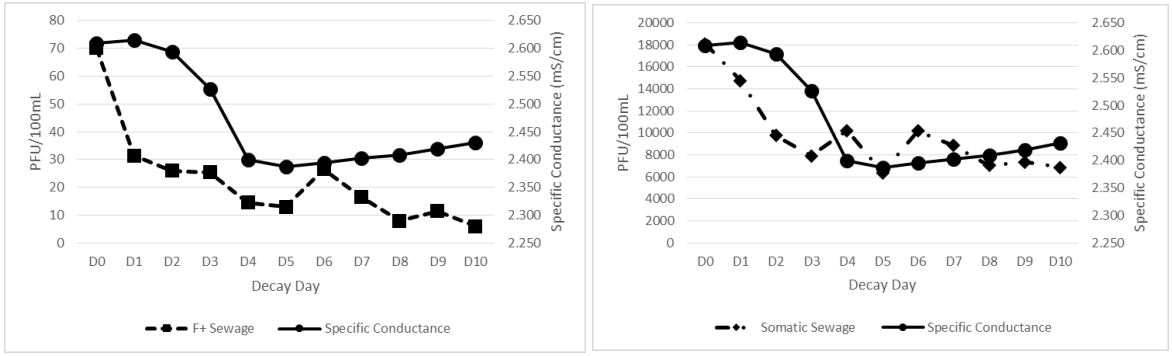

Figure 15. Specific conductance versus F+ coliphage (left) and somatic coliphage (right) during the winter decay study.

Turbidity, water temperature, and dissolved oxygen concentrations were positively associated with the decay rates, although the association did not appear as strong as salinity and specific conductance (results not shown).

Several of the measured water parameters during the winter decay experiment were found to affect the decay of two bacterial indicators. Salinity and specific conductance were found to affect the decay of total coliforms and *E. coli* (results not shown). Turbidity, water temperature, and dissolved oxygen concentrations were positively associated with the bacterial decay rates (results not shown). Chlorophyll-a concentrations had no observed effect on bacterial decay rates (results not shown).

V.iii.6. Decay of coliphages and bacterial indicators in winter:R1 and R2 averaged

The decay of coliphages in winter were compared to the decay bacterial indicators. Coliphage and traditional fecal indicator bacteria concentrations were measured, which included F+ and somatic coliphages, as well as total coliforms and *E. coli*. There was no significant difference in decay rates between somatic coliphages and total coliforms or *E. coli* bacterial indicators, but the F+ coliphages did decay at a faster rate than somatic coliphages, total coliforms, and *E. coli* (Figure 9). The coliphage decay rate for F+ coliphages was 0.075 per day, and for somatic coliphages the decay rate was 0.033 per day. The bacterial decay rates for total coliforms was 0.23 per day and for *E. coli* the decay rate was 0.31 per day. Somatic coliphages, total coliforms, and E. coli were found to persist for a significantly longer time in the water than F+ coliforms.

CHAPTER 6. DISCUSSION

Environmental factors were found to significantly affect the occurrence of coliphages in recreational water systems in this research. Rainfall and water temperature in particular were found to significantly affect coliphage occurrence. Previous research has indicated conflicting results regarding temperature and its effect on coliphage concentrations. Lee and Sobsey (2011) and Wang et al. (2015) did not see significant associations between temperature and somatic coliphage concentrations, which agrees with this research. Other research has seen associations between temperature and coliphage concentrations and rates of inactivation. Yang and Griffiths (2013) found that temperature is a major factor affecting FRNA phages in river water and that persistence was dependent on temperature. Ravva and Sarreal (2016) also found that water temperature played a significant role in persistence for FRNA phages in Salinas Valley, California. The phages survived for significantly longer in colder waters (10°C) than warmer waters (25°C). Herrig et al. (2015) found that there were significant correlations between somatic coliphages and temperature, results which differed from the results of this research.

The environmental factors of turbidity, tidal stage, surf height, sea state, salinity, kelp coverage, tide height, and wind speed were not significantly associated with coliphage occurrence in this study of recreational beach waters in San Diego, California.

We observed several unexpected findings. The type of tide was not found to have a significant effect on coliphage occurrence. This indicates that the number of coliphages during high tide events was not significantly different than the number of coliphages seen during low tide events. When the tide is low, additional water is pulled from the ground than when the tide is high due to lower hydrostatic pressure (Burnett and Dulaiova 2003). Therefore, if pathogens are present in the groundwater, we would expect the presence of pathogens to increase during low tide, as has been

seen with groundwater and FIB in the surf zone (Boehm et al. 2004). This lack of association may indicate that the groundwater at the sampling locations did not contain coliphages or nutrients necessary for pathogen growth. However, as we did not sample groundwater, we were unable to confirm these hypotheses. Future research should test both groundwater and recreational water for coliphages during tidal events.

The location of the sampling sites in the beach water study was significantly different from one another, however sampling sites located closer to the discharge sites did not have higher coliphage concentrations than sampling sites further away from the discharge sites during rainfall events. This was unexpected as we hypothesized that sampling sites closer to the discharge sites would result in higher coliphage concentrations during rainfall events due to runoff entering the San Diego River and Tourmaline Creek.

When examining potential source locations of the fecal contamination in the tributary study, we did not observe an increase in coliphage concentrations as samples were taken further downstream (closer to the discharge site). However, we did observe several locations with countable coliphage plaques in the water samples. Seven of the 12 sites had F+ coliphages present, and 11 of the 12 sites had somatic coliphages present. These sites could contribute to the coliphage concentrations seen in the San Diego River discharge site rather than contamination coming from the city of San Diego, however we cannot definitively confirm whether this explanation was the cause of coliphages. Wang et al. (2015) also saw significant differences in somatic coliphage concentrations between sampling sites. The absence of coliphages at the San Diego River discharge site could indicate the coliphages die-off before reaching the discharge site. Based on an analysis of the data, it does not appear that bacterial and coliphage indicators are predictive of one another, therefore it may be that bacterial indicators are not protective of viral pathogens.

During the winter decay study, slight increases were seen in F+ coliphage concentrations on days 3 and 9, and a larger increase on day 6 in R1 samples, and days 2 and 5 through 7 in R2 samples. During the winter decay study, somatic coliphage counts increased on days 4 and 6 in R1 samples,

and days 6 and 8 through 10 in R2 samples. Lysing of *E. coli* cells could account for increases in coliphage concentrations, rather than actual replication (Noble 2015). Lysis affects whether the coliphages are inside the cell or outside of the cell. It is also possible that coliphages replicate in the natural environment with temperature and host (Noble 2015). Additionally, the starting concentrations in summer and winter differed. The starting concentration could have an effect on the overall rate of decay, as a high starting concentration may give different decay rates than those observed in the decay study.

Holding time was also found to not affect the persistence of coliphages despite unideal holding conditions and holding times of up to four days. Future research should run a controlled experiment to determine if holding times and conditions significantly affect coliphage persistence.

In general, there are limitations of coliphages and viral indicators. Viral indicators cannot protect against all potential pathogens that make humans sick, such as cryptosporidium and giardia (protozoan parasites), and coliphages cannot detect all enteric viruses (Payment and Franco 1993). Additionally, the current literature is not conclusive on whether coliphages are associated with the presence of human viruses or if coliphages can be used as indicators of water quality. Havelaar et al. (1993) and Ballester et al. (2005) support that coliphages are correlated with the presence of human viruses, while Jiang et al. (2007) and Viau et al. (2011) found no significant correlations between coliphages and enteric viruses. Lee et al. (1997) observed a relationship between F-specific bacteriophages and illness incidence, concluding that F-specific RNA bacteriophages were an indicator of risk in fresh water, while van Asperen et al. (1998) did not find a relationship between faecal streptococci, enteroviruses, and F-specific RNA bacteriophages, therefore no indication of coliphages as water quality indicators. These differences have been attributed to different uses of detection methods and coliphages or specific pathogens being tested for, the number and volume of samples being taken, the location of the sampling environment, waste management practices and level of contamination, presence of the host populations and the carriage rates and shedding patterns among them, environmental factors such as rainfall and time of year the sampling occurred, resistance to

growth and environmental stressors, transport characteristics, and the statistical analysis preformed on the data (EPA 2015c). Therefore, making generalizations is difficult. Coliphages are not currently approved as water quality indicators for recreational waters, however they have been approved for groundwater use, and they are under review for use as ambient water quality indicators.

Previous research has suggested that water parameters, such as temperature, pH, salinity, and sunlight can affect the incidence and survival of coliphages. Feng et al. (2003) observed that higher temperatures had higher MS2 inactivation rates, Jonczyk et al. (2011) found that for lysogenic phages, temperature plays a role in attachment, penetration, multiplication, and length of the latent period and determines the occurrence, viability, and storage of bacteriophages, and Sinton et al. (1999) concluded that somatic coliphages were more persistent in sunlit seawater than fecal coliforms, enterococci, and F-RNA phages. Silverman et al. (2013) found that sunlight inactivation of viruses is affected by environmental, water quality, and viral factors, such as sunlight spectrum and intensity, pH, DO, ionic strength, and genome type. The results of the decay study performed in summer agree with this previous research, as sunlight in summer was observed to decay coliphages at a faster rate than shaded treatments. These results also agree with previous research performed by Noble et al. (2004), which saw significantly higher inactivation rates when samples were incubated at high-light than low-light, indicating that solar irradiation plays a significant role in F+ coliphage inactivation.

The results of the winter decay study disagree with previous research on the association between coliphage decay, salinity, and conductivity, in which salinity and conductivity were found to correlate with F+ and somatic coliphage concentrations. Jonczyk et al. (2011) observed that osmotic shock inactivated bacteriophages, indicating salinity affected them. Sinton et al. (2002) observed that with higher salinity, inactivation of sewage F-RNA phages increased. Herrig et al. (2015) did observe that somatic coliphages and conductivity were significantly associated with one another, along with pH, turbidity, chlorophyll-a, and oxygen. The results of the winter decay study showed that salinity, conductance, and coliphage concentrations were associated with one another, with concentrations

increasing as salinity and conductivity increased and coliphage concentrations decreasing with lower salinity and conductivity levels. This association was seen for both F+ and somatic coliphages.

Previous research has found that pH also affects coliphage inactivation. Feng et al. (2003) observed that a pH of 6 to 9 had lower MS2 inactivation rates, while Jonczyk et al. (2011) found that the effect of pH varied for different phages. As the winter decay study did not examine the effects of pH on coliphage concentrations, it was not possible to corroborate previous research.

There were several limitations associated with this research. The beach water samples were only collected during the winter wet season for 3 months, and not year-round. The samples were also only collected in one area on the west coast, and it is not known if these results can be applied to other areas of the United States such as the east coast or inland waters. Additionally, the residuals in the regression models showed that the models were not a good fit for the data, indicating that the models were not robust and may change with more data. Also, only 100 mL of water was processed, rather than a larger volume, which may have depressed coliphage detection. Despite these limitations, this research still adds to the body of knowledge about the measurability of coliphages in ambient waters, and the effect of environmental parameters on coliphage concentrations. These data are important in considering whether coliphages would be appropriate recreational water quality indicators.

CHAPTER 7. SUMMARY AND CONCLUSIONS

Certain environmental factors influence coliphage occurrence in marine environments. Sample location, rainfall events, and water temperature were significantly associated with coliphage occurrence. Surf height, sea state, salinity, tide type, kelp coverage, tide height, wind speed, and turbidity were not significantly associated with coliphage occurrence. Coliphages were observed upstream of San Diego, however it appeared that these coliphages may die off before reaching sites further downstream that are closer to the San Diego River discharge site. Our research showed unexpected findings that holding times of up to four days did not appear to affect coliphage occurrence. The decay study results indicate that coliphage can persist longer in winter than in summer.

The generalizability of our findings is uncertain, as the research was only conducted during one season in one location of the United States. Additional studies could determine if different environmental dynamics exist and affect coliphage occurrence at different times of year and locations in the United States. These future research options could help determine if coliphages would be appropriate water quality indicators.

This research will provide more information on how environmental parameters affect coliphage concentrations in marine environments. The results will be useful for understanding the timing and conditions when people are exposed to enteric viruses, as well as for evaluating the feasibility of incorporating viral measures for monitoring programs and source water protection.

APPENDIX I. DATA FOR BEACH WATER STUDY

Table 1. Coliphage counts during winter rainfall events in San Diego at the San Diego River discharge site.

Coliphage Type	Collection Date	PFU or copies per 100mL
F+ coliphage	1/11/2015	1
	1/12/2015	2
	1/13/2015	1
	1/14/2015	1
	2/23/2015	<1
	2/23/2015	<1
	2/24/2015	<1
	2/25/2015	<1
	2/26/2015	<1
	3/1/2015	9
	3/2/2015	16
	3/3/2015	1
	3/4/2015	1
Somatic coliphage	1/11/2015	25
	1/12/2015	42
	1/13/2015	32
	1/14/2015	76
	2/23/2015	96
	2/23/2015	63
	2/24/2015	29
	2/25/2015	110
	2/26/2015	54
	3/1/2015	90
	3/2/2015	549
	3/3/2015	211
	3/4/2015	77

Table 2. Coliphage counts during winter rainfall events in San Diego at the Tourmaline Creek discharge site.

Coliphage Type	Collection Date	PFU or copies per 100mL
F+ coliphage PFU/100mL	1/11/2015	3
	1/12/2015	2
	1/13/2015	1
	1/14/2015	<1
	2/23/2015	<1
	2/23/2015	<1
	2/24/2015	<1
	2/25/2015	<1
	2/26/2015	<1
	3/1/2015	5
	3/2/2015	10
	3/3/2015	<1
	3/4/2015	<1
Somatic coliphages PFU/100mL	1/11/2015	12
	1/12/2015	53
	1/13/2015	3
	1/14/2015	<1
	2/23/2015	9
	2/23/2015	10
	2/24/2015	301
	2/25/2015	106
	2/26/2015	63
	3/1/2015	236
	3/2/2015	28
	3/3/2015	60
	3/4/2015	4

Table 3. Coliphage counts recorded in San Diego during winter at additional sampling sites.

Sample Name	Coliphage	Collection Date	Tide	Rainfall	PFU/100mL
FM010	F+	1/11/2015		Y	1
		1/12/2015		Y	1
		1/13/2015		Y	<1
		1/14/2015		Y	<1
		1/19/2015	H	N	1
		1/19/2015	L	N	<1
		1/20/2015	L	N	2
		1/20/2015	H	N	2
		1/21/2015	H	N	<1
		1/21/2015	L	N	2
		2/1/2015	H	N	1
		2/1/2015	L	N	<1
		2/2/2015	H	N	<1
		2/2/2015	L	N	<1
		2/3/2015	L	N	<1
		2/3/2015	H	N	1
		2/17/2015	H	N	1
		2/17/2015	L	N	<1
		2/18/2015	H	N	<1
		2/18/2015	L	N	<1
		2/19/2015	L	N	<1
		2/19/2015	H	N	<1
		2/23/2015		Y	<1
		2/24/2015		Y	<1
		2/25/2015		Y	<1
		2/26/2015		Y	<1
		3/1/2015		Y	3
		3/2/2015	H	Y	3
		3/3/2015	H	Y	<1
		3/4/2015	H	Y	<1
		3/18/2015	L	N	<1
		3/18/2015	H	N	<1
		3/19/2015	L	N	<1
		3/19/2015	H	N	<1
		3/20/2015	L	N	<1
		3/20/2015	H	N	<1
FM010	Somatic	1/11/2015		Y	13
		1/12/2015		Y	113

		1/13/2015		Y	24
		1/14/2015		Y	26
		1/19/2015	H	N	<1
		1/19/2015	L	N	4
		1/20/2015	L	N	1
		1/20/2015	H	N	<1
		1/21/2015	H	N	1
		1/21/2015	L	N	2
		2/1/2015	H	N	<1
		2/1/2015	L	N	<1
		2/2/2015	H	N	<1
		2/2/2015	L	N	1
		2/3/2015	L	N	<1
		2/3/2015	H	N	<1
		2/17/2015	H	N	<1
		2/17/2015	L	N	<1
		2/18/2015	H	N	1
		2/18/2015	L	N	8
		2/19/2015	L	N	1
		2/19/2015	H	N	2
		2/23/2015		Y	11
		2/24/2015		Y	19
		2/25/2015		Y	28
		2/26/2015		Y	56
		3/1/2015		Y	1
		3/2/2015	H	Y	1
		3/3/2015	H	Y	<1
		3/4/2015	H	Y	<1
		3/18/2015	L	N	2
		3/18/2015	H	N	<1
		3/19/2015	L	N	<1
		3/19/2015	H	N	<1
		3/20/2015	L	N	<1
		3/20/2015	H	N	<1
FM030	F+	1/11/2015		Y	2
		1/12/2015		Y	1
		1/13/2015		Y	1
		1/14/2015		Y	<1
		1/19/2015	H	N	<1
		1/19/2015	L	N	<1
		1/20/2015	L	N	3

		1/20/2015	H	N	2
		1/21/2015	H	N	<1
		1/21/2015	L	N	<1
		2/1/2015	H	N	<1
		2/1/2015	L	N	<1
		2/2/2015	H	N	<1
		2/2/2015	L	N	1
		2/3/2015	L	N	<1
		2/3/2015	H	N	1
		2/17/2015	H	N	<1
		2/17/2015	L	N	<1
		2/18/2015	H	N	<1
		2/18/2015	L	N	<1
		2/19/2015	L	N	<1
		2/19/2015	H	N	<1
		2/23/2015		Y	<1
		2/24/2015		Y	<1
		2/25/2015		Y	<1
		2/26/2015		Y	<1
		3/1/2015		Y	<1
		3/2/2015	H	Y	<1
		3/3/2015	H	Y	<1
		3/4/2015	H	Y	<1
		3/18/2015	L	N	<1
		3/18/2015	H	N	<1
		3/19/2015	L	N	<1
		3/19/2015	H	N	<1
		3/20/2015	H	N	<1
FM030	Somatic	1/11/2015		Y	<1
		1/12/2015		Y	<1
		1/13/2015		Y	<1
		1/14/2015		Y	<1
		1/19/2015	H	N	<1
		1/19/2015	L	N	<1
		1/20/2015	L	N	<1
		1/20/2015	H	N	<1
		1/21/2015	H	N	<1
		1/21/2015	L	N	<1
		2/1/2015	H	N	<1
		2/1/2015	L	N	<1
		2/2/2015	H	N	<1

		2/2/2015	L	N	<1
		2/3/2015	L	N	<1
		2/3/2015	H	N	<1
		2/17/2015	H	N	<1
		2/17/2015	L	N	<1
		2/18/2015	H	N	<1
		2/18/2015	L	N	<1
		2/19/2015	L	N	<1
		2/19/2015	H	N	1
		2/23/2015		Y	<1
		2/24/2015		Y	<1
		2/25/2015		Y	<1
		2/26/2015		Y	<1
		3/1/2015		Y	<1
		3/2/2015	H	Y	<1
		3/3/2015	H	Y	1
		3/4/2015	H	Y	<1
		3/18/2015	L	N	<1
		3/18/2015	H	N	<1
		3/19/2015	L	N	<1
		3/19/2015	H	N	<1
		3/20/2015	H	N	4
OB Pier	F+	1/11/2015		Y	<1
		1/12/2015		Y	<1
		1/13/2015		Y	<1
		1/14/2015		Y	<1
		1/19/2015	H	N	<1
		1/19/2015	L	N	<1
		1/20/2015	L	N	1
		1/20/2015	H	N	<1
		1/21/2015	H	N	<1
		1/21/2015	L	N	<1
		2/1/2015	H	N	1
		2/1/2015	L	N	<1
		2/2/2015	H	N	<1
		2/2/2015	L	N	<1
		2/3/2015	L	N	1
		2/3/2015	H	N	<1
		2/17/2015	H	N	<1
		2/17/2015	L	N	<1
		2/18/2015	H	N	<1

		2/18/2015	L	N	<1
		2/19/2015	L	N	<1
		2/19/2015	H	N	<1
		2/23/2015		Y	<1
		2/24/2015		Y	<1
		2/25/2015		Y	<1
		2/26/2015		Y	<1
		3/1/2015		Y	<1
		3/2/2015	H	Y	15
		3/3/2015	H	Y	<1
		3/4/2015	H	Y	<1
		3/18/2015	L	N	<1
		3/18/2015	H	N	<1
		3/19/2015	L	N	<1
		3/19/2015	H	N	<1
		3/20/2015	L	N	<1
		3/20/2015	H	N	<1
OB Pier	Somatic	1/11/2015		Y	5
		1/12/2015		Y	1
		1/13/2015		Y	1
		1/14/2015		Y	2
		1/19/2015	H	N	<1
		1/19/2015	L	N	<1
		1/20/2015	L	N	<1
		1/20/2015	H	N	<1
		1/21/2015	H	N	<1
		1/21/2015	L	N	<1
		2/1/2015	H	N	<1
		2/1/2015	L	N	<1
		2/2/2015	H	N	<1
		2/2/2015	L	N	<1
		2/3/2015	L	N	<1
		2/3/2015	H	N	<1
		2/17/2015	H	N	<1
		2/17/2015	L	N	<1
		2/18/2015	H	N	<1
		2/18/2015	L	N	<1
		2/19/2015	L	N	<1
		2/19/2015	H	N	<1
		2/23/2015		Y	<1
		2/24/2015		Y	1

		2/25/2015		Y	<1
		2/26/2015		Y	<1
		3/1/2015		Y	<1
		3/2/2015	H	Y	1
		3/3/2015	H	Y	3
		3/4/2015	H	Y	1
		3/18/2015	L	N	<1
		3/18/2015	H	N	<1
		3/19/2015	L	N	<1
		3/19/2015	H	N	<1
		3/20/2015	L	N	<1
		3/20/2015	H	N	1
PL100	F+	1/11/2015		Y	2
		1/12/2015		Y	1
		1/13/2015		Y	<1
		1/14/2015		Y	<1
		1/19/2015	H	N	1
		1/19/2015	L	N	<1
		1/20/2015	L	N	1
		1/20/2015	H	N	1
		1/21/2015	H	N	<1
		1/21/2015	L	N	<1
		2/1/2015	H	N	<1
		2/1/2015	L	N	<1
		2/2/2015	H	N	2
		2/2/2015	L	N	<1
		2/3/2015	L	N	<1
		2/3/2015	H	N	<1
		2/17/2015	H	N	<1
		2/17/2015	L	N	<1
		2/18/2015	H	N	<1
		2/18/2015	L	N	<1
		2/19/2015	L	N	<1
		2/19/2015	H	N	<1
		2/23/2015		Y	<1
		2/24/2015		Y	<1
		2/25/2015		Y	<1
		2/26/2015		Y	<1
		3/1/2015		Y	<1
		3/2/2015	H	Y	<1
		3/3/2015	H	Y	<1

		3/4/2015	H	Y	<1
		3/18/2015	L	N	<1
		3/18/2015	H	N	<1
		3/19/2015	L	N	<1
		3/19/2015	H	N	<1
		3/20/2015	L	N	<1
		3/20/2015	H	N	<1
PL100	Somatic	1/11/2015		Y	<1
		1/12/2015		Y	2
		1/13/2015		Y	<1
		1/14/2015		Y	<1
		1/19/2015	H	N	<1
		1/19/2015	L	N	<1
		1/20/2015	L	N	<1
		1/20/2015	H	N	1
		1/21/2015	H	N	<1
		1/21/2015	L	N	1
		2/1/2015	H	N	<1
		2/1/2015	L	N	<1
		2/2/2015	H	N	3
		2/2/2015	L	N	<1
		2/3/2015	L	N	<1
		2/3/2015	H	N	<1
		2/17/2015	H	N	<1
		2/17/2015	L	N	<1
		2/18/2015	H	N	<1
		2/18/2015	L	N	1
		2/19/2015	L	N	1
		2/19/2015	H	N	1
		2/23/2015		Y	<1
		2/24/2015		Y	<1
		2/25/2015		Y	<1
		2/26/2015		Y	<1
		3/1/2015		Y	<1
		3/2/2015	H	Y	<1
		3/3/2015	H	Y	1
		3/4/2015	H	Y	<1
		3/18/2015	L	N	<1
		3/18/2015	H	N	3
		3/19/2015	L	N	<1
		3/19/2015	H	N	<1

		3/20/2015	L	N	<1
		3/20/2015	H	N	<1
PL110	F+	1/11/2015		Y	2
		1/12/2015		Y	<1
		1/13/2015		Y	<1
		1/14/2015		Y	<1
		1/19/2015	H	N	<1
		1/19/2015	L	N	<1
		1/20/2015	L	N	2
		1/20/2015	H	N	<1
		1/21/2015	H	N	<1
		1/21/2015	L	N	1
		2/1/2015	H	N	<1
		2/1/2015	L	N	<1
		2/2/2015	H	N	<1
		2/2/2015	L	N	<1
		2/3/2015	L	N	3
		2/3/2015	H	N	<1
		2/17/2015	H	N	<1
		2/17/2015	L	N	<1
		2/18/2015	H	N	<1
		2/18/2015	L	N	<1
		2/19/2015	L	N	<1
		2/19/2015	H	N	<1
		2/23/2015		Y	<1
		2/24/2015		Y	<1
		2/25/2015		Y	<1
		2/26/2015		Y	<1
		3/1/2015		Y	1
		3/2/2015	H	Y	2
		3/3/2015	H	Y	<1
		3/4/2015	H	Y	<1
		3/18/2015	L	N	<1
		3/18/2015	H	N	<1
		3/19/2015	L	N	<1
		3/19/2015	H	N	<1
		3/20/2015	L	N	<1
		3/20/2015	H	N	<1
PL110	Somatic	1/11/2015		Y	<1
		1/12/2015		Y	<1
		1/13/2015		Y	2

		1/14/2015		Y	<1
		1/19/2015	H	N	<1
		1/19/2015	L	N	1
		1/20/2015	L	N	1
		1/20/2015	H	N	<1
		1/21/2015	H	N	<1
		1/21/2015	L	N	<1
		2/1/2015	H	N	<1
		2/1/2015	L	N	2
		2/2/2015	H	N	<1
		2/2/2015	L	N	<1
		2/3/2015	L	N	<1
		2/3/2015	H	N	<1
		2/17/2015	H	N	<1
		2/17/2015	L	N	<1
		2/18/2015	H	N	1
		2/18/2015	L	N	<1
		2/19/2015	L	N	<1
		2/19/2015	H	N	<1
		2/23/2015		Y	5
		2/24/2015		Y	1
		2/25/2015		Y	6
		2/26/2015		Y	<1
		3/1/2015		Y	<1
		3/2/2015	H	Y	4
		3/3/2015	H	Y	<1
		3/4/2015	H	Y	1
		3/18/2015	L	N	2
		3/18/2015	H	N	<1
		3/19/2015	L	N	<1
		3/19/2015	H	N	<1
		3/20/2015	L	N	<1
		3/20/2015	H	N	<1
San Diego River	F+	1/11/2015		Y	1
		1/12/2015		Y	2
		1/13/2015		Y	1
		1/14/2015		Y	1
		2/24/2015		Y	<1
		2/25/2015		Y	<1
		2/26/2015		Y	<1
		3/1/2015		Y	9

		3/2/2015	H	Y	16
		3/3/2015	H	Y	1
		3/4/2015	H	Y	1
San Diego River #1		2/23/2015		Y	<1
San Diego River	Somatic	1/11/2015		Y	25
		1/12/2015		Y	42
		1/13/2015		Y	32
		1/14/2015		Y	76
		2/24/2015		Y	29
		2/25/2015		Y	110
		2/26/2015		Y	54
		3/1/2015		Y	90
		3/2/2015	H	Y	549
		3/3/2015	H	Y	211
		3/4/2015	H	Y	77
San Diego River #1		2/23/2015		Y	96
San Diego River #2		2/23/2015		Y	63
Tourmaline Creek	F+	1/11/2015		Y	3
		1/12/2015		Y	2
		1/13/2015		Y	1
		1/14/2015		Y	<1
		2/24/2015		Y	<1
		2/25/2015		Y	<1
		2/26/2015		Y	<1
		3/1/2015		Y	5
		3/2/2015	H	Y	10
		3/3/2015	H	Y	<1
		3/4/2015	H	Y	<1
Tourmaline Creek #1		2/23/2015		Y	<1
Tourmaline Creek #2		2/23/2015		Y	<1
Tourmaline Creek	Somatic	1/11/2015		Y	12
		1/12/2015		Y	53
		1/13/2015		Y	3
		1/14/2015		Y	<1
		2/24/2015		Y	301
		2/25/2015		Y	106
		2/26/2015		Y	63
		3/1/2015		Y	236
		3/2/2015	H	Y	28
		3/3/2015	H	Y	60
		3/4/2015	H	Y	4

Tourmaline Creek #1	Somatic	2/23/2015		Y	9
Tourmaline Creek #2	Somatic	2/23/2015		Y	10
Tourmaline South	F+	1/11/2015		Y	<1
		1/12/2015		Y	<1
		1/13/2015		Y	<1
		1/14/2015		Y	<1
		1/19/2015	H	N	<1
		1/19/2015	L	N	<1
		1/20/2015	L	N	<1
		1/20/2015	H	N	<1
		1/21/2015	H	N	<1
		1/21/2015	L	N	<1
		2/1/2015	H	N	<1
		2/1/2015	L	N	<1
		2/2/2015	H	N	<1
		2/2/2015	L	N	<1
		2/3/2015	L	N	<1
		2/3/2015	H	N	1
		2/17/2015	H	N	<1
		2/17/2015	L	N	<1
		2/18/2015	H	N	1
		2/18/2015	L	N	<1
		2/19/2015	L	N	<1
		2/19/2015	H	N	<1
		2/23/2015		Y	<1
		2/24/2015		Y	<1
		2/25/2015		Y	<1
		2/26/2015		Y	<1
		3/1/2015		Y	<1
		3/2/2015	H	Y	3
		3/3/2015	H	Y	<1
		3/4/2015	H	Y	<1
		3/18/2015	L	N	<1
		3/18/2015	H	N	<1
		3/19/2015	L	N	<1
		3/19/2015	H	N	<1
		3/20/2015	H	N	<1
Tourmaline South	Somatic	1/11/2015		Y	<1
		1/12/2015		Y	<1
		1/13/2015		Y	1
		1/14/2015		Y	2

		1/19/2015	H	N	<1
		1/19/2015	L	N	<1
		1/20/2015	L	N	<1
		1/20/2015	H	N	<1
		1/21/2015	H	N	<1
		1/21/2015	L	N	1
		2/1/2015	H	N	<1
		2/1/2015	L	N	<1
		2/2/2015	H	N	<1
		2/2/2015	L	N	<1
		2/3/2015	L	N	<1
		2/3/2015	H	N	<1
		2/17/2015	H	N	<1
		2/17/2015	L	N	<1
		2/18/2015	H	N	<1
		2/18/2015	L	N	6
		2/19/2015	L	N	<1
		2/19/2015	H	N	<1
		2/23/2015		Y	<1
		2/24/2015		Y	<1
		2/25/2015		Y	<1
		2/26/2015		Y	<1
		3/1/2015		Y	85
		3/2/2015	H	Y	2
		3/3/2015	H	Y	<1
		3/4/2015	H	Y	<1
		3/18/2015	L	N	<1
		3/18/2015	H	N	<1
		3/19/2015	L	N	<1
		3/19/2015	H	N	<1
		3/20/2015	H	N	2

Table 4. Additional water parameters recorded in San Diego during winter at additional sampling sites.

Sample Name	Collection Date	Holding Time (Days)	Water Temp (^{0}C)	Salinity (ppt)	Turbidity	Wind Speed
FM010	1/11/2015	2	15.5	27.4	Turbid	2.3
	1/12/2015	1	15.9	23.1	Clear	0
	1/13/2015	1	15.3	22.1	Slightly Turbid	0.9
	1/14/2015	1	13.5	23.9	Clear	0
	1/19/2015	1	15	32.9	Turbid	0
	1/19/2015	1	18.3	30.6	Clear	4.9
	1/20/2015	1	17.6	31.4	Slightly Turbid	2
	1/20/2015	1	15.9	33.4	Slightly Turbid	1
	1/21/2015	1	16.2	33.3	Slightly Turbid	0
	1/21/2015	1	18	32.1	Clear	8.4
	2/1/2015	2	15.6	33.6	Slightly Turbid	2.4
	2/1/2015	2	19	31.8	Slightly Turbid	5.2
	2/2/2015	1	15.6	33.6	Clear	0
	2/2/2015	1	18.4	32.3	Slightly Turbid	4.8
	2/3/2015	1	18.9	32.1	Clear	4.7
	2/3/2015	1	16.1	33.5	Clear	0
	2/17/2015	1	17	33.4	Slightly Turbid	0
	2/17/2015	1	20.1	32.2	Clear	4.6
	2/18/2015	1	17.2	33.4	Clear	0
	2/18/2015	1	19.2	32.6	Clear	5.8
	2/19/2015	2	19.6	31.9		1.4
	2/19/2015	2	17.3	33.4	Turbid	0.1
	2/23/2015	1	15.6	31	Slightly Turbid	7.5
	2/24/2015	1	15.7	28.8	Turbid	0
	2/25/2015	2	15.5	28.1	Turbid	3.2
	2/26/2015	4	16.5	29.2	Clear	3.5
	3/1/2015	3	16.2	33.1	Clear	2.4

	3/2/2015	2	15.6	33.5	Clear	N/A
	3/3/2015	2	15.7	33.6		0.6
	3/4/2015	2	16	33.3	Slightly Turbid	N/A
	3/18/2015	1	20.1	33.2	Slightly Turbid	6.8
	3/18/2015	1	17.7	33.4	Turbid	3.4
	3/19/2015	1	20.3	32.1	clear	5.8
	3/19/2015	4	17.9	33.3	Turbid	2.9
	3/20/2015	1	21.7	33.1	Clear	4.1
	3/20/2015	3	17.7	33.4	Slightly Turbid	N/A
FM030	1/11/2015	2	15.9	33.2	Clear	0.4
	1/12/2015	1	15.8	33	Clear	0
	1/13/2015	1	15.8	33	Clear	0
	1/14/2015	1	15.3	33.5	Clear	0
	1/19/2015	1	15.7	33.5	Turbid	
	1/19/2015	1	18.1	32.9	Slightly Turbid	4.8
	1/20/2015	1	16.8	33.3	Slightly Turbid	4.4
	1/20/2015	1	16.6	33.2	Slightly Turbid	0
	1/21/2015	1	16.4	33.4	Clear	0
	1/21/2015	1	17.1	33.3	Clear	0
	2/1/2015	2	15.9	33.5	Slightly Turbid	0
	2/1/2015	2	18.4	33.5	Clear	7
	2/2/2015	1	15.5	33.6	Clear	0
	2/2/2015	1	18.2	33.4	Clear	4.6
	2/3/2015	1	17.5	33.5	Clear	5.9
	2/3/2015	1	16.2	33.5	Clear	0
	2/17/2015	1	17	32.9	Clear	0
	2/17/2015	1	19.3	33.3	Clear	5.1
	2/18/2015	1	17.3	33.1	Clear	0
	2/18/2015	1	18.2	33.4	Clear	3.3
	2/19/2015	2	18.2	33.3	Clear	5
	2/19/2015	2	17.1	33	Clear	0
	2/23/2015	1	15.5	33.1	Clear	0
	2/24/2015	1	16	33.6	Clear	0
	2/25/2015	2	15.6	33.4	Clear	0
	2/26/2015	4	16.2	33.7	Clear	0

	3/1/2015	3	16.4	30.9	Turbid	0
	3/2/2015	2	16	33.2	Clear	N/A
	3/3/2015	2	15.2	33.3	Clear	0
	3/4/2015	2	16	33.4	Slightly Turbid	N/A
	3/18/2015	1	18.8	27.2	Clear	4.2
	3/18/2015	1	17.9	33.5	Clear	1.1
	3/19/2015	1	20.2	33.2	clear	4.1
	3/19/2015	4	17.2	33.1	Clear	0.1
	3/20/2015	3	17.5	33.4	clear	N/A
OB Pier	1/11/2015	2	16	33.3	Turbid	1.6
	1/12/2015	1	16.2	33.1	Clear	0
	1/13/2015	1	16.1	33.3	Slightly Turbid	0
	1/14/2015	1	15.2	33.5	Turbid	5.3
	1/19/2015	1	15.7	33.3	Clear	0
	1/19/2015	1	17.6	33.1	Clear	4.2
	1/20/2015	1	16.6	33.4	Slightly Turbid	0.1
	1/20/2015	1	16.2	33.4	Slightly Turbid	0
	1/21/2015	1	16.1	33.4	Clear	3.6
	1/21/2015	1	17.6	33.3	Slightly Turbid	5.9
	2/1/2015	2	16	33.5	Turbid	0
	2/1/2015	2	17.6	33.5	Turbid	2
	2/2/2015	1	16	33.5	Slightly Turbid	0
	2/2/2015	1	17.4	33.4	Slightly Turbid	3
	2/3/2015	1	17.4	33.4	Slightly Turbid	3.3
	2/3/2015	1	16.1	33.5	Slightly Turbid	0
	2/17/2015	1	17.2	31.5	Slightly Turbid	0
	2/17/2015	1	18.4	33.2	Clear	3.4
	2/18/2015	1	17.2	33.3	Clear	2.5
	2/18/2015	1	18.1	33.2	Clear	4.2
	2/19/2015	2	17.9	33.4	Clear	0.9
	2/19/2015	2	17.3	33.5	Slightly Turbid	0

	2/23/2015	1	16.6	33.6	Clear	5.5
	2/24/2015	1	16.7	33.3	Clear	1.8
	2/25/2015	2	16.7	33.5	Clear	0
	2/26/2015	4	17.1	33.5	Clear	1.3
	3/1/2015	3	16.4	33.4	Clear	1.9
	3/2/2015	2	16.1	33.2	Clear	N/A
	3/3/2015	2	16	33.5	Clear	N/A
	3/4/2015	2	16.3	33.3	Slightly Turbid	0.2
	3/18/2015	1	18.6	33.3	Clear	6.9
	3/18/2015	1	17.6	33.5		4.2
	3/19/2015	1	19.2	33.1	Slightly Turbid	4
	3/19/2015	4	18	33.4	clear	1.3
	3/20/2015	1	19.1	33.5	Slightly Turbid	1.3
	3/20/2015	3	17.8	33.2		N/A
PL100	1/11/2015	2	15.9	33.3	Clear	1.4
	1/12/2015	1	16.2	32	Clear	0
	1/13/2015	1	16.1	33.3	Clear	0
	1/14/2015	1	15.1	33.5	Clear	1.8
	1/19/2015	1	15.6	33.4	Clear	0
	1/19/2015	1	17.2	33.3	Clear	4.7
	1/20/2015	1	16.7	33.4	Clear	1
	1/20/2015	1	16.2	33.4	Slightly Turbid	0.8
	1/21/2015	1	16.1	33.2	Slightly Turbid	0
	1/21/2015	1	17.4	32.7	Clear	9.5
	2/1/2015	2	15.9	33.6	Clear	0.8
	2/1/2015	2	17.9	33.4	Slightly Turbid	2.1
	2/2/2015	1	16	33.5	Slightly Turbid	0
	2/2/2015	1	17.7	33.4	Clear	3.1
	2/3/2015	1	17.5	33.3	Clear	5.3
	2/3/2015	1	16.1	33.5	Clear	0
	2/17/2015	1	17.2	33.4	Slightly Turbid	1.1
	2/17/2015	1	18.8	33.2	Clear	6.8
	2/18/2015	1	17.2	33.3	Clear	0
	2/18/2015	1	18.5	33.3	Clear	5.5

	2/19/2015	2	19	33.2	Clear	2.4
	2/19/2015	2	17.3	33.5	Clear	0.3
	2/23/2015	1	16.5	33.5		5.2
	2/24/2015	1	17	33.4		0.8
	2/25/2015	2	16.8	33.5	Clear	1.7
	2/26/2015	4	17.1	33.4	Clear	2.8
	3/1/2015	3	16.7	33.5	Slightly Turbid	0
	3/2/2015	2	16.1	33.6	Clear	N/A
	3/3/2015	2	16.3	33.4		0
	3/4/2015	2	16.4	33.3	Clear	N/A
	3/18/2015	1	19	33.3	Slightly Turbid	6.2
	3/18/2015	1	17.7	33.5	Slightly Turbid	6.2
	3/19/2015	1	19.5	33.2	Slightly Turbid	4.6
	3/19/2015	4	18	33.3	Slightly Turbid	1.2
	3/20/2015	1	19.7	33.3	Slightly Turbid	5.3
	3/20/2015	3	17.8	33.2	Slightly Turbid	N/A
PL110	1/11/2015	2	15.8	33.1	Clear	1.3
	1/12/2015	1	16.3	33.1	Clear	0
	1/13/2015	1	16.1	31.9	Clear	0
	1/14/2015	1	15.4	32.7	Slightly Turbid	2.5
	1/19/2015	1	15.5	33.5	Turbid	0
	1/19/2015	1	17.5	31.1	Clear	6.2
	1/20/2015	1	17	33	Clear	0.6
	1/20/2015	1	16.2	33.3	Slightly Turbid	1.8
	1/21/2015	1	16	33.4	Slightly Turbid	0
	1/21/2015	1	17.3	33.2	Slightly Turbid	7.5
	2/1/2015	2	15.7	33.6	Slightly Turbid	3.4
	2/1/2015	2	18.3	32.3	Clear	5.1
	2/2/2015	1	15.6	33.3	Slightly Turbid	0

	2/2/2015	1	18	33.1	Slightly Turbid	6.3
	2/3/2015	1	17.7	32.6	Slightly Turbid	4.6
	2/3/2015	1	16.1	33.5	Slightly Turbid	0
	2/17/2015	1	16.9	33.4	Slightly Turbid	3.1
	2/17/2015	1	18.7	33.4	Clear	6.5
	2/18/2015	1	17.8	33.3	Clear	0
	2/18/2015	1	18.1	33.3		4.3
	2/19/2015	2	19.1	33.2	Clear	5.1
	2/19/2015	2	17.3	33.5	Clear	2.5
	2/23/2015	1	16.2	33.1	Clear	7.5
	2/24/2015	1	16.7	31.8	Slightly Turbid	2.4
	2/25/2015	2	16.3	32.3	Clear	1.5
	2/26/2015	4	17	33.5	Clear	2.5
	3/1/2015	3	16.5	33.4	Slightly Turbid	1.7
	3/2/2015	2	15.8	33.6	Clear	N/A
	3/3/2015	2	16.1	33.5	Clear	0.6
	3/4/2015	3	16.4	33.3	Slightly Turbid	0.1
	3/18/2015	1	19.8	33	Slightly Turbid	6.2
	3/18/2015	1	17.7	33.4	Slightly Turbid	3.7
	3/19/2015	1	19.8	33	clear	6
	3/19/2015	4	17.8	33.3	Slightly Turbid	1.5
	3/20/2015	1	19.9	33.3	Clear	5.5
	3/20/2015	3	17.8	33.3	clear	1.7
Tourmaline South	1/11/2015	2	15.8	32.9	Slightly Turbid	0
	1/12/2015	2	15.8	33.3	Clear	0
	1/13/2015	1	15.3	33.2	Clear	0
	1/14/2015	1	14.6	33.5	Clear	0
	1/19/2015	1	15.1	33.3	Slightly Turbid	
	1/19/2015	1	17.3	33.3	Slightly Turbid	5.7

	1/20/2015	1	16.7	33.1	Clear	3.7
	1/20/2015	1	16.3	32.7	Slightly Turbid	0
	1/21/2015	1	16	33.5	Slightly Turbid	0
	1/21/2015	1	17.2	33.3	Clear	8.5
	2/1/2015	2	15.5	33.6	Slightly Turbid	0.9
	2/1/2015	2	17.6	32.8	Slightly Turbid	9.4
	2/2/2015	1	14.9	33.6	Slightly Turbid	0
	2/2/2015	1	17.8	33.5	Slightly Turbid	6.5
	2/3/2015	1	17.3	33.5	Clear	8.9
	2/3/2015	1	15.8	33.6	Clear	0.3
	2/17/2015	1	16.8	33.4	Clear	0
	2/17/2015	1	19	33.4	Slightly Turbid	4.8
	2/18/2015	1	17.8	33.4	Clear	0
	2/18/2015	1	18.1	33.4	Slightly Turbid	6.4
	2/19/2015	2	18	33.5	Slightly Turbid	4.6
	2/19/2015	2	16.8	33.4	Clear	0
	2/23/2015	1	16.1	33.1	Clear	0
	2/24/2015	1	16	33.7	Slightly Turbid	0.3
	2/25/2015	2	16.2	33.6	Slightly Turbid	3.2
	2/26/2015	4	16.4	33.7	Slightly Turbid	0
	3/1/2015	3	15.6	19	Turbid	0
	3/2/2015	2	15.7	33.1	Clear	N/A
	3/3/2015	2	15.4	33.5	Slightly Turbid	N/A
	3/4/2015	3	15.6	33.5	Slightly Turbid	N/A
	3/18/2015	1	18.8	33.3	Slightly Turbid	8.3
	3/18/2015	1	17.5	33.5	Slightly Turbid	3.3
	3/19/2015	1	19.2	33.2	clear	7.8

	3/19/2015	4	17.7	33.2	clear/slightly turbid	N/A
	3/20/2015	3	17.5	33.4	clear	N/A

Statistical output for Model 1 for F+:

Regression Analysis: log (PFU+1) versus Sample Name, Rainfall Entry, Tide Type Entry

Method

Categorical predictor coding (1, 0)
Rows unused 74

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	9	1.31764	0.146404	3.76	0.000
Sample Name	7	0.65570	0.093671	2.41	0.023
Rainfall Entry	1	0.14002	0.140019	3.60	0.060
Tide Type Entry	1	0.00032	0.000320	0.01	0.928
Error	156	6.07567	0.038947		
Lack-of-Fit	10	0.49237	0.049237	1.29	0.243
Pure Error	146	5.58330	0.038242		
Total	165	7.39331			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.197349	17.82%	13.08%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.1103	0.0418	2.64	0.009	
Sample Name					
FM030	-0.0557	0.0542	-1.03	0.306	1.66
OB Pier	-0.0419	0.0537	-0.78	0.437	1.67
PL100	-0.0688	0.0537	-1.28	0.202	1.67
PL110	-0.0511	0.0537	-0.95	0.343	1.67
San Diego River	0.402	0.128	3.14	0.002	1.24
Tourmaline Creek	0.138	0.128	1.08	0.281	1.24
Tourmaline South	-0.0741	0.0542	-1.37	0.174	1.66
Rainfall Entry					
Y	0.0986	0.0520	1.90	0.060	1.43
Tide Type Entry					
L	-0.0030	0.0331	-0.09	0.928	1.14

Regression Equation

log (PFU+1) = 0.1103 + 0.0 Sample Name_FM010 - 0.0557 Sample Name_FM030
- 0.0419 Sample Name_OB Pier - 0.0688 Sample Name_PL100

- 0.0511 Sample Name_PL110 + 0.402 Sample Name_San Diego River
+ 0.138 Sample Name_Tourmaline Creek - 0.0741 Sample Name_Tourmaline South
+ 0.0 Rainfall Entry_N + 0.0986 Rainfall Entry_Y + 0.0 Tide Type Entry_H
- 0.0030 Tide Type Entry_L

Statistical output for Model 1 for somatic coliphages:

Regression Analysis: log (PFU+1) versus Sample Name, Rainfall Entry, Tide Type Entry

Method

Categorical predictor coding (1, 0)
Rows unused 74

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	9	19.1687	2.12986	49.89	0.000
Sample Name	7	13.6078	1.94398	45.54	0.000
Rainfall Entry	1	0.2440	0.24403	5.72	0.018
Tide Type Entry	1	0.0359	0.03586	0.84	0.361
Error	156	6.6594	0.04269		
Lack-of-Fit	10	0.7977	0.07977	1.99	0.039
Pure Error	146	5.8616	0.04015		
Total	165	25.8281			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.206611	74.22%	72.73%	66.93%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.1525	0.0437	3.49	0.001	
Sample Name					
FM030	-0.1310	0.0568	-2.31	0.022	1.66
OB Pier	-0.1254	0.0562	-2.23	0.027	1.67
PL100	-0.0696	0.0562	-1.24	0.217	1.67
PL110	-0.0753	0.0562	-1.34	0.182	1.67
San Diego River	2.037	0.134	15.22	0.000	1.24
Tourmaline Creek	1.033	0.134	7.72	0.000	1.24
Tourmaline South	-0.1002	0.0568	-1.77	0.079	1.66
Rainfall Entry					
Y	0.1302	0.0544	2.39	0.018	1.43
Tide Type Entry					
L	0.0318	0.0347	0.92	0.361	1.14

Regression Equation

log (PFU+1) = 0.1525 + 0.0 Sample Name_FM010 - 0.1310 Sample Name_FM030
- 0.1254 Sample Name_OB Pier - 0.0696 Sample Name_PL100

- 0.0753 Sample Name_PL110 + 2.037 Sample Name_San Diego River
+ 1.033 Sample Name_Tourmaline Creek - 0.1002 Sample Name_Tourmaline South
+ 0.0 Rainfall Entry_N + 0.1302 Rainfall Entry_Y + 0.0 Tide Type Entry_H
+ 0.0318 Tide Type Entry_L

Statistical output for Model 2 for F+ coliphages:

Regression Analysis: log (PFU+1) versus Water Temp (, Sample Name, Rainfall Ent, ...

Method

Categorical predictor coding (1, 0)
Rows unused 86

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	12	0.77884	0.064904	2.17	0.016
Water Temp (^{0}C)	1	0.42193	0.421925	14.10	0.000
Sample Name	5	0.24331	0.048662	1.63	0.157
Rainfall Entry	1	0.05957	0.059568	1.99	0.161
Tide Type Entry	1	0.18178	0.181785	6.07	0.015
Turbidity	4	0.02519	0.006298	0.21	0.932
Error	141	4.21982	0.029928		
Lack-of-Fit	137	4.11410	0.030030	1.14	0.522
Pure Error	4	0.10572	0.026431		
Total	153	4.99867			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.172997	15.58%	8.40%	*

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	1.262	0.327	3.86	0.000	
Water Temp (^{0}C)	-0.0650	0.0173	-3.75	0.000	2.60
Sample Name					
FM030	-0.1038	0.0506	-2.05	0.042	1.85
OB Pier	-0.0888	0.0503	-1.77	0.079	1.77
PL100	-0.1046	0.0501	-2.09	0.039	1.81
PL110	-0.0860	0.0496	-1.73	0.085	1.78
Tourmaline South	-0.1406	0.0523	-2.69	0.008	1.98
Rainfall Entry					
Y	0.0709	0.0503	1.41	0.161	1.21
Tide Type Entry					
L	0.1039	0.0422	2.46	0.015	2.26
Turbidity					
Clear	-0.0242	0.0701	-0.35	0.731	6.29
clear/slightly turbid	0.028	0.187	0.15	0.880	1.16
Slightly Turbid	-0.0316	0.0702	-0.45	0.653	6.21
Turbid	-0.0764	0.0945	-0.81	0.420	2.26

Regression Equation

log (PFU+1) = 1.262 - 0.0650 Water Temp (^{0}C) + 0.0 Sample Name_FM010
- 0.1038 Sample Name_FM030 - 0.0888 Sample Name_OB Pier
- 0.1046 Sample Name_PL100 - 0.0860 Sample Name_PL110
- 0.1406 Sample Name_Tourmaline South + 0.0 Rainfall Entry_N
+ 0.0709 Rainfall Entry_Y + 0.0 Tide Type Entry_H + 0.1039 Tide Type Entry_L
+ 0.0 Turbidity_clear - 0.0242 Turbidity_Clear + 0.028 Turbidity_clear/slightly turbid - 0.0316 Turbidity_Slightly Turbid - 0.0764 Turbidity_Turbid

Statistical output for Model 2 for somatic coliphages:

Regression Analysis: log (PFU+1) versus Water Temp (, Sample Name, Rainfall Ent, ...

Method

Categorical predictor coding (1, 0)
Rows unused 86

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	12	0.71693	0.059744	1.57	0.107
Water Temp (^{0}C)	1	0.00342	0.003424	0.09	0.765
Sample Name	5	0.34691	0.069382	1.82	0.112
Rainfall Entry	1	0.23706	0.237058	6.23	0.014
Tide Type Entry	1	0.00414	0.004141	0.11	0.742
Turbidity	4	0.10024	0.025060	0.66	0.621
Error	141	5.36120	0.038023		
Lack-of-Fit	137	5.36120	0.039133	*	*
Pure Error	4	0.00000	0.000000		
Total	153	6.07814			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.194994	11.80%	4.29%	*

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.132	0.369	0.36	0.722	
Water Temp (^{0}C)	0.0059	0.0195	0.30	0.765	2.60
Sample Name					
FM030	-0.1538	0.0570	-2.70	0.008	1.85
OB Pier	-0.1403	0.0567	-2.48	0.014	1.77
PL100	-0.0848	0.0565	-1.50	0.136	1.81
PL110	-0.0814	0.0559	-1.46	0.147	1.78
Tourmaline South	-0.1040	0.0590	-1.76	0.080	1.98
Rainfall Entry					
Y	0.1415	0.0567	2.50	0.014	1.21
Tide Type Entry					
L	0.0157	0.0475	0.33	0.742	2.26
Turbidity					
Clear	-0.0443	0.0790	-0.56	0.576	6.29
clear/slightly turbid	-0.131	0.210	-0.62	0.534	1.16
Slightly Turbid	-0.0874	0.0791	-1.10	0.271	6.21
Turbid	-0.106	0.107	-1.00	0.319	2.26

Regression Equation

log (PFU+1) = 0.132 + 0.0059 Water Temp (^{0}C) + 0.0 Sample Name_FM010
- 0.1538 Sample Name_FM030 - 0.1403 Sample Name_OB Pier
- 0.0848 Sample Name_PL100 - 0.0814 Sample Name_PL110
- 0.1040 Sample Name_Tourmaline South + 0.0 Rainfall Entry_N
+ 0.1415 Rainfall Entry_Y + 0.0 Tide Type Entry_H + 0.0157 Tide Type Entry_L
+ 0.0 Turbidity_clear - 0.0443 Turbidity_Clear - 0.131 Turbidity_clear/slightly turbid - 0.0874 Turbidity_Slightly Turbid - 0.106 Turbidity_Turbid

APPENDIX II. TRIBUTARY STUDY

Table 1. Coliphage counts for tributary experiments in San Diego.

Sample name	Coliphage type	Sample Date	Processing Date	Total PFU/100mL
01-Ingraham (22:53)	F+	1/31/2016	2/4/2016	<1
01-Ingraham (Time)	F+	1/31/2016	2/4/2016	1
02-Morena	F+	1/31/2016	2/4/2016	14
03-Fashion Valley	F+	1/31/2016	2/4/2016	<1
04-Murphy Canyon	F+	1/31/2016	2/4/2016	<1
05-SD Mission Rd	F+	1/31/2016	2/6/2016	<1
06-Alvarado	F+	1/31/2016	2/6/2016	<1
07-Mission Trails	F+	1/31/2016	2/4/2016	12
08-Sycamore Canyon	F+	1/31/2016	2/6/2016	3
09-Forrester	F+	1/31/2016	2/2/2016	582
10-Carlton Hills	F+	1/31/2016	2/4/2016	5
11-Los coches	F+	1/31/2016	2/4/2016	8
12-Upper Euchlid Hills (13:35)	F+	1/31/2016	2/4/2016	<1
12-Upper Euchlid Hills (Time)	F+	1/31/2016	2/4/2016	<1
01-Ingraham (22:53)	Somatic	1/31/2016	2/2/2016	39
01-Ingraham (Time)	Somatic	1/31/2016	2/4/2016	21
02-Morena	Somatic	1/31/2016	2/4/2016	19
03-Fashion Valley	Somatic	1/31/2016	2/4/2016	55
04-Murphy Canyon	Somatic	1/31/2016	2/2/2016	389
05-SD Mission Rd	Somatic	1/31/2016	2/6/2016	5
06-Alvarado	Somatic	1/31/2016	2/2/2016	<1
07-Mission Trails	Somatic	1/31/2016	2/2/2016	289
08-Sycamore Canyon	Somatic	1/31/2016	2/6/2016	12
09-Forrester	Somatic	1/31/2016	2/2/2016	1
10-Carlton Hills	Somatic	1/31/2016	2/4/2016	152
11-Los coches	Somatic	1/31/2016	2/4/2016	105
12-Upper Euchlid Hills (13:35)	Somatic	1/31/2016	2/2/2016	3
12-Upper Euchlid Hills (Time)	Somatic	1/31/2016	2/2/2016	2

APPENDIX III. DATA FOR DECAY STUDY

Table 1. F+ and somatic coliphage counts in water samples during summer decay study.

	F+ (count/100ml)				**Somatic (count/100ml)**			
Sample name/date	*R1Sun*	*R1Shade*	*R2Sun*	*R2Shade*	*R1Sun*	*R1Shade*	*R2Sun*	*R2Shade*
SEW-D0-R1	943				55300			
Ambient_1RWD_D0	<1				9			
D1	972	3590	1447	8872	18300	19100	12400	13400
D2	93	226	124	138	1150	5140	3120	7470
D3	84	67	59	71	550	320	280	2030
D4	44	36	19	36	512	1262	112	790
D5	14	3	2	20	30	598	130	384
D6	9	22	1	<1	44	212	57	290
D7	20	8	2	2	8	213	<1	274
D8	<1	<1	1	<1	<1	6	<1	2
D9	3	<1	<1	<1	7	11	<1	7
D10	<1	<1	1	<1	<1	2	6	4

Table 2. F+ and somatic coliphage counts in water samples during winter decay study.

Sample Name	Decay Day	Collection Date	F+ PFU/100 mL	Somatic PFU/100 mL
IRWD F+	-	1/8/2015	10	8900
	-	1/9/2015	30	23900
	-	1/9/2015	10	7600
Sewage R1 F+	D0	1/9/2015	70	18100
	D1	1/10/2015	35	16200
	D2	1/11/2015	19	11800
	D3	1/12/2015	24	9400
	D4	1/13/2015	17	13760
	D5	1/14/2015	12	6930
	D6	1/15/2015	28	9790
	D7	1/16/2015	7	9320
	D8	1/17/2015	8	8310
	D9	1/18/2015	14	7550
	D10	1/19/2015	8	5310
Sewage R2 F+	D0	1/9/2015	70	18100
	D1	1/10/2015	28	13300
	D2	1/11/2015	33	7700
	D3	1/12/2015	27	6400
	D4	1/13/2015	12	6680
	D5	1/14/2015	14	5800
	D6	1/15/2015	25	10560
	D7	1/16/2015	26	8390
	D8	1/17/2015	8	5740
	D9	1/18/2015	9	7180
	D10	1/19/2015	4	8410

Table 3. Additional water parameters in water samples during the winter decay study.

Sample Name	Decay Day	Turbidity (NTU)	Temp (^{0}C)	Specific Conductance (mS/cm)	Salinity (ppt)	Chl (ug/L)	ODOsat (%)	ODO (mg/L)
IRWD F+	-	18.2	12.260	2.615	1.361	17.990	76.804	8.159
	-	16.19	12.581	2.608	1.360	7.984	67.949	7.174
	-	16.19	12.581	2.608	1.360	7.984	67.949	7.174
Sewage R1 F+	D0	16.19	12.581	2.608	1.360	7.984	67.949	7.174
	D1	10.19	12.893	2.615	1.360	5.449	51.275	5.378
	D2	23.5	14.136	2.594	1.347	6.651	38.135	3.878
	D3	14.45	14.764	2.527	1.312	6.986	35.786	3.600
	D4	13.39	14.754	2.400	1.242	8.236	36.530	3.678
	D5	13.32	13.894	2.387	1.236	6.084	30.742	3.154
	D6	12.01	13.135	2.395	1.240	5.173	28.925	3.017
	D7	17.39	12.726	2.402	1.244	4.985	29.974	3.155
	D8	34.1	12.694	2.409	1.250	4.869	30.447	3.207
	D9	15.97	12.825	2.420	1.254	4.826	35.995	3.779
	D10	13.74	12.777	2.431	1.260	5.391	42.620	4.483
Sewage R2 F+	D0	16.19	12.581	2.608	1.360	7.984	67.949	7.174
	D1	10.19	12.893	2.615	1.360	5.449	51.275	5.378
	D2	23.5	14.136	2.594	1.347	6.651	38.135	3.878
	D3	14.45	14.764	2.527	1.312	6.986	35.786	3.600
	D4	13.39	14.754	2.400	1.242	8.236	36.530	3.678
	D5	13.32	13.894	2.387	1.236	6.084	30.742	3.154
	D6	12.01	13.135	2.395	1.240	5.173	28.925	3.017
	D7	17.39	12.726	2.402	1.244	4.985	29.974	3.155
	D8	34.1	12.694	2.409	1.250	4.869	30.447	3.207
	D9	15.97	12.825	2.420	1.254	4.826	35.995	3.779
	D10	13.74	12.777	2.431	1.260	5.391	42.620	4.483
IRWD Somatic	-	18.2	12.260	2.615	1.361	17.990	76.804	8.159
	-	16.19	12.581	2.608	1.360	7.984	67.949	7.174
	-	16.19	12.581	2.608	1.360	7.984	67.949	7.174
Sewage R1 Somatic	D0	16.19	12.581	2.608	1.360	7.984	67.949	7.174
	D1	10.19	12.893	2.615	1.360	5.449	51.275	5.378
	D2	23.5	14.136	2.594	1.347	6.651	38.135	3.878
	D3	14.45	14.764	2.527	1.312	6.986	35.786	3.600
	D4	13.39	14.754	2.400	1.242	8.236	36.530	3.678
	D5	13.32	13.894	2.387	1.236	6.084	30.742	3.154
	D6	12.01	13.135	2.395	1.240	5.173	28.925	3.017
	D7	17.39	12.726	2.402	1.244	4.985	29.974	3.155

	D8	34.1	12.694	2.409	1.250	4.869	30.447	3.207
	D9	15.97	12.825	2.420	1.254	4.826	35.995	3.779
	D10	13.74	12.777	2.431	1.260	5.391	42.620	4.483
Sewage R2 Somatic	D0	16.19	12.581	2.608	1.360	7.984	67.949	7.174
	D1	10.19	12.893	2.615	1.360	5.449	51.275	5.378
	D2	23.5	14.136	2.594	1.347	6.651	38.135	3.878
	D3	14.45	14.764	2.527	1.312	6.986	35.786	3.600
	D4	13.39	14.754	2.400	1.242	8.236	36.530	3.678
	D5	13.32	13.894	2.387	1.236	6.084	30.742	3.154
	D6	12.01	13.135	2.395	1.240	5.173	28.925	3.017
	D7	17.39	12.726	2.402	1.244	4.985	29.974	3.155
	D8	34.1	12.694	2.409	1.250	4.869	30.447	3.207
	D9	15.97	12.825	2.420	1.254	4.826	35.995	3.779
	D10	13.74	12.777	2.431	1.260	5.391	42.620	4.483

Table 4. Bacterial counts for winter decay study conditions.

Sample Name	**Decay Day**	**Bacteria**	**Winter (log10MPN/100 ml)**
Sewage R1	D0	Total Coliform	7
			7
	D1		8
	D2		8
			8
	D3		6
			6
	D4		5
			6
	D5		5
			5
	D6		5
			5
	D7		5
			5
	D8		5
			5
	D9		5
			5
	D10		4
			4
			5
			5
Sewage R2	D0	Total Coliform	6
			6
	D1		6
			6
	D2		8
			8
	D3		6
			6
	D4		6
			6
	D5		5
			5
	D6		5

			5
	D7		4
			4
	D8		5
			6
	D9		5
			5
	D10		5
			5
Sewage R1	D0		6
			6
	D1		6
			6
	D2		7
			7
	D3		5
			5
	D4		4
			4
	D5		4
			4
	D6	E. coli	4
			4
	D7		4
			4
	D8		4
			4
	D9		4
			4
	D10		2
			2
			3
			3
Sewage R2	D0		6
			6
	D1		8
	D2	E. coli	7
			7
	D3		6

			5
	D4		4
			5
	D5		4
			4
	D6		4
			4
	D7		4
			3
	D8		4
			4
	D9		4
			4
	D10		4
			4

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