

Gardner-Webb University

Digital Commons @ Gardner-Webb University

Undergraduate Honors Theses

Honors Program

2020

Analysis of Bacterial DNA and Water Quality: Surface Water Sampling at Gardner-Webb University

Celsea Reeder

Follow this and additional works at: <https://digitalcommons.gardner-webb.edu/undergrad-honors>



Part of the [Biology Commons](#), [Microbiology Commons](#), and the [Pharmacology, Toxicology and Environmental Health Commons](#)

Citation Information

Reeder, Celsea, "Analysis of Bacterial DNA and Water Quality: Surface Water Sampling at Gardner-Webb University" (2020). *Undergraduate Honors Theses*. 40.

<https://digitalcommons.gardner-webb.edu/undergrad-honors/40>

This Thesis is brought to you for free and open access by the Honors Program at Digital Commons @ Gardner-Webb University. It has been accepted for inclusion in Undergraduate Honors Theses by an authorized administrator of Digital Commons @ Gardner-Webb University. For more information, please see [Copyright and Publishing Info](#).

Analysis of Bacterial DNA and Water Quality: Surface Water Sampling at Gardner-Webb University

An Honors Thesis
Presented to
The University Honors Program
Gardner-Webb University
2 April 2020

by

Celsea Reeder

Accepted by the Honors Faculty

Dr. David Campbell, Thesis Advisor

Dr. Tom Jones, Associate Dean, Univ.
Honors

Prof. Frank Newton, Honors Committee

Dr. Christopher Nelson, Honors Committee

Dr. Bob Bass, Honors Committee

Dr. Shea Stuart, Honors Committee

A sincere thank you to the Natural Sciences Department and the Honors Program of Gardner-Webb University for the generous funding of this research. Thank you to my thesis advisor, Dr. David Campbell, for your help, as well as family and friends for encouraging me to never give up in the pursuit of knowledge and dreams.

Table of Contents	Page #'s
1. Abstract.....	5
2. Introduction.....	6-25
I. Freshwater Environments.....	7
II. Defining Pollution.....	8-11
i. Point Sources.....	8-9
ii. Diffuse (Nonpoint Source).....	9
iii. Chronic Pollution.....	9-10
iv. Episodic Pollution.....	10
v. Plastic Pollution.....	10-11
III. Effects on Wildlife.....	11-14
IV. Lotic vs. Lentic Water Systems.....	14-16
V. Bacteria.....	16-18
VI. Extraction of 16S rRNA.....	18-21
VII. Water Quality & Tested Factors in the “HACH® Surface Water Test Kit”.....	21-25
i. About pH.....	22
ii. About Temperature.....	22-23
iii. About Nitrate.....	23
iv. About Ammonia.....	24
v. About Orthophosphate.....	24
vi. About Dissolved Oxygen.....	25

3. Methods.....	25-39
I. Surface Water Sample Collection.....	25-29
II. Vacuum Filtration & Utilization of the <i>Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit”</i>	29-32
III. Utilizing the “ <i>HACH® Surface Water Test Kit</i> ”.....	33-38
i. Determining pH.....	33
ii. Determining Temperature.....	34
iii. Determining Nitrate Levels.....	34-35
iv. Determining Ammonia Levels.....	35-36
v. Determining Orthophosphate Levels.....	36
vi. Determining Dissolved Oxygen Levels.....	37-38
IV. Sending & Receiving Test Results for the <i>Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit”</i>	38-39
V. DNA Sequence Analysis.....	39
4. Results.....	40-56
I. Results for <i>Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit”</i>	40-54
i. <i>BR1 Results</i>	44-46
ii. <i>BR2 Results</i>	46-47
iii. <i>BR3 Results</i>	47-48
iv. <i>BR4 Results</i>	48
v. <i>BS1 Results</i>	49-50
vi. <i>BS2 Results</i>	50

vii.	<i>CR1 Results</i>	50
viii.	<i>CR2 Results</i>	50-51
ix.	<i>Phylogenetic Analysis of the DNA Samples</i>	51-54
II.	Results for “HACH® Surface Water Test <i>Kit</i> ”	55-56
5.	Discussion.....	56-74
I.	Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit”.....	56-68
i.	<i>BR1</i>	56-58
ii.	<i>BR2</i>	58-59
iii.	<i>BR3</i>	59-61
iv.	<i>BR4</i>	61-63
v.	<i>BS1</i>	63-65
vi.	<i>BS2</i>	65
vii.	<i>CR1</i>	65-66
viii.	<i>CR2</i>	66
ix.	<i>Phylogenetic Analysis of the DNA Samples</i>	66-68
II.	“HACH® Surface Water Test Kit”.....	68-74
6.	Conclusion.....	74-77
7.	Bibliography.....	78-88

Abstract:

Part 1 of this experiment was to explore the bacterial types within the surface water on the campus of Gardner-Webb University, located in Boiling Springs, NC. Two water samples were collected from four locations on campus, including the boiling spring, Lake Hollifield, and an adjacent creek. Using the Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit,” the bacterial DNA within these samples was isolated and sent to Psomagen, Inc. for analysis. The resulting DNA sequences were analyzed through BLAST, and subsequently interpreted. For the second part of this experiment, one surface water sample was taken from the boiling spring, and another was taken from Lake Hollifield. These samples were analyzed using the “HACH® Surface Water Test Kit” to determine the water quality. The factors tested for included pH, temperature, and nitrate, ammonia, orthophosphate, and dissolved oxygen levels.

A variety of bacterial strains were identified within this study. While the percent identity was not 100%, a mixture of bacterial types ranging from *Limnohabitans*, *Clostridiales*, *Curvibacter*, *Rhodoferrax*, *Vibrio*, and *Acidovorax* displayed a percent identity ranging from approximately 80% to 99.5%, among dozens of other genera. The phylogenetic study revealed that the bacterial types discovered within all collection sites showed similarity to one another. Finally, it was revealed that the temperature and nitrate, ammonia, and dissolved oxygen levels were within the normal ranges for the two collected samples. However, the pH was slightly acidic, and the orthophosphate levels were above average. These results could yield information pertaining to present pollutants and their environmental impact.

Introduction

Bacterial DNA isolation, sequencing, and analysis can yield information about the bacterial life that is present in various environments such as freshwater ecosystems, or within the depths of the Earth's soil or snow. Characterization of the different bacterial types within a certain location can help one to determine if the site is contaminated, or if the bacteria present could pose a threat to other living organisms within the same environment. Additionally, further factors pertaining to the quality of a body of water could influence the various bacterial types that are present within that location. Some of these factors which reflect water quality include pH, temperature, bacterial content, and nitrate, ammonia, orthophosphate, and dissolved oxygen levels, which were all elaborated upon in this extensive study.

The purpose of this study was to examine and characterize bacterial DNA isolations from eight total surface water samples on the campus of Gardner-Webb University, located in Boiling Springs, North Carolina. From these samples, the 16S ribosomal RNA was extracted and sequenced from bacterial specimens within the eight collected surface water samples. Additionally, two other surface water samples were collected and tested to determine their pH, temperature, and nitrate, ammonia, orthophosphate, and dissolved oxygen levels. One sample was collected from the boiling spring while the other sample was collected from the northeastern side of Lake Hollifield. Before examining the results of this study, one must first have an understanding of the properties of the bodies of water utilized for testing, as well as a conceptual grasp of how the previously listed test factors play a role in the quality of a water source.

Freshwater Environments

For the purpose of this study, samples from freshwater sources were taken and utilized (the methods of sample collection will be later discussed). These freshwater sources included a “boiling” spring, a lake, and a creek, all within relative proximity of one another and on the same college campus grounds. Freshwater locations include reservoirs, lakes, rivers, and groundwater, while saltwater (saline) sources include, for example, coastal waters and estuaries. In both freshwater and saltwater systems, pathogen contamination is a major problem that can result in the transmission of diseases to humans and animals alike. Given that pathogens are any organisms (such as a virus, bacterium, or other microorganism) that cause disease, it is imperative to test water sources of interest to ensure that they do not contain any pathogens to ensure the safety of those living around or drinking the water (Pandey et al., 2014).

The surface water locations utilized for research were tested for the purpose of determining if they contained any known pathogens that would pose a direct risk to the wildlife that lives in them. Additionally, I was interested in studying the bacterial life within the freshwater locations on the campus of Gardner-Webb University. This study in conjunction with further experimentation to determine the pH, temperature, and nitrate, ammonia, orthophosphate, and dissolved oxygen levels of the surface water samples would yield information pertaining to the overall quality of the freshwater locations of Gardner-Webb University.

Defining Pollution

Various types of water pollution can affect both saltwater and freshwater ecosystems alike. Environmentally speaking, pollution can be defined as,

“the introduction by man into the environment of substances or energy liable to cause hazards to human health, harm to living resources and ecological systems, damage to structure or amenity, or interference with legitimate uses of the environment” (Mason, 2002, p. 3).

Historically speaking, the Industrial Revolution was the beginning of the introduction of new water and air pollutants. This introduction led to a series of changes that would forever shape sanitary conditions, waste collection, waste treatment, and laws concerning public health. The Industrial Revolution from 1760-1840 was a time in England where labor shifted from primarily agrarian to machinery. As a result, large factories were being created to keep up with the mass production of goods. These factories that were created ultimately discharged carbon dioxide into the atmosphere, in addition to damaging water sources through chemical runoff and dumping (Warren, 1971). Over time, scientists learned about the results and effects of various pollutants. Additionally, several different types of pollution were identified and studied in-depth. These pollution sources are man-made or natural, and can be defined as point sources, diffuse, chronic, or episodic in nature, which will be discussed at length.

Point Sources

Some forms of pollutants derive from what are known as “point sources.” These are localized outputs that have available treatments at hand. For example, factory waste or the discharge from a sewage plant are point sources. State and federal agencies

typically regulate point source pollution. As the name implies, point sources are easily identifiable and recognizable. These point sources can pollute the environment by spewing toxic particles and chemicals into the air and waterways, such as sulfur dioxide, heavy metals, carbon monoxide, nitrogen dioxide, *etc.* (“Point Source vs. Nonpoint Source Pollution,” 2020).

Diffuse (Nonpoint Source)

Diffuse sources of pollution (also known as “nonpoint sources”) occur where there exist various contaminants and pollutants from a broad range of sources. Nonpoint-sources are not easily identifiable like point sources are. An example of a nonpoint-source is runoff, in which water is carried toward lakes and streams as it runs down land in instances of heavy rainfall or flooding. In this manner, wastewater that contains harmful chemicals can be unloaded into adjacent creeks, streams, and other bodies of water. Through runoff, an abundance of nutrients and harmful microbes can be introduced to other water systems. Therefore, abundant algae growth could be a result of the nutrient abundance within the water, which could lead to further pollution of the water source (Mason, 2002, p. 3-4).

Chronic Pollution

Chronic pollution (also known as “steady state” pollution) can be defined as pollution that a waterway constantly receives. The pollution received from wastewater within urban or rural areas, or various farming practices that cause pollution through the form of pesticides and fertilizer are prime examples of chronic pollution sources. The vast majority of the pollution that contaminates waterways is chronic in nature. An example of chronic pollution occurred from 1932 to 1966, in which methylmercury was

released from a chemical plant off of the coast of Minimata Bay in Japan on a daily basis. This exceptionally toxic substance contaminated the food chain by entering the fish and sea life living within the waters. As a result, local consumers were poisoned by the methylmercury that was present within the seafood that ate the contaminated substance from the waters (Masazumi, 1995, pp. 1-24).

Episodic Pollution

A type of pollution which is variable in nature is known as episodic pollution. Episodic pollution is also known as “intermittent” pollution due to the fact that it is mostly random and cannot be predicted ahead of time. Examples of episodic pollution include accidents; for example, if someone crashes a road tanker near a river, releasing harmful chemicals into the water. Other examples of episodic pollution include heavy rainfall which upturns acid from soils or causes waste systems to overflow and pollute the surrounding areas. Episodic pollution is of the utmost concern to water managers, as one random event or accident can critically damage and contaminate a body of water (Mason, 2002, p. 3-4).

Plastic Pollution

Various plastics introduced by humans have devastated freshwater and saltwater sources over the decades. These plastic pollutants originate from several different locations, including litter and waste dropped from towns and cities, fishing equipment that has been lost, litter discarded by individuals at beaches, debris that is sewage-related, landfill sites and bins near the coast that are poorly managed, and even shipping equipment and materials that have been accidentally dropped overboard into the water. The introduction of plastics and other waste into freshwater and saltwater locations has

caused a dramatic impact on the surrounding wildlife that lives around or within the water, as well as affecting water quality (“Plastic Pollution-Facts and Figures,” 2020).

Two types of plastic pollution are currently recognized: microplastic and macroplastic. Microplastics are defined as small pieces of plastic less than 5 millimeters in size that pollute the environment. Macroplastics, on the other hand, are generally larger than 5 millimeters in size. Studies have shown that, while the effects of macroplastic pollution can be directly observed, microplastic pollution is still being studied to determine the effects on surrounding wildlife and water quality (Bucci, K., et al., 2019).

Effects on Wildlife

Pollutants in and around water sources can have a devastating effect on local wildlife. Macroplastics such as plastic bags and soda straws, for example, can be choking hazards for wildlife. Sea turtles are commonly observed swallowing plastic bags, as they mistake the bag for jellyfish. Straws can become lodged within the nasal passages of seals and turtles, and fishing nets can become wrapped around the fragile legs of seagulls and other waterfowl. A study in 2015 estimated that there are over 15 trillion plastic trash pieces littering the Earth’s oceans every year, and this number is constantly increasing (“Ocean Plastic & Sea Turtles,” 2020).

Interestingly, recent research reveals that many different animals such as turtles and avian wildlife are consuming plastic found within oceans because it even smells like food to them. A chemical called dimethyl sulfide is released into the air when phytoplankton within the water are crushed or consumed. This chemical is what attracts some animals to a specific food source. While dimethyl sulfide may smell like oysters or seaweed to humans, this chemical smells like “dinner” to various wildlife. When plastic

is discarded into oceans, phytoplankton are able to grow on these pollutants. Studies show that birds that hunt by primarily utilizing scent to locate dimethyl sulfide ate more plastic pollutants than other bird species that do not hunt through primarily utilizing their sense of smell. The most obvious way to avoid macroplastic pollution and to prevent animals from ingesting harmful plastics is through cleaning up litter, using biodegradable products, and through using reusable products and bags (Yeager, 2019). Some of the toxic chemicals and compounds within the water are known as endocrine disruptors. Pollution within the water can disrupt the endocrine system of aquatic life as the toxic chemicals affect the natural hormones of the body. This can, in turn, negatively affect an organism's growth, health, and reproductive abilities (Quesada-Calderón, et al., 2017).

Apart from the observable effects on wildlife, various microscopic pollutants within the water could be detrimental to the animals living within. Fertilizer that is meant to increase the fertility of soil can runoff into lakes, streams, and creeks during periods of rainfall. As a result, the fertilizer present in the water promotes the growth of various types of algae. This process is known as eutrophication, in which the water becomes excessively rich in nutrients such as phosphates and nitrates that promote the growth of algae and other plant life. While this may seem harmless, the plants and algae can grow and ultimately take over other species. The consumers living within the water take up most of the oxygen during respiration, as can decay. This can be harmful, as aquatic life within the water relies on the oxygen to survive. With its rapid depletion, animal life is threatened. Additionally, as the plant life within the water dies eventually, they sink near the bottom of the body of water, where the various decomposing bacteria also use up any

additional oxygen in the water. Crustaceans and other life within will die as a result of the oxygen depletion (Walls-Thumma, 2018).

The excessive growth of algae can also be fatal if consumed by animals. Blue-green algae that is typically present within small lakes and ponds can become toxic if the water is stagnant, hot, and there is little to no rain present. Depending on the algae type, it can appear to be blue, brown, red, or green in coloration. Only a few algal types can produce toxins. In response to high temperatures, excessive light, and the nutrient levels within the water, the algae will begin to produce toxins that can be harmful to fish, fowl, and even humans. Some examples of toxins produced include saxitoxins, brevetoxins, domoic acid, and microcystin. These toxins have different results on wildlife and can affect the nervous system, respiratory system, liver, and muscles if ingested (“Algal Blooms,” 2020).

In the summer of 2019, three dogs within the same family went swimming in a local pond polluted with algae in Wilmington, North Carolina. Within 15 minutes of swimming in the water, the dogs began suffering from seizures, and within a few hours, all three dogs had passed away. Unfortunately, the toxins produced from blue green algae are extremely potent and have been proven to harm animals swimming in and ingesting the water. In order to reduce the amount of toxins produced by the algae, it is important to limit fertilizer runoff and excessive nutrient presence within the water (Thompson, 2019).

It is therefore pertinent to have a balance between the amount of nutrients in the water with the wildlife living within, as some wildlife can even contribute to the production of toxins within the water. Interestingly, one single goose can produce 1.5

pounds of waste each day. Imagining an entire lake with an abundance of geese, it is apparent that lakes, streams, creeks, and ponds can easily suffer if an excessive amount of feces is present within the water. This is because fecal coliform bacteria living within the feces are excreted into the water. Fecal coliform bacteria can contribute to the proliferation of water borne diseases such as gastroenteritis, dysentery, typhoid fever, and ear infections. Nutrients present within the feces, such as nitrogen and phosphorus, contribute to plant and algae growth, thus reducing oxygen amounts within the water. Balance between the water contents and the wildlife within is important for ecosystems to thrive (Blount, 2019).

Lotic vs. Lentic Water Systems

Continental waters can ultimately be divided into two major categories. These categories are lotic and lentic water systems, and ecologists choose to divide bodies of water in this way based on whether the water within is flowing or still. A lotic water system includes water that is flowing and constantly moving, such as is present within rivers, creeks, streams, or brooks. Lentic water systems are still and non-moving, and include lakes, ponds, and marshes, to name a few. The water sampled for experimentation was therefore comprised of both lotic and lentic water systems (Sharma, 2018).

The samples collected from the boiling spring were collected from a lotic water system, as the spring was constantly bubbling and moving. It should be noted that this “boiling spring” was not actually hot in temperature but is considered “boiling” due to its constant and vigorous bubbling of the water. When collecting water from the lake, this source was identified as a lentic water system, as the water was still and not constantly

flowing. However, some movement was generated within the lake from the large fountain present at the heart of Lake Hollifield. Regardless, this source was still considered a lentic water source. Samples collected from the creek adjacent to the Broyhill Adventure Course were considered as being collected from a lotic water system, as the creek was constantly moving and flowing.

Collecting from both lotic and lentic water sources allows for variation between the bacterial types present within each source, as well as variation between the calculated quantity of nitrate, ammonia, orthophosphate, and dissolved oxygen levels when determining water quality. For this experiment, I chose to sample a variety of lotic and lentic water sources; when determining water quality, I decided to compare the water quality between the lotic source of the boiling spring and the lentic source of Lake Hollifield. While both lotic and lentic water sources can be equally contaminated with pollutants from fertilizers, runoff, sewage water, pesticides, and various plastics, one factor that I wished to study in this experiment was whether the water quality between lotic and lentic water sources on the campus of Gardner-Webb University differed in their contents and bacterial types (Sharma, 2018).

As previously stated, runoff can have a major effect on the water quality of both lotic and lentic water systems. On one occasion during sample collection from Lake Hollifield, heavy rain was present and could be observed running off into the lake and nearby creek. Throughout the spring semester, Boiling Springs had several periods of heavy rainfall, which potentially washed waste, fertilizer, and other pollutants into the lotic and lentic water systems of the university. One period of excessive runoff on the campus of Gardner-Webb University can be viewed on the following page. Most of the

water within the creek in the center of the picture originates from the nearby storm drains. The brown color of the water reflects the significant sediment runoff into the system.



Figure 1. Photograph depicting runoff into the adjacent boiling spring, the creek running into Lake Hollifield, and into Lake Hollifield on the campus of Gardner-Webb University (Campbell, 2020).

Bacteria

The types of bacteria present within a body of water can be an indicator of the overall quality of the water itself. While some bacteria are beneficial in nature, others are linked to disease and illness in humans and animals alike. *Escherichia coli* is a type of bacteria that is widespread and diverse. Some strains of *E. coli* are relatively harmless, but others can cause serious illness, urinary tract infections, pneumonia, and respiratory problems. Typically, water-borne illness is the result of coming into contact with or consuming bacteria from water sources or the feces of animals. It should be noted that, while some bacterial strains may cause mild symptoms in some, the same strain can prove to be deadly to individuals with compromised immune systems, the elderly, or young children (“Bacteria and *E. Coli* in Water,” 2019).

Bacteria are also present in the residential setting, and can be found on kitchen countertops, faucets, fans, and other household items. The primary concern with bacteria in the kitchen is the possibility of illness through foodborne disease. One published scientific study revealed that, in a household setting, 34 bacterial phyla and archaeal phyla were identified, especially phyla Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes. Few genera were found that were related to foodborne illness but some were discovered in locations including faucet water and countertops. Human skin was discovered to be the primary source of the bacteria within the parameters of this study conducted in 2012. For my experimental study, it was interesting to discover bacterial types within those phyla; these were discovered within the various freshwater locations on the campus of Gardner-Webb University (Flores, Gilbert, et al., 2013).

As it has been proven that various bacterial phyla contaminate kitchen settings, generally microbial risks are associated with the ingestion of human or animal feces through contaminated water. In the United States alone, it is estimated that approximately 560,000 individuals suffer from diseases that are waterborne, and there are nearly 12,000 deaths each year as a result. Some of the main bacterial diseases that are transmitted through water sources include gastroenteritis, cholera, acute diarrheas, bacillary dysentery, and serious salmonellosis or typhoid fever. The bacterial species known to cause gastroenteritis are *Vibrio parahaemolyticus* and *Escherichia coli*. Additionally, *Vibrio cholerae* is known to cause cholera, *Escherichia coli* causes acute diarrheas, and several strains of *Shigella* (*Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, *Shigella sonnei*) are known to cause bacillary dysentery or shigellosis. Several *Salmonella* strains are known to cause typhoid fever and salmonellosis (Cabral, 2010).

For this study, I was interested if any of these bacterial strains were present within the various freshwater locations sampled on the campus of Gardner-Webb University. Given that numerous geese and duck species are present in and around the water sources on campus, I was interested if any bacterial types associated with avian feces and known to cause diseases in humans and animals would be discovered. Many students and local individuals walk their dogs around Lake Hollifield and across campus as well. Therefore, runoff could be distributing feces from pets into the surface water locations, in addition to the feces of other local animals including squirrels and other rodents, or other mammalian and avian species. It was therefore a possibility and a question as to whether or not harmful bacterial species would be discovered in the sampled sources.

Extraction of 16S rRNA

To identify various Archaea and Bacteria types, 16S ribosomal RNA is utilized in a laboratory setting. When studying bacterial taxonomy and phylogeny, 16S rRNA is used, mainly because it is present in all bacteria, its function has not changed, and because it is large enough to yield informative results (Janda & Sharon, 2007).

Ribosomal RNA (ribonucleic acid) makes up part of the ribosome, which is an organelle within cells that produces proteins and can be found abundantly within the cytoplasm.

(Cheriyedath, 2018).

Types of rRNA differs in size. The smaller sized and larger sized ribosomal RNA units combine with ribosomal proteins. These combinations form small and large ribosomal subunits. Within bacteria, these are known as the 30S and 50S ribosomal subunits, with the “S” standing for “Svedberg,” which is a unit of measurement for the sedimentation rate. The sedimentation rate refers to the rate in which particles or

molecules move towards the bottom portion of a test tube after centrifugation. Svedberg units are measurements of time and can be defined as 10^{-13} seconds. This measurement is influenced by the density, mass, and shape of the molecules or particles themselves (Kaiser, 2019).

Eukaryotic ribosomal subunits have sedimentation rates of 40S and 60S, as their contents of proteins and ribosomal RNA molecules differ from prokaryotes. For the isolation of bacterial DNA, the 16S rRNA of the bacteria should be extracted and utilized for analysis to get the best results. The 16S rRNA is made of about 1,540 nucleotides and is a part of the prokaryotic 30S subunit, in addition to 21 proteins (Kaiser, 2019). An illustration depicting the 16S rRNA within the bacterial species *Escherichia coli* can be viewed on the next page.

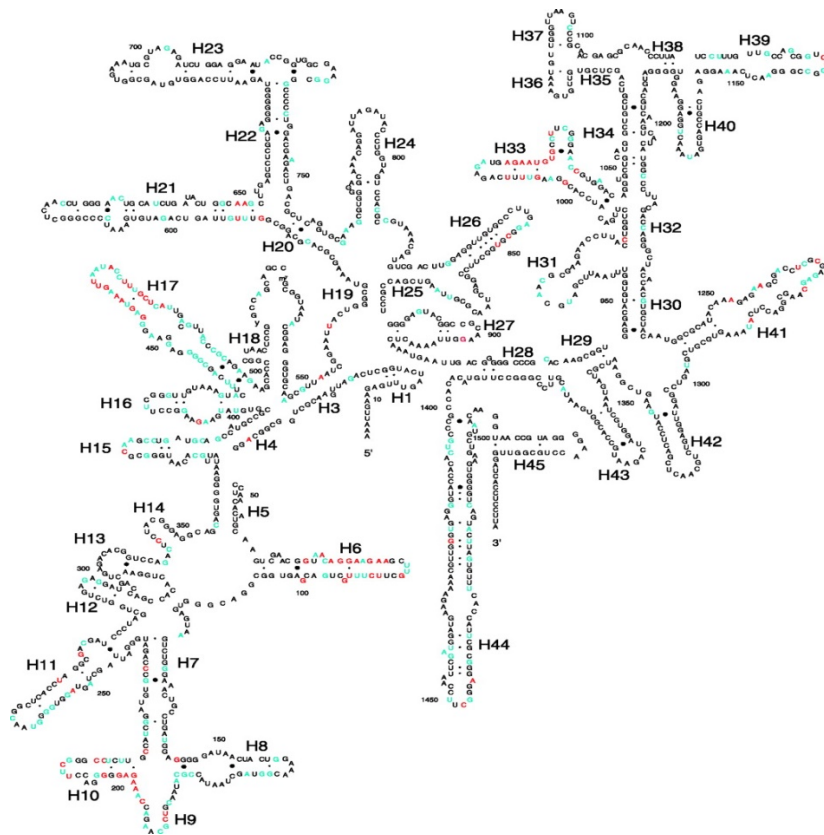


Figure 2. Illustration depicting the 2-D structure of the 16S rRNA within *Escherichia coli*. The part of the gene analyzed for experimentation can be seen from the H18 to the H24 loop region (Kaiser, 2019).

Genomic DNA of bacterial samples must be purified if one desires to perform molecular analysis or to study the bacterial strains present within a particular sample. Typically, this process is accomplished through commercially available kits, such as the Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit” that was purchased and used for this research. While these kits are expensive, they are user-friendly and readily packaged. These kits make the isolation of 16S rRNA from bacterial samples a much easier task (Wright, et al., 2017). The Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit” is designed to rapidly isolate bacterial DNA from tough-to-lyse cells (“Quick-DNA Fungal/Bacterial Miniprep Kit,” 2019).

The utilization of 16S rRNA allows for an abundance of a myriad of taxa to be easily identified for analysis. 16S rRNA is found in prokaryotes, mitochondria, and chloroplasts, so all organisms will have an equivalent sequence. This is because the isolation of 16S rRNA is easier than direct DNA-DNA hybridization techniques, which are difficult and complex. DNA hybridization assays are more definitive compared to using 16S rRNA, however, but they are extremely expensive and take much time to complete. In most cases, the utilization of 16S rRNA for bacterial samples allows for species and genus identification; unfortunately, this identification may only be of a low percentile (Janda & Sharon, 2007).

Some reasons as to why there are difficulties in identification of species and genus include the fact that some species may have identical or similar 16S rRNA sequences, difficulty in recognition of novel taxa, or the fact that many bacterial species have never been studied and had their sequences submitted to the nucleotide databases. Furthermore, the presence of genomovars can make identification difficult. Genomovar is a term denoting types of bacteria that belong to a particular genus that cannot be phenotypically differentiated but are distinct genotypically. This term is commonly used within the genera *Agrobacterium* and *Burkholderia* (Janda & Sharon, 2007).

Water Quality & Tested Factors in the “HACH® Surface Water Test Kit.”

The “HACH® Surface Water Test Kit” tests for factors concerning water quality, such as pH, dissolved oxygen, orthophosphate levels, ammonia, nitrate, temperature, and chloride levels. Each of these components uniquely reflect the quality of a sampled water source; therefore, it is pertinent to understand each term and to have a grasp of what each factor encompasses.

About pH

When determining the pH of a water sample, one is essentially determining how acidic or basic a water source is. The pH range extends from 0 to 14, with 0 being the most acidic rating and 14 being the most basic. Some extreme solutions can have a negative pH, or a pH extending beyond 14. A rating of 7 is neutral on the pH scale. Essentially, the pH of something is the measurement of the amount of hydroxyl and hydrogen ions within the water. Samples that are more acidic contain more hydrogen ions, while basic samples contain more hydroxyl ions than hydrogen ions. The pH is recorded in logarithmic units and is ultimately an excellent indicator of the quality of a water source (“pH and Water,” 2020).

Freshwater sources typically have a pH ranging from 6-8. Generally, the pH of water sources is higher if the water is sampled closer to the surface. If the pH of a water source is extremely high or low, the effects could be detrimental to humans or animals that are consuming or living within the water. An extremely basic pH can cause drinking water to taste bitter and can cause deposits to become encrusted in pipes and appliances that utilize water. Various pollutants, natural soil variations, and some rock types can also change the pH of a water source, which can be harmful to the wildlife living within the water (“pH and Water,” 2020).

About Temperature

Temperature is the measure of hotness or coldness on a scale, or the thermal energy of the particles within a substance. The outside temperature can affect the biological and chemical characteristics of surface water locations. Interestingly, the ambient temperature within an environment can alter the temperature of a water source,

which in turn can affect photosynthetic rates of aquatic plants, dissolved oxygen concentration within the water, disease, sensitivity to pollution, and the metabolic rates of the different aquatic organisms living within the water (“Water Quality Assessment: Physical: Temperature,” 2004).

When warmer water is introduced into another body of water that has a colder temperature, this is known as “thermal pollution.” An example of this occurs near power plants, in which hot water that has been used to cool the equipment is directly discharged into other sources of water such as streams. Urban runoff is another form of thermal pollution, in which water is heated as it flows across hot parking lots and sidewalks on hot days and into nearby water sources. Warm water cannot dissolve as much oxygen. Increased temperatures thus increase the metabolic rates of the aquatic plants within the water, therefore increasing their demand for oxygen. Unfortunately, if water temperatures are too high, then any aquatic organisms are left weakened, as dissolved oxygen levels subsequently decrease (“Water Quality Assessment: Physical: Temperature,” 2004).

About Nitrate

Nitrates are inorganic compounds that are formed when nitric acid loses a proton. Nitrates occur naturally at low concentrations but can be high due to pollution. They consist of three oxygen atoms that surround a central nitrogen atom. If present within water sources, nitrates can be toxic to the plant and aquatic life within (“National Center for Biotechnology Information,” 2019). Fertilizers, manure, and septic systems can introduce nitrates into water sources through runoff. Nitrates are the oxidized form of dissolved nitrogen, and if present in high quantities in water, can be toxic to the aquatic life living in the water (“Nitrate Contamination,” 2020).

About Ammonia

Ammonia is essentially a nutrient that is made up of hydrogen and nitrogen. It is pertinent for plant growth and has the ability to be converted to nitrate and nitrate through bacteria. This, in turn, is utilized by plants. Animals of all species secrete ammonia through their excrement. Additionally, ammonia is produced when animals and plants decompose after death. While relatively common, ammonia is a pollutant that has the capacity of being toxic if present in high levels. Too much ammonia in water sources can cause decreased plant growth and death in wildlife and aquatic animals. The un-ionized form of ammonia is extremely toxic (more-so than the ionized form of ammonia), and higher temperatures are likely to contain the un-ionized form. Toxicity also increases as the pH increases (“Ammonia,” 2019).

About Orthophosphate

Orthophosphates are formed naturally, but are also created through sewage waste, agricultural site runoff, and the utilization of lawn fertilizers. They are one of the three forms of phosphates, with the other two forms being organically bound phosphate and metaphosphate. Orthophosphates are present in decaying and living animals and plants, chemically bound to aqueous systems, and within sediments, soils, and rocks. Phosphates can be used by plants for growth and can even aid in preventing corrosion. Typically, phosphates are retained by the soil and are present in very low quantities in freshwater sources that are well-oxygenated. However, if orthophosphate is present in high levels, it can be toxic to plants and animals (Oram, 2014).

About Dissolved Oxygen

Dissolved oxygen is a measurement of the amount of oxygen that is dissolved within a water source. This is the amount of oxygen that is free for aquatic organisms and plants to use. Oxygen primarily enters water sources through the atmosphere and through discharge from groundwater. Zooplankton and fish especially utilize the dissolved oxygen for respiration. Stagnant water systems contain less dissolved oxygen compared to moving water systems. Additionally, bacteria present within the water can consume some dissolved oxygen. Lower water temperatures are associated with higher dissolved oxygen content, and vice versa. Water sources rich in nutrients such as phosphorus and nitrates are typically oxygen deficient. The presence of decay and respiration by consumers can deplete the amount of dissolved oxygen present within the water, and this is therefore toxic for the aquatic life living within the water, as they will eventually not have a sufficient amount of dissolved oxygen to utilize for respiration (“Dissolved Oxygen and Water,” 2019).

Methods

Surface Water Sample Collection

During the Fall 2019 semester, surface water samples for experimentation were collected. The place of collection was at four different locations on the campus of Gardner-Webb University in Boiling Springs, NC. These included collection at the boiling spring adjacent to Lake Hollifield (label 1), at the red bridge adjacent to the boiling spring within the northeastern side of Lake Hollifield (label 2), next to the second bridge on the southern side of Lake Hollifield (closest to the Broyhill Adventure Course;

label 3), and in the adjacent creek to the Broyhill Adventure Course (label 4). These sites are labeled 1-4 respectively, as shown within Figure 3 below.



Figure 3. Map displaying collection sites. (“Gardner-Webb University Map,” 2020).

In total, eight surface water samples were collected for experimentation with the Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit,” after vacuum filtration, with two samples being collected at each of the four labeled locations in Figure 3. For collection, 1,000 mL Pyrex Media Storage Bottles were autoclaved to ensure sterilization and to prevent contamination. After sterilization, the 1,000 mL Pyrex Media Storage Bottles were manually removed from the autoclave using non-sterile nitrile gloves and placed into a cardboard box for transportation purposes. The bottles were labeled by pencil prior to sample collection.



Figure 4. Picture showing a 1,000 mL Pyrex Media Storage Bottle filled with a surface water sample from the boiling spring (Reeder, 2019).

The samples collected at the boiling spring (location #1 in Figure 1) were labelled “BS1” and “BS2,” the samples collected adjacent to the red bridge closest to the boiling spring (location #2 in Figure 1) were labelled “BR1” and “BR2,” the samples collected adjacent to the bridge of Lake Hollifield closest to the football stadium (location #3 in Figure 1) were labelled “BR3” and “BR4,” and the two samples collected by the creek adjacent to the Broyhill Adventure Course (location #4 in Figure 1) were labelled “CR1” and “CR2.”

Sample collection for experimentation with the vacuum filtration and utilization of the Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit” occurred on six different occasions during the fall semester. The amount filtered, date of collection, time of collection, outside condition, and sample appearance was recorded for each collected sample. This data was used for discussion after the results of experimentation had been identified. The dates of collection were October 28th, October 30th, and November 11th of

2019. Additionally, on October 14th, 2019, two 1,000 mL samples were collected from location #1 and location #2. These samples were used to practice filtration and to ensure that adequate results would be achieved by following the methods for using the *Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit.*



Figure 5. Picture of two 1,000 mL Pyrex Media Storage Bottles, reflecting the difference in sample appearance. The clear sample (left) was collected at location #1 in Figure 1, while the cloudy sample (right) was collected at location #2 in Figure 1 (Reeder, 2020).

For the utilization of the “*HACH® Surface Water Test Kit,*” a total of two samples were collected for experimentation and water quality testing. These samples were collected in the same manner as previously described. One sample was collected at the boiling spring (location #1 in Figure 1), and subsequently labeled as “BSQ1.” The second sample was collected at the red bridge near the boiling spring within the northeastern side of Lake Hollifield (location #2 in Figure 1) and was labeled as “BRQ1.” On three separate occasions, these samples were collected. Sample BSQ1 was collected on November 13th, 2019, and sample BRQ1 was collected November 20th, 2019. The third collection date was for the re-collection of a 1,000 mL sample for BSQ1 to re-test the

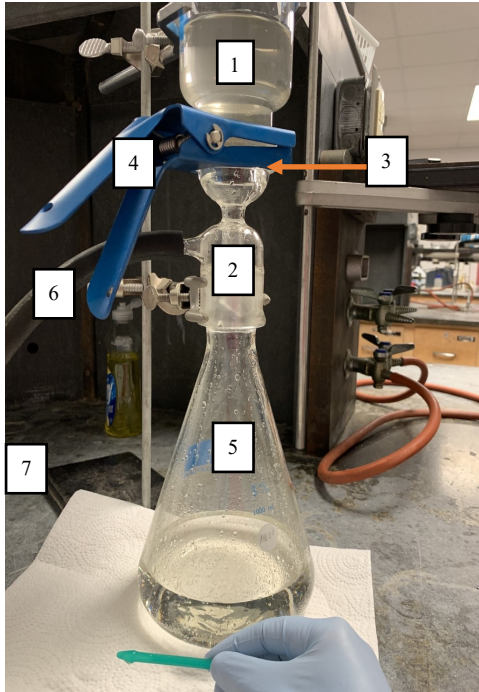
dissolved oxygen levels. For each of these collections, the amount collected, date of collection, time of collection, outside condition, and sample appearance was recorded for each collected sample.

It should be noted that all samples collected for utilization of the *Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit”* and the *“HACH® Surface Water Test Kit”* were obtained with the same manual technique. The 1,000 mL Pyrex Media Storage Bottle for each sample was firmly grasped with one hand. Using aseptic technique, the lid of the bottle was carefully unscrewed. Care was taken to ensure that the interior of the bottle and lid did not touch any other surface. Then, the opening of the bottle was placed halfway down into the surface of the body of water. Slowly, water was allowed to fill the entirety of the bottle. Using a sweeping motion, water was collected from the surface. As the bottle became fuller, its base was lowered into the water to ensure that the sample filled the bottle to the 1,000 mL indentation. If more than 1,000 mL of water was obtained per sample, the extra was carefully poured down the laboratory sink. When collecting the sample, it was ensured that the water sample filled the bottle at or greater than the 1,000 mL indentation on the bottle’s interior.

Vacuum Filtration & Utilization of the Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit”

After the collection of the surface water samples, a vacuum filtration apparatus (VFA) was properly assembled. The VFA parts included the filtering cup, the filtering head, sterile filter paper, a metal clamp, conical flask, rubber tubing, and the laboratory sink, which can be seen on the following page.

Key:



1	Filtering Cup
2	Filtering Head
3	Sterile Filter Paper
4	Metal Clamp
5	Conical Flask
6	Rubber Tubing
7	Laboratory Sink (not pictured)

Figure 6. Setup of the Vacuum Filtration Apparatus (left) with subsequent key pictured (right) (Reeder, 2020).

This apparatus was assembled for the purpose of filtering the collected water samples prior to DNA isolation. Additionally, a new disc of sterile filter paper was placed in the VFA for bacterial collection for each sample. Approximately 1,000 ml was vacuum filtered for BS1 and BS2; 500 ml was filtered for BR1, BR2, BR3, and BR4; 1,000 ml was filtered from CR1; and approximately 990 ml was filtered from CR2. Initially, it was thought that approximately 1,000 ml of each sample should successfully filter utilizing the VFA. However, some samples that contained excessive debris did not successfully filter the 1,000 ml. For this reason, the amount filtered from each sample was not consistent. Non-sterile nitrile gloves were worn during vacuum filtration to prevent contamination. Safety glasses were worn for personal protection.

Prior to assembling the VFA, the glassware of the apparatus was sterilized by first being washed with soap and distilled water. Then, ethanol was poured over the interior of the glass and a Bunsen Burner was lit with a lighter. The flame was used to sterilize the glassware prior to assembling the vacuum filtration apparatus and in between the filtration of samples from differing locations. Sterilized pestles were used to scrape debris from the sterile filtration discs to aid in efficient filtration. After filtration was complete, each sterile filtration disc was placed into a labelled ZR BashingBead™ Lysis Tube using sterilized forceps (“Quick-DNA Fungal/Bacterial Microprep Kit,” 2019).

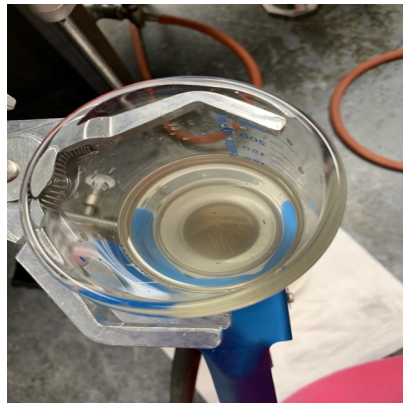


Figure 7. Filtration of a sample through the sterile filter paper, with debris along the bottom. This was scraped with a sterile pestle to promote proper filtration (Reeder, 2020).

The Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit” was then utilized. At a dilution of 0.5%, beta-mercaptoethanol was added to the Genomic Lysis Buffer. Using a micropipette, 750 µl of BashingBead™ Buffer was added to each of the ZR BashingBead™ Lysis Tubes. Because a bead beater was not available, the tubes were mixed for 20 minutes using a vortex mixer. The ZR BashingBead™ Lysis Tubes were centrifuged in a microcentrifuge at 10,000 x g for 1 minute. Then, up to 400 µl of supernatant was transferred into labelled Zymo-Spin™ III-F Filter in a collection tube and

centrifuged at 8,000 x g for 1 minute (“Quick-DNA Fungal/Bacterial Microprep Kit,” 2019).

Next, 1,200 µl of Genomic Lysis Buffer (containing the 0.5% of the added beta-mercaptoethanol) was added to the filtrate from the previous step. It was then that 800 µl of this mixture was pipetted into a Zymo-Spin™ IICR Column in a Collection Tube and centrifuged at 10,000 x g for 1 minute. The flow through from the Collection Tube was discarded and this process was repeated, in which 800 µl of the previous mixture was transferred to the same Zymo-Spin™ IICR Column in a Collection Tube and centrifuged at 10,000 x g for 1 minute (“Quick-DNA Fungal/Bacterial Microprep Kit,” 2019).

Afterwards, 200 µl of DNA Pre-Wash Buffer was added to the Zymo-Spin™ IICR Column in a new Collection Tube and centrifuged at 10,000 x g for 1 minute. Then, 500 µl of g-DNA Wash Buffer was added to the Zymo-Spin™ IICR Column and centrifuged at 10,000 x g for 1 minute. The Zymo-Spin™ IICR Column was transferred to a clean 1.5 ml microcentrifuge tube. Next, 100 µl of DNA Elution Buffer was added directly to the column matrix. This was centrifuged at 10,000 x g for 30 seconds to elute the DNA. It was at this point that the ultra-pure bacterial DNA was isolated from the samples (“Quick-DNA Fungal/Bacterial Microprep Kit”). The labelled microcentrifuge tubes containing the bacterial DNA extracts were then mailed to *Psomagen Corp.* for polymerase chain reaction (PCR), purification, and sequencing. Results from the sequencing were received via e-mail for the bacterial identification of each collected sample (“Quick-DNA Fungal/Bacterial Microprep Kit,” 2019).

Utilizing the “*HACH® Surface Water Test Kit*”

The “*HACH® Surface Water Test Kit*” was used for the purpose of determining the pH, temperature, and the levels of nitrate, ammonia, orthophosphate, and dissolved oxygen in the collected surface water samples for experimentation. These tests were completed after the bacterial DNA isolation procedure with newly collected samples. During collection, non-sterile nitrile examination gloves were worn. Afterwards, a new pair of non-sterile nitrile examination gloves were donned for testing. Two autoclaved 1,000 ml Pyrex Media Storage Bottles were used to collect 1,000 ml water sample. The time of collection, climate during collection, and appearance of the water samples was noted following collection.

Determining pH

The pH of each of the two water samples was determined by utilizing the “Pocket Pro pH Tester” that was included in the “*HACH® Surface Water Test Kit*.” The pH tester was first calibrated using the provided buffer packet and deionized water. A Kimwipe® and deionized water were used to clean the end piece of the tester that was placed into the sample during testing. Once calibration and cleaning of the device was complete, sample water from each sample was tested one at a time. Sample water was poured into the cap of the pH tester up to the fill line. The tester was turned on and the lid was placed on the tip of the tester end. After a few seconds, the pH of the sample was electronically displayed on the tester screen, and the pH was recorded. The cap and the end of the pH tester was cleaned with deionized water, following the directions contained within the “*HACH® Surface Water Test Kit*” (“Surface Water Test Kit,” 2019).

Determining Temperature

To determine the temperature of both of the collected water samples, the cap of the “Pocket Pro pH Tester” and the testing end of the device was first cleaned with deionized water and wiped with Kimwipes[®]. Then, the lid was filled to the fill line with sample water. The device was turned on and the end of the pH tester was capped so that the tip rested in the sample water. After a few seconds, the temperature in °C was displayed electronically on the screen of the device. This temperature was recorded for each sample. In between uses, the cap and the end of the pH tester was cleaned with deionized water, following directions in the “*HACH[®] Surface Water Test Kit*” instructions (“Surface Water Test Kit,” 2019).

Determining Nitrate Levels

The nitrate levels of each of the two water samples was determined by first washing two test tubes with sample water. Afterwards, the two rinsed test tubes were filled up to the first line with sample water, with a total of 5 ml of sample in each tube. One of the tubes was inserted into the left opening compartment of the provided color comparator box. The nitrate nitrogen color disc was placed into the color comparator box prior to testing. One NitraVer 5 Nitrate Reagent Powder Pillow was added to the other tube filled with 5 ml of sample. The cap was placed on the tube, and the tube was vigorously shaken for one minute. A timer was set for one minute for this (“Surface Water Test Kit,” 2019).

After a minute had passed, an amber color appeared within the tube. The second tube was then placed in to the second compartment within the color comparator box, next to the first tube. The color comparator box containing the two test tubes was held in front

of a light source, which was a window with sunlight beaming into the laboratory. Next, the color disc was turned to find the correct color match. The result in mg/L was read through the scale window, and this measurement was recorded for each sample. All test tubes were cleaned with deionized water in between testing, as depicted within the *“HACH® Surface Water Test Kit”* directions (*“Surface Water Test Kit,”* 2019).

Determining Ammonia Levels

To determine the ammonia levels in both water samples, two test tubes were first rinsed with sample water. Next, the two tubes were filled with 5 mL of sample water, up to the first indicator line. One test tube was placed into the left compartment of the color comparator box. The ammonia nitrogen color disc was placed into the color comparator box. One Ammonia Salicylate Reagent Powder Pillow was added to the other test tube. The stopper was placed on the tube and the tube was shaken until all the powder dissolved. A timer was set for 3 minutes while the tube was placed on the counter and held still (*“Surface Water Test Kit,”* 2019).

Then, one Ammonia Cyanurate Reagent Powder Pillow was placed into the same tube. The stopper was placed on the tube and the tube was shaken until all the powder dissolved. This time, a timer was set for 15 minutes while the tube was sitting still on the countertop. It was noted that a green color began to develop. Once the 15 minutes had passed, this tube was placed into the color comparator box. The color comparator box was held in front of a light source (a window with ample sunlight beaming through). Finally, the color disc was turned until a color match was found. The result was read in mg/L through the scale window. Deionized water was used to rinse supplies after use. Additionally, the calculation chart in the instructions was used to calculate final ammonia

levels. These instructions were followed according to the provided “*HACH® Surface Water Test Kit*” directions (“Surface Water Test Kit,” 2019).

Determining Orthophosphate Levels

The orthophosphate levels of both of the collected water samples was determined by first rinsing two test tubes and one provided bottle with sample water prior to testing. Then, the long path adaptor was inserted into the color comparator box. One of the clean tubes was filled to the top line with sample water. This tube was placed into the leftmost compartment of the color comparator box. Next, the bottle was filled to the 20 mL mark with sample water (“Surface Water Test Kit,” 2019).

One PhosVer 3 Phosphate Reagent Powder Pillow was added to the bottle, and the bottle was swirled to mix. A timer was set for 8 minutes, and the bottle was left to sit for the duration of this time. It was noted that the sample must be read within 10 minutes, or else it would need to be discarded. After 8 minutes had passed, a second tube was filled to the top line with the prepared sample within the bottle. This tube was placed into the second compartment of the color comparator box (“Surface Water Test Kit,” 2019).

The color comparator box was then held below a light source for evaluation. In this case, the light source was a fluorescent ceiling fixture that was present within the laboratory. The color disc was turned to find a match in color to the test tube containing only sample water. Finally, the value was read looking through the scale window. The number that was indicated on the color disc was divided by 50 to get the result in mg/L. In between tests, the used test tubes and testing bottle were rinsed and cleaned with deionized water, in accordance to the “*HACH® Surface Water Test Kit*” instructions (“Surface Water Test Kit,” 2019).

Determining Dissolved Oxygen Levels

To determine the dissolved oxygen levels of both of the collected water samples, the high range (1 to 20 mg/L) test procedure was utilized. First, the two measuring tubes and the BOD bottle was washed with sample water prior to testing. After testing each water sample, all used measuring tubes and the BOD bottle were rinsed with deionized water. The BOD bottle (the round bottle with the glass stopper) was then filled with sample water. This was done in such a way so that sample water overflowed from the bottle for 3 minutes straight. A timer was used to ensure that the water overflowed for 3 minutes. Care was taken to avoid turbulence when filling; the water was slowly poured to avoid the creation of air bubbles within the bottle (“Surface Water Test Kit,” 2019).

After 3 minutes had passed, the bottle was slightly inclined, and the glass stopper was carefully placed on the bottle to prevent the trapping of air bubbles. For both times utilizing the dissolved oxygen test, no observable air bubbles were viewed as being trapped when placing the stopper onto the bottle. However, this does not mean that air bubbles were not present. If air bubbles were trapped, the sample would have been discarded and the test would have been repeated (“Surface Water Test Kit,” 2019).

Next, the stopper was removed, and one Dissolved Oxygen 1 Reagent Powder Pillow was added to the BOD bottle in addition to one Dissolved Oxygen 2 Reagent Powder Pillow. The stopper was placed back onto the bottle and the bottle was inverted several times to ensure that the powders dissolved properly. Flocculent (floc) precipitate formed within the bottle; each of the tested samples revealed a brownish-orange colored precipitate, which indicated that oxygen was present within the sample. The floc was allowed to settle into the bottom half of the bottle. Slow settling would indicate high

chloride presence; this was not the case for the samples tested. Then, the bottle was inverted several times and allowed to mix again. The floc settled in the same manner as before (“Surface Water Test Kit,” 2019).

The stopper was removed from the bottle and one Dissolved Oxygen 3 Reagent Powder Pillow was added. Nail clippers were used to open the packet. The bottle was stoppered and inverted several times. The flocculent dissolved and the sample turned yellow in color for both of the samples tested. This indicated the presence of oxygen. One full measuring tube was filled with sample from the BOD bottle. Sodium Thiosulfate Solution was added dropwise to the measuring tube. The bottle was swirled with the addition of each drop; the drops added were counted until the yellow-colored solution turned clear in color. Results for the Dissolved Oxygen Test were indicated by the number of drops of Sodium Thiosulfate Solution that was added to the measuring. The number of drops added indicated the test result in mg/L. The instructions provided by the “*HACH® Surface Water Test Kit*” were followed for this procedure (“Surface Water Test Kit,” 2019).

Sending & Receiving Test Results for the Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit”

The eight labeled microcentrifuge tubes containing the bacterial DNA isolate were shipped to Psomagen Inc., based out of Rockville, Maryland. These tubes were placed into a suitable package, shipped via USPS, and received by Psomagen Inc. on November 20th, 2019. The requested process for Psomagen Inc. was, “8 gDNA sample(s) for PCR, purification and sequencing for 16 reaction(s).” A few days later, the sequences from the bacterial DNA isolates was received via e-mail by Dr. David Campbell, in

addition to the supplemental electropherograms. These results were shared and utilized for further analysis. The BioEdit Program (Hall, 1999) was used to check and correct the sequences. It should be noted that a total of 31 hours and 5 minutes was spent in the laboratory setting performing the previously described methodology.

DNA Sequence Analysis

After compiling and analyzing the DNA sequences from the bacterial DNA isolates resulting from using the Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit,” computerized programs were used to input all of the sequence results for comparison to one another. The BLAST program (Zhang et al., 2000) was used to identify similar sequences in GenBank, the DNA sequence database. Similar sequences that were identified were saved for further analyses. Many sequences were only identified as “uncultured environmental bacterium” and were not included in analyses. This was because they do not provide any information about what type of bacterium was found.

Because two of the sequences were very different from the others and much longer, they were analyzed separately. The sequences were aligned using the online MAFFT program (Kazutaka et al., 2019). The Gblocks program (Castresana, 2000) was used to eliminate uncertain parts of the alignment. Parsimony analyses in PAUP*4.0a167 (Swofford, 2002) and TNT (Goloboff, et al. 2000) were used to generate a phylogenetic tree to compare all of the bacterial strains identified from the DNA sequences, in addition to diatoms, other algae, and unidentified eukaryote sequences that were identified as similar to the new sequences.

Results

Results for Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit”

Upon collecting the eight samples for bacterial DNA isolation, the day of collection, time of collection, outside conditions, and sample appearance were recorded and compiled into a corresponding table. These data would be later utilized for discussion when analyzing the bacterial DNA results. After filtering each sample using vacuum filtration, the amount of sample that was filtered was recorded. Some samples did not have the complete 1,000 ml filtered due to containing an abundance of debris that made filtration difficult, or due to accidentally pouring too much sample down the sink when attempting to ensure that the collected sample was approximately 1,000 ml. This table can be viewed on the following page.

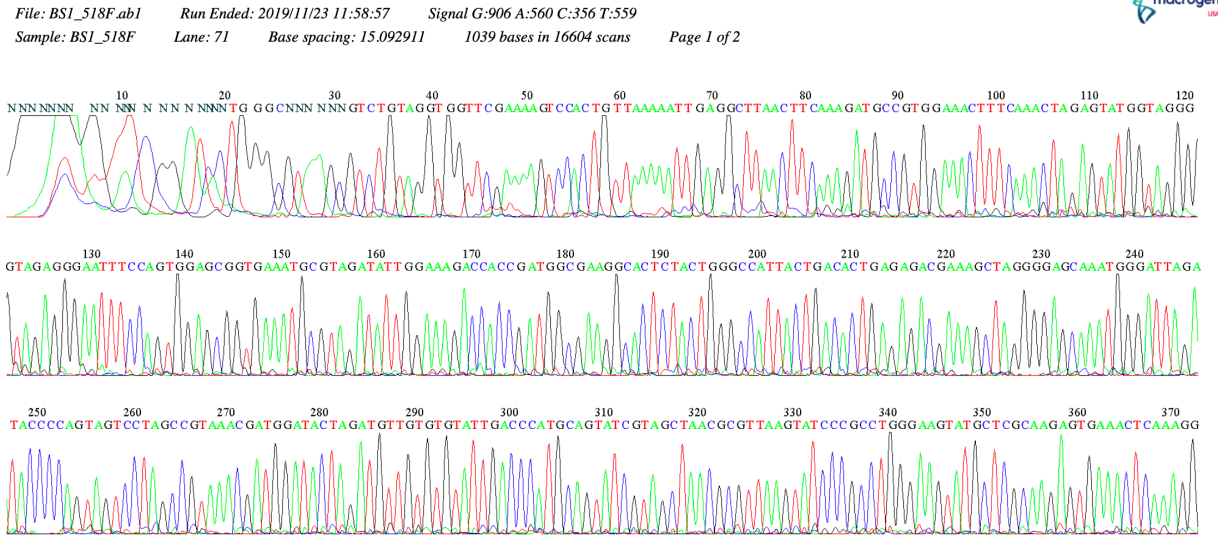
Table 1. Depicting the Collected Samples Initial Data for Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit.”

	Amount Filtered	Date of Collection (Month-Day-Year)	Time of Collection	Outside Condition	Sample Appearance
BS1	1,000 ml	10-28-2019	12:15 pm	72°F, sunny	Clear
BS2	1,000 ml	10-28-2019	12:15 pm	72°F, sunny	Clear
BR1	500 ml	10-30-2019	1:12 pm	63°F, raining	Tan coloration, cloudy
BR2	500 ml	11-11-2019	12:15 pm	61°F, sunny	Tan coloration, cloudy
BR3	500 ml	10-30-2019	1:18 pm	63°F, raining	Tan coloration, green tint, cloudier than BR4
BR4	500 ml	10-30-2019	1:18 pm	63°F, raining	Tan coloration, green tint, cloudy
CR1	1,000 ml	11-11-2019	12:30 pm	61°F, sunny	Clear
CR2	990 ml	11-11-2019	12:30 pm	61°F, sunny	Clear

After all sequences were sent to *Psomagen, Inc.* for analysis, the corresponding DNA sequences and electropherograms were received within a few days for each of the eight collected samples. Two DNA sequences were received from each sample- one with the 800F primer and one with the 518R primer. One corresponding electropherogram from each of the eight collected samples was received for analysis. An electropherogram is essentially a chart plotting various DNA fragment sizes. This chart is created through electrophoresis automatic sequencing and depicts the four different nucleotides within a

DNA sequence, which are cysteine, thymine, guanine, and adenine. A photo depicting the appearance of an electropherogram can be seen below.

Figure 8. Electropherogram Example from BS1 Data (Psomagen Inc., 2019).



Each DNA sequence was analyzed extensively by hand using BioEdit (Hall, 1999). Several nucleotide bases were unknown and were represented by an “N” displayed in the corresponding electropherograms. For each “N,” the nucleotide was determined from analyzing the height and color of the corresponding peak in addition to the relation of the peak to the adjacent peaks. Some peaks could not be assigned definitely as being cysteine, thymine, adenine, or guanine. For these peaks, other letters were assigned based on their appearance. An International Union of Pure and Applied Chemistry (IUPAC) Nucleotide Nomenclature Table was utilized to properly assign letters to each unknown peak in the electropherograms. The IUPAC Nucleotide Nomenclature Table that was used for this is displayed on the following page.

Table 2. The IUPAC Nucleotide Nomenclature Table used for nucleotide identification and identification for electropherogram analysis and completion of DNA sequences for the eight collected samples (Fox, et al., 2010).

IUPAC Nucleotide Nomenclature Table			
symbol	base	symbol	base
A	adenosine	M	A C (amino)
C	cytidine	S	G C (strong)
G	guanine	W	A T (weak)
T	thymidine	B	G T C
U	uridine	D	G A T
R	G A (purine)	H	A C T
Y	T C (pyrimidine)	V	G C A
K	G T (keto)	N	A G C T (any)

The length of each of the DNA sequences was first checked. As expected, most of these sequences were relatively short in length, as expected for typical bacterial sequences using these primers, reaching approximately 250 bases. However, two longer sequences were obtained. Due to their extensive length and difference from each other, the data for the two sequences were separately analyzed. MAFFT (Kazutaka, et al., 2019) was a program used for aligning the DNA sequences, using default values for all of the present parameters. This program was used for analysis because the 16S gene for different species can have missing or additional DNA bases. Therefore, it is necessary to analyze the sequences to determine which bases of one sequence correspond to the bases of other sequences.

Afterward, Gblocks (Castresana. 2000) was a program that was used to eliminate any base positions that were, unfortunately, not reliably aligned. This program helps to

analyze the alignment, working to identify any parts where the data could be aligned in more than one way. In this way, associated uncertainty is eliminated. The options for less stringent alignments were utilized. The programs PAUP*4.0a167 (Swofford, 2002) which can be used to calculate percent differences) and TNT were then used to analyze the resulting alignments. PAUP* was used to format the alignments, to calculate percent similarities between sequences, and to perform parsimony analyses on the data for the longer sequences. Analyzing more, shorter sequences is a much greater computational challenge, so the faster program TNT was used to for parsimony analysis of the data set with all of the normal-length sequences.

BR1 Results

The length of bacterial DNA isolation used for analysis for BR1 was 247 base pairs long. After analyzing and editing the DNA sequence from the subsequent electropherogram, the sequence for the BR1 sample was placed into BLAST for analysis. It was determined that the isolated DNA from experimentation reflected an 89.43% identity with “Uncultured bacterium gene for 16S rRNA, partial sequence.” It should be noted that the percent identity is a number depicting the similarity between the query (sample) sequence and the target sequence. Additionally, there was an 89.02% identity with “Uncultured Rhodocyclaceae bacterium clone,” an 89.02% identity with “Uncultured *Propionivibrio* sp. clone,” an 89.02% identity with “Uncultured beta proteobacterium clone,” an 88.62% identity with two other strains of “Uncultured *Propionivibrio* sp. clone,” and an 88.66% and 88.62% identity with two other strains of “Uncultured beta proteobacterium clone” (BLAST: Basic Local Alignment Search Tool, (n.d.)).

In addition, the BLAST analysis for the isolated DNA sequence reflected an 88.62% identity with “*Thauera aminoaromatica* strain,” an 88.62% identity with “*Thauera chlorobenzoica* strain,” an 88.52% identity for two strains of “Beta proteobacterium 16S small subunit ribosomal RNA gene, partial sequence,” and an 88.62% identity with another strain of “Beta proteobacterium.” An 88.62% identity was reflected for “Uncultured alpha proteobacterium clone, 16S ribosomal RNA gene, partial sequence,” an 88.62% identity for both “Uncultured *Thauera* sp. clone, 16S ribosomal RNA gene, partial sequence” and “*Thauera* sp. R5 gene for 16S ribosomal RNA, partial sequence” (BLAST: Basic Local Alignment Search Tool, (n.d.)).

Again, an 88.62% identity was discovered for two other strains of “Uncultured betaproteobacterium clone” and “Beta proteobacterium 16S ribosomal gene, partial sequence,” an 88.62% identity was reflected for two more strains of “*Thauera chlorobenzoica* strain, 16S ribosomal RNA gene, partial sequence,” and a percent identity of 88.57% was reflected for “Uncultured *Propionivibrio* sp. clone, 16S ribosomal RNA gene, partial sequence.” Interestingly, there was an 88.62% identity with “Uncultured *Candidatus Accumulibacter* sp. clone, 16S ribosomal RNA gene, partial sequence. Finally, an 87.70% identity was reflected for another sequence of “Beta proteobacterium 16S small subunit ribosomal RNA gene, partial sequence” (BLAST: Basic Local Alignment Search Tool, (n.d.)).

Some strains such as “Uncultured beta proteobacterium” and “Uncultured *Propionivibrio*” exhibited multiple strains that reflected the same percent identity with the isolated DNA samples from experimentation. Other strains not reflected, but still displayed a percent identity of approximately 87% or higher, include “Uncultured

bacterium clone” and “Uncultured bacterium partial 16S rRNA gene, clone/partial sequence.” These sequences will not be elaborated upon, as they are too vague in nature. All listed results are from 16S rRNA genes (BLAST: Basic Local Alignment Search Tool, (n.d.)).

BR2 Results

For the results of the bacterial isolates of BR2, the 800-primer strand was utilized, which was 288 base pairs in length. The 518-primer strand was not used because it yielded fewer and similar results to the 800-primer strand, but with lower percent identity. A percent identity of 81.34% was discovered for “Uncultured Burkholderiaceae bacterium clone...16S ribosomal RNA gene, partial sequence” a percent identity of 80.92% was revealed for two results titled “Uncultured Rhodoferax sp. clone; 16S ribosomal RNA gene, partial sequence,” a percent identity between 80.57% and 80.92% was reflected for several strains titled “Uncultured beta proteobacterium gene for 16S ribosomal RNA, partial sequence” while a percent identity of 80.92% and 80.57% was reflected for two strains titled “Beta proteobacterium...16S small subunit ribosomal RNA gene, partial sequence” (BLAST: Basic Local Alignment Search Tool, (n.d.)).

There was a percent identity of 80.57% for “Limnohabitans species...16S ribosomal RNA gene, partial sequence” and a percent identity of 80.92% for “Uncultured Limnohabitans sp. clone...16S ribosomal RNA gene, partial sequence,” 80.92% for “Uncultured Limnohabitans sp. partial 16S rRNA gene...” and 80.92% for four different strains of “Limnohabitans sp. strain; complete genome/small subunit ribosomal RNA gene, partial sequence.” A percent identity of 80.92% was discovered for four different

results titled “Uncultured *Curvibacter* sp. clone...16S ribosomal RNA gene, partial sequence” (BLAST: Basic Local Alignment Search Tool, (n.d.)).

In addition, a percent identity of 80.92% was revealed for three strains of “Uncultured *Comamonadaceae* clone...16S ribosomal RNA gene, partial sequence,” and a percent identity between 80.57% and 80.92% was discovered for 17 different strains of beta proteobacterium, titled “Beta proteobacterium...16S small subunit ribosomal RNA gene, partial sequence,” “Uncultured beta proteobacterium clone...16S ribosomal RNA gene, partial sequence,” and “Uncultured beta proteobacterium isolate...16S ribosomal RNA gene, partial sequence” (BLAST: Basic Local Alignment Search Tool, (n.d.)).

BR3 Results

It was determined through BLAST analysis that the isolated bacterial DNA samples for BR3 reflected an 83.92% identity with “*Xanthomonadaceae* bacterium partial 16S rRNA gene,” an 82.74% identity with “*Vibrio* sp. THAF191c chromosome, complete genome,” an 81.96% identity with “Uncultured sulfate-reducing bacterium clone,” and percent identities ranging from approximately 79% to 81% for multiple strains of “Uncultured gamma proteobacterium partial 16S rRNA gene.” Of interest, there was an 80.35% identity with “16s rDNA sequence amplified from human fecal sample” (BLAST: Basic Local Alignment Search Tool, (n.d.)).

Additionally, the experimental isolated bacterial DNA sequences for BR3 had an 80.09% identity for “Uncultured *Rhizobiales* bacterium clone,” a 79.91% identity for “Bacterium symbiont of *Calyptogena valdiviae* partial 16S rRNA gene,” a 79.91% identity for “Uncultured *Clostridiales* bacterium clone,” and a 79.48% identity for “Uncultured *Thiotrichaceae* bacterium clone.” All sequence results were 16S rRNA

genes; other results not depicted were too vague for analysis. It should be noted that sequenced utilized for BLAST analysis had a length of 229 base pairs (BLAST: Basic Local Alignment Search Tool, (n.d.)).

BR4 Results

The DNA isolate for the BR4 samples had a total base pair length of 249. Through analysis using BLAST, it was determined that the isolated bacterial DNA from the BR4 samples had an 85.08% identity to “*Vibrio* sp. THAF191c chromosome, complete genome” (of multiple strains), and percent identities ranging from approximately 82% to 84% for multiple strains of “Uncultured gamma proteobacterium partial 16S rRNA gene, partial sequence.” There was an 84.68% identity to “Uncultured Firmicutes bacterium clone...16S ribosomal RNA gene, partial sequence,” and a percent identity of 83.47% for five different sequences of “Uncultured proteobacterium clone...ribosomal RNA gene, partial sequence” (BLAST: Basic Local Alignment Search Tool, (n.d.)).

BR4 also displayed a percent identity between 83% and 85% for “Uncultured delta proteobacterium gene...16S rRNA, partial sequence, clone,” “*Thalassomonas* sp.,” “*Thalassotalea eurytherma* strain,” “*Eubostrichus diana*e epibacterium,” “*Thalassotalea piscium* partial 16S rRNA gene, isolate,” “Enterobacteriaceae bacterium symbiont of *Paracoccus marginatus* isolate MEPMAR genome assembly, chromosome: I,” “*Synechococcus* sp.,” and “*Thalassomonas* sp.” (BLAST: Basic Local Alignment Search Tool, (n.d.)).

BS1 Results

The analyzed bacterial DNA isolate for BS1 samples contained a base pair length of 1,454. BLAST analysis revealed a percent identity between 94.41% and 99.46% for 16 different strains titled “Uncultured eukaryote clone...16S ribosomal RNA gene, partial sequence.” There was a percent identity between 94.92% and 99.31% for 6 different strains titled “Uncultured Cyanobacterium sp. clone...16S ribosomal RNA gene, partial sequence” and a percent identity of 98.62% for “Uncultured Oscillatoriales cyanobacterium clone...16S ribosomal RNA gene, partial sequence,” and a percent identity of 94.57% for “*Vaucheria litorea* chloroplast, complete genome” (BLAST: Basic Local Alignment Search Tool, (n.d.)).

Additionally, this sequence had a 92.05% identity with “*Bacillaria paxillifer* clone...16S ribosomal RNA gene, partial sequence; chloroplast,” a percent identity of 91.10% for three results titled “*Fucus vesiculosus* var. *spiralis* voucher UC 2050586 plastid, complete genome,” “*Fucus vesiculosus* complete chloroplast genome,” and “*Fucus vesiculosus* 16S ribosomal RNA gene, partial sequence; chloroplast.” There was a percent identity of 90.61% for “Uncultured diatom clone...16S ribosomal RNA gene, partial sequence; chloroplast,” and a percent identity of 90.68% and 90.28% for two strains titled “*Coscinodiscus radiatus* chloroplast, complete genome” (BLAST: Basic Local Alignment Search Tool, (n.d.)).

A percent identity of 90.47% was present for “*Ectocarpus siliculosus* plastid, complete genome,” and also 90.47% for “*Ectocarpus siliculosus* chloroplast, complete genome” Finally, a percent identity of 90.42% was present for two different DNA isolates titled “*Guinardia striata* chloroplast, complete genome.” Unless otherwise stated,

all results were for 16S ribosomal RNA from bacterial DNA isolates within the BLAST system for comparison (BLAST: Basic Local Alignment Search Tool, (n.d.)).

BS2 Results

Unfortunately, due to possible experimental error (which will be later discussed), the resulting electropherogram and bacterial DNA isolate for the BS2 samples could not be analyzed. The DNA sequence contained mainly unknown bases, and the electropherogram was disorganized and unable to be deciphered. Therefore, the results for BS2 are inconclusive.

CR1 Results

The bacterial DNA isolate for CR1 was determined to have a length of 247. A percent identity of 91.87% was present for “Comamonadaceae bacterium strain...16S ribosomal RNA gene, partial sequence,” and a percent identity of 91.87% was present for “Uncultured beta proteobacterium gene for 16S ribosomal RNA, partial sequence, clone...” In addition, a percent identity between approximately 89% and 93% was present for “*Mitsuaria chitosanitabida* partial 16S rRNA gene,” “Uncultured *Simplicispira* sp. clone,” “*Delftia* sp.,” “*Comamonas testosteroni* strain,” “*Oryzomicrobium terrae* strain,” and “Uncultured *Rhodocyclaceae* bacterium clone” (BLAST: Basic Local Alignment Search Tool, (n.d.)).

CR2 Results

From the analysis of the bacterial DNA isolates of CR2, it was determined that the analyzed sequence was 245 base pairs in length. A percent identity of 91.46% was present for “*Acidovorax* species...gene for 16S rRNA, partial sequence,” a percent identity of 91.46% was present for “Comamonadaceae bacterium strain...16S ribosomal

RNA gene, partial sequence,” and a percent identity of 91.46% was present for “Limnohabitans sp. Rim 11 genomic DNA containing partial 16S rRNA gene.” This same percent identity was present for seven other strains of “Limnohabitans.” Further, a percent identity of 91.46% was depicted for “Uncultured Limnohabitans sp. clone...16S ribosomal RNA gene, partial sequence” (BLAST: Basic Local Alignment Search Tool, (n.d.)).

Phylogenetic Analysis of the DNA Samples

Any sequences that were identified as being similar through BLAST were subsequently analyzed in PAUP*4.0a167. With this program, the Pairwise Base Differences function was used to generate a table reflecting the genetic differences for comparison between the different sequences. Parsimony analysis of the shorter sequences used the program TNT, using the 500 random replicates with all of the four “new technology” options that were present. For the longer sequences, the number of the sequences was fortunately small enough to compose a branch and bound search in PAUP*. A branch and bound search is mathematically guaranteed to find the optimum trees, but it is computationally intensive. For larger data sets, that can exceed a computer’s capacity to calculate. Instead, approximate searches are used that find relatively good trees and then test a huge number of variations on those trees to see if any better trees can be found. These were all parsimony analyses, which aim to discover the phylogenetic trees that require the fewest changes in the DNA. Additionally, the majority-rule consensus tree for the shorter sequences is shown in Figure 9 on the following page.

Majority-rule consensus tree

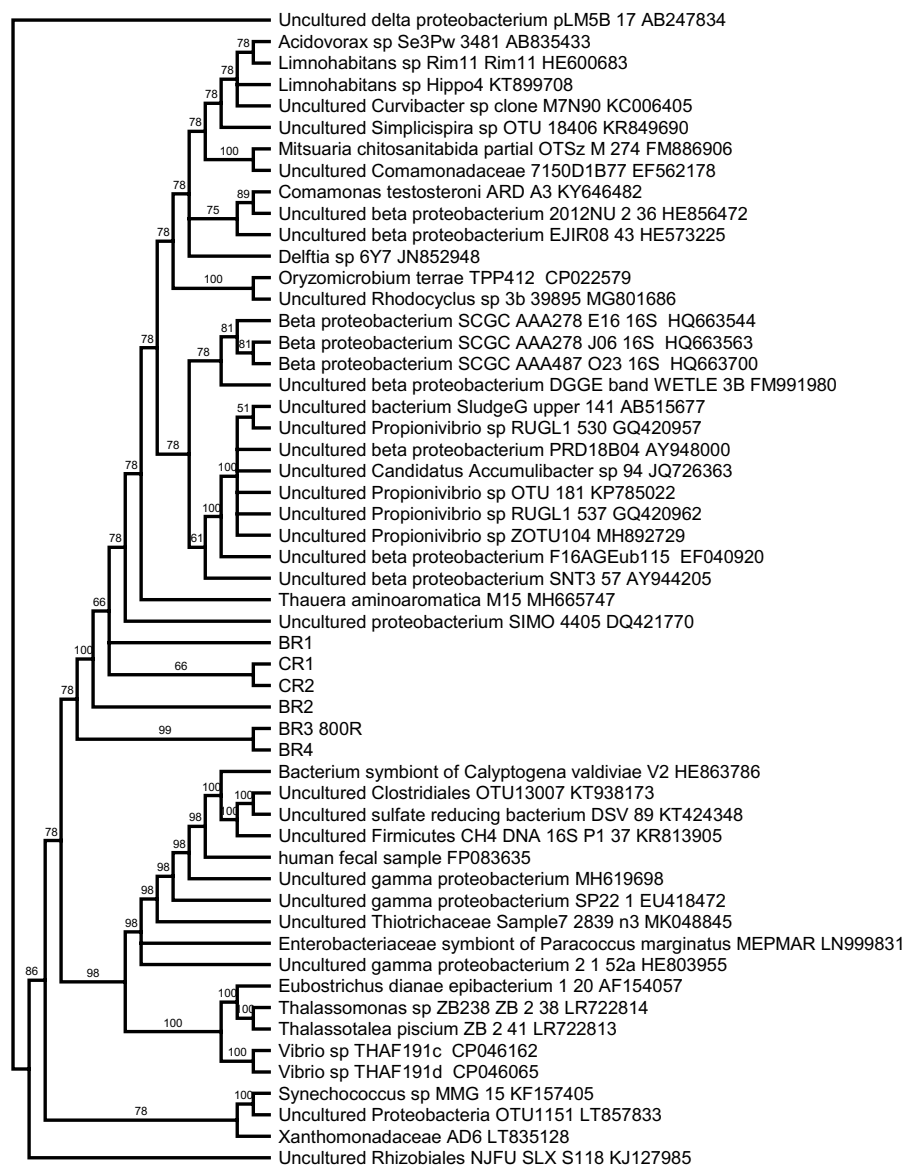


Figure 9. Majority-rule consensus tree of the most parsimonious trees for the shorter sequences. Numbers on the branches are the percentage of the most parsimonious trees that supported each branch.

In addition, a phylogram was constructed which displayed the strict consensus for the sequences that were shown to be similar to BR3 518. While Figure 9 displayed a

cladogram, Figure 10 displays a phylogram which shows the amount of difference between sequences through the individual branch lengths of the tree. For example, the relatively long branch for BR3 518F shows that it had more changes in DNA than in the short branch for *Aulacoseira ambigua*.

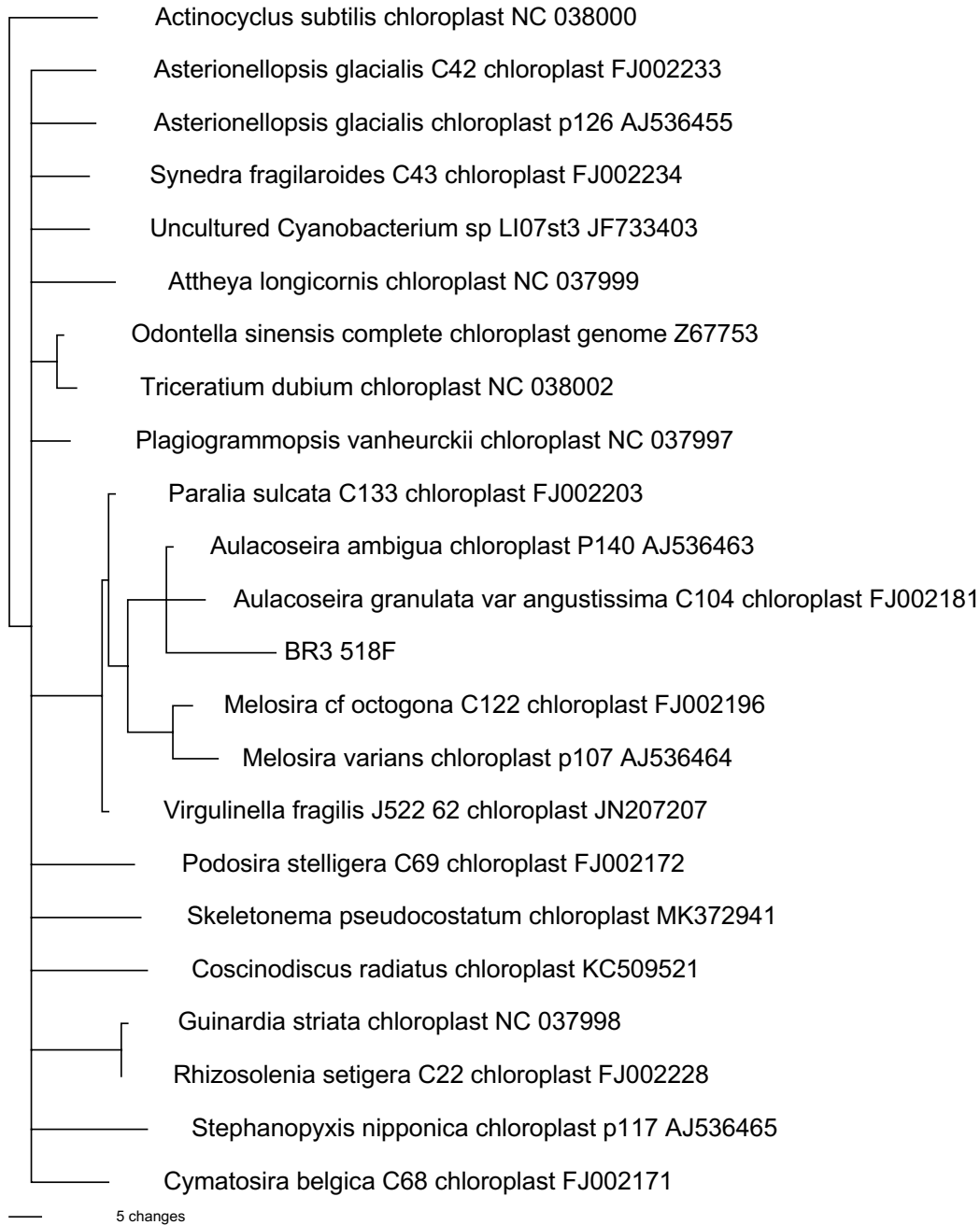


Figure 10. Phylogram of the strict consensus for the sequences similar to BR3 518.

Another phylogram was constructed, displaying the strict consensus of the most parsimonious trees for the longer sequences matching BS1. This phylogram can be viewed below.

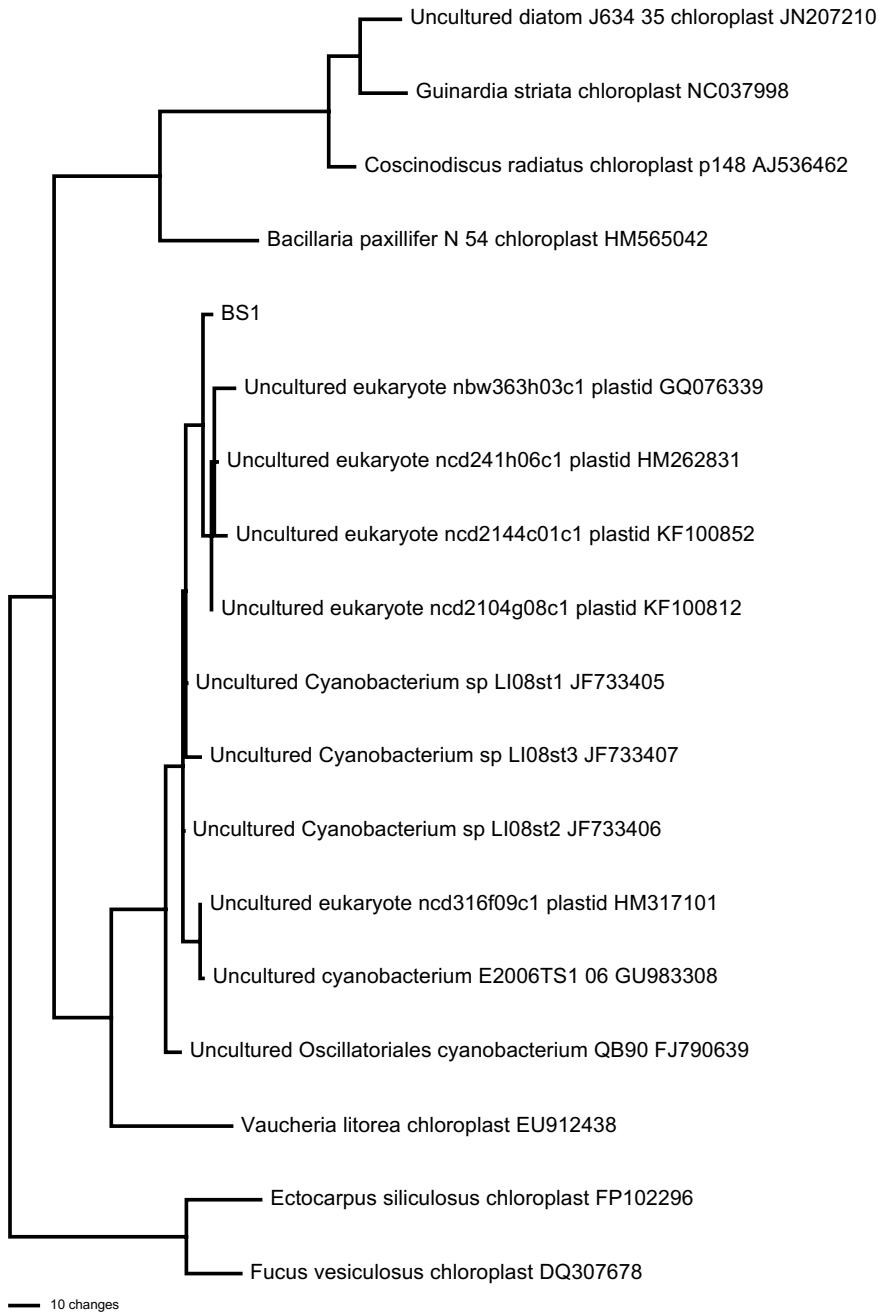


Figure 11. Phylogram of the strict consensus of the most parsimonious trees for sequences matching BS1.

Results for “HACH® Surface Water Test Kit”

Upon collection of the two samples for utilization of the “HACH® Surface Water Test Kit,” data were collected and compiled into a subsequent chart. The collected data include the amount of sample collected, the date of collection, time of collection, the outside conditions during collection, and the appearance of the sample at the time of collection. This data was composed within a table, which was later utilized for the analysis pertaining to the water quality of the two collected samples. This data table can be viewed below.

Table 3. Depicting the Collected Samples Initial Data for the “HACH® Surface Water Test Kit.”

	Amount Collected	Date of Collection (Month-Day-Year)	Time of Collection	Outside Condition	Sample Appearance
BSQ1	1,000 ml	11-13-2019; 11-20-2019 to re-do the dissolved oxygen test.	1:40 pm; 1:30 pm	38°F, cloudy; 58°F, cloudy	Clear
BRQ1	1,000 ml	11-20-2019	1:40 pm	58°F, cloudy	Cloudy; slight yellow- green tint

In addition to this, the pH, temperature, nitrate, ammonia, orthophosphate, and dissolved oxygen levels for both collected samples was determined and recorded. These values were determined using the “HACH® Surface Water Test Kit.” A table illustrating these results is depicted on the following page. It should be noted that BSQ1 refers to the sample collected at the boiling spring, and BRQ1 refers to the sample collected within the northeastern side of Lake Hollifield.

Table 4. Depicting the Results from Utilizing the “HACH® Surface Water Test Kit.”

	pH	Temperature	Nitrate	Ammonia	Orthophosphate	Dissolved Oxygen
BSQ1	5.4	19.0°C (66.2°F)	15.4 mg/L	0.0 mg/L	0.1 mg/L	19.0 mg/L
BRQ1	6.7	18.6°C (65.48°F)	8.8 mg/L	0.0 mg/L	0.08 mg/L	21.0 mg/L

Discussion

Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit.”

BR1

Many strains related to BR1 were identified as being “uncultured” bacterium genes. The term “uncultured” refers to the fact that many of the related strains to the bacterial DNA isolate for BR1 were not grown on anything inside of a laboratory, and thus exist naturally within the soil or natural water sources, for example (Lewis, 2010). The sequence for BR1 was related to Rhodocyclaceae, which is a family of bacteria consisting of a wide array of bacterial types. Some bacteria within the family Rhodocyclaceae degrade various carbon sources, while others are nitrogen-fixing aerobes that fix nitrogen that is present within the air and convert it to ammonia. Bacterial strains from the family Rhodocyclaceae have been discovered from a wide variety of environments, including sewage treatment plants, both unpolluted and polluted water sources, plant roots, or within soil (Oren, 2013).

The isolate for BR1 also showed similarity to the genus *Propionivibrio*. This genus represents bacteria that are straight or curved rods in shape and are gram-negative. They are typically motile and able to move through the utilization of a singular flagellum.

Additionally, they are non-spore forming, multiple through binary fission, and are chemoorganotrophic, meaning that they use chemical compounds as a source of energy (*Bergey's Manual of Systematics of Archaea and Bacteria*, 2015). BR1 also showed similarity to betaproteobacteria, which are a class of bacteria that are gram-negative and aid in nitrogen fixation, essentially oxidizing ammonium to produce nitrate, which is a chemical that is vital for plant function. Betaproteobacteria are commonly found in various environmental samples such as surface water samples and soil samples (“Betaproteobacteria,” 2019).

The experimental bacterial DNA isolates for BR1 were also similar to a *Thauera aminoaromatica* strain. This particular strain belongs to the genus *Thauera*, and within the family Rhodocyclaceae. The *Thauera aminoaromatica* strain is characterized by the ability to produce an abundance of exopolysaccharide. Additionally, this strain can degrade aromatic compounds of nitrates, which can help the processing of organic matter by the environment (Jiang, *et al.*, 2012). BR1 was also related to the *Thauera chlorobenzoica* strain, which is a strain of bacteria characterized by being gram-negative, short, and rod-shaped, and being motile during early growth through peritrichous flagellation (Song, *et al.*, 2001).

BR1 also displayed a relation to alphaproteobacterium, which is a class of bacteria that are one of the most abundant and diverse in nature. Some alphaproteobacterium live inside the cells that are present within other organisms; in fact, mitochondria evolved from bacteria that are present within this group. Others will act as beneficial symbionts within some cells, or even parasites (Sergio, *et al.*, 2019). Finally, BR1 showed relation to the *Candidatus accumulibacter species*, which is a bacterial

species that can beneficially remove phosphorus from wastewater systems in both aerobic and anaerobic environments. This bacterial strain is typically found in wastewater treatment plants with the ability to remove phosphorus and nitrogen from wastewater (Camejo, et al., 2019). Because the similarity values were low and below the mid-90's range, in addition to the disparate assortment of bacteria, it can be concluded that the sequences for BR1 were not particularly close to any species within the database.

BR2

This sample was related to the family Burkholderiaceae, which is a family composed of bacteria that are extremely diverse, ecologically speaking. Bacteria within this family obtain nutrients through the breakdown of organic matter, such as dead or decaying material. These bacteria are known as being “saprophytic.” Other bacterial species within family Burkholderiaceae contain pathogens that can cause illness in both humans and animals alike, as well as phytopathogens which cause disease in plants (Coenye, 2013).

The BR2 sample was also related to *Rhodoferrax*, which is a genus of betaproteobacteria that are gram-negative, short, and rod-shaped. *Rhodoferrax* belongs within the order Burkholderiales. Interestingly, BR2 showed relation to the family Comamonadaceae, which is a family of bacteria belonging within the genus *Rhodoferrax*. The bacterial DNA isolates from BR2 also showed a percent identity related to *Curvibacter*, which is yet another genus of bacteria belonging within the family Comamonadaceae (Kaden, et al., 2014).

Some bacterial types within the family Comamonadaceae are pathogenic and can cause disease in plants specifically. Additionally, bacteria within this family are gram-negative and are aerobic. Individuals of this family belong within the class beta proteobacteria (“Harvard Catalyst Profiles,” 2020). The bacterial DNA isolates from BR2 also showed percent identity of *Limnohabitans*, which is a bacterial genus representing bacteria that are present in freshwater environments. Members of *Limnohabitans* typically prefer habitats that are non-acidic and with an increased pH. Further, they are the bacterial component of plankton, and exhibit high growth and substrate uptake rates (Kasalicky, 2013).

BR3

The bacterial DNA isolates from the BR3 samples displayed a percent identity related to Xanthomonadaceae, a bacterial family characterized by bacteria that are gram-negative and non-spore forming. Some members of Xanthomonadaceae have been known to be human pathogens, causing disease through bloodstream infections (Rocco, 2007). BR3 was also related to the genus *Vibrio*, which is characterized by bacteria that are present typically in coastal waters, being present in high concentration between the months of May and October. Nearly one dozen *Vibrio* species can cause illness in humans. These illnesses are known as “vibriosis,” and is typically the result of eating undercooked or raw shellfish. Some species of *Vibrio* can cause skin infections when open wounds are exposed to brackish water or saltwater (“Questions and Answers,” 2019).

In addition, the bacterial DNA isolates of BR3 also showed a percent identity indicating a possible relation to uncultured sulfate-reducing bacteria. Sulfate-reducing

bacteria are common within habitats that are oxygen-deprived. These bacteria uptake sulfate instead of oxygen, and thus reduce sulfate to hydrogen sulfide to provide the energy that they need. Unfortunately, hydrogen sulfide can cause health problems in humans through drinking water contamination and carry a smell similar to that of rotten eggs. On the other hand, sulfate-reducing bacteria can be beneficial, as they reduce the risk of contaminants of groundwater through converting contaminants like chromium and uranium from their soluble to the insoluble form (“Study reveals new insights into sulfate-reducing bacteria,” 2014).

The BR3 bacterial isolates showed relation to uncultured gamma proteobacterium. Gamma proteobacterium are a class of bacteria characterized by being gram-negative, and within the phylum Proteobacteria. Additionally, gamma proteobacteria are genera-rich, including some well-known bacterial species such as *E. coli*, and pathogens such as *Salmonella*, *Vibrio*, and *Pseudomonas*, to name a few (Williams, et al., 2010). One of the most interesting results was the relativity to a human fecal sample for BR3. The presence of human waste within Lake Hollifield on the campus of Gardner-Webb University could be due to sewage runoff or waste running into the water during periods of heavy rainfall. Human and animal waste can lead to harmful bacterial growth and the transmission of pathogens for humans and animals alike if not corrected and treated properly.

Rhizobiales is an order of bacteria that displayed some percent identity to the BR3 bacterial DNA isolates. Bacteria within this order are nitrogen-fixing, act as precursors for some metabolites utilized by plants, and work to provide essential nutrients for plant growth. This order is common on lichens, which are plants that grow slowly and typically form on rocks and trees. Lichen is a composite organism, formed of fungus and algae,

and can arise from cyanobacteria (Erlacher, et al., 2015). *Calyptogena valdiviae* is a genus of bivalves that live in habitats with symbiotic sulfur-oxidizing bacteria. It was particularly interesting to discover that BR3 was related to a symbiont of *Calyptogena valdiviae*, as this genus is typically present within whale carcasses and even hydrothermal vents (Krylova, et al., 2006).

The isolates for BR3 also showed a percent identity reflecting a relation to the order Clostridiales, which is composed of bacteria that are obligate anaerobes that typically perish within normal oxygen atmospheric concentrations. Some species of Clostridiales are known for causing illnesses, such as *Clostridioides difficile*, which is known to cause severe diarrhea and dehydration. Other members of this order are important in human health, with some acting as probiotics and other Clostridiales species being present within the animal gut (Nie, et al., 2019). BR3 also showed a percent identity for Thiotrichaceae, which is a family of proteobacteria that are colorless. This family of bacteria is one of the largest-known, where some species are mobile through the utilization of a flagella (Harvard Catalyst Profiles, 2020).

BR4

Interestingly, the bacterial DNA isolates for the BR4 samples displayed a percent identity for the genus *Vibrio*, in addition to gamma proteobacterium and uncultured proteobacterium, which were previously discussed. BR3 was also related to Firmicutes, which is a phylum of bacteria that is made up of over 200 different genera. The various genera within this phylum include members of *Clostridium*, *Lactobacillus*, and *Enterococcus*, to name a few. These bacteria are typically gut microbiota, which are prominent within the digestive system (Rinninella, et al., 2019).

The bacterial DNA isolate for BR4 showed relation to an uncultured delta proteobacterium gene. Delta proteobacteria is a class of bacteria that are typically gram-negative, and are composed of predominantly aerobic species, with the exception of one branch of strictly anaerobic bacteria that are sulfate and sulfur-reducing (“Deltaproteobacteria, 2019). There was also a relation to *Thalassomonas sp.* which is a genus of bacteria that are gram-negative and have been known to cause some coral diseases (Olonade, et al., 2015). Within the genus *Thalassotalea*, the bacterial strain *Thalassotalea eurytherma* was identified as being related to BR4. This bacterial strain is gram-negative, facultatively anaerobic, and, interestingly, common in marine water. *Thalassotalea piscium* is another similar species belonging within the genus *Thalassotalea* (Hou, et al., 2015).

BR4 was also related to *Eubostrichus diana*, which is a marine nematode. This nematode lives in the pore space within sediment and seeks concentrations of high sulfide. Typically, these worms are covered in bacteria which serve as the main nutrition source for the nematode. This could explain why *Eubostrichus diana* was a result from the BR4 bacterial DNA isolation (Polz, et al., 1999). An Enterobacteriaceae bacterium symbiont of *Paracoccus marginatus* was another result for BR4 analysis. Enterobacteriaceae are a family of bacteria that are gram-negative. Many of the bacteria within this family are symbionts, which means that they reside in a mutual relationship with another organism. The Enterobacteriaceae bacterium symbiont result for BR4 was a symbiont of *Paracoccus marginatus*, which is a small insect within the mealybug family that is native to Central America (“*Paracoccus marginatus*,” 2020). Finally, the bacterial

DNA isolate for BR4 showed a percent identity to *Synechococcus sp.*, which is a strain of cyanobacterium that is typically found in marine environments (Ruffing, et al., 2016).

BS1

Concerning the BS1 bacterial DNA isolates, the resulting bacterial DNA had a percent identity between 94.41% and 99.46% reflecting an uncultured eukaryote clone. Eukaryotes are organisms excluding archaeobacteria and eubacteria, consisting of one or more cells, and including fungi, plants, animals, and essentially all organisms except for viruses, bacteria, and some blue-green algae (William, 2018). In addition, eukaryotic DNA may have resulted instead of bacterial DNA due to the endosymbiotic origin of chloroplasts. The Endosymbiotic Theory is the theory that states that present mitochondria are the result of the endosymbiosis of aerobic bacteria. Endosymbiosis is the process of cell eating; however, the cells are engulfed but not digested, thus left to live in a mutual relationship. According to the theory, present chloroplasts are the result of the endocytosis of photosynthetic bacteria. In this way, anaerobic bacteria ingested aerobic bacteria, living symbiotically with one another. Chloroplasts and mitochondria thus contain their own DNA. Based on the Endosymbiotic Theory, some of the organelles within today's eukaryotes once belonged to prokaryotic microbes. This would explain why the attempt to isolate bacterial DNA would actually result in eukaryotic DNA being isolated in the process ("The Endosymbiotic Theory," 2002).

However, the identification of these sequences as eukaryotes is uncertain. All of these "uncultured eukaryote" sequences were generated by a set of studies on bacteria living on human skin. They were identified as eukaryotic several years ago by inputting them into a program that searches for matches to a 16S sequence (Grice, et al., 2009). In

turn, the results from that program are only as good as the available data for comparison. All of the definitively identified sequences that were more closely related to BS1 were for cyanobacteria.

BS1 was also shown to be related to Cyanobacterium, which is a phylum of bacteria that are photosynthetic and aquatic. Cyanobacteria are often referred to as “blue-green algae,” due to the ability of Cyanobacteria to make their own food through photosynthesis; however, Cyanobacteria are distinct from algae (“Introduction to the Cyanobacteria,” 2020). In addition, BS1 was related to the Oscillatoriales cyanobacterium clone. Oscillatoriales is an order that belongs within the phylum Cyanobacteria. The cells of Oscillatoriales are cylindrical or coin-like in shape, joining end-to-end to create long filaments. Within Oscillatoriales, there are many different species; only a few of these species are known to form planktonic cyanobacteria blooms. Some members of Oscillatoriales are toxic, producing and releasing saxitoxins, microcystins, and anatoxins that target the nerves and the liver. These toxins can be degraded by other species of bacteria, or if the water pH is above neutral (Matthews, 2020).

Interestingly, the DNA isolate for BS1 showed similarity to chloroplasts of *Vaucheria litorea*, *Coscinodiscus radiatus*, *Ectocarpus siliculosus*, *Bacillaria paxillifer*, *Fucus vesiculosus* var. *spiralis*, and *Guinardia striata*. *Vaucheria litorea* is a type of yellow-green algae, while *Coscinodiscus radiatus* is a type of diatom, *Ectocarpus siliculosus* is a species of brown algae, and *Guinardia striata* and *Bacillaria paxillifer* are species of diatoms. *Fucus vesiculosus* is a brown alga species (“NCBI: Taxonomy”). As

previously stated, the Endosymbiotic Theory could account for the reason why chloroplast of algae and diatoms were isolated instead of bacterial DNA.

BS2

The results of the bacterial DNA isolates from the BS2 samples was inconclusive, resulting in an incomplete and disorganized electropherogram with a subsequent sequence that was extremely short in length and virtually unidentifiable. Sources of error that potentially contributed to this include human error when conducting the methodology of the experiment, error in performing the steps utilizing the Zymo Research “Quick-DNA™ Fungal/Bacterial Miniprep Kit,” or perhaps the sample did not contain bacterial DNA that could be properly isolated. Additionally, the water within the boiling spring is most likely to be relatively clean, containing few nutrients. This could therefore explain the low abundance in bacteria.

CR1

The bacterial DNA isolates of CR1 reflected a percent identity similar to a *Comamonadaceae* bacterium strain, *Rhydocyclaceae* bacterium, and beta proteobacteria, which were previously described. *Oryzomicrobium terrae*, which belongs within the family *Rhydocyclaceae*, was also related to CR1 (Oren, 2013). In addition, the sequence for CR1 was related to *Mitsuaria chitosanitabida*, which is a member of Betaproteobacteria. *Simplicispira* sp. was also related to CR1, and is a gram-negative, facultatively anaerobic bacteria that is motile and rod-shaped, and can be found within sludge (Siddiqi, et al., 2019).

CR1 was also similar to *Delftia sp.*, which is a bacterial strain that is gram-negative and commonly found within affected soil (Vásquez-Piñeros, et al., 2018). *Comamonas testosteroni* is a strain of bacteria that CR1 was related to. This bacterial strain shows resistance to drugs and heavy metals and is typically found within environmental samples that are polluted. *Comamonas testosteroni* are motile and aerobic, having the ability to perform nitrate reduction (Liu, et al., 2015).

CR2

The bacterial DNA isolates for CR2 showed a percent identity of *Comamonadaceae bacterium* and *Limnohabitans sp.*, both of which have been previously discussed. In addition, CR2 showed relation to the *Acidovorax species*. In one study, four different *Acidovorax* strains of bacteria were identified within sampled soil of North Carolina. *Acidovorax* is a genus of bacteria in which the bacterial strains grow best in low salinity conditions, within a neutral pH and under aerobic conditions. Additionally, *Acidovorax* strains have been found in a variety of environments, including within plants, soils, and even in wastewater treatment plants. Some members of this genus have been known to biodegrade some contaminants, or to have denitrification properties (Singleton, et al., 2018).

Phylogenetic Analysis of the DNA Samples

When constructing the cladogram and phylograms to compare the related sequences from the bacterial DNA isolations, the identity of each bacterial sample was more pronounced. The majority-rule consensus tree in Figure 9 showed sequences BR1, BR2, BR3 800, BR4, CR1, and CR2, with the analysis pointing toward these sequences

being related to a type of betaproteobacteria. Because the support levels were not extremely high, it was possible that this is a type of betaproteobacteria that had not been previously studied. The tree also showed that BR3 800 and BR4 were closer relatives to one another when compared to the other sequences within the tree. In turn, the group of BR3 and BR4 are most closely related to the group of sequences displayed from *Acidovorax* to BR2.

Concerning BR2, this sequence was likely related to the group from *Acidovorax* through CR1 and CR2 on the tree. Based on the majority-rule consensus tree in Figure 9, it was noted that CR1 and CR2 are more closely related to one another. From these results, it can be determined that the bacterial samples BR1, BR2, BR3 800, BR4, CR1, and CR2 are some form of betaproteobacteria. While there was no definite match to a particular bacterial strain or pathogen, these sequences showed similarity to betaproteobacteria based on the constructed majority-rule consensus tree.

When analyzing Figure 10 reflecting the phylogram of the strict consensus for the sequences similar to BR3 518, it was determined that the BR3 sequence was within the group containing *Aulacoseira* species, within the diatoms. While the results are not 100% definite, it can be determined from this tree that BR3 518 seems to belong in the diatom genus *Aulacoseira*, which in turn appears to be closely related to *Melosira*.

Upon analyzing Figure 11 containing the phylogram of the strict consensus of the most parsimonious trees for sequences matching BR1, it was determined that BR1 resembles the sequences titled “uncultured eukaryote.” These uncultured eukaryote sequences can be shown to resemble cyanobacteria. They are also more distantly similar to the chloroplast DNA from the various groups of algae. It is possible that the type of

cyanobacteria that is related to BR1 is a non-photosynthetic type, as the “uncultured eukaryote” sequences were obtained from human skin, which is often in the dark. However, they could have been picked up by accidental contact and not from actually living there.

“HACH® Surface Water Test Kit.”

In 2015, Gardner-Webb University student Whitley Bowman completed a comprehensive study pertaining to the quality of Lake Hollifield on the campus of Gardner-Webb University. With her research, she tested two different locations of Lake Hollifield for the presence of metals, inorganic non-metals, hardness, orthophosphate, chloride, ammonia, nitrate-nitrite, and phosphorus. The two locations of collection were referred to as the “yellow star” and “red diamond” locations and can be viewed below.



Figure 12. Map displaying the collection locations from Whitley Bowman’s thesis research in 2015. Both collection locations are shown with the yellow star and the red diamond (“Gardner-Webb University Map,” 2020).

Concerning Bowman's study, two samples were collected at both the yellow star and red diamond location to sample for metals. Additionally, three samples were collected at the yellow star location and two samples were collected at the red diamond location to test for inorganic non-metals. For hardness, two samples were collected at each of the two locations. To test for orthophosphate and chloride levels, two samples were collected at each location. Finally, three samples were collected at each of the two locations to test for orthophosphate, ammonia, and nitrate-nitrite levels. Bowman sent all of her collected samples to the Shealy Environmental Services, Inc. for analysis.

For my research, I wanted to compare my results to Bowman's results collected in 2015, as I was wondering if there had been a change in the water quality of Lake Hollifield within the past five years. Interestingly, the two locations that Bowman selected for sampling were almost identical to the locations that I chose to test for water quality. Bowman's yellow star location is adjacent to my sample labeled "BRQ1" that was collected within the northeastern side of Lake Hollifield, and her red diamond location was adjacent to my sample labeled "BSQ1" that was collected at the boiling spring. For this reason, Bowman's yellow star location results were compared to the results for BRQ1, and Bowman's red diamond location results were compared to the results for BSQ1.

Before comparing Bowman's results to my own, it should be noted that discrepancies could be the result of samples not being collected in the same location. Bowman and I had sampled in adjacent locations; however, Bowman did not sample in the boiling spring directly, but within the water adjacent to the boiling spring and running into Lake Hollifield. In addition, Bowman chose to utilize the Shealy Environmental

Services, Inc. for testing her samples, while I used the “HACH® Surface Water Test Kit” to test samples by hand. Because professional testing in a laboratory is far more accurate and precise compared to testing by hand, this likely accounts for the differences in my results compared to Bowman’s. When comparing my results to those of Bowman, I only compared our results for testing orthophosphate, ammonia, nitrate-nitrite, and orthophosphate. Bowman’s results for these tests can be viewed below.

Table 5. Whitley Bowman’s results of testing for orthophosphate, ammonia, nitrate-nitrite, and orthophosphate levels within the yellow star location (Bowman, 2015).

Parameter	Result	Units
Orthophosphate	0.010	mg/L
Ammonia-N (phenate)	0.47	mg/L
Nitrate-Nitrite	0.53	mg/L

Table 6. Whitley Bowman’s results of testing for orthophosphate, ammonia, nitrate-nitrite, and orthophosphate levels within the red diamond location (Bowman, 2015).

Parameter	Result	Units
Orthophosphate	0.012	mg/L
Ammonia-N (phenate)	0.27	mg/L
Nitrate-Nitrite	1.9	mg/L

First, Bowman’s yellow star location results were compared to the results for BRQ1. It was determined that the orthophosphate levels for BRQ1 were 0.07 mg/L higher than Bowman’s yellow star location results in 2015. Additionally, the ammonia level for Bowman was 0.47 mg/L higher than BRQ1. Finally, the nitrate level for BRQ1 was a shocking 8.27 mg/L higher than Bowman’s results for the yellow star location (Bowman, 2015).

Next, the results for Bowman's red diamond location and my BSQ1 location were compared. It was determined that the orthophosphate level for BSQ1 was 0.088 mg/L higher than the red diamond location. Concerning the ammonia levels, the red diamond location was 0.27 mg/L higher than BSQ1. Finally, the nitrate level for BSQ1 was 13.5 mg/L higher than the red diamond location. It was interesting to note that the orthophosphate level for both BRQ1 and BSQ1 were higher than Bowman's results, the ammonia levels for BRQ1 and BSQ1 were both lower than Bowman's results, and the nitrate levels for BRQ1 and BSQ1 were both higher than Bowman's results.

After comparing the results, it appeared that, since 2015, the orthophosphate, ammonia, and nitrate levels within the boiling spring and Lake Hollifield have increased. However, several factors and potential sources of error could have affected the obtained results from the "HACH® Surface Water Test Kit." As previously described, some differences in results could be due to the fact that Bowman did not test in the exact same locations as BRQ1 and BSQ1, and the fact that Bowman utilized a laboratory for testing while I chose to test manually. In addition, Bowman's samples were taken at a different time of year, in which the differences in water movement and chemical content could affect the results.

Within freshwater systems, the average temperature is between 65°F and 75°F in the summer, and between 35°F and 45°F in the winter season. Because the BRQ1 and BSQ1 samples were collected during the fall, their respective temperatures of 65.48°F and 66.2°F were considered normal for the season. If the samples were not taken at the surface and were instead taken at a deeper point within the water, the temperatures would have been decreased. Warm water contains less dissolved oxygen than cool water, so it is

important that the temperature of the boiling spring and Lake Hollifield be within normal range for the health of the organisms living within the water systems (Rinkesh, 2016).

The normal range for pH within systems of surface water is typically between 6.0 and 8.5. While the pH for BRQ1 fell within the normal range, the pH of BSQ1 (5.4) fell slightly below the normal range. Therefore, the pH of the sample collected from the boiling spring was determined to be slightly acidic. If the pH of a water source is acidic, the water within could be corrosive or soft. Unfortunately, the water could leach harmful metal ions such as manganese, copper, zinc, lead, and iron ions that could originate from underground piping, or even from aquifers or fixtures from plumbing. Water containing a low pH could damage metal piping as well; if one drinks the water, a sour or metallic taste would form in the mouth. Acidic water is typically low in sodium and oxygen, which can be harmful to the animals within. A popular mode of treatment for water sources with a low pH is to place a neutralizer within the water. One common chemical used for neutralization is soda ash (Oram, 2014).

In addition, the nitrate levels of the BSQ1 and BRQ1 samples was analyzed. According to the World Health Organization, the nitrate level within surface water is typically low (between 0-18 mg/L). Levels at this rate typically pose no harm to the organisms within the water. Therefore, it was determined that the nitrate levels of the boiling springs and Lake Hollifield on the campus of Gardner-Webb University were within the normal range (Cotruvo, 2011). It was also determined that the ammonia levels of the boiling spring and Lake Hollifield were within the normal range. To maintain a normal ammonia level and healthy aquatic life, the ammonia level within a freshwater

environment should not exceed 0.02 mg/L. In this way, the ammonia levels of the boiling spring and Lake Hollifield were within the normal range (Strange, 2020).

The orthophosphate levels for the BSQ1 and the BRQ1 samples was unfortunately high. A healthy phosphate range within freshwater environments is between 0.005-0.05 mg/L. The orthophosphate level for BSQ1 was 0.1 mg/L, and the orthophosphate level for BRQ1 is 0.08 mg/L. Therefore, the orthophosphate levels of the boiling spring and Lake Hollifield was 0.05-0.03 mg/L higher than the orthophosphate maximum normal level for freshwater environments. In addition, phosphorus levels that are between 0.08 and 0.1 mg/L have been known to trigger algal blooms within the water. Over time, this can lead to decreased dissolved oxygen levels, thus being detrimental to the fish and other organisms living within the water (Oram, 2014).

Fortunately, the dissolved oxygen levels of the BSQ1 and BRQ1 samples were within a healthy range, thus indicating that the increased orthophosphate levels could be the result of human error, or that this increase is temporary and not consistent for the boiling spring and Lake Hollifield environments. A healthy dissolved oxygen concentration within freshwater environments are above 6.5-8.0 mg/L, or in other words, between 80% and 120% (“Dissolved Oxygen and Water,” 2019).

In 1966, a study was conducted to determine the quality of the groundwater in Cleveland County. Several samples were taken of the groundwater; this was the only record discovered for groundwater or surface water samples that had been taken to determine water quality prior to the year 2015 and near Gardner-Webb University. GWU is within Cleveland County, so it was interesting to compare the results nearly 54 years

later. Within the parameters of this study, 49 samples were taken to determine the water quality of the groundwater of Cleveland County (Duncan, 1966).

Of these samples, there was a nitrogen content ranging from 0 to 33 mg/L, with a median of 0.8 mg/L. The pH of the samples ranged from 5.3 to 8.3, with a median of 6.2. The dissolved oxygen concentration, temperature, and ammonia concentration of the samples was not determined within this study. Compared to the results of this experiment, the nitrate concentration was significantly higher (though, the Cleveland County study measured nitrogen, and not nitrate). The pH of the water was similar and not significantly different from the pH of the samples taken within this experiment (Duncan, 1966).

Conclusion

In conclusion, the results from the bacterial DNA isolation and analysis indicate that there was a relation to many different genera of bacteria, including *Limnohabitans*, *Clostridiales*, *Curvibacter*, *Rhodospirillum rubrum*, *Vibrio*, and *Acidovorax*, to name a few. The isolated DNA also showed some relation to various types of algae and chloroplasts. As previously explained, the Endosymbiotic Theory states that present chloroplasts are the result of the endocytosis of photosynthetic bacteria. Therefore, the DNA of algae chloroplasts was extracted instead of the bacterial DNA.

The resulting phylogenetic trees and cladograms constructed as a parsimony analysis using the computer programs TNT and PAUP* helped in narrowing down what the bacterial DNA isolates were related to. While MAFFT and GBlocks were used for preparing the data for analysis, TNT and PAUP* helped to provide the actual analysis. It can be concluded that the BR1, BR2, BR3 800, BR4, CR1, and CR2 sequences are all

related to a type of betaproteobacteria. Additionally, BR3 518 was within the group containing *Aulacoseira* species, within the diatoms. The BR1 sequence resembled other sequences titled “uncultured eukaryote.” These uncultured eukaryote sequences were shown as resembling cyanobacteria, which are distantly similar to the chloroplast DNA from algal groups. Therefore, it can be concluded that BR1 is related to a type of cyanobacteria that is likely non-photosynthetic.

Unfortunately, the bacterial types in the surface water of Gardner-Webb University cannot be identified with 100% certainty due to the lack of 100% identity. This could be the result of some species having identical or similar 16S rRNA sequences, difficulty in the recognition of novel taxa, genomovar presence, or the fact that many types of bacteria have never been analyzed and submitted into the nucleotide databases. Additionally, the bacterial types within the surface water of Gardner-Webb University have never been studied before. It is therefore possible that the bacterial DNA isolations were that of new strains that had not been previously identified. Further research pertaining to the bacterial presence in the surface water on campus is necessary before arriving at definite conclusions pertaining to bacterial identification. From the parsimony analysis, one has a general but not definite idea of what the bacterial isolates showed relation to.

The results of the water quality study indicate that, while the temperature and nitrate, ammonia, and dissolved oxygen levels were within the normal ranges for the sampled sources, the pH was slightly acidic for the boiling spring location, and the orthophosphate levels were slightly elevated for both locations. When compared to samples taken in similar locations in 2015, it was revealed that the orthophosphate,

ammonia, and nitrate levels within the boiling spring and Lake Hollifield have increased. In a study conducted in 1966, these values for sampled groundwater were similar. However, these changes in orthophosphate, ammonia, and nitrate levels over time could be attributed to the fact that the water samples were not taken in the exact same locations as the 2015 study, the 1966 study did not sample in the same location as this study, and the values for water quality for this study were calculated manually as opposed to within a laboratory setting. While a laboratory would have yielded more accurate results, the process of conducting water quality testing by hand was cheaper and more interesting.

The slightly acidic pH of the boiling spring within this study could cause the water to become corrosive and soft. In addition, the water may contain metal ions that could damage underground pipes and plumbing structures in the future. Given that the sample collected from Lake Hollifield was within the normal pH range, this threat is not of great prominence currently. In the future, it would be wise to frequently test the pH of the natural water sources on campus to ensure that the pH remains within a normal range.

The elevated orthophosphate levels within the boiling spring and Lake Hollifield could be the result of fertilizer and animal waste running off into the water. Several different species of animals have been seen living around the sampled locations. These animal species include mallard ducks, Muscovy ducks, Pekin ducks, Canada geese, gray squirrels, and other avian and mammalian species. The waste from these animals, in addition to the fertilizer placed around the sampled locations, runs into the water during heavy rainfall. As a result, the elevated orthophosphate levels from this runoff could result in increased algal blooms within the water. As the excess of algae eventually die, bacteria decay the algae. This process of decay depletes the amount of dissolved oxygen

that is present within the water. Increased algal blooms thus increases the bacterial presence within the water. Some bacterial strains can produce toxins within the water that could be fatal to the animals within. Essentially, the depleted dissolved oxygen levels in addition to the increase in bacteria would result in the death of animals within the water, or animals that consume the water. Therefore, I recommend that the natural water sources on the campus of Gardner-Webb University be frequently tested in a laboratory setting to ensure that it is of good quality to maintain suitable living conditions for the animals within the water, as well as those consuming the water.

Bibliography

Algal Blooms. (2020). Retrieved from

<https://www.niehs.nih.gov/health/topics/agents/algal-blooms/index.cfm>.

“Ammonia.” *Waterboards*, Water Resources Control Board, 2019,

https://www.waterboards.ca.gov/water_issues/programs/swamp/docs/cwt/guidance/3310en.pdf.

“Bacteria and E. Coli in Water.” *USGS*, U.S. Geological Survey's Water Science School,

2019, https://www.usgs.gov/special-topic/water-science-school/science/bacteria-and-e-coli-water?qt-science_center_objects=0#qt-science_center_objects.

Bergey's Manual of Systematics of Archaea and Bacteria. (2015). doi:

10.1002/9781118960608.gbm01002.

Betaproteobacteria. (2019, September 25). Retrieved March 20, 2020, from

[https://bio.libretexts.org/Bookshelves/Microbiology/Book:_Microbiology_\(Boundles\)/8:_Microbial_Evolution,_Phylogeny,_and_Diversity/8.07:_Proteobacteria/8.7C:_Betaproteobacteria](https://bio.libretexts.org/Bookshelves/Microbiology/Book:_Microbiology_(Boundles)/8:_Microbial_Evolution,_Phylogeny,_and_Diversity/8.07:_Proteobacteria/8.7C:_Betaproteobacteria).

BLAST: Basic Local Alignment Search Tool. (n.d.). Retrieved from

<https://blast.ncbi.nlm.nih.gov/Blast.cgi>.

Blount, Sarah. “Waterfowl and Water Quality.” *NEEF*, National Environmental

Education Foundation, 2019, <https://www.neefusa.org/nature/water/waterfowl-and-water-quality>.

Bowman, Whitley. *An Examination of the Water Quality of Lake Hollifield*. 2015.

- Bucci, K., et al. "What Is Known and Unknown about the Effects of Plastic Pollution: A Meta-Analysis and Systematic Review." *Ecological Society of America*, 23 Nov. 2019, 10.1002/eap.2044.
- Cabral, João P S. "Water microbiology. Bacterial pathogens and water." *International journal of environmental research and public health*, vol. 7, no. 10, 2010, pp. 3657-703. NCBI, 10.3390/ijerph7103657.
- Camejo, et al. (2019, February 26). Integrated Omic Analyses Provide Evidence that a "Candidatus Accumulibacter phosphatis" Strain Performs Denitrification under Microaerobic Conditions. Retrieved March 20, 2020, from <https://msystems.asm.org/content/4/1/e00193-18>.
- Campbell, D. (Photograph). (2020, February 6).
- Castresana. (2000, April). Selection of Conserved Blocks from Multiple Alignments for Their Use in Phylogenetic Analysis. *Molecular Biology and Evolution*, 17(4), 540-552. <https://doi.org/10.1093/oxfordjournals.molbev.a026334>.
- Cheriyedath, S. (2018, November 12). Types of RNA: mRNA, rRNA and tRNA. Retrieved from <https://www.news-medical.net/life-sciences/-Types-of-RNA-mRNA-rRNA-and-tRNA.aspx>.
- Coenye, Tom. (2013). The Family Burkholderiaceae-The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. 759-776. 10.1007/978-3-642-30197-1_239.
- Cotruvo. (2011). *Nitrate and nitrite in drinking-water*. World Health Organization.

Deltaproteobacteria. (2019, September 25). Retrieved April 1, 2020, from [https://bio.libretexts.org/Bookshelves/Microbiology/Book:_Microbiology_\(Boundless\)/8:_Microbial_Evolution,_Phylogeny,_and_Diversity/8.07:_Proteobacteria/8.7F:_Deltaproteobacteria](https://bio.libretexts.org/Bookshelves/Microbiology/Book:_Microbiology_(Boundless)/8:_Microbial_Evolution,_Phylogeny,_and_Diversity/8.07:_Proteobacteria/8.7F:_Deltaproteobacteria).

“Dissolved Oxygen and Water.” *Dissolved Oxygen and Water*, USGS, 2019, https://www.usgs.gov/special-topic/water-science-school/science/dissolved-oxygen-and-water?qt-science_center_objects=0#qt-science_center_objects.

Duncan, Donald A. “Ground-Water Resources of Cleveland County, North Carolina State Publications II.” *Ground-Water Resources of Cleveland County, North Carolina State Publications II*, State Archives of North Carolina, 1966, <http://digital.ncdcr.gov/cdm/ref/collection/p16062coll9/id/226614/>.

Erlacher, et al. (2015). Rhizobiales as functional and endosymbiotic members in the lichen symbiosis of *Lobaria pulmonaria* L. *Frontiers in microbiology*, 6, 53. <https://doi.org/10.3389/fmicb.2015.00053>.

Flores, Gilberto E et al. “Diversity, distribution and sources of bacteria in residential kitchens.” *Environmental microbiology*, vol. 15, no. 2, 2013, pp. 588-96. 10.1111/1462-2920.12036.

Fox, et al., *Reduced codon mutagenesis*. 2010.

“Gardner-Webb University Map,” 2020.

Goloboff, P.A., and S. Catalano. 2016. TNT, version 1.5, including a full implementation of phylogenetic morphometrics. *Cladistics* 32:221-238. DOI 10.1111/cla.12160.

Grice, et al. Topographical and temporal diversity of the human skin microbiome. *Science* 324 (5931), 1190-1192 (2009).

- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95-98.
- Harvard Catalyst Profiles. (2020). Retrieved March 25, 2020, from <https://connects.catalyst.harvard.edu/Profiles/display/Concept/Comamonadaceae>.
- Harvard Catalyst Profiles. (2020). Retrieved March 26, 2020, from <https://connects.catalyst.harvard.edu/Profiles/display/Concept/Thiotrichaceae>.
- Hou, et al.. (2015). *Thalassotalea marina* sp. nov., isolated from a marine recirculating aquaculture system, reclassification of *Thalassomonas eurytherma* as *Thalassotalea eurytherma* comb. nov. and emended description of the genus *Thalassotalea*. *International Journal of Systematic and Evolutionary Microbiology* , 65(12). doi: <https://doi.org/10.1099/ijsem.0.000637>.
- Introduction to the Cyanobacteria. (2020). Retrieved March 27, 2020, from <https://ucmp.berkeley.edu/bacteria/cyanointro.html>.
- Janda, Michael J., and Sharon L. Abbott. “16S RRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory: Pluses, Perils, and Pitfalls.” *American Society for Microbiology*, vol. 45, no. 9, Sept. 2007, pp. 2761–2764. 10.1128/JCM.01228-07.
- Jiang, K., Sanseverino, J., Chauhan, A. *et al.* Complete genome sequence of *Thauera aminoaromatica* strain MZ1T. *Stand in Genomic Sci* 6, 325–335 (2012). <https://doi.org/10.4056/sigs.2696029>.

- Kaden, et al. (2014). *Rhodoferax saidenbachensis* sp. nov., a psychrotolerant, very slowly growing bacterium within the family Comamonadaceae, proposal of appropriate taxonomic position of *Albidiferax ferrireducens* strain T118T in the genus *Rhodoferax* and emended description of the genus *Rhodoferax*. *International Journal of Systematic and Evolutionary Microbiology*, 64(Pt 4), 1186–1193. doi: 10.1099/ijs.0.054031-0.
- Kaiser, G. E. (2019, February). The Sedimentation Rate of Ribosomal Subunits. Retrieved from http://faculty.ccbcmd.edu/courses/bio141/lecguide/unit1/proeu/density_rRNA.html.
- Kasalicky, et al. (2013). The Diversity of the Limnohabitans Genus, an Important Group of Freshwater Bacterioplankton, by Characterization of 35 Isolated Strains. *PLoS One*, 8(3). Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3591437/>.
- Kazutaka, et al. (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics*, 20 (4), 1160. <https://doi.org/10.1093/bib/bbx108>.
- Krylova, et al. (2006). Recent Bivalve Molluscs of the Genus *Calyptogena* (Vesicomidae). *Journal of Molluscan Studies*, 72, 359. Retrieved from <https://academic.oup.com/mollus/article-abstract/72/4/359/288318>.
- Lewis, K. (2010, July 12). The Uncultured Bacteria. Retrieved March 15, 2020, from <https://schaechter.asmblog.org/schaechter/2010/07/the-uncultured-bacteria.html>.

- Liu, et al. (2015). High correlation between genotypes and phenotypes of environmental bacteria *Comamonas testosteroni* strains. *BMC genomics*, 16(1), 110.
<https://doi.org/10.1186/s12864-015-1314-x>.
- Masazumi, Harada. “Minamata Disease: Methylmercury Poisoning in Japan Caused by Environmental Pollution.” *Critical Reviews in Toxicology*, vol. 25, no. 1, 1995, pp. 1-24. 10.3109/10408449509089885.
- Mason, Christopher. *Biology of Freshwater Pollution*. 4th ed., Pearson Education Limited, 2002.
- Matthews, R. (2020). Oscillatoriales. Retrieved March 27, 2020, from https://www.inaturalist.org/guide_taxa/751676.
- National Center for Biotechnology Information. PubChem Database. Nitrate, CID=943, 4 Dec. 2019, <https://pubchem.ncbi.nlm.nih.gov/compound/Nitrate>.
- NCBI: Taxonomy. (n.d.). Retrieved April 1, 2020, from <https://www.ncbi.nlm.nih.gov/taxonomy/?term=>.
- Nie, et al. (2019). Genomic reconstruction of σ^{54} regulons in Clostridiales. *BMC genomics*, 20(1), 565. <https://doi.org/10.1186/s12864-019-5918-4>.
- Nitrate Contamination. (2020). Retrieved from <https://www.watereducation.org/aquapedia/nitrate-contamination>.
- Ocean Plastic & Sea Turtles. (2020). Retrieved February 7, 2020, from <https://www.seeturtles.org/ocean-plastic>.
- Olonade, et al. (2015). Draft Genome Sequences of Marine Isolates of *Thalassomonas viridans* and *Thalassomonas actiniarum*. *Genome announcements*, 3(2), e00297-15. <https://doi.org/10.1128/genomeA.00297-15>.

- Oram, B. (2014). "Phosphate in Surface Water, Streams, and Lakes." *Water Research Center*, Water Research Watershed Center. Retrieved March 20, 2020, from <https://water-research.net/index.php/phosphate-in-water>.
- Oram, B. (2014). "The pH of Water." *Water Research Center*, Water Research Watershed Center. Retrieved March 31, 2020, from <https://www.water-research.net/index.php/ph>.
- Oren, A. (2013). *The prokaryotes: Alphaproteobacteria and betaproteobacteria*. doi: 10.1007/978-3-642-30197-1.
- Pandey, Pramod K et al. "Contamination of water resources by pathogenic bacteria." *AMB Express*, vol. 4, no. 51, 28 Jun. 2014. NCBI, 10.1186/s13568-014-0051-x.
- Paracoccus marginatus* (2020). Retrieved April 1, 2020, from <https://www.cabi.org/isc/datasheet/39201>.
- pH and Water. (2020). Retrieved from https://www.usgs.gov/special-topic/water-science-school/science/ph-and-water?qt-science_center_objects=0#qt-science_center_objects.
- Plastic Pollution-Facts and Figures. (2020). Retrieved March 10, 2020, from <https://www.sas.org.uk/our-work/plastic-pollution/plastic-pollution-facts-figures/>.
- "Point Source vs. Nonpoint Source Pollution." Water Education Foundation, 2020, <https://www.watereducation.org/aquapedia-background/point-source-vs-nonpoint-source-pollution>.

- Polz, et al. (1999). Diversity and heterogeneity of epibiotic bacterial communities on the marine nematode *Eubostrichus diana*. *Applied and environmental microbiology*, 65(9), 4271–4275.
- Psomagen Inc. (Photograph). (2020).
- Quesada-Calderón, et al. (2017). The multigenerational effects of water contamination and endocrine disrupting chemicals on the fitness of *Drosophila melanogaster*. *Ecology and evolution*, 7(16), 6519–6526.
<https://doi.org/10.1002/ece3.3172>.
- Questions and Answers. (2019, March 5). Retrieved March 26, 2020, from <https://www.cdc.gov/vibrio/faq.html>.
- “Quick-DNA Fungal/Bacterial Microprep Kit.” *Zymo Research*, Zymo Research, 2019, <https://www.zymoresearch.com/collections/quick-dna-fungal-bacterial-kits/products/quick-dna-fungal-bacterial-microprep-kit>.
- Reeder, C. (Photograph). (2019, November 20).
- Rinkesh. (2016, December 25). Freshwater Biome: Location, Climate, Plants, Animals and Threats. Retrieved February 20, 2020, from <https://www.conserve-energy-future.com/freshwater-biome.php>.
- Rinninella, et al. (2019). What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. *Microorganisms*, 7(1), 14. <https://doi.org/10.3390/microorganisms7010014>.
- Rocco, et al. (Feb. 2007). *Journal of Clinical Microbiology*, 45 (2) 641-644. DOI: 10.1128/JCM.01938-06.

- Ruffing, et al. (2016). Genetic tools for advancement of *Synechococcus* sp. PCC 7002 as a cyanobacterial chassis. *Microbial cell factories*, 15(1), 190.
<https://doi.org/10.1186/s12934-016-0584-6>.
- Sergio, et al. (2019, February 21). An updated phylogeny of the Alphaproteobacteria reveals that the parasitic Rickettsiales and Holosporales have independent origins. Retrieved March 20, 2020, from <https://elifesciences.org/articles/42535>.
- Sharma P, Giri A. “Productivity evaluation of lotic and lentic water body in Himachal Pradesh, India.” *MOJ Eco Environ Sci*. vol. 3, no. 5, 2018, pp. 311–317.
10.15406/mojes.2018.03.00105.
- Siddiqi, et al. (2019). *Simplicispira hankyongi* sp. nov., a novel denitrifying bacterium isolated from sludge. *Antonie Van Leeuwenhoek* , 113(3), 331–338. doi:
<https://doi.org/10.1007/s10482-019-01341-0>.
- Singleton, et al. (2018). Polyphasic characterization of four soil-derived phenanthrene-degrading *Acidovorax* strains and proposal of *Acidovorax carolinensis* sp. nov. *Systematic and applied microbiology*, 41(5), 460–472.
<https://doi.org/10.1016/j.syapm.2018.06.001>.
- Song, et al. (2001). Taxonomy browser (*Thauera chlorobenzoica*). Retrieved March 20, 2020, from
<https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=96773>.
- Strange, R. (2020). Water Quality: Ammonia. Retrieved January 10, 2020, from
<http://web.utk.edu/~rstrange/wfs556/html-content/05-ammonia.html>.

Study reveals new insights into sulfate-reducing bacteria. (2014, March 20). Retrieved March 25, 2020, from <https://phys.org/news/2014-03-reveals-insights-sulfate-reducing-bacteria.html>.

“Surface Water Test Kit.” *HACH*, Water Quality Products for Government Buyers, 2019, <https://www.hach.com/surface-water-test-kit/product?id=7640218498&callback=qs>.

Swofford, D.. (2002). PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10. <https://doi.org/10.1111/j.0014-3820.2002.tb00191.x>.

The Endosymbiotic Theory. (2002, January 14). Retrieved April 1, 2020, from <https://www.biology.iupui.edu/biocourses/N100/2k2endosymb.html>.

Thompson, Dennis. “Toxic Pond Algae is Killing Dogs-How to Protect Your Pooch.” *Phys.org*, Phys.org, 16 Aug. 2019, <http://phys.org/news/2019-08-toxic-pond-algae-dogshow-pooch.html>.

Vásquez-Piñeros, et al. (2018). Delftia sp. LCW, a strain isolated from a constructed wetland shows novel properties for dimethylphenol isomers degradation. *BMC microbiology*, 18(1), 108. <https://doi.org/10.1186/s12866-018-1255-z>.

Walls-Thumma, D. (2018, December 27). How Can Water Pollution Affect Animals, Homes and Health? Retrieved from <https://homeguides.sfgate.com/can-water-pollution-affect-animals-homes-health-79201.html>.

Warren, Charles E. *Biology and Water Pollution Control*. W.B. Saunders Company, 1971.

Water Quality Assessment: Physical: Temperature. (2004, November 10). Retrieved from <http://www.cotf.edu/ete/modules/waterq3/WQassess4h.html>.

- William, S. (2018, December 4). Definition of Eukaryote. Retrieved March 27, 2020, from <https://www.medicinenet.com/script/main/art.asp?articlekey=3336>.
- Williams, et al. (April 2010). *Journal of Bacteriology*, 192 (9), pp. 2305-2314; DOI: 10.1128/JB.01480-09.
- Wright, Mitchell Henry et al. "Bacterial DNA Extraction Using Individual Enzymes and Phenol/Chloroform Separation." *Journal of microbiology & biology education*, vol. 18, no. 2, 1 Sep. 2017. NCBI, 10.1128/jmbe.v18i2.1348.
- Yeager, A. (2019, December 3). Food-like smell on plastic may lure seabirds to eat it. Retrieved from <https://www.sciencenewsforstudents.org/article/food-smell-plastic-may-lure-seabirds-eat-it>.
- Zhang, et al. (2000), "A greedy algorithm for aligning DNA sequences," *J Comput Biol* 2000; 7(1-2):203-14.