WATER QUALITY INDICATORS IN WATERSHED SUBBASINS WITH MULTIPILE LAND USES

by

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Abstract

The wide use and accessibility of surface waters leads to multiple sources of contamination. The two main forms of pollution are from point and nonpoint sources. Point sources are regulated by the federal government; however, nonpoint sources are more difficult to regulate since there is no defined origin. Due to this problem, surface water monitoring is performed by state agencies which can include the testing of several different water quality indicators chosen by the state. This thesis examines several water quality indicators from two watershed subbasins with different land uses. The types of contamination and sources were evaluated from the data, which was analyzed based on sampling site, season, and two statistical tests.

The water quality indicators that were examined in this study included physical, chemical, and microbiological indicators. The two subbasins that were monitored were located in the Wachusett reservoir watershed in central Massachusetts. One subbasin, Malagasco Brook, was located south of the reservoir. Six sampling sites were chosen in proximity to a swampy area, a nursery, and condominium housing complex. The second subbasin, Beaman Pond, was located to the northwestern side of the reservoir and was monitored at three sites. These sites were located in a residential area in addition to a special use two acre farm. Analyses were performed by site and by season to find trends in the data. Statistical correlation and ANOVA analyses were performed in order to better understand the relationships of the water quality indicators.

From these analyses, it was determined that organic carbon and human sources of contamination were significant in the Malagasco Brook subbasin. Organics originated in the headwaters and nursery area, and the residential area was a possible source of microorganisms. The Beaman Pond subbasin was found to be affected by both human and animal sources of contamination. Downstream of the farm, animal contamination was found and supported by measurement of microbial source tracking indicators. The other two sites were affected by human sources, a result of septic systems. Strong correlations were found between several water quality parameters, including temperature

and dissolved oxygen, turbidity and particle counts, and fecal coliforms and enterococcus. Based on data usefulness and ease of measurement, it is recommended that temperature, DO, conductivity, pH, dissolved organic carbon, turbidity or particle counts, and fecal coliforms be included in a routine watershed monitoring program.

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Chapter 1

Introduction

Surface waters are used for a number of purposes including potable water sources, recreation, transportation, and aesthetics. With so many uses, water bodies are susceptible to affects that can degrade water quality. Therefore, mechanisms to protect surface waters, maintain current water quality, or reduce the degradation of surface water bodies are important.

Protection of water bodies is governed by both federal and state agencies. Federal protection of surface water bodies is under the control of the United States Environmental Protection Agency (U.S. EPA). The U.S. EPA regulates point sources of pollution such as discharges from municipal wastewater treatment plants or discharges from factories. State programs vary for individual states but generally require some form of a water quality monitoring program. The state government manages nonpoint source pollution, which is a difficult pollution source to control.

Nonpoint source pollution is difficult to monitor due to the nature of the type of discharge. Nonpoint sources of pollution are not specific sources like point sources, but include mainly contamination from surface runoff. Since runoff from surfaces is the largest contributing source to nonpoint source pollution, the type of land use that a watershed contains significantly influences the type of contamination in the runoff. For instance, an agricultural land use may contribute high concentrations of fertilizers and pesticides to a surface water body, while urban areas may contribute contaminants such as grease, oil, and trash.

In order to help control the impact of land uses on surface water bodies, monitoring programs are utilized to determine the quality of the water and potential sources of contamination. Several water quality parameters can be measured. Physical measurements include, temperature and suspended solids. Chemical measurements

include pH, phosphorous, nitrogen, and biological oxygen demand (BOD). Lastly, biological measurements include microbiological indicator organisms, plant and animal measurements, and classification of plants and animals.

Not only are there numerous parameters that can be measured, there are also several factors that affect the reliability and outcome of these measurements. Water quality can vary with season and can be significantly affected by precipitation events. Therefore watershed managers need to design year-round, comprehensive sampling plans. It is therefore important to determine the appropriate water quality indicators to measure based on time, need, and cost.

The objective of this thesis was to evaluate the usefulness of various water quality parameters and the effect of land use on water quality. The subbasins are located in central Massachusetts and are part of the watershed that flows into the Wachusett Reservoir, which is used as a drinking water source for Boston, Massachusetts and numerous surrounding communities. The watershed is managed by the Department of Conservation and Recreation (DCR). Samples were collected from several locations over four seasons and analyzed for water quality.

This was accomplished by examining water quality in two watershed subbasins with different land uses. The collected data was analyzed to determine contaminant sources within each subbasin. Statistical analyses were used to assess correlations between water quality parameters, and water quality differences among sampling sites and among seasons. This research focused on water quality parameters as these measurements are routinely taken by the DCR, but did not evaluate flows nor the effect of flows on water quality. Finally, recommendations are provided for selecting which water quality parameters to measure in a watershed sampling plan.

The next chapter describes the current literature on federal and state programs for monitoring and regulation, water quality parameters, and the affects of land uses on surface water quality. Chapter 3 discusses the methods used for collecting and analyzing

water quality as well as statistical analyses conducted on the data. Finally, the results of this study are presented along with the recommendations on the usefulness of water quality indicators in monitoring surface waters.

Chapter 2

Literature Review

Many constituents are examined when determining the quality of surface waters. Constituents include physical, chemical, and microbiological characteristics of water. This chapter examines the background of water pollutants, regulations regarding surface waters, and factors affecting surface water quality. A discussion of monitoring and the relationship of the water characteristics concludes this chapter.

2.1 Pollution

Pollution can put our nation's surface waters at great risk. Pollution is a waste that originates from residential, industrial, municipal, and agricultural discharges to water (U.S. EPA, 2004d). Surface water contamination includes microbial, inorganic, organic, and radioactive contaminants (U.S. EPA, 2004c). Microbial contaminants are viruses, bacteria and protozoa found in surface waters. Rather than measuring individual pathogens, indicator organisms such as *E. coli* and fecal coliforms are used to indicate the presence or absence of pathogens. Common inorganic contaminants found in source waters are nitrite and arsenic, originating from natural sources. In addition to naturally occurring inorganic contaminants, a number of inorganic contaminants originate from anthropogenic sources such as industrial and domestic waste discharges. Organic chemical contaminants are synthetic or volatile chemicals such as oil and grease. These are often a result of leaks from cars or automotive repair shops. Pesticides and herbicides are also a type of organic chemical contaminant typically transported to surface waters by runoff from agricultural areas. Home use of commercial pesticides and herbicides is another source of these contaminants. Radioactive contaminants are naturally occurring and are also produced during oil and gas processes.

2.1.1 Point Source Pollution

Pollutants that originate from an established source are considered point source pollution. Point source pollution, as defined by the U.S. EPA, is "any discernible, confined and discrete conveyance, such as a pipe, ditch, channel, tunnel, conduit, discrete fissure, or

container …from which pollutants are or may be discharged" (U.S. EPA, 2004b). Wastewater facilities and industrial factories discharging waste directly into surface waters are a form of point source pollution. Since point source pollution is a known form of pollution, the discharge can be regulated as discussed in Section 2.2.1.

2.1.2 Nonpoint Source Pollution

A second form of pollution to surface waters is through nonpoint source discharges. Nonpoint source pollution comes from many diffuse sources, where contaminants on land surfaces are transported by stormwater or snowmelt runoff into waterbodies. Examples of nonpoint source pollution include agricultural runoff and runoff from highly urbanized areas where a majority of the surfaces are paved. These sources of pollution are not regulated and are considered the leading remaining cause of water quality problems reported by state officials (U.S. EPA, 2004d).

Effects from nonpoint source pollutants include excess sediment accumulating in water bodies, high levels of nutrients, and bacterial contamination. Sediment transport into water bodies is greatly affected by construction sites with little or no erosion control measures. High levels of nutrients are produced by runoff transporting pesticides, manure, and other nutrient containing wastes into waterbodies. Nutrients affect water quality by providing excess nitrogen or phosphorous, leading to extreme plant and algal growth. Bacterial contamination can result from wildlife, domestic, or livestock feces contaminating water, or from overburdened or deteriorating septic systems.

2.1.3 Municipal Storm Sewers

Many cities have separate municipal storm sewers where runoff from storms is directed into a separate pipe specifically for stormwater, which is different from the sanitary sewer infrastructure. This structure of stormwater sewers is typical in newly developed cities and towns where it was easier and less costly to build separate systems than it would be to retrofit existing infrastructure. Several pollutants can be directed from separate storm water systems into surface waters. These include pesticides, sediments, grease and oil, fertilizers, and debris.

The other form of stormwater infrastructure is combined sewer systems. Combined sewer and stormwater systems are typically found in older cities where stormwater is directed into the sanitary sewer infrastructure to be treated at wastewater facilities. Approximately 772 communities still have combined sewer systems (U.S. EPA, 2002a). Problems with combined sewer systems are found during storms with high flows. The stormwater mixes with the sanitary sewerage, increasing the flow. If the flow is high enough, a portion of the flow will be diverted through an outlet pipe, which directs excess flows to the receiving water. This decreases the flow to the municipal treatment plant, so it may operate properly, but also contaminates the surface water with some sewage.

2.2 Regulations

Historically, high quality surface waters have been used for drinking water sources and desired for recreational waters. As recently as 25 years ago, only one third of the nation's waters were safe for recreational use (U.S. EPA, 2004b). The other two thirds of the nation's waters were polluted from several sources, including contamination from runoff (U.S. EPA, 2004b). In 1972, the Federal Water Pollution Control Act (now known as the Clean Water Act) was promulgated to inform Congress and the public about the water conditions of the nation and implement a course of action.

2.2.1 National Pollutant Discharge Elimination System

In accordance to Title IV of the Clean Water Act, a system for permitting wastewater discharges was created called the National Pollutant Discharge Elimination System (NPDES). This system required all facilities that discharged pollutants from point sources to obtain a permit that regulated technological requirements and quantitative limits on the water discharged. Since its origination, this permitting system has significantly reduced the amount of point source pollution entering surface waters, preventing billions of pounds of pollution from entering surface waters every year (U.S. EPA, 2004b; 1998). Currently, two thirds of the nation's surface waters are now safe for fishing and swimming (U.S. EPA, 2004d).

2.2.1.1 Phase I

The final rule for NPDES Phase I was published in November 1990, regulating point source discharges. The two major groups of dischargers were industrial facilities and municipal separate storm sewers, abbreviated by the U.S. EPA as MS4s. Municipal separate storm sewers are defined by 40 CFR 122.26(b)(8) as "a conveyance or system of conveyances…owned or operated by a State, city, town, borough, county, parish, district, association, or other public body…designed or used for collecting or conveying storm water…which is not a combined sewer (and) not part of a publicly owned treatment works (POTW)" (U.S. EPA, 2000b). Phase I required industrial facilities and MS4s within an incorporated place or county with populations greater than 100,000 people to obtain a permit for discharge. Approximately 900 MS4s are regulated under Phase I (U.S. EPA, 2000b).

Publicly owned treatment works are regulated under NPDES through secondary treatment standards. The technology-based requirements on the secondary treatment of the POTW requires the effluent quality to meet both five-day biological oxygen demand (BOD_5) and total suspended solids (TSS) requirements. These limits are 30 mg/L BOD_5 and 30 mg/L TSS on a 30-day averaging period, and 45 mg/L for each when averaged over 7 days.

When technology-based regulations are not sufficient to protect water qualities, water quality based standards can be used. Based on section 303(d) of the Clean Water Act, the state may rank the priority of surface waters and develop a total maximum daily load (TMDL) for the highest ranked surface waters. A TMDL is the amount of a pollutant from point, nonpoint, and natural sources allowable in a surface water that will maintain the water quality standards of the surface water, including a factor of safety (U.S. EPA, 2004b).

2.2.1.2 Phase II

Phase II of the NPDES was promulgated in 2000, requiring regulated small MS4s to obtain permits for discharge. Regulated small MS4s include the MS4s not regulated

under Phase I, which meet one of the three designations: automatic nationwide designation, potential designation by the NPDES permitting authority by evaluation, or physical connection (U.S. EPA, 2000b). Automatic nationwide designated small MS4s include all operators of MS4s located within boundaries as defined by the Bureau of the Census as "urbanized areas." Urbanized areas include one or more places and the adjacent densely settled surrounding area that collectively have a residential population of 50,000 and an overall population density of at least 1,000 people per square mile (U.S. EPA, 2000b). The NPDES permitting authority may also determine if small MS4s located outside of an urbanized area serving a population of at least 10,000 should be included in Phase II regulations. The third designation is by physical connection of a small MS4 substantially contributing to the pollutant loading of a regulated NPDES MS4.

There are six measures that Phase II required to be integrated into the management program for all MS4s. Two required measurements involved the inclusion of the public, in the forms of outreach education and participation. A third measure is to detect, map, and prohibit illicit discharges within the stormwater infrastructure. Illicit discharges are any discharges not comprised of storm water with the exception of permitted sources and fire-fighting activities.

During construction projects, Phase II requires measures to control the runoff from disturbed sites greater than or equal to one acre. Site plans are reviewed for proper erosion and sediment control measures. Other measures include post-construction stormwater management for new developments and redevelopments using structural best management practices (BMPs). Structural BMPs are physical structures that accept storm water flow and can hold or remove sediments from the flow ultimately reducing the pollutants in the runoff. The final measure required under Phase II regulations is the development of a pollution prevention/good housekeeping operation for municipal operations, mainly through employee training (U.S. EPA, 2000a).

2.2.2 Safe Drinking Water Act

Congress passed the Safe Drinking Water Act in 1974 to protect public health by protecting the nation's drinking water supply. Under the 1996 Amendments, states were required to implement Source Water Assessment Programs (SWAPs). These programs would determine existing and possible threats to the water quality of drinking water supplies. The required actions of these programs were to assess a delineated watershed using a model of existing contaminants. Based on the model, the susceptibility of the water supply was determined and presented to the public.

2.3 Impact of Land Use on Water Quality

Pollution from nonpoint sources can vary significantly due to the types of land uses within a watershed. Several studies have been performed to determine the relationship between land uses and various parameters of water quality. These studies are discussed in the following sections.

2.3.1 Urban Land Use

Urban land uses are defined within this thesis as areas of increased impervious surfaces. However, urban land uses are not limited only to problems incurred by impervious surfaces. Other land uses classified under urban uses may include residential, industrial, and commercial areas, as well as roads and highways. The land's natural tendency to filter water is reduced when natural land is converted into impervious surfaces, such as roads, driveways, parking lots, and rooftops (Mallin *et al*., 2001).

Bannerman *et al*. (1993) studied critical urban source areas, which were defined as areas that produced large contaminant loading. Loading was evaluated by sampling from stormwater outfalls that were representative of runoff from urban land uses. The samples were analyzed for 16 constituents including total and dissolved phosphorous, total and suspended solids, total and recoverable cadmium, chromium, copper, lead, and zinc, hardness, and fecal coliform bacteria. The geometric mean was analyzed in order to compare the data to loads outside of the sample area. The locations observed consisted of a residential area with 66.7% pervious surfaces, a commercial area with no pervious

surfaces, and an industrial area with 38.4% pervious surfaces. Runoff from the streets had the largest mean concentrations in 10 out of the 16 contaminants studied in this test. Total recoverable zinc concentrations were highest in runoff collected from industrial roofs, and phosphorus was found in highest concentrations from lawns. Lawns contributed significant amounts of phosphorous loading, 2 – 18 times greater than other residential runoff sources (Bannerman *et al*., 1993).

Basnyat *et al*. (1999) studied areas with different urban uses that contributed nonpoint source pollution to Weeks Bay, which eventually connects with the Gulf of Mexico. The water quality within the watershed was effected negatively due to urban land use activities. Residential land uses observed in this study produced the greatest amount of nitrate in runoff (Basnyat *et al.,* 1999).

Rooftops from urban areas were found to be a significant source of contamination by Van Metre and Mahler (2003). The researchers studied contaminant loadings from galvanized metal and asphalt shingles. These two types of rooftops are typically found within urban land uses. Twenty-two pollutants were measured, including, zinc, lead, pyrene, and chrysene. Rooftops were found to significantly contribute to loading of zinc and lead. Twenty percent and 18 % of the total watershed load of zinc and lead, respectively, came from rooftops. The concentrations of zinc were in the range of 141 - 6200 mg/kg and lead ranged from 36 - 390 mg/kg for the 22 sampling dates (Van Metre and Mahler, 2003).

Additional studies have shown increases in bacterial loading in urban areas. A study by Kelsey *et al*. (2004) suggested that proximity to septic tanks was an important predictor of fecal coliform counts. However, contamination from domestic cats and dogs was more likely the source of the contamination, contributing a load of 1.36×10^{14} FCU/day where human contribution was only 0.0048×10^{14} FCU/day (Kelsey *et al*., 2004). Mallin *et al.* (2000) established that fecal coliform abundance was correlated to watershed-impervious surface coverage. A study performed by Gannon and Busse (1989) found that urban stormwater runoff significantly affected the levels of bacterial indicator organisms. Samples taken from storm drains in Ann Arbor, Michigan were found to have elevated

levels of *E. coli* and enterococci. Fecal coliform levels were the highest downstream of four collective storm drain discharge pipes at 41,000/100 mL (73% - 99% greater than the concentrations at the individual pipes upstream). Enterococci levels were found to be the highest at the Traver Creek drain at 80,000/100 mL (58% - 99% greater) (Gannon and Busse, 1989). The U.S. EPA standards at the time of this study were set at 126/100 mL for *E. coli* and 33/100 mL for enterococci.

2.3.2 Agricultural Land Use

Agricultural land has been found to have significant, long lasting affects on water quality. Agricultural land uses include land used for the growth of crops, orchards, pastures, and sod, as well as livestock management.

A study performed by Basnyat *et al*. (1999) showed that agricultural land uses could have significant impacts on surface water, particularly from nonpoint source pollution. Sediments, animal wastes, plant nutrients, and pesticides can all affect water quality negatively. Proximity of agricultural land to the surface water was found to impact water quality degradation (Basnyat *et al*., 1999). It was observed that a decrease in agricultural land use would produce decreases in nitrogen loading.

Buck *et al*. (2003) studied areas in New Zealand, which were primarily used as agricultural lands for farming sheep and deer. Total nitrogen and nitrate were found to have a 99% statistical significance for the two study areas, as well as with turbidity and total phosphorous. High levels of bacterial indicators were observed during the high flood seasons in July reaching concentrations of 88,000 cfu/100 mL, 2000 times higher than the May sampling (Buck *et al*., 2003).

Large amounts of fertilizer and manure are put on agricultural lands in order to increase nutrients for crop growth. Elevated levels of nitrogen were observed in the Sumas River located in Washington state and British Columbia, resulting in surplus values averaging 120 kg/ha·yr. Levels 20 to 70 percent greater than the tolerance level (100 kg N/ha) were observed by Berka *et al*. (2000). Agricultural land also has the capability to contribute

sediments through runoff, which is the second highest contribution of nitrate in water (Basnyat *et al*., 1999).

In a later study performed by Mallin *et al*. (2001), stream fecal coliform counts were significantly higher in rural areas where animal production was located when compared to swampy areas with animal production. Mallin *et al*. (2001) concluded that waterborne microbial pathogens could be reduced through the reduction of impervious surfaces. This was confirmed by Tufford and Marshall (2002), who showed that fecal coliform abundance was correlated to large areas of impervious surfaces.

2.3.3 Other Land Use Factors

Buck *et al*. (2003) showed that the influence of land use on water quality was scale-dependant. The conclusions from this study showed that local land use was a significant factor for smaller streams where upstream land use was a significant factor for larger streams. Not only can land use affect the quality of water, but also the distance of the land use from the water source can affect quality. This was shown from 95% statistically significant correlations of land use and sediment phosphorous levels 4000 meters away from the source (Houlahan and Findlay, 2004).

In addition to land use, seasonal differences also impact receiving water quality (Berka *et al*., 2000). In this study, increased activity (26% increase of agricultural land use) was correlated to an increase in water pollution. In conjunction, a high amount of nitrates was found in the tributary during the winter (57% - 75% higher than summer seasons). Ammonia, phosphate, and coliforms were elevated during wet winters (Berka *et al*., 2000).

2.4 Monitoring

Due to the affects of pollution on surface waters, regulations have been created to ensure the monitoring of surface water quality. Monitoring is done to characterize water streams over time, provide solutions for pollution prevention, maintain the integrity of natural waters, and respond to emergency problems (EPA, 2004d). As recently as the 1980s,

monitoring was based on meeting certain standards or goals set for water quality in single water bodies (Waite, 1984; Canter, 1985; Biswas, 1997; EPA, 2002b). Under new amendments to existing legislation, such as the Clean Water Act and the Safe Drinking Water Act, water-monitoring programs are evolving to meet national monitoring requirements. These new methods for water quality monitoring take into account whole watersheds or subbasins, and include a larger number of quality indicators.

Under the Clean Water Act, grants for pollution control programs are only given to states that monitor the quality of navigable waters and provide annual reports under CWA Section 305 (CWA, Section 106(e)(1)). In order to aid in the completion of the required monitoring and reporting, the U.S. EPA released a document with the guidelines to meet this legislation (U.S. EPA, 2003a). This document, *Elements of a State Water Monitoring and Assessment Program,* recognizes the differences in water quality monitoring between states and looks for conformity in implementation and reporting by 2013. Biennial, national reports of the outcomes of the water quality monitoring are published for the purpose of informing Congress of the conditions. These 305(b) reports are also updated annually by each state, the District of Colombia, and territories.

In order to develop a monitoring program, state's observe the overall quality of all water bodies and how they have changed over time. Determining problem areas and areas that need protection allows a framework of objectives to be established. A number of sampling sites are chosen for further analysis at priority locations within water bodies. These sites are chosen as representative location to observed the characteristics of each water body. Biological, chemical, and physical indicators are measured including pH, temperature, dissolved oxygen (DO), suspended solids, pathogens, and various other indicators depending on the use of the water body. Uses are divided into four categories: aquatic life and wildlife, recreation, drinking water, and fish/shellfish consumption. Along with these baseline indicators, supplemental indicators, such as sediment toxicity, hazardous chemicals, and hydrophilic pesticides, can be used when a specific pollutant is present and it is desirable to monitor it further (U.S. EPA, 2003a).

After a monitoring plan is developed based on a core of indicators, the data can be stored based on an U.S. EPA provided data system called STORET (U.S. EPA, 2003a). This data is then analyzed and reported to Congress as described previously.

2.4.1 Water Quality Parameters

Several parameters are analyzed when determining water quality of public water sources. This section describes the parameters analyzed and their meaning. These parameters include physical, chemical, and microbiological constituents that were analyzed on the water samples for this thesis.

2.4.1.1 Particulate Matter

Turbidity is an aggregate measure of water clarity based on the amount of particles within the sample. The composition of materials that contribute to turbidity are suspended and colloidal matter including, clay, silt, finely divided organic matter, plankton, and other microscopic organisms (APHA *et al*., 1998). Solids may also be analyzed by counting the concentration of particles in a water sample. This method quantifies the number of particles within certain size ranges.

Particulate matter found in water can affect the aesthetics of water by decreasing clarity and also by contributing to tastes and odors. Turbid water can affect water treatment processes, including disinfection and coagulation, as well as adding costs to treatment from the extra demand for removal of the particles. In addition, solids can be harmful to aquatic species, as turbid waters inhibit respiratory processes and reduce visibility (Vigil, 2003).

2.4.1.2 Temperature

Changes in temperature largely affect the chemical characteristics of water. Overall increased temperatures in water bodies can cause increased chemical and biological reaction rates, mineral solubility, and growth of aquatic organisms. Higher temperatures also decrease gas solubility and respiration rates (Tchobanoglous, 1985). Warmer waters have a lower dissolved oxygen solubility. Low DO levels negatively affect plant and

aquatic species within the water and change the character of a water body (Wilber, 1969; APHA *et al*., 1998; Kailasam and Sivakami, 2004).

Energy producing and industrial facilities can cause increases in surface water temperatures. These facilities use large volumes of water used for cooling processes, and then discharge the heated water to surface waters. Runoff in urban areas can also be heated from hot asphalt and pavement, increasing temperature (WOW, 2004). In riparian areas, waterbodies can be protected from temperature changes from shading cause by plant life on the edges of the water body. Other causes of temperature changes are due to seasonal variations and daily temperature changes. Seasonal variations are slower processes, especially in larger water bodies where the deeper water experiences little change in temperature due to ground insulation (Spellman and Drinan, 2000). This result also indicates that sunlight and wind can affect the speed of the temperature change.

2.4.1.3 pH

The acidic or basic characteristics of a water body are described by pH (APHA, 1998). pH is measured on a scale from 1.0 – 14.0 with no unit, where more basic solutions have a higher pH and more acidic solutions have a lower pH. The pH scale measures the logarithmic concentration of hydrogen (H^+) and hydroxide (OH^-) ions as shown in equation 2.1 (U.S. EPA, 2003b).

$$pH = -\log [H^+] \qquad \text{(Equation 2.1)}$$

Several factors can be affected by the pH of water, including biological availability and solubility of elements in water (WOW, 2001). Growth and reproduction of freshwater aquatic species of fish are found to be ideal within a pH range of 6.5 to 8.5; however, they may thrive slightly outside this range as well (Wilber, 1969). The ability of water to resist changes in pH is based on the buffering capacity of the water body.

2.4.1.4 Conductivity

Conductivity describes the ability of an aqueous solution to carry an electric current (APHA *et al.*, 1998). The amount of ions or total dissolved salts in water is an indicator

of conductivity, meaning conductivity increases as the concentration of ions increases (Tchobanoglous, 1985). Conductivity is typically reported in microsiemens per centimeter (μS/cm). Solutions with mostly inorganic compounds tend to be better conductors while solutions with organic compounds do not conduct currents well (APHA *et al.*, 1998). The type of rock and soil within the watershed affects conductivity. Watershed size is also a factor, as contact time with the rocks and soils increases with increasing watershed size.

2.4.1.5 Dissolved Oxygen

Dissolved oxygen (DO) is a measurement of the amount of oxygen gas dissolved in water, and available for use by plant and aquatic species. Oxygen gas naturally mixes with water through surface interaction. Fast moving waters typically have a higher DO due to mixing with air when the water hits debris such as rocks and logs (Vigil, 2003). Dissolved oxygen can be depleted by the demand from organic decomposition and use from plant and animal respiration.

Aquatic populations exposed to low dissolved oxygen concentrations may be more susceptible to adverse effects of other stressors such as disease or effects of toxic substances. Different varieties of fish need different amounts of DO to thrive. Based on the U.S. EPA's water quality criteria, the one-day minimum for cold-water species is 5.0 mg/L in early development stages and 4.0 mg/L for other stages. For warm water species, 5.0 mg/L and 3.0 mg/L is needed in early and other stages, respectively (U.S. EPA, 1986).

2.4.1.6 Organic Matter

Organic matter can come from both natural and anthropogenic sources. Organic compounds are defined by the presence of carbon. They may also include elements such as hydrogen, oxygen, nitrogen, phosphorous, and sulfur. Natural organic contributions to waters include decaying vegetation and microorganisms (Tchobanoglous, 1985). There are greater than 100,000 types of synthetic organic products that can contribute to organic loading in water. These include paints, herbicides, synthetic fertilizers, dyes, and fuels (Spellman and Drinan, 2000). The affects of organic matter in a water source are odors,

colors, tastes, oxygen depletion, and the formation of halogenated compounds with the addition of chlorine for disinfection (Tchobanoglous, 1985).

Total organic carbon (TOC) is an analysis of the concentration of organic matter. Organic matter can be found in both particulate and dissolved forms. Measurement of TOC is used as a substitute for the traditional five-day biological oxygen demand (BOD) analysis. A correlation between TOC and the absorbance of ultraviolet light at a wavelength of 254 nm has been observed (MacCraith *et al*., 1993; Sung, 2003; Westphal *et al*., 2004). Based on the strong correlation between the two measurements, UV_{254} has been suggested as an inexpensive and easy way to determine the organic content within a water sample (MacCraith *et al*., 1993; Westphal *et al*., 2004).

2.4.2 Pathogens

The microbiological quality of water can be evaluated by the absence of pathogens in the water sample. The main types of microorganisms found in water are bacteria, protozoa, viruses, and helminths. Not all bacteria are pathogenic; many forms of bacteria are helpful such as bacteria in the human gut. Examples of bacteria that are known to cause disease are *Salmonella typhi* and pathogenic *Escherichia coli*, which cause typhoid fever and gastroenteritis, respectively. Protozoa are single-celled organisms, which have a feeding strategy similar to humans (Spellman and Drinan, 2000). The most commonly known waterborne pathogenic protozoa are *Giardia lamblia* and *Cryptosporidium. Giardia lamblia* can cause giardiasis, which causes symptoms of nausea, anorexia, and severe diarrhea. Cryptosporidiosis is an illness caused by ingestion of the pathogen *Cryptosporidium.* Symptoms include headache, abdominal cramps, nausea, and vomiting. Both illnesses can be self-limiting in individuals with healthy immune systems. However, cryptosporidiosis can be fatal to people with compromised or weak immune systems.

Viruses are the smallest known infectious life form. Viruses need a host in order to reproduce; however, only one virus is needed to infect the host. Approximately 100 viruses are found in human feces (Spellman and Drinan, 2000). Waterborne viruses include infectious hepatitis, polio virus, and Norwalk agent. Lastly, helminths are

intestinal worms such as *Ascaris lumbricoides* (stomach worms) and *Necator americanus* (hookworm).

The enumeration of individual pathogens found within a water source is not an ideal method of detection. Determining every individual bacteria, protozoa, virus, and helminth is not practical due to the difficulty, time, and expense to perform the enumerations (Mardon and Stretch, 2004). Many watershed management agencies and water utilities do not have the instrumentation or personnel needed. As a result of the complexity of quantifying individual pathogens, indicator organisms are measured on a routine basis to assess water safety.

2.4.3 Indicator Organisms

The alternative to classifying each individual pathogen in a water sample is to enumerate indicator organisms. An indicator organism is an organism that is more easily measured than pathogens, and indicates the potential presence of a pathogen. Ideal indicator organisms have certain characteristics that make them desirable to use. The indicator organism should be present when the pathogen is present and absent when the pathogen is absent (Noble *et al*., 2003). The indicator should be easier to enumerate than individual pathogens, as well as safer to handle. Lastly, the indicator should be present in high numbers in fecal matter so it is easy to detect.

2.4.3.1 Total and Fecal Coliforms

Fecal coliform and total coliform counts are the most commonly used indicator organisms. Coliforms are part of the Enterobacteriaceae family (Noble *et al*., 2003; Mallin, 2000; APHA *et al.*, 1998). Fecal coliforms are a subset of total coliforms, which can grow at an elevated temperature of 44.5°C (AWWA, 1999). The growth of fecal coliforms is an indication that pathogens found from the intestines of warm-blooded mammals may be present. Total coliforms include all coliforms and are used because the absence of all coliforms indicates an absence of fecal coliforms. Total coliforms can originate from the intestines of warm-blooded mammals as well as from the environment.

Total and fecal coliforms are commonly measured in two ways: the multiple tube fermentation (MTF) technique and membrane filtration (MF) technique (APHA *et al.*, 1998). The multiple tube fermentation method relies on dilution to the point of extinction. The presence of coliforms is determined by production of gas during fermentation of lactose. A series of test tubes are inoculated with a water sample at different dilutions, and the resulting positive and negative test tubes are correlated to the most probable number (MPN) of organisms present through statistical analyses. Membrane filtration is the process of filtering volumes of sample water through a membrane with a pore size small enough to trap bacteria. The membrane is incubated on nutrient media to grow coliform colonies. After incubation, the colonies are counted and reported in colonies per 100 mL.

Fecal and total coliform enumeration for water treatment facilities must have lower than five percent of the samples positive in a month. For facilities that collect fewer than 40 samples a month, no more than one sample can be positive for total coliforms (U.S. EPA, 2004a).

The use of coliforms as an indicator organism has been questioned as far back as 1922 (AWWA *et al.*, 1999). Pathogens have been isolated when there have been low concentrations of fecal coliforms (Waite 1984). This indicates that fecal coliform counts may not be an accurate indicator. Other studies have shown that pathogens such as viruses and protozoan cysts are more resistant to disinfection than coliforms (AWWA, 1999). Some studies have shown a better correlation between pathogens and other indicators. For example, *E. coli* has been shown to be a better indicator of bather illness in recreational waters (Noble, 2003).

2.4.3.2 Fecal Streptococcous/Enterococcus

An indicator that may be more specific than coliforms is fecal streptococcous, which includes enterococci. *Enterococcus faecalis* has been found in humans, dogs, and chickens, and may or may not be limited to other warm-blooded animals (Wheeler *et al*., 2002). Enterococci are measured in a similar method as coliforms by membrane

filtration. A volume of sample water is filtered through a membrane that will retain the bacteria. The membrane is then incubated on an agar that will allow enterococci colonies to develop while inhibiting other microorganisms. The membrane is then transferred to another agar that confirms the presence of the colonies. Results are presented as colonies per 100 mL.

The U.S. EPA limitations on enterococcus given for recreational waters are 61 cfu/100 mL for single sample advisory limits. The five-day geometric mean should not exceed 33 cfu/100 mL (Kinzelman *et al*., 2003).

Enterococcus has been found to be a better indicator of pathogenic pollution in marine environments, especially when indicators are found at relatively low concentrations (Mardon and Strecth, 2004). For example, at polluted beaches in South America, the *E. coli* limit of 126 cfu/100 mL was never exceed. However, the 33 cfu/100 mL of enterococcus was exceed between 3% and 10% of the time. At more polluted beaches, there were only slight differences in detection of the two indicators above regulatory limits. Studies in both the United States and the United Kingdom have determined high correlations between the presence of enterococci and gastrointestinal symptoms in marine waters and slightly lower correlations between the two in fresh waters (Nuzzi and Burhans, 1997).

Conversely, studies have also showed that *E. coli* and enterococci have no statistical comparison (Kinzelman *et al*., 2003). Kinzelman *et al*. found that based on U.S. EPA single-event guidelines, the threshold was exceeded 20 and 46 times for enterococci and *E. coli*, respectively. The results for the 5-day geometric mean showed the levels would be exceeded 33 and 26 times for enterococci and *E. coli*, respectively.

2.4.3.3 FC/FS Ratio

Some studies have shown that the ratio of fecal coliforms to fecal streptococci (FC/FS) may more accurately signify human contamination than individual measurements. The concentration of fecal coliforms and fecal streptococci are different within humans and

animals (Tchobanoglous, 1985). Ratios determined by Mara (1976) show that humans had a ratio of 4.4, and chickens, cows, ducks, pigs, sheep, and turkeys had ratios ranging from 0.4 – 0.1. Therefore, a FC/FS ratio greater than 4 is associated with human waste, while ratios less than 0.7 are indicative of animal sources (Brion and Lingireddy, 1998; Gannon and Busse, 1989; Daby *et al*., 2002). This method is problematic because contamination sources are uncertain for ratios between 0.7 and 4 (Tchobanoglous, 1985). The two microbial indicators have differing survival rates, adding to the uncertainty of the method (Brion and Lingireddy, 1998). Studies have shown that the FC/FS ratio can increase over a one day period (with a statistical significance of 0.01) from the rapid disappearance in fecal streptococci over fecal coliforms (Gannon and Busse, 1989).

In order to determine the FC/FS ratio of a sample, analysis must be performed at a pH between 4 and 9 in order to eliminate the adverse affects of pH on microorganisms. The analysis is not as reliable if the sample is not fresh or if the sample has indicator concentrations less than 100 cfu/100 mL (Brion and Lingireddy, 1998). A study performed by Brion and Lingireddy, (1998) examined the correlation between the FC/FS ratio and origination of the pollution source from an urban area or an agricultural area. Average FC/FS ratios were indicative of animal contamination; however, the presence of human contamination could not be excluded without additional specialized analysis. The researchers concluded that the data was highly variable and not appropriate to differentiate between urban or agriculturally impacted areas.

2.5 Summary of Literature

Since the 1970s, the U. S. EPA has been concerned with the nation's watersheds. This is evidenced by the Clean Water Act, which requires regulatory and non-regulatory means of action for point and nonpoint source pollution to surface waters. More recently, the Safe Drinking Water Act amendments require monitoring of the quality of the nation's waters and monitoring pollutant sources from adjacent land uses. Within a watershed, land uses include urban, agricultural, residential, and other land uses. These different land uses impact the quality of water from runoff of pavement and treated soils contributing to increased pollutant constituents. Watershed agencies monitor water quality and the

impacts of land uses on water quality by measuring several physical, chemical, and microbiological constituents. In this project, several of these water quality parameters were measured and results statistically analyzed to determine correlations between land use, seasonal changes, and water quality. In addition, enterococci was evaluated for use in determining the source of fecal contamination. The following chapter describes the methods used to meet this purpose.

Chapter 3

Methodology

In order to determine the physical, chemical and microbiological quality of source waters, specific methods must be utilized for analysis. This chapter first describes the experimental plan, which includes field sampling conducted over a 12-month period, field measurements and laboratory measurements. The chapter concludes with experimental procedures, which describe the methodologies used to perform the sampling and water quality analyses.

3.1 Experimental Design

The quality of surface waters, including chemical, physical and microbiological characteristics, is affected by several factors. These factors include contaminant inputs from point and nonpoint sources, climate, and characteristics of the watershed including stream size and flow. As discussed in Chapter 2, nonpoint source contamination is affected by land use, precipitation, and seasonal changes. The impact of nonpoint sources on a particular surface water body can be determined through laboratory analyses and field observations. A complete analysis of water quality includes observations and data from all four seasons as well as wet and dry weather sampling. In this thesis, several indicators of water quality were measured and analyzed for two subbasins in the Wachusett Reservoir watershed in Massachusetts. The land use around both of the surface waterbodies was also determined. Correlations between water quality parameters were developed. Lastly, the usefulness of various water quality parameters for contamination sourcing was determined.

Field and laboratory data were gathered from two subbasins in the Wachusett Reservoir watershed: Malagasco Brook subbasin and Beaman Pond subbasin. Historical records from DCR showed periodic elevated fecal coliform measurements in the tributaries of these subbasins. In the Malagasco Brook subbasin, located in West Boylston, elevated fecal coliform concentrations were observed at the confluence with the Wachusett Reservoir.

Numerous upstream land uses could have contributed to the elevated bacterial levels. The predominant land uses near Malagasco Brook are residential use and forest, as shown in Figure 3.1. Six sample sites were chosen along Malagasco Brook for water sampling. The first site was located at the intersection of the headwaters of the water body and a private street of a local plant nursery. The section that was sampled is owned by the DCR. The second sample site was located downstream of the nursery and upstream of a development of condominiums. The third and fourth sampling sites were located in the condominium development. The fifth sampling site was located downstream of the condominiums. The sixth sampling site was at the confluence with the Wachusett Reservoir. Details on the sampling sites are provided in Section 4.1.1.

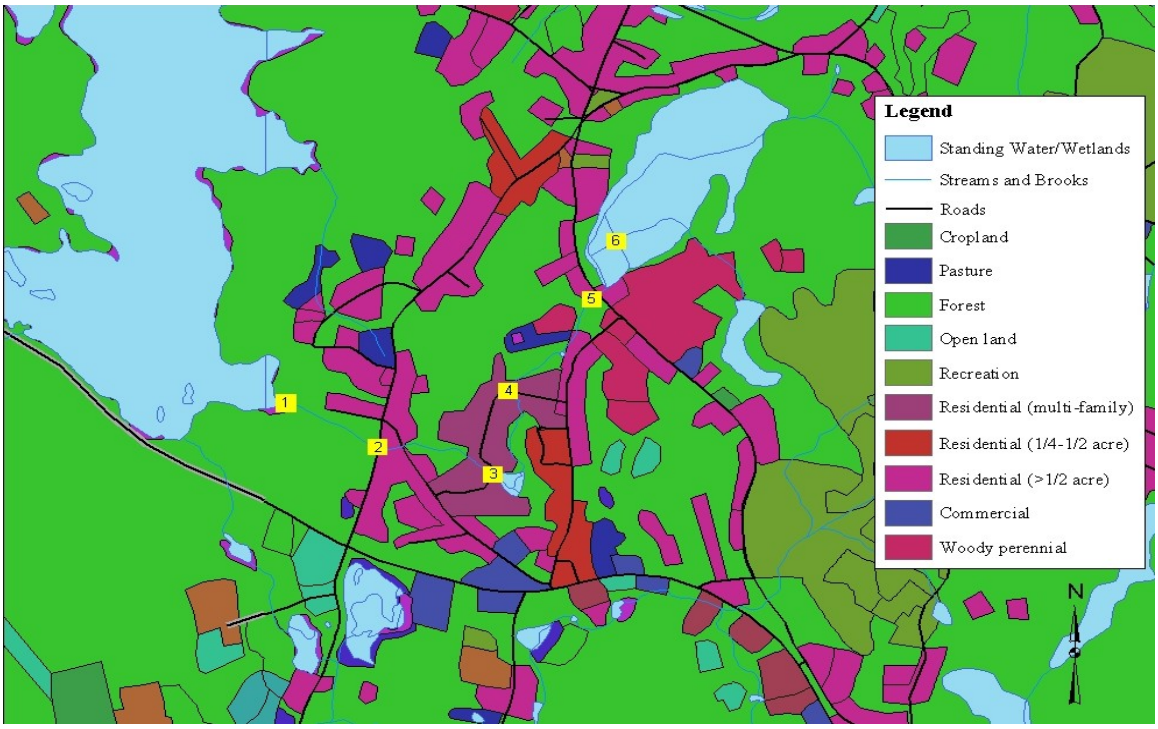

Figure 3.1. Malagasco Brook Sampling Sites

The Beaman Pond subbasin is located in Boylston, Massachusetts. Several possible sources of contamination were identified along the brook including domestic animals and septic systems. Land uses within the Beaman Pond Brook area are shown in Figure 3.2. Alongside the brook, the predominant use is residential housing, on ¼ to ½ acre lots.

Three sample sites were selected on the Lily Ponds along Route 110. The first site was located on DCR property upstream of a small horse farm. Several residences are located upstream of this site. The second sampling site was downstream of a small horse farm with 4 horses. The third sampling site was located downstream of a residential area served by septic systems. A full description of sampling sites is provided in Section 4.1.2.

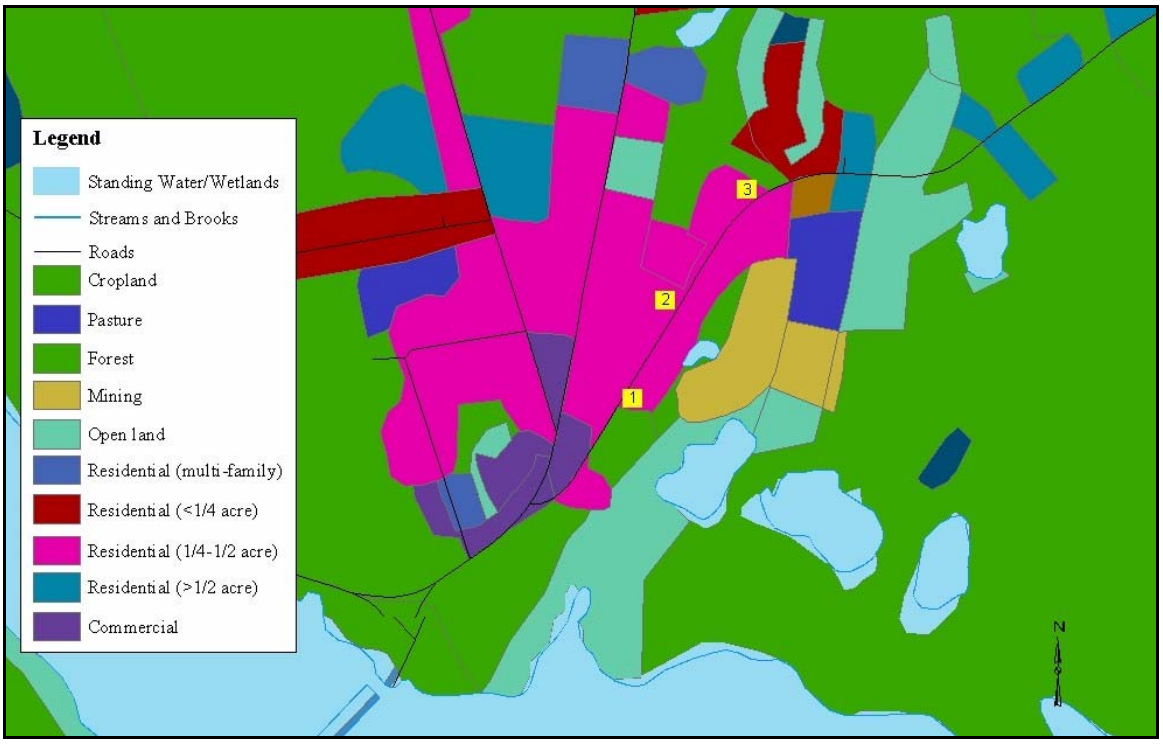

Figure 3.2. Beaman Pond Brook Sampling Sites

For both subbasins, sampling sites were selected to identify sources of contamination as best as possible. Selection of sample sites was made at upstream and downstream locations of certain land uses that can affect water quality, in order to isolate the problem areas. All sites were located at or near the intersection of a road and the water source, so the sites were more accessible.

All sites were sampled at least once during every season, with two samples taken in the fall. Seasons were defined astronomically where four divisions are made according to the equinoxes and solstices. Spring occurs between March 22 and June 21, summer from

June 22 until September 22, fall from September 23 to December 22, and winter from December 23 to March 21. Sampling during every season allowed for evaluation of seasonal changes in temperature, land use, and foliage in and around the water.

The samples were taken aseptically from the stream in a manner so as not to disturb the sediments in the streambed. This was important in order to ensure that the samples were a good representation of the water source. Samples were also taken first from the site that was most downstream and then sequentially up the stream. All samples were collected in autoclaved sampling bottles and labeled with the appropriate sample site number. In order to keep the sample uncontaminated by external elements other than the sample water, the lid was kept closed until the sample was taken and immediately closed again. The sampler's hands were also sprayed with a 50% alcohol solution before handing the bottles.

Conductivity, dissolved oxygen, and temperature were measured at each sample site using field meters. The meters were standardized on the day of use. The probes were placed downstream of where the water sample was collected so as not to influence the sample quality. Conductivity was measured in μS according to Standard Method 2510 B (APHA *et al*., 1998). Temperature was measured in 0.1°C increments using Standard Method 2550 B (APHA *et al*., 1998). Both conductivity and temperature were measured with an YSI 30 Salinity-Conductivity-Temperature field meter (YSI, Yellow Springs, OH). Dissolved oxygen was measured by the Membrane Electrode Method, Standard Method 4500-O G (APHA *et al*., 1998), using an YSI 95 DO field meter (YSI, Yellow Springs, OH). The probes were placed in the water until a stable reading was achieved.

The samples were first taken back to the laboratory at the DCR office in West Boylston, MA, where each sample was split aseptically into two water samples. One sample was transported to the University of Massachusetts, Amherst and the second to Worcester Polytechnic Institute (WPI). This study was performed in conjunction with the University of Massachusetts (UMass) as a larger study of microbial source tracking. The WPI samples were taken back to the Environmental Engineering Laboratory at the WPI campus. Each sample was split into two sterile sample bottles in a laminar flow hood.

The first set of bottles was kept in a refrigerator or cooler and used for microbiological analyses. The second set of bottles was allowed to warm to room temperature and was used for physical and chemical analyses. The specific laboratory methods are documented in Section 3.2.

3.2 Experimental Procedures

This section provides the analytic methods performed in the laboratory. All analyses were performed in accordance with Standard Methods and were conducted within allowable holding times according to Standard Methods (APHA *et al*., 1998). All physical and chemical water quality parameters were measured in duplicate and average results are reported in Chapter 4. For microbiological measurements, a minimum of four dilutions and/or volumes were plated, with three replicates per dilution. Again, average results (for counts in the appropriate ranges as specified by Standard Methods) are reported. Positive and negative controls were also completed.

3.2.1 Turbidity

Turbidity was measured in accordance with Standard Method 2130B (APHA *et al*., 1998) with a Hach Turbidimeter 2100N (Hach Company, Loveland, CO). The turbidimeter was calibrated every four months using Stabl Cal Calibration standards of less than 0.1, 20, 200, 1000 and 4000 ntu (Hach Calibration Standards Catalog Number 26621-05). To measure the turbidity of the water samples collected from the tributaries, the samples were allowed to warm to room temperature. Each sample was gently inverted three times before it was transferred into a cleaned and oiled turbidity sample cell. The turbidity cell was placed in the turbidimeter, and an average reading was taken from the first 30 seconds that the sample cell was placed in the turbidimeter. The measurement was recorded in units of ntu.

3.2.2 Particle Counts

The concentration of particles in the sample water was determined in accordance with Standard Method 2560C (APHA *et al*., 1998) using a light blockage particle counter in the laboratory (PC 2400 PS, Chemtrac Systems Inc., Norcross, GA). The particle counter can measure up to 16,000 particles per mL in sizes ranging from 2 to 400 μm. The data

are categorized into a maximum of 16 size ranges, as specified by the user. The software (Grabbit 3.11) was set up for each sample site using the values shown in Table 3.1. Each sample was analyzed in duplicate using 50 mL per run and categorized into 15 size ranges. The size ranges were focused on the smaller sizes (<10 μm) as it was anticipated that counts would be highest in these ranges.

Table 3.1. Grabbit 3.11 Software Values for Particle Counts.

Sample Volume (mL)	50
Sample Number	2
Purge Volume (mL)	25
Size Channels (μm)	2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9, 9-10, 10-20, 20-30, 30-40, 40-50, 50-75, 75-100, 100 and greater

The file created in Grabbit 3.11 was downloaded from the computer to the particle counter. The particle counter was set to grab sample mode and the flow rate calibrated to 100 mL/min using a graduated cylinder and stopwatch. The cleaned intake tube was placed in a beaker containing approximately 150 mL of the sample and the output tube was put in a waste bucket for disposal. Each sample was then analyzed based on the software settings. An analysis of E-pure water was performed as a baseline indicator. E-pure samples were also run in between each water sample to ensure that carry over of the samples did not occur. After all samples were processed, the information was then uploaded back to the computer and compiled into a spreadsheet.

3.2.3 Ultraviolet Absorption

Ultraviolet absorption at 254 nanometers, also known as UV_{254}, was analyzed according to Standard Method 5910 B (APHA *et al*., 1998). UV_{254} is a surrogate measure for the concentration of organic matter in the sample, as many organic compounds absorb UV light. The samples were filtered as follows. First, glass microfiber filters (Whatman GF/F, 0.7 μm retention) were pre-washed with 30 mL of E-pure water. Then the room temperature sample was passed through the filter. The first 2 - 5 mL of the sample was wasted and approximately 20 mL of sample water was filtered and retained for analysis. The spectrophotometer (UV-Visible Spectrophotometer CARY 50, Varian, Mulgrave,

Victoria, Australia) was set to a wavelength of 254 nm. Approximately 4 mL of the filtered sample was put into a quartz glass spectrophotometer cell (10 mm, Varian, part # 6610001100) and then placed in the spectrophotometer for analysis. A duplicate measurement was analyzed for each water sample.

3.2.4 pH

A measurement of pH by the electrometric method (Standard Method 4500-H^+ B, APHA *et al*., 1998) is an analysis of hydrogen ion activity in a sample. A pH meter (AB15 pH meter, Fisher Scientific, Hampton, NH) was calibrated with 4.00, 7.00, and 10.01 buffer solutions. Approximately 20 mL of the sample water was poured into a small glass beaker. The pH and temperature probes were suspended in the liquid until the pH meter indicated a stable reading was achieved.

3.2.5 Total and Dissolved Organic Carbon

Water samples were analyzed for total organic carbon (TOC) and dissolved organic carbon (DOC) according to Standard Method 5310B (APHA *et al*., 1998). All glassware was washed with soap and water and then acid washed in 20% sulfuric acid before use. The glassware was then dried in a 50°C oven and wrapped with aluminum foil until use. For DOC samples, glass microfiber filters (Whatman GF/F, 0.7 μm retention) were first pre-washed with 30 mL of E-pure water. Then the water sample was passed through the filter. The first 5 - 10 mL of sample was wasted and approximately 40 mL was then filtered into a 40 mL acid washed vial. For TOC analysis, samples were poured into 40 mL acid washed vials without filtration. For both TOC and DOC analyses, the samples were then acidified with 40 μL of 6 N HCl and the vials were capped with open top screw caps with TFE lined septa. All samples were retained at 4°C up to two weeks before analysis. Analysis was completed on a TOC analyzer using potassium hydrogen phthalate standards as described in the following sections.

3.2.5.1 Primary and Intermediate Stock Standard

In order to produce the primary stock standard, approximately 0.75 g of potassium hydrogen phthalate (KHP) was dried in an oven between 103 - 110°C for 30 minutes and

cooled in a desiccator for 30 minutes. Exactly 0.5314 g of the KHP was weighed and dissolved in E-pure to a total volume of 250 mL in a volumetric flask. This primary stock, with a concentration of 1,000 mg/L TOC, was stored in an amber acid-washed bottle at 4°C and used within 4 weeks. The primary stock standard was used to make the intermediate standard. The intermediate standard of 100 mg/L was produced by diluting 10 mL of the primary stock up to 100 mL with E-pure. This standard was made on the day the TOC and DOC samples were analyzed, stored at 4°C, and discarded after two days.

3.2.5.2 Working Standards

An estimate of the concentrations needed for the working standards was determined by the UV_{254} value. The standards were made in acid washed 100 mL volumetric flasks. Each flask received 100 μL of 6 N HCl. The volume of the intermediate standard to be added was determined by the concentration desired. For a 10 mg/L standard, 10 mL of the intermediate standard was added and then diluted up to 100 mL with E-pure. A standard of 0 mg/L was made directly in the TOC sample vials by adding 40 μL of 6 N HCl to the vial and filling it with E-pure water. Two sets of calibration curves made of three standards each were prepared in order to produce a curve with the best fit to the samples analyzed.

3.2.5.3 Analysis

The standards and samples were analyzed on a TOC analyzer (TOC-5000A, Shimadzu, Kyoto, Japan), connected to an autosampler (ASI-V, Shimadzu, Kyoto, Japan). The gas (ultra zero grade air) and furnace were turned on. While the furnace warmed, all samples and standards were placed in Shimadzu autosampler vials for analysis. Each vial was capped with parafilm and a plastic Shimadzu cap. The standards were placed around the inner circle of the auto sampler tray labeled S1-S8 and the samples were placed around the outer ring, which was numbered from 1-16. The instrument was inspected before use to ensure proper operation. This inspection included checking the rinse water bottle, humidifier, dehumidifier drain container, IC reagent container, carrier gas pressure gauge, carrier and sparge gas flow meters, and microliter syringe.

The analyzer software was programmed to measure the standards, create calibration curves and determine the concentration of the samples in mg/L. All standards and samples were sparged for five minutes before analysis to remove any carbon dioxide. Then, each standard and sample was analyzed three to five times. After three measurements, the standard deviation and coefficient of variation are calculated. If the values are within acceptable limits (200 for standard deviation and 2.0% for coefficient of variation), then the sample analysis is complete. If not, a fourth or fifth measurement is taken and the best three measurements selected by the instrument. Two calibration curves were produced and the instrument selected the best curve for determining the concentration of each sample.

3.2.6 Microbiological Analysis

Two types of microbial analyses were performed to determine water quality of the source waters: the enumeration of fecal coliforms and enterococci. Samples were analyzed maintaining aseptic techniques as described in the following sections.

3.2.6.1 Aseptic Technique

All microbiological supplies and equipment were sterilized prior to use or were purchased presterilized. Serological pipettes and Petri dishes were purchased presterilized. Other glassware, plasticware, and metalware used for microbiological testing were washed with warm soapy water, rinsed with tap water, and rinsed with E-pure. Material was wrapped or capped with aluminum foil or capped with autoclaveable screw caps as appropriate and then autoclaved at 121°C for between 15 - 45 minutes, depending on contents and volume of the contents.

Aseptic conditions were maintained during analyses by several means. All laboratory benches and surfaces used during microbiological analyses were sprayed with a 50% alcohol solution. The hands of the people performing the experiments were also sprayed with the 50% alcohol solution. Items such as tweezers were dipped in 95% alcohol and passed through a flame prior to each use. Bottle caps and necks were flamed between each use. Many steps of microbiological experiments were performed in a laminar flow

hood where filtered air flows toward the person performing the experiment so organisms from the person or air cannot contaminate the sample.

3.2.6.2 Fecal Coliforms

Fecal coliforms were enumerated using the membrane filtration technique, Standard Method 9222D (APHA *et al*., 1998). A sterile 0.45 μm membrane filter (Millipore, 47 mm gridded sterile membrane, Billerica, MA) was placed on a filter funnel. If the sample volume to be filtered was less than 10 mL, at least 10 mL of buffered water was added to the filter tower. The appropriate volume of the sample was then added and the vacuum turned on. After the sample was passed through the filter, the tower was rinsed with 20 to 30 mL of buffered water, and vacuum maintained until all liquid had passed. Using flamed tweezers, the filter was transferred to a 50 mm Petri dish with m-FC agar. Each filter tower was rinsed with 20 to 30 mL of buffered water between each sample.

For each sample site, four volumes of water were filtered with three replicates of each volume taken, along with pre-negative and post-negative plates. Pre-negative and post-negative plates consisted of 10 mL of filtered buffered water processed before water samples were filtered and after all samples were finished, respectively. These plates were incubated to insure that no contaminants were on the filter towers or in the buffered water before filtering samples and that all contaminants were rinsed from the tower after the samples were filtered. The ability of the media to grow colonies (positive controls) was also checked by filtering dilutions of *E. coli* and incubating to observe growth. All Petri dishes were incubated upside down in a water bath (Coliform Incubator Bath, Precision, Winchester, MA) for 24 ± 2 hours at 44.5 ± 0.2°C. All samples were analyzed by counting the blue colonies under 10 – 15 times magnification. Background counts were also taken which included any non-fecal coliform colonies, observed by their gray to cream color.

3.2.6.3 Enterococci

Enterococci colonies were counted as a possible indicator of the source of contamination. This test was performed by membrane filtration, Standard Method 9230C (APHA *et al*., 1998). This method is similar to fecal coliform membrane filtration, except that peptone

is used instead of buffered water and the filters are initially placed on ME agar. Four different volumes of each sample were filtered with three replicates of each volume. Petri dishes were incubated for 48 hours at 41 ± 0.5°C. After 48 hours, the filters were transferred to Esculin Iron agar plates and retained at room temperature for 20 – 30 minutes. The plates were incubated for another 20 minutes at 41 ± 0.5°C. The samples were analyzed by counting the pinkish red colonies that had developed a black precipitate on the underside of the filter. This was observed by using a fluorescent lamp and a magnifying glass.

3.2.6.4 Positive Control

A positive control of *E. coli* growth on media was analyzed in order to confirm growth on the m-FC agar. This test was performed by membrane filtration, Standard Method 9222D (APHA *et al*., 1998). Three dilutions of *E. coli* in the tryptic soy broth were filtered through a sterile filter tower. The filtered volumes were 1 mL of 10^{-8} dilution, 0.1 mL of 10^{-6} dilution, and 1 mL of 10^{-6} dilution. The Petri dishes were incubated at 44.5 ± 0.2°C for 24 ± 2 hours. The plates were enumerated by counting the blue colonies that had grown during the incubation period.

3.3 Reagents

This section describes in detail the methods used to make the reagents used in this research.

3.3.1 Reagent Grade Water

Reagent grade water (E-pure) was used for all laboratory measurements (E-pure deionizer, Barnstead/Thermolyne, Dubuque, IA). Water treated by the E-pure system is feed from an ROpure ST system (Series 631, Barnstead/Thermolyne, Dubuque, IA). The ROpure ST is a reverse osmosis treatment system where salts and synthetic organic compounds are removed by a membrane. Two cartridges are used in this system, cellulose acetate tri-acetate membrane (Catalog Number D6317) and a thin film composite membrane cartridge (Catalog Number D6318). E-pure is deionized water, where positively charged ions (cations) and negatively charged ions (anions) are

exchanged for hydrogen (H^+) and hydroxyl (OH^-) ions. This process removes impurities such as calcium and sodium. A series of four cartridges are utilized to produce E-pure water, a macropure filter (Catalog Number D0836), high capacity two-bed filter (Catalog Number D0803), ultrapure mixed bed filter (Catalog Number D5027), and an organic free filter (Catalog Number D5021). The water also passes through a 0.2 μm filter. E-pure is made on site in the WPI laboratory.

3.3.2 Agars

Membrane filtration for fecal coliforms calls for the filter to be incubated on m-FC agar. Since this agar is not autoclaved, all glassware used to make the agar was autoclaved before use. Exactly 52 g of m-FC powder was suspended in 1 L of E-pure in an Erlenmeyer flask and boiled for one minute. A 1% rosalic acid solution of 0.1 g of stock rosalic acid dissolved in 10 mL of 0.2 N NaOH solution was added to the agar and boiled for an additional minute. The agar was cooled to 47°C in a water bath (Isotemp 110, Fisher Scientific, Pittsburgh, PA). The agar was dispensed in 50 x 9 mm petri dishes (5 to 6 mL per dish) and allowed to cool. The dishes were stored in sealed plastic bags upside down in a 4°C refrigerator. The pH was checked using pH paper to verify it was approximately 7.4.

Enterococci membrane filtration requires that the filter be incubated on mE agar. A solution of 71.2 g mE powder suspended in 1 L of E-pure water was boiled until it dissolved. The agar was autoclaved in a media bottle at 121°C and then cooled to 47°C in a water bath. Two chemicals where then added to the agar. The first was nalidixic acid, for which 0.25 g was dissolved in 5 mL of E-pure water and several drops of 0.2 N NaOH were added to the mixture to help dissolve the nalidixic acid. The second chemical added was 0.15 g of 2,3,5 – triphenyl tetrazolium chloride. The agar was dispensed into petri dishes (5 to 6 mL per 50 x 9 mm petri dish), cooled, and stored upside down in a sealed bag at 4°C. Before use, the plates were checked for a pH of approximately 7.1.

After a 48-hour incubation period, the enterococci filters were transferred to Esculin Iron agar (EIA). Exactly 16.5 g of EIA powder was suspended in 1 L of E-pure water. The

mixture was boiled and autoclaved in a media bottle. The agar was cooled in a water bath to 47°C and approximately 5 mL was dispensed into petri dishes. The cooled dishes were stored upside down in a sealed bag at 4°C. The pH was checked to be 7.1 before use.

3.3.3 Dilution Waters

Buffered water is used in the fecal coliform membrane filtration method. Buffered water is a solution that neither prohibits nor enhances growth of the organisms. A stock of magnesium chloride was made by dissolving 20.275 g of $MgCl_2 \cdot 6H_2O$ to a total volume of 250 mL of E-pure. A stock phosphate buffer was made by suspending 8.5 g of KH_2PO_4 up to 125 mL of E-pure. The buffered water was produced by diluting 1.25 mL of the stock phosphate buffer and 5 mL of the stock magnesium chloride up to 1 L of E-pure. The solution was autoclaved at 121°C in both squeeze bottles and media bottles.

Peptone is a similar solution to buffered water in that the water neither prohibits nor enhances growth. Peptone was used for enterococci membrane filtration. In order to make an 0.1% peptone solution, 1 g of the dry powder was dissolved in E-pure water to a total volume of 1 L. The solution was mixed and then autoclaved in squeeze bottles and media bottles and stored at 4°C until use.

3.3.4 Tryptic(ase) Soy Broth

In order to test the ability of the m-FC media to grow fecal coliforms, *E. coli* was filtered and incubated using the same method as used for samples. *E. coli* was grown in tryptic soy broth (TSB) overnight for laboratory use (Standard Methods, 9211 D, APHA *et al*., 1998). Thirty grams of TSB powder was dissolved in 1 L of E-pure water in a beaker. The solution was warmed on a hot plate and gently mixed until the powder was completely dissolved. Fifty mL of the solution was poured into capped shaker flasks and autoclaved for 15 minutes at 121°C. The TSB flasks were retained at 4°C and warmed to 35°C before use.

3.3.4.1 Tryptic Soy Broth Inoculation

Using aseptic techniques, the TSB flasks were inoculated with *E. coli*. A portion of the frozen stock *E. coli* was transferred using a sterilized metal transfer ring. The inoculated flasks were incubated overnight at 35°C on a shaker table at 100 rpm.

3.4 Statistical Methods

Two statistical methods were utilized for analyzing data collected from the sampling sites. Correlation analyses were performed on the individual water quality parameters to identify relationships within each subbasin. ANOVA analyses were completed to determine differences between different sites within each of the subbasins as wells as between seasons.

3.4.1 Correlation Analysis

Pearson's method of correlation analyses is a statistical test to determine the linear association between two pairs of the data. The analysis is not dependant on the units of the data, meaning the data must be standardized before running the analysis. The data pairs can be standardized using equation 3.1 and 3.2.

$$X_i' = (X_i - \overline{X})/S_X \qquad \text{(Equation 3.1)}$$

$$Y_i' = (Y_i - \overline{Y})/S_Y \qquad \text{(Equation 3.2)}$$

The correlation coefficient, R, is a value of the linear relationship between the data pairs. Correlation coefficient values range from –1.00 to +1.00, where the negative sign indicates a negative correlation and zero indicates no correlation. The coefficient of determination is the magnitude of the relationship between two variables (Statsoft, 2003). The statistical significance of the analysis is determined using a correlation coefficient table (see Appendix C). This table takes two variables into consideration when determining that the correlation coefficient was not calculated based on pure chance. The two variables are the desired confidence level and number of data pairs. The P-value is a measure how reliable the data is. The P-value commonly used on research is 0.05, which

is borderline significant. A “statistically” significant correlation would have a P-value of ≤ 0.01 and a highly significant correlation would be ≤ 0.005.

Using the data analysis tool pack in Microsoft Excel, correlation analyses were performed on the data from the two subbasins. The correlation analysis was chosen from the Data Analysis Tools as seen in Figure 3.3. The data was input in a worksheet where the data for each of the water quality constituents was arranged in columns, as shown in Figure 3.4.

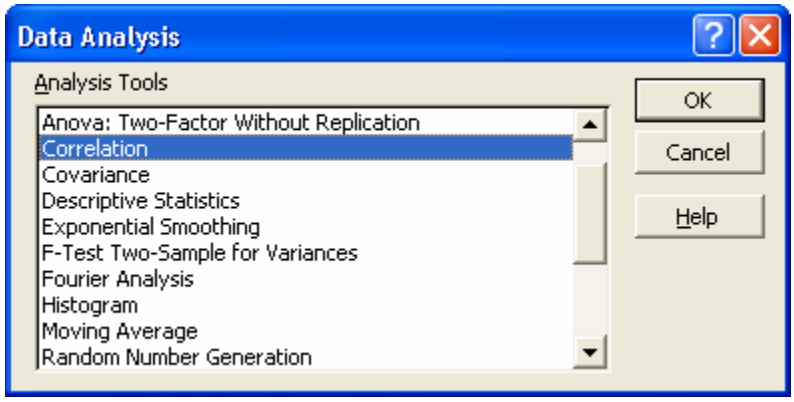

Figure 3.3. Statistical analyses available on Microsoft data analysis tool pack.

The correlation analysis output was directed to another sheet where a table was generated giving the correlation coefficients for the pairs of constituents measured. As shown in Figure 3.5, only one half of the table is filled because the correlation corresponding to the temperature from the first column and the DO from the second row is the same as the temperature from the first row and the DO from the second column. The correlation between the same two constituents is always 1. The correlation coefficients from Excel were compared to R-values in the correlation coefficient table to determine if the relationships were significant at the 95% confidence level.

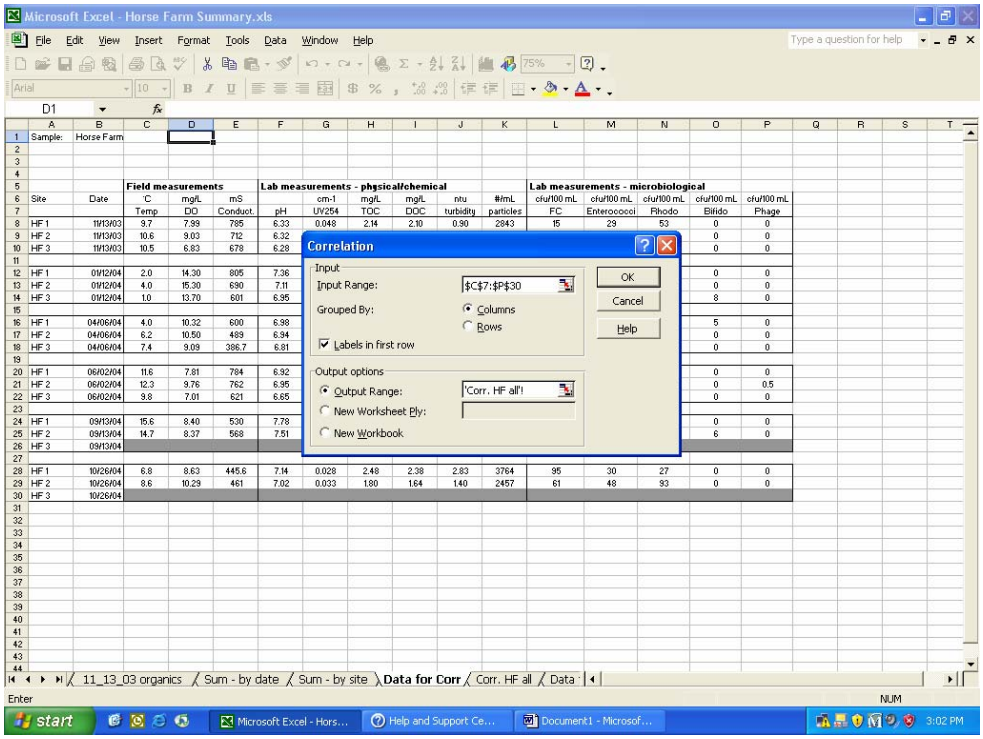

Figure 3.4. Input for correlation analysis.

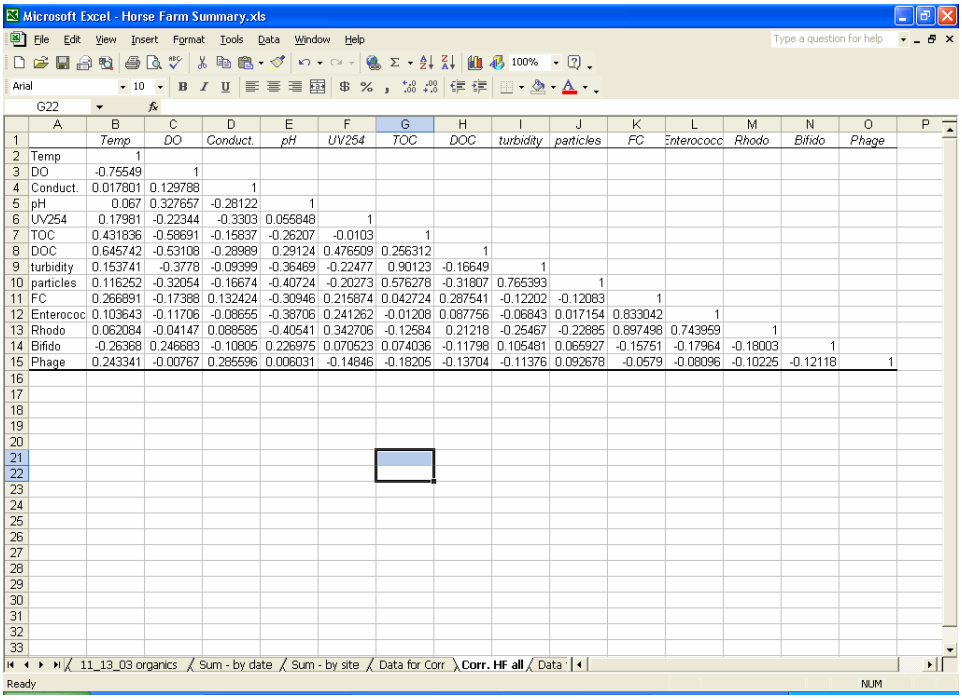

Figure 3.5. Table output from correlation analysis.

3.4.2 ANOVA Analysis

The analysis of variance (ANOVA), also known as the F-test, is a method to determine the variation of the means of a group of data or variables to evaluate statistical significance. This method, when comparing two means, is similar to the t-test for independent samples. The single factor ANOVA test assumes a null hypothesis, H_0, which states there is no difference between the groups within the population, as shown in Equation 3.3.

$$H_0 : \beta_1 = \beta_2 = \cdots = \beta_q = 0 \qquad \text{(Equation 3.3)}$$

If the analysis is found to be statistically significant, then the null hypothesis is rejected for the alternative hypothesis. The alternative hypothesis states that the means of the groups in the population are different. For this research, a P-value of ≤ 0.05 was used to determine statistical significance.

Microsoft Excel's data analysis tool pack was used to conduct the ANOVA analyses. The single factor test was chosen for analysis, as shown in Figure 3.6. The data was arranged in two ways for analyses. The first was by sampling site and the second by season. The configuration for testing differences between sampling sites is shown in Figure 3.7.

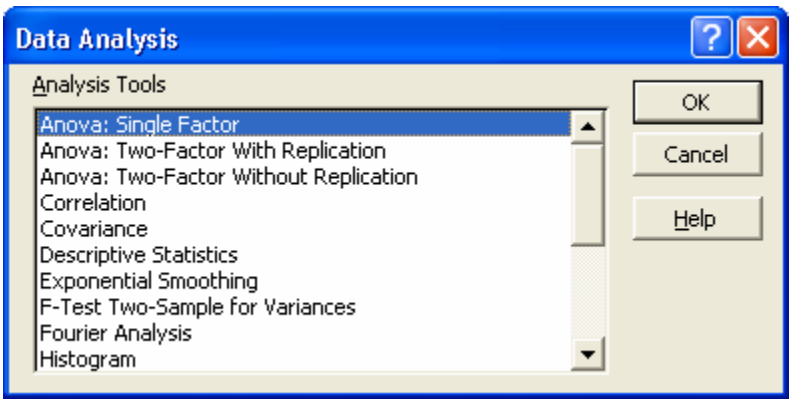

Figure 3.6. Statistical analysis on Microsoft Excel used for ANOVA method.

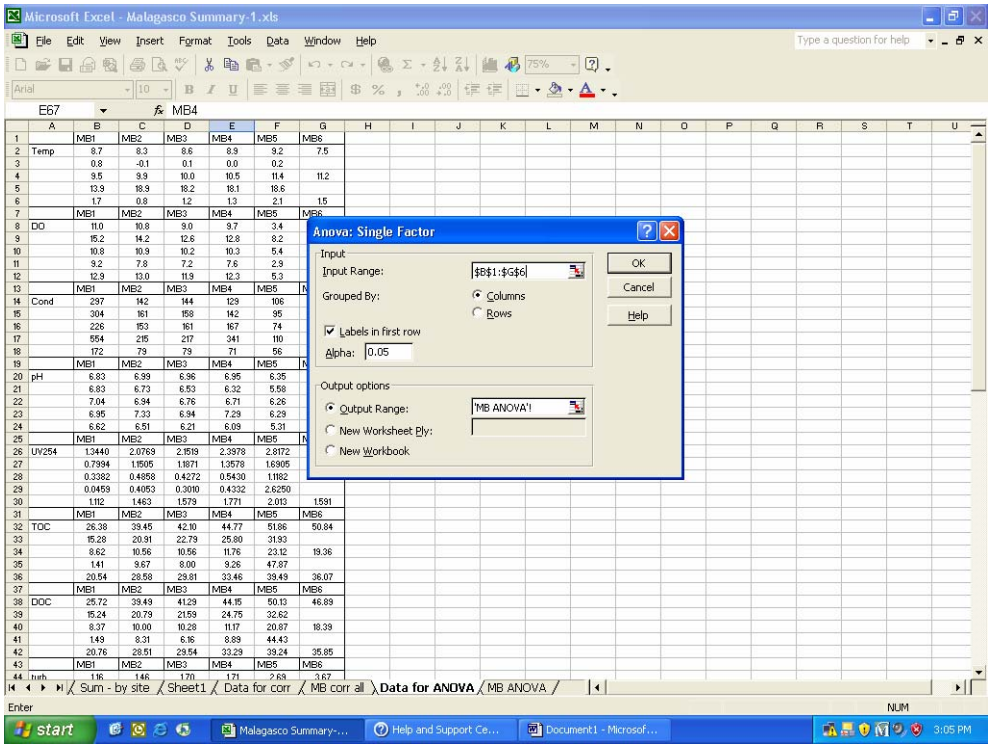

Figure 3.7. Input data for ANOVA analysis based on each Malagasco sampling site.

The output from the analysis breaks down the sum, mean, and variance from each sampling group. For example, Figure 3.8 shows output for the temperatures for each of the six sampling sites from Malagasco Brook (see "Summary" table in Figure 3.6). The second table ("ANOVA" table) gives statistical values between groups and within groups, including the sums of squares (SS), the degrees of freedom (df), the mean squares (MS), the variable under questioning (F), probability (P-value), and the critical value of F (F-critical). These values were computed by using the equations shown in Table 3.2. All data analysis outputs for the ANOVA testing are found in Appendix D.

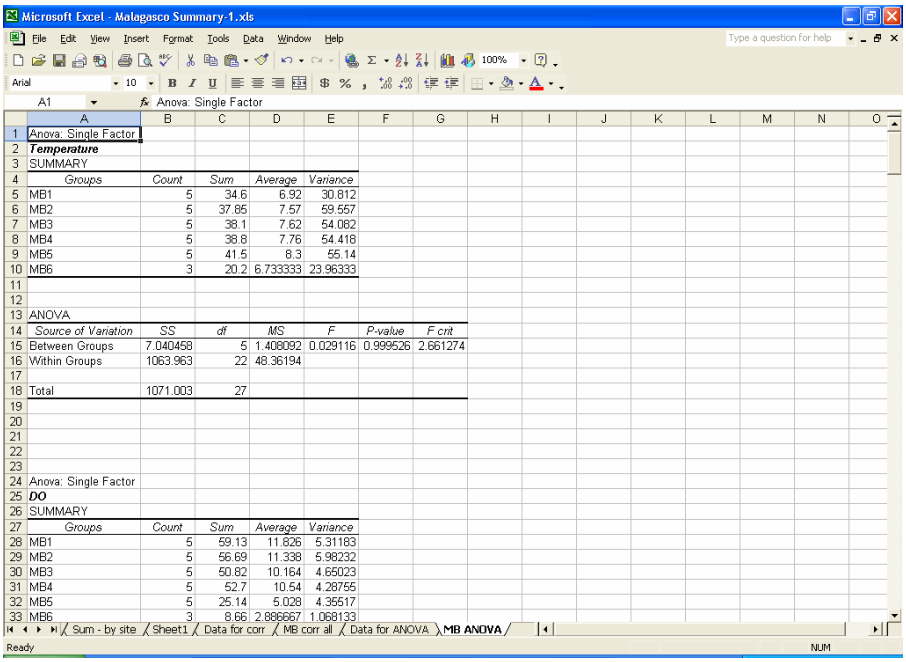

Figure 3.6. Output from ANOVA analysis.

Table 3.2. ANOVA Equations.

Name	Equation
Total sums of squares	$SS_T = \sum x^2 - \frac{\left(\sum x_T\right)^2}{N}$
Sums of squares between groups	$SS_b = \sum \frac{\left(\sum x\right)^2}{n} - \frac{\left(\sum x_T\right)^2}{N}$
Sums of squares within groups	$SS_W = SS_T - SS_b$
Degrees of freedom between groups	$df_b = (\text{number of groups} - 1)$
Total degrees of freedom	$df_T = (\text{number of groups} - 1)$
Degrees of freedom within groups	$df_W = df_T - df_b$
Mean squares between groups	$MS_b = \frac{SS_b}{df_b}$
Mean squares within groups	$MS_W = \frac{SS_W}{df_W}$
Critical value of F	$F = \frac{MS_b}{MS_W}$

Chapter 4

Results

An analysis of all data collected over a 12-month period was performed in order to draw conclusions about physical, chemical, and microbiological water quality in two subbasins of the Wachusett Reservoir watershed in Massachusetts. This chapter first describes the site locations where water samples were collected. Then, the water quality data is analyzed by site and by season, and data collected at the University of Massachusetts, Amherst is summarized. Lastly, is a discussion of the statistical correlation and ANOVA analyses performed on the data. Data for all sampling events is provided in Appendix A.

4.1 Sampling Site Descriptions

All sampling sites analyzed in this thesis were located within the Wachusett Reservoir watershed. This watershed is located in central Massachusetts and is comprised of 71,000 acres. This research focused on two subbasins: the Malagasco Brook subbasin and the Beaman Pond subbasin. Site locus maps are provided in Appendix B. These subbasins were chosen based on water quality concerns and based on the diversity of land uses within the subbasins, which can affect receiving water quality.

4.1.1 Malagasco Brook Subbasin

The Malagasco Brook subbasin is located in the south end of the town of Boylston and just south of the Wachusett Reservoir. Six sites were chosen for sampling within the subbasin. Each site is referred to by the abbreviation MB for Malagasco Brook and a number referring to the order in which the samples were collected. Sample MB1 is the most downstream site and MB6 the most upstream.

The most upstream sampling site (MB6) was located at the headwaters of the stream, and upstream of a nursery. The nursery is shown in Figure 4.1. This site is located within Pine Swamp, which is owned by the Department of Conservation and Recreation (DCR), near Boylston Center. The water at the head of the stream seeps up from the ground with no

apparent flow path. Organic material was found in and around the stream from the heavily wooded area surrounding the site.

The next site, MB5, is located downstream of the nursery approximately 0.14 miles from MB6. A large plot of land adjacent to the sampling site is used for growing trees for the nursery. This site is at the intersection of Malagasco Brook and School Street. The sample was collected downstream of the street on private property. Three single-family homes are adjacent to the site.

Figure 4.1. Nursery near sites MB6 and MB5.

The following sampling site, MB4, is located within a condominium development along Edgebrook Drive. This sampling site is approximately 0.31 miles from the MB5. The development, which is named Timberbrook Condominiums, has a range of six to 12 residences in each of the 21 buildings. Figure 4.2 is a photograph of one of the condominium units near MB4. The brook is located approximately 50 feet back from the street and down a small hill. Two condominium units, each with six residences, are located on the right side of the sampling site. Three condominium units are adjacent to the left of the sampling site.

MB3 is also located within the condominium complex on Edgebrook Drive, 0.30 miles from the last site. The site is located approximately 25 feet from the curb. Two condominium units are located on each side of the sampling site. The units each have six residences along with garages. At this site, the stream is diverted through a concrete

structure and flows over a weir (see Figure 4.3). This structure allows the water to pool. The sample was collected from the pool upstream of the weir.

Figure 4.2. Condominium complex near site MB4.

Figure 4.3. Sample site MB3 with weir.

The following sampling site, MB2, is located downstream of the condominiums along East Temple Street approximately 0.22 miles from MB3. The brook flows adjacent to the road, approximately 20 feet below the grade of the street. Five single-family houses are located adjacent to the sampling site. The land around the brook is heavily wooded with a steeply graded slope.

The last site, MB1, is located at the confluence with the Wachusett Reservoir and approximately 0.26 miles from MB2. The site is located at the end of East Temple Street past a protected access street for the reservoir. One single-family house is located at the end of East Temple Street. The stream is piped under the access street and flows through

a gauging station to the reservoir. The gauging station is shown in Figure 4.4. The land adjacent to the reservoir is heavily wooded.

Figure 4.4. Gauging station at site MB1 at the confluence of the Wachusett Reservoir.

4.1.2 Beaman Pond Subbasin

The Beaman Pond subbasin is located on the north side of the Wachusett Reservoir. Three samples were taken from a stream along Lancaster Street, which is MA Route 110. The most upstream sample, HF3, was taken on DCR property, upstream of a small horse farm (see Figure 4.5). The sampling location is approximately 50 feet behind DCR's Field Maintenance Headquarters in a slightly swampy area. The sample was collected next to a chain-linked fence, which separated the DCR property and the horse farm. The site typically had a low flow which made sampling hard or impossible at certain times.

The second site, HF2, is located just downstream of a horse farm approximately 0.06 miles from HF3 (see Figure 4.6). This horse farm housed four horses until they were removed on July 7, 2004. One horse was spotted on the property on September 23, 2004. This site location was typically overgrown during warmer seasons and the owners of the property maintained a pile of refuse leaves and animal waste near the embankment. The owners were asked in 2004 by DCR to remove the horses or install best management practices in order to protect the water stream. The septic system for the house was installed in 1973 and consisted of a tank, distribution system, and soil absorption system.

Figure 4.5. Site HF3 upstream of the horse farm.

Figure 4.6. Photographs near site HF2:
Left: View of the horse barn and field, surrounded by a fence. Right: Stock pile of refuse material adjacent to stream.

Figure 4.7 shows the last sampling site, HF1, which was located downstream of the horse farm site approximately 0.26 miles. The stream is located approximately 100 feet behind a house located on Lancaster Street. A septic system was installed in 1959 and consisted of two cesspools and an overflow cesspool. The sample was taken just downstream of a small footbridge within a lightly wooded area. The flow was typically low so the deepest location across the width of the stream was located for collecting samples.

Figure 4.7. Site HF1 during the spring.

4.2 Data Analysis

Analysis of the water quality data was performed by site and by season. For analyses performed by site, trends in data at different sites or changes from the most upstream sampling location to the most downstream site were determined. Analysis of the data by season was completed by grouping sampling dates into four seasons based on the astronomical calendar. Spring included dates from March 22 to June 21, summer from June 22 until September 22, fall from September 23 to December 22, and winter from December 23 to March 21. One exception was made for the seasonal analysis. Data from the samples taken on December 9, 2003 from Malagasco Brook were considered to be winter samples. This was done since there were no samples taken within the appropriate astronomical calendar and because the measurements made were more consistent with winter characteristics than fall ones. The samples taken at Horse Farm on January 12, 2004 had an average temperature of 2.3 °C and the Malagasco Brook samples taken on December 9, 2003 had and average temperature of 0.2 °C. The measurements of dissolved oxygen were also more consistent with winter measurements where the average of the December 9, 2003 Malagasco Brook samples was 12.6 mg/L and the winter Horse Farm average of samples was 14.4 mg/L. In contrast, fall samples at Malagasco Brook averaged 7.7 mg/L and 9.6 mg/L dissolved oxygen for sampling dates October 21, 2003 and November 10, 2004, respectively.

4.2.1 Site Analysis – Malagasco Brook

The following section describes the data observed for the Malagasco Brook subbasin based on variations in the measurements by site.

4.2.1.1 Organic Carbon

Data on organic carbon concentrations included total organic carbon, dissolved organic carbon, and UV_{254} measurements. At the Malagasco Brook sampling sites, organic carbon concentrations generally showed a decreasing trend from upstream locations to downstream sites from site MB5 to MB1. TOC, DOC, and UV_{254} levels at site MB6 (the most upstream location) were similar to, but slightly less than levels at MB5. For example, in the fall of 2003, TOC was 50.8 mg/L at MB6, increased slightly to 51.9 mg/L at MB5, and then decreased progressively downstream to a low of 26.4 mg/L at MB1 (see Figure 4.8). DOC and UV_{254} followed similar trends.

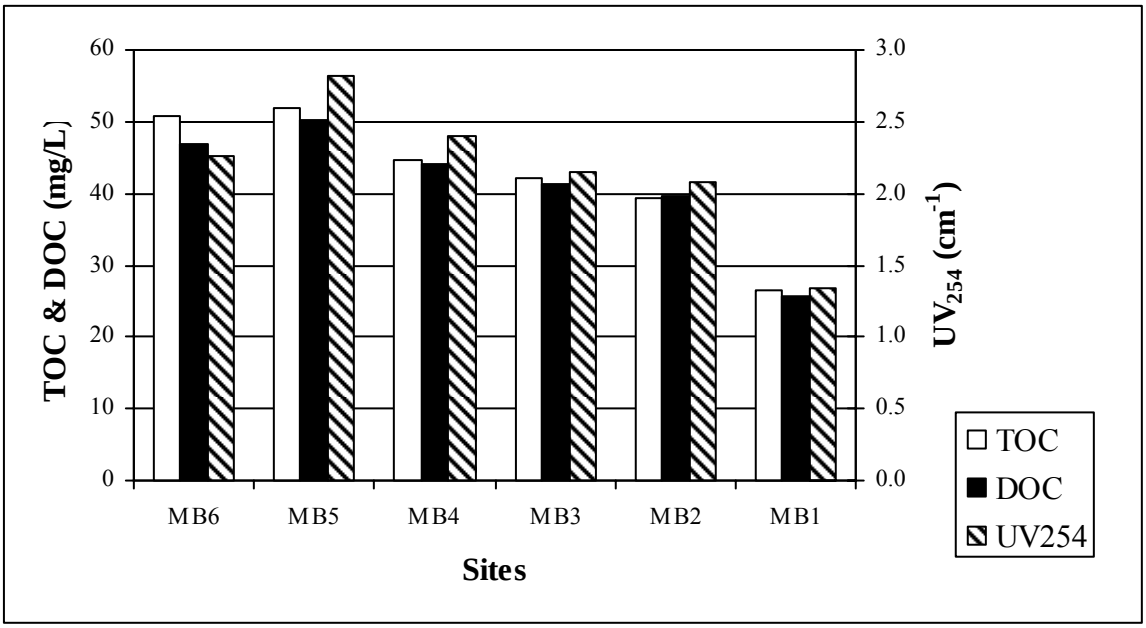

Figure 4.8. Organic carbon concentrations in Malagasco Brook, October 21, 2003.

Similar trends were observed in other seasons, with the highest TOC and DOC at MB5, and the lowest levels of organics at MB1. In the winter of 2003, the highest level of TOC was observed at MB5 (31.9 mg/L), and concentrations decreased in order to 15.3 mg/L at

MB1 (see Figure 4.9). DOC concentrations were within 0.3% – 5.3% of TOC concentrations for this sampling date. In the fall and spring sampling events, TOC and DOC peaked at MB5 with concentrations ranging from 23.1 to 51.9 mg/L for TOC and 20.9 to 50.1 mg/L for DOC. Concentrations consistently decreased downstream by 0% to 49 %. Although trends were similar in the summer, absolute values were different. TOC peaked at 47.9 mg/L at MB5, and decreased to 9.3 mg/L at MB4. The concentration was only 1.4 mg/L at MB1, a 97% decrease from MB5.

These high concentrations of organic carbon may be attributed to extra plant material from the nursery facility. Runoff of chemicals used in the production of a nursery also enters surface water bodies inadvertently increasing growth of other plant life found in the water. In addition, the swampy headwaters at MB6 were a likely source of organic matter, especially in the fall when leaves from the wooded areas fell in or near the water.

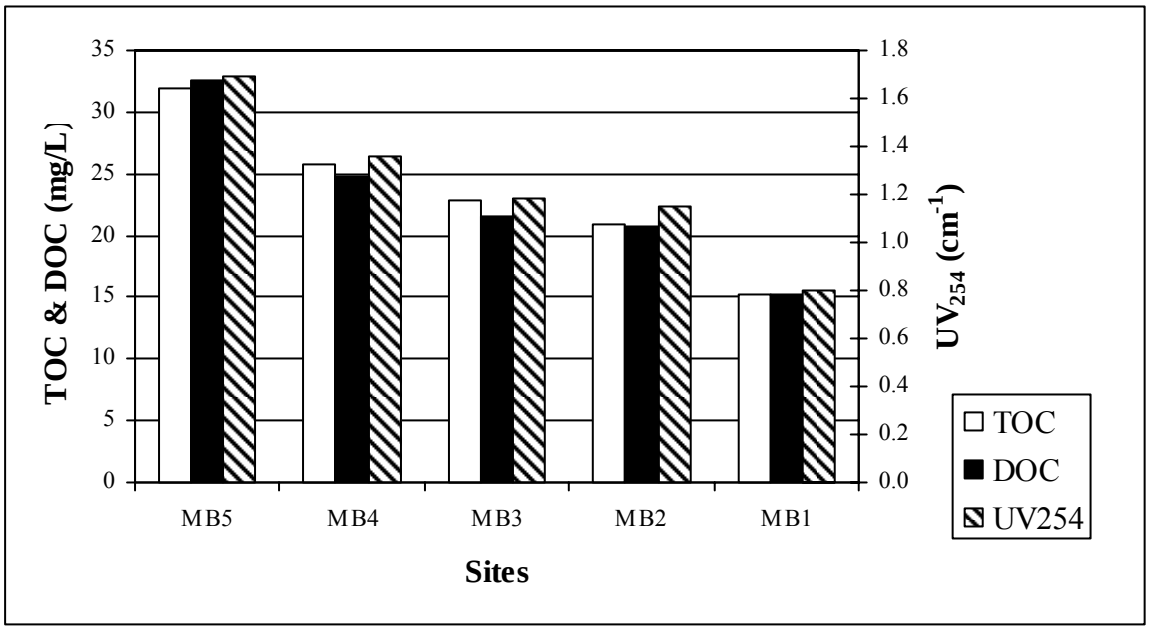

Figure 4.9. Malagasco Brook organics data, December 9, 2003.

UV_{254} levels followed the same trends as TOC and DOC. During the fall 2003 sampling date, a level of 2.26 cm^{-1} was observed at MB6, then increased slightly to 2.82 cm^{-1} at MB5, then decreased by 52% from MB5 to MB1. All sampling dates showed the highest

concentrations for UV_{254} at site MB5. The difference in concentrations from site MB5 to the most downstream site (MB1) ranged from 45% to 98%.

4.2.1.2 Physical and Chemical Water Quality

Physical and chemical parameters measured on the water quality samples included temperature, conductivity, dissolved oxygen, and pH. In the Malagasco Brook subbasin, the temperature and pH were relatively constant along the water body. For example, during the spring 2004 sampling, the temperature levels ranged from 11.4 – 9.5 °C, a difference of only 1.9 °C (see Figure 4.10). On August 11, 2004, the temperature at MB1 was 13.9 °C and the other measurements ranged from 18.1 – 18.9 °C. However, temperature differences among sites for all other sampling dates was 0.9 - 1.9 °C . pH values ranged from a low of 5.31 at MB5 in the fall of 2004 to a high of 7.33 at MB2 on August 11, 2004. A 3 percent average of variation in pH was observed in all sites for all sampling dates.

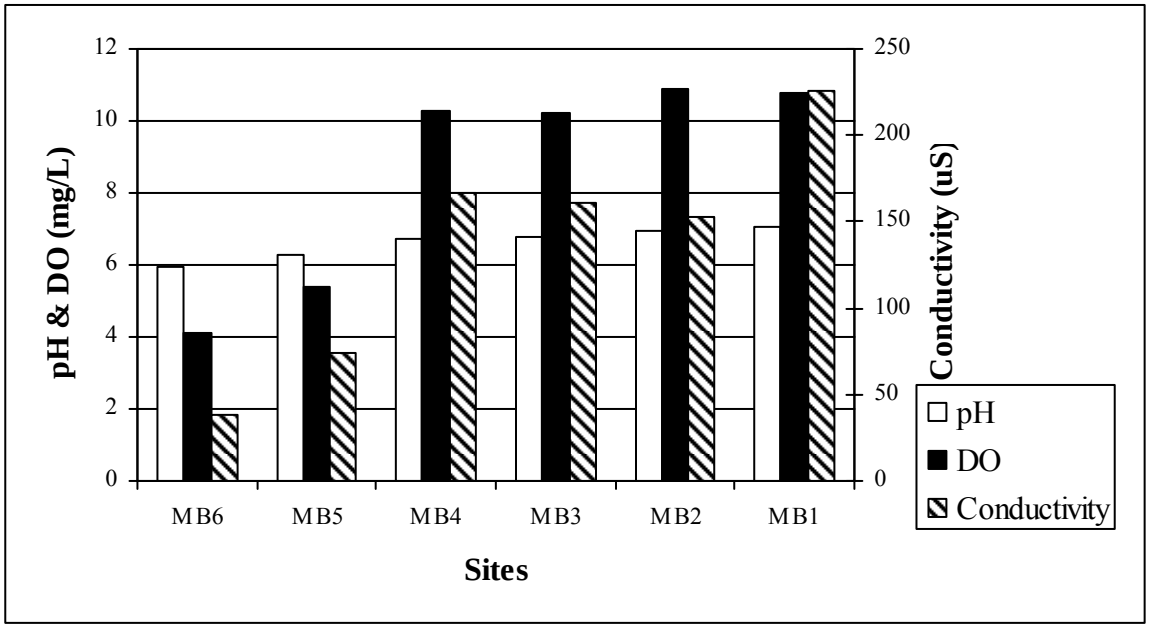

Figure 4.10. DO, pH, and conductivity in Malagasco Brook, May 4, 2004.

The dissolved oxygen levels within the Malagasco Brook subbasin showed a relatively consistent increase from upstream to downstream locations. During the spring 2004

sampling, the lowest level was observed at MB6 at 4.1 mg/L and the second lowest of 5.4 mg/L at MB5. Levels increased to >10 mg/L for all other downstream sites. The same trend was observed for all other sampling dates with minimal outliers. In the fall 2004 sampling date, levels varied from 11.9 – 13.0 mg/L at the four most downstream sites with highest DO observed at MB2. The difference between sites was only 1.1 mg/L, showing consistent levels of DO as the water moves more downstream.

This leveling of DO indicates that the water quality of the brook is increasing following the downstream flow of water. One source of this increased water quality can be a result of turbulence along the flow of the brook, in particular going over the weir between sample sites MB3 and MB2. The DO can also be related to the concentration of organics found in the water. Increased levels of organic matter at the upstream sites require oxygen from the water for degradation. This demand decreases as TOC and DOC levels decrease, allowing for increasing DO levels due to reaeration.

The conductivity at Malagasco Brook increased from MB6 to MB1. Overall, large increases were observed from MB6 through MB4 and then would level out until another large increase occurred from MB2 to MB1. For example, the fall 2004 sampling conductivity was 30 μS at MB6 and increased to 56 μS at MB5. The conductivity then increased to 71 μS at MB4, increasing only slightly until another large increase was observed from MB2 to MB1. The difference between these two sites was 116%. On average, the difference between MB5 to MB4 was 86% and 103% between MB2 and MB1.

High levels of conductivity are associated to increased inorganic dissolved solids such as chloride and nitrate. The areas where high levels were observed were located in areas that were highly wooded and had elevated amounts of plant material in the water body. These excessive amounts of debris result in decay of plant material which increase the dissolved solids which pass an electrical current. The brook passes through a granite gauging station at MB1 which can account for the increase in conductivity from the decay of the station's materials.

4.2.1.3 Particulate Matter

No observable trend in particulate matter was found on the sites along Malagasco Brook. Highs and lows of both turbidity and particle counts were observed at several different sampling sites out of the five times the water body was sampled. For example, the turbidity and particle counts for both the December 9, 2003 and August 11, 2004 sampling dates showed the highest level of particles at site MB3. However, the highest turbidity (1.23 ntu) was observed at site MB2 on the fall of 2004 sampling date and the highest particle count (3012/mL) was observed at MB6 on the same sampling date. Low levels of turbidity and particle counts also showed no observable variation based on site. Over all, the difference between the lowest and highest levels of turbidity ranged from 33% to 97% and the differences in particle counts ranged from 20% to 84%.

Due to the variable locations of highs and lows of the two constituents, no clear correlation can be made between the land use and measurement of the solids. Differences in measured levels could have been due to sudden turbulence in the water mixing up the sediments in the brook bed.

4.2.1.4 Indicator Organisms

The two indicator organism analyses performed on the water samples were fecal coliforms and enterococci. The analysis performed on Malagasco Brook did not show a consistent trend in high levels fecal coliforms. However, higher levels were generally found in and just downstream of the housing development (sites MB4-MB2). Four out of the five samples indicated the lowest levels of fecal coliforms at site MB1 (see Table 4.1). The range between highest and lowest concentrations of fecal coliforms on a given sampling date was found to be from 76% to 96%. Table 4.2 shows that no discernable relationship between high and low concentrations of enterococci were found by site. However, it is again seen that high concentrations tended to be in the housing development. Ranges from 36% to 99% were found for the differences in high and low concentrations of enterococci, at different sites on a given sampling date.

Table 4.1. Fecal Coliform concentrations at Malagasco Brook by sampling date.

	Maximum Concentration		Minimum Concentration	
Date	Location	cfu/100mL	Location	cfu/100mL
October 21, 2003	MB3	75	MB1	16
December 9, 2003	MB4	46	MB1	2
May 4, 2004	MB6	734	MB1	175
August 11, 2004	MB2	2459	MB1	152
November 10, 2004	MB4	26	MB6	2

Table 4.2. Enterococci concentrations at Malagasco Brook by sampling date.

	Maximum Concentration		Minimum Concentration	
Date	Location	cfu/100mL	Location	cfu/100mL
October 21, 2003	MB4	129	MB3	83
December 9, 2003	MB3	13	MB5	3
May 4, 2004	MB5	1061	MB6	31
August 11, 2004	MB3	3233	MB1	45
November 10, 2004	MB4	41	MB5	1

During two sampling dates, the lowest concentrations of fecal coliforms and enterococci were observed during the lowest levels of particulate matter. Many times microorganisms attach to particulates in a water sample, so the less solids in a water sample could produce a lower level of indicator organisms. Another notable trend was the increased measurement of fecal coliforms and enterococci at site MB4. This site was located immediately after the first section of condominiums. Increased microbiological concentrations could be an indication of failing septic systems within this area. Continued increased fecal coliforms and enterococci concentrations at MB3, also located within a condominium development, could indicate problems with septic systems as well.

4.2.2 Seasonal Analysis – Malagasco Brook

The following sections describe the results and analyses of the data found in the Malagasco Brook subbasin based on different seasons.

4.2.2.1 Organic Carbon

Comparing TOC, DOC, and UV_{254} for each of the four seasons, organic matter concentrations were highest during the fall season. As shown in Figure 4.11, DOC at site 1 was 25.7 mg/L in the fall of 2003 and 20.5 mg/L in the fall of 2004, compared to 15.2 mg/L, 8.4 mg/L, and 1.5 mg/L during the winter, spring, and summer, respectively. Total

organic carbon concentrations followed similar trends, with fall of 2003 values being 38.4% to 47.0% higher than values in winter, 55.4% to 74.9% higher than values in the spring and 7.7% to 94.7% higher than values in the summer. UV_{254} values also showed similar seasonal trends, which are expected, as UV_{254} is a surrogate measure for organic matter. Sites MB2-MB6 had UV_{254} values greater than 2.0 cm^{-1} in the fall of 2003. Only twice out of the other 22 samples was the value of UV_{254} greater than or equal to 2.0 cm^{-1}. This occurred during the fall of 2004 sampling at MB5 (2.0 cm^{-1}) and in the summer of 2004 at MB5 (2.6 cm^{-1}).

High concentrations of organics are typical during the fall due to the increased amounts of plant material in the water body. Since several sections of the brook are within highly wooded areas, the fall would produce higher levels of organic carbon. Sections that are not near wooded areas are also affected by the fall foliage since plant material is carried down the brook.

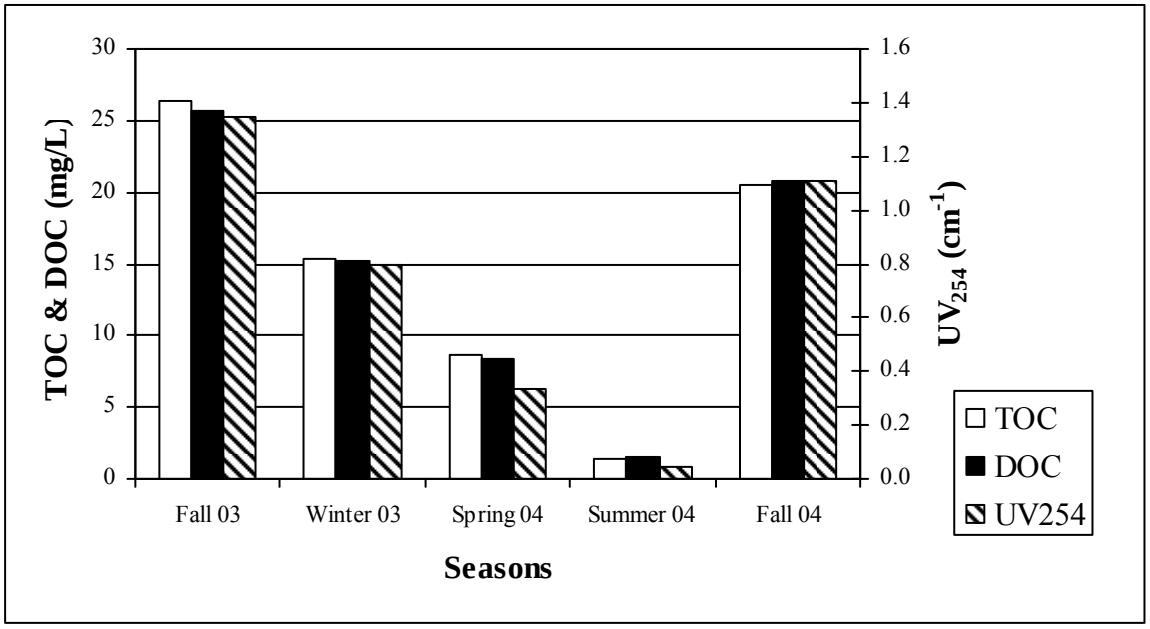

Figure 4.11. Organic carbon seasonal concentrations at MB1.

4.2.2.2 Physical and Chemical Water Quality

Temperatures in Malagasco Brook were appropriate for each season. Temperatures of 7.5 – 9.2°C were measured in the fall, -0.1 – 0.8°C in the winter, 9.5 – 11.4°C in the spring,

and 13.9 – 18.9°C in the summer. Dissolved oxygen and temperature have an inverse relationship, which is depicted in Figure 4.12 where the temperature for the winter is lowest and the DO is highest, and vice versa for the summer data. For example, the temperature at MB4 during the winter was measured at 0°C and the DO was 12.8 mg/L. Conversely the temperature was 18.1°C at MB2 during the summer and the DO of the same site was 7.6 mg/L. The pH of the water bodies was consistently neutral, where the difference between the highest and lowest levels was always less than 1.4 units during every season. The conductivity of the water bodies showed few patterns based on seasonal differences. The only observed pattern with conductivity based on season was that the summer levels were higher than all other seasons (see Table 4.3). The conductivity during the summer was 25.6% – 59.2% greater than the spring, 13.5% – 58.4% greater than the winter, and 3.6% – 62.2% greater than the fall conductivities. The difference in conductivity based on seasonal analysis could be an indication of waste contamination. However, high levels of conductivity would also be assumed to occur in late winter/early spring due to runoff from salted roads throughout the condominiums.

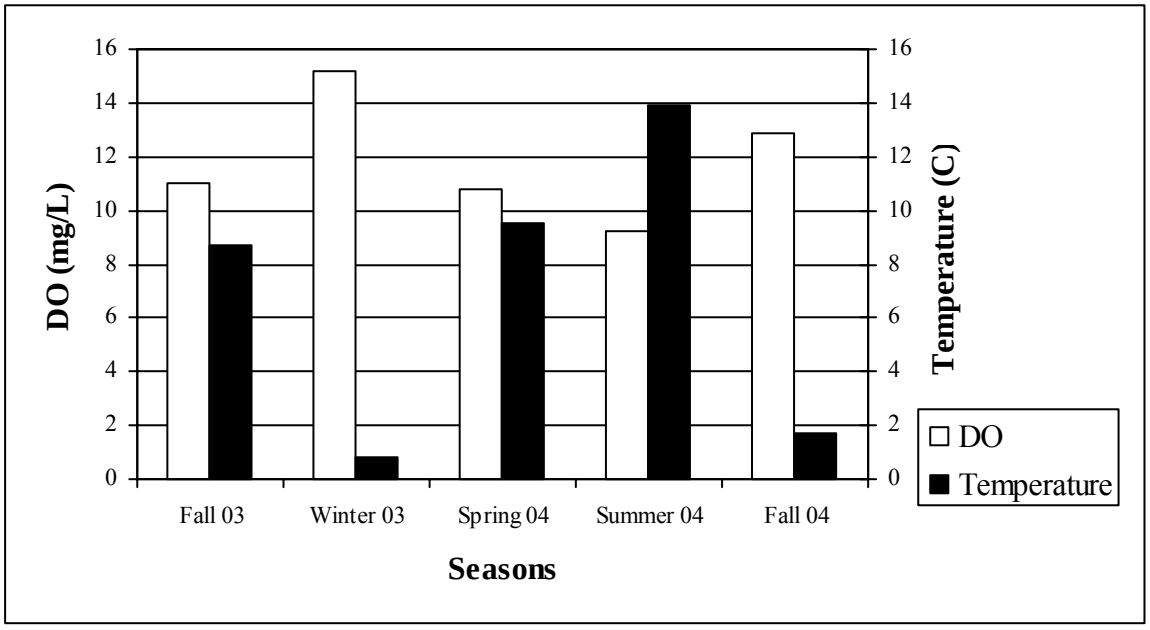

Figure 4.12. Temperature and DO seasonal measurements at MB1.

Table 4.3. pH and Conductivity levels at Malagasco Brook by season.

	pH			Conductivity (µS)		
Season	Maximum	Average	Minimum	Maximum	Average	Minimum
Fall 2003	6.99	6.70	6.09	297	146	58
Winter	6.83	6.40	5.58	304	172	95
Spring	7.04	6.61	5.95	226	137	38
Summer	7.33	6.96	6.29	554	287	110
Fall 2004	6.62	6.06	5.31	172	81	30

4.2.2.3 Particulate Matter

The turbidity and particle counts were found to be higher during the summer season than during all other seasons, with the exception of the most downstream sampling site (MB1). The turbidity was the lowest at MB1 during the summer compared to all other seasons (see Figure 4.13). The turbidity for the summer was an average of 68% greater than fall values, 55% greater than winter R-values, and 42% greater than the spring values for turbidity. Averages for particle counts were 71% greater than fall values, 47% greater than winter R-values, and 51% greater than the spring values.

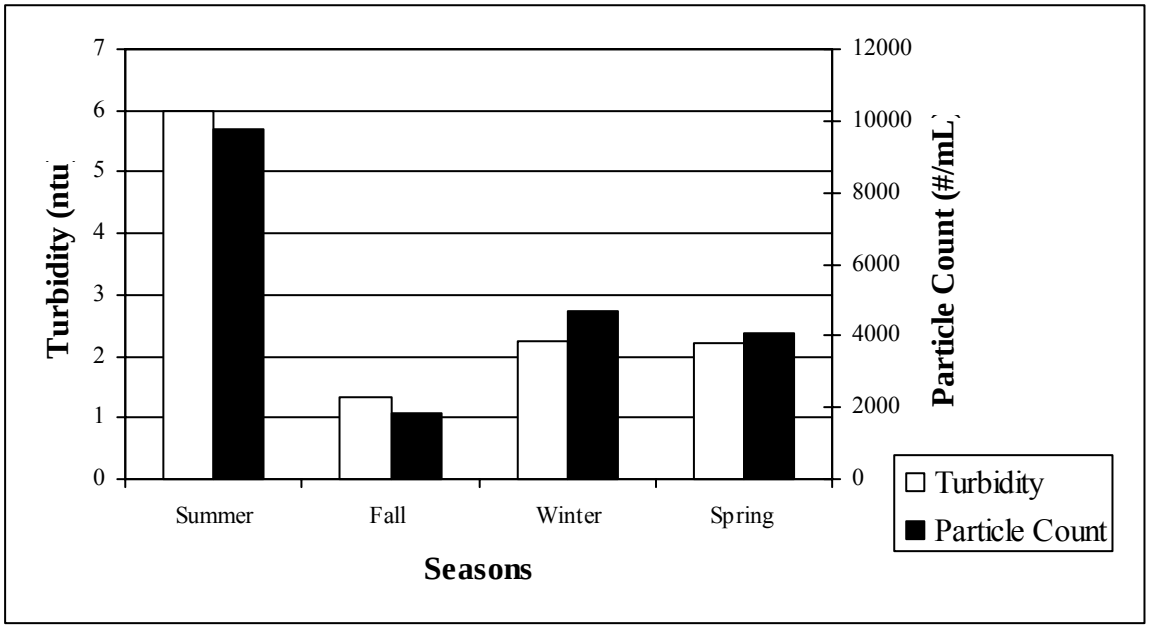

Figure 4.13. Particulate matter seasonal measurements at MB2.

4.2.2.4 Indicator Organisms

The fecal coliform and enterococci colony counts were highest during the summer with a maximum of 2459 cfu/100 mL for fecal coliforms and 3233 cfu/100 mL for enterococci, both taken during the August 11, 2004 sampling date. The lowest colony counts were found during the winter at 2 cfu/100 mL for fecal coliforms and 3 cfu/100 mL for enterococci, taken on the December 9, 2003 sampling date. The summer maximum levels of fecal coliforms are 70% greater than the spring levels, 97% and 99% greater than the fall levels, and 98% greater than the winter levels. The enterococci summer maximum concentrations for Malagasco Brook are 67% greater than the spring levels, 96% and 99% greater than the fall levels, and 99% greater than the winter levels. Higher levels of microbiological indicator concentrations would be likely during the summer because the warmer temperatures aid in the growth and reproduction of the organisms, where colder temperatures kill the organisms so detection would not be observed.

4.2.3 Site Analysis – Beaman Pond

The following sections describe the data and analyses performed on the Beaman Pond subbasin based on differences in site.

4.2.3.1 Organic Carbon

Total organic carbon, DOC, and UV_{254} levels were measured at three sites at the Beaman Pond subbasin. Total organic carbon concentrations generally decreased in the downstream direction. For example, the TOC concentrations for the November 13, 2003 sampling were 6.11 mg/L at site HF3, 2.22 mg/L at site HF2, and 2.14 mg/L at site HF1 (see Figure 4.14). However, on October 26, 2004, the TOC level was 1.80 mg/L at HF2 and increased to 2.48 mg/L downstream at HF1. HF3 could not be sampled on this date, as there was no flow. In contrast to TOC, DOC tended to increase in the downstream direction. In the fall of 2003 DOC concentrations increased from 1.31 mg/L at HF3 to 2.10 mg/L at HF1. Typically, the UV_{254} levels were greatest at the second sampling site. The fall 2003 sampling had a low level at HF3 of 0.027 cm^{-1}, which increased to a high of 0.058 cm^{-1} at HF2 and then decreased to 0.048 cm^{-1} at HF1.

High organic levels at site HF2 could be attributed to the stock pile of clippings and refuse waste located adjacent to the stream. During rainstorms, this waste can be easily washed into the brook altering the natural characteristics.

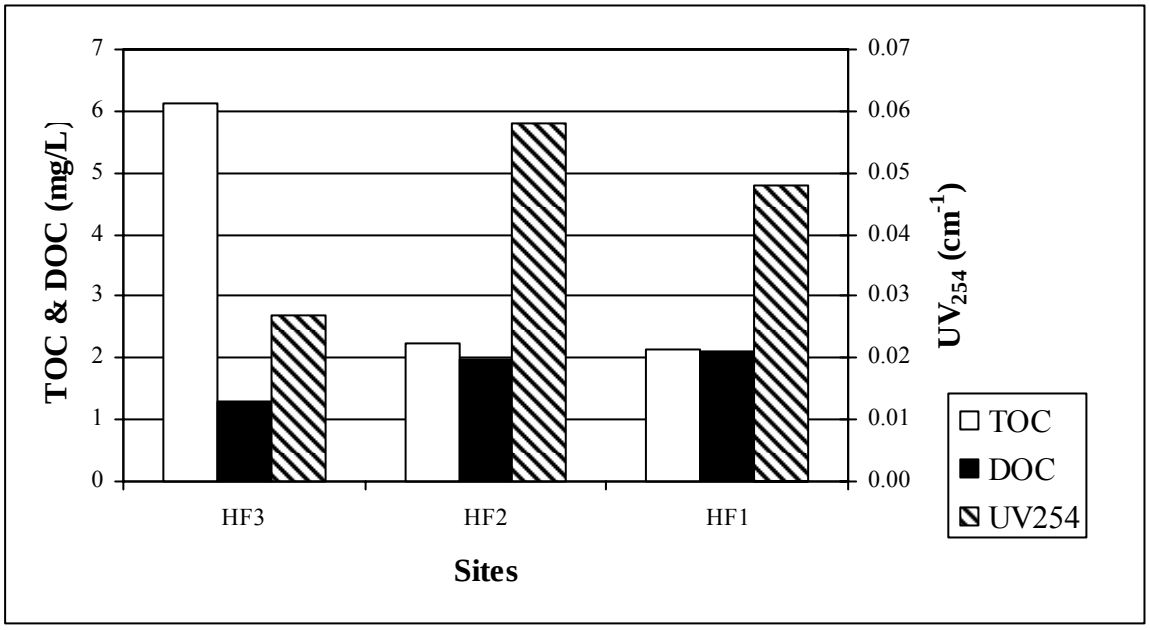

Figure 4.14. Organic carbon concentrations in Beaman Pond, November 13, 2003.

4.2.3.2 Physical and Chemical Water Quality

Physical and chemical water quality parameters included temperature, conductivity, dissolved oxygen, and pH. The temperature and pH were relatively consistent along the water body. In the fall and summer, temperature varied by only 2 °C among the sampling sites. In the winter and spring, temperatures varied by 3.0 - 3.4 °C. The pH was reasonably consistent, varying by 0.05 – 0.40 for a particular sampling date. For example, in fall of 2003 the highest pH (6.33) was found at HF1 and the lowest (6.28) at HF3, a difference of only 0.05. Dissolved oxygen levels in the Beaman Pond subbasin had the lowest level upstream at HF3, increased to a high at HF2, then decreased again at HF1. On November 13, 2003, DO was 6.83 mg/L at HF3, 9.03 mg/L at HF2, and 7.99 mg/L at HF1 (see Figure 4.15). The October 26, 2004 sampling showed a similar trend of a high of 10.29 mg/L at HF2 and a lower level at HF1 of 8.63 mg/L, however the sample for HF3 was not taken due to no observable flow. On September 13, 2004, DO levels were virtually identical at HF1 and HF2, measuring 8.40 mg/L and 8.37 mg/L, respectively.

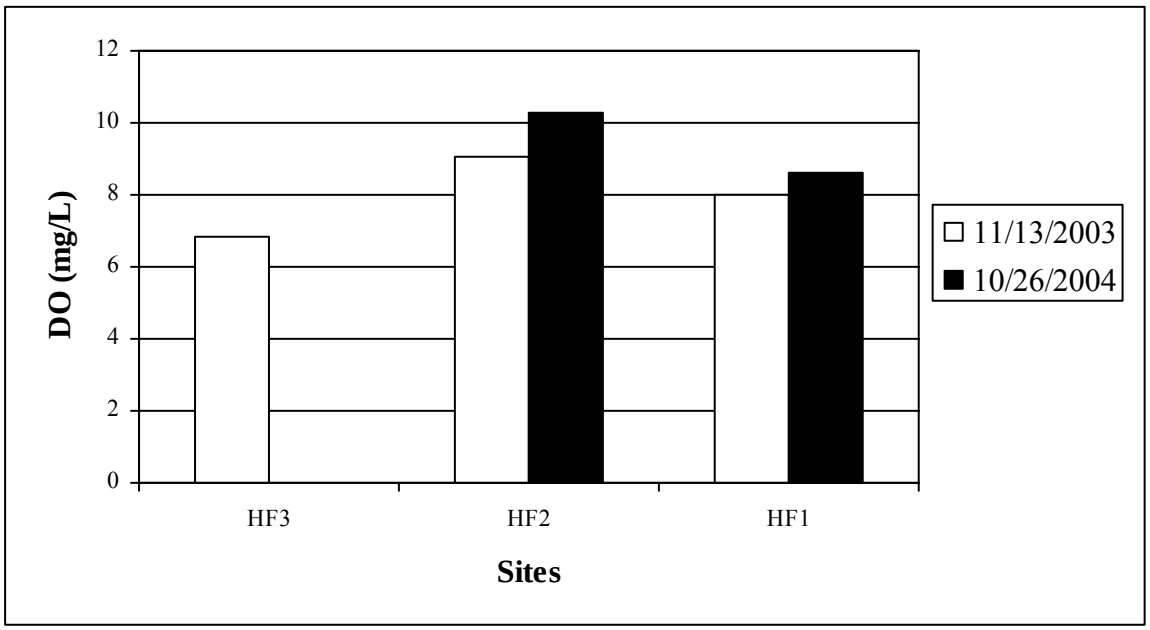

Figure 4.15. Dissolved oxygen fall concentrations in Beaman Pond.

The conductivity levels increased downstream for four out of the six sampling dates. On the dates that conductivity increased downstream, a 3% to 21% increase was found between each sampling site for a particular sampling date. The two sampling dates that decreased downstream (September 13, 2004 and October 26, 2004) only had a 3% and 7% decrease between sites. These sampling dates had two sampling sites (HF1 and HF2) because HF3 had no or low flow on the day of sampling.

Higher levels of conductivity may be due to septic systems that are leaching waste. This waste would introduce ions that would increase the ability to pass a charge in the water.

4.2.3.3 Particulate Matter

The turbidity and particle counts showed a decreasing trend in the Beaman Pond subbasin except for the samples taken on October 26, 2004, where the downstream samples were greater than the upstream samples. In the fall of 2003, the turbidity was 17.75 ntu at HF3, 1.34 ntu at HF2, and 0.90 ntu at HF1. Similarly, the particle concentration decreased from a high of 6881/mL at HF3 to 3137/mL at HF2 and 2843/mL at HF1. On October 26,

2004, the levels upstream were lower than the levels downstream. Site HF2 had a turbidity of 1.40 mg/L and a particle count of 2457/mL. The turbidity and particle counts at HF1 were 2.83 mg/L and 3764/mL, respectively. Site HF3 was not sampled on the October 26 sampling date due to no flow.

It appears that the large stock pile of plant material located adjacent to site HF2 was not a significant source of particulate matter. Higher levels found at HF3 may be due to the fact that there was consistently low flow at the site. Due to the low flow it was hard to take samples from the water body without disturbing the sediment on the bottom of the brook.

4.2.3.4 Indicator Organisms

The two indicator organism analyses performed on the water samples were fecal coliforms and enterococci. Within the Beaman Pond subbasin, elevated concentrations of both fecal coliforms and enterococci were detected at site HF2 or HF3 until the September 13, 2004 sampling date where the highest colony enumeration was observed at site HF1 (see Table 4.4 and Table 4.5). Large increases in both indicator organism concentrations were found from HF3 to HF2 on the November 13, 2003 sampling date with a 95% increase in fecal coliform concentration and a 98% increase in enterococci concentrations.

The elevated concentrations at HF2 on November 13, 2003 (fecal coliforms = 1364 cfu/100 mL, enterococci = 1636 cfu/100 mL) were 93% – 98% greater than the fecal coliform concentrations and 98% greater than the enterococci concentrations taken at HF1 and HF3. These elevated levels would indicate contamination from the horse farm upstream of the sampling site; however, fecal coliforms were maximum at this site once out of the six sampling dates and three out of six times for enterococci. This means that the horses were a source of water quality contamination, but not a recurring problem. The elevated levels at HF1 would indicate contamination from a septic system, while contamination at HF3 could be caused from stagnant water with little turnover.

Table 4.4. Fecal Coliform concentrations at Beaman Pond by sampling date.

	Maximum Concentration		Minimum Concentration	
Date	Location	cfu/100mL	Location	cfu/100mL
November 13, 2003	HF2	1364	HF1	15
January 12, 2004	HF3	42	HF1	6
April 6, 2004	HF3	57	HF1	11
June 2, 2004	HF3	115	HF2	78
September 13, 2004	HF1	283	HF2	69
October 26, 2004	HF1	95	HF2	61

Table 4.5. Enterococci concentrations at Beaman Pond by sampling date.

	Maximum Concentration		Minimum Concentration	
Date	Location	cfu/100mL	Location	cfu/100mL
November 13, 2003	HF2	1636	HF3	33
January 12, 2004	HF2	85	HF1	25
April 6, 2004	HF3	1000	HF1	32
June 2, 2004	HF3	118	HF1	59
September 13, 2004	HF1	103	HF2	38
October 26, 2004	HF2	48	HF1	30

4.2.4 Seasonal Analysis – Beaman Pond

The following sections describe the data and results of the analyses on the Beaman Pond subbasin based on seasonal changes.

4.2.4.1 Organic Carbon

The measured organic carbon for Beaman Pond showed fewer relationships based on seasonal variations than Malagasco Brook. High concentrations of TOC and DOC were observed during the summer, and lowest concentrations were found in the winter. Table 4.6 shows the maximum, average, and minimum concentrations of the three organic carbon water quality parameters. The fall and spring data is based on two sampling events and the winter and summer data is based on one sampling event. The summer of 2004 and fall of 2004 were only sampled at HF1 and HF2 since there was no flow at HF3. Higher levels of organics would be appropriate for the summer due to increased activity outside with plant care and full growth of plant life. However, even higher levels would have been expected during the fall because of the increased amounts of foliage and

plant material falling off of trees. The low levels of organic carbon would also be appropriate for the winter because of the low growth of plant life during this season.

Table 4.6. Organic carbon concentrations at Beaman Pond by season.

	TOC (mg/L)			DOC (mg/L)			UV_{254} (cm^{-1})		
Season	Max	Avg	Min	Max	Avg	Min	Max	Avg	Min
Fall*	6.11	2.95	1.80	2.38	1.88	1.31	0.058	0.039	0.027
Winter	2.11	1.35	0.94	0.98	0.89	0.81	0.023	0.020	0.017
Spring*	2.34	1.85	1.44	1.57	1.39	1.04	0.083	0.049	0.023
Summer	3.61	3.22	2.83	2.89	2.57	2.24	0.059	0.056	0.052

* Data based on two sampling events per season

4.2.4.2 Physical and Chemical Water Quality

Physical and chemical parameters such as temperature, turbidity and particle counts, pH, conductivity, and DO showed seasonal relationships. The ranges in temperature were consistent with the associated seasons. The range of temperatures for the spring was 4.0 – 12.3 °C, summer temperatures ranged from 14.7 – 15.6 °C, fall from 6.8 – 10.6 °C, and winter from 1.0 – 4.0 °C. The range in temperatures is highly variable during the spring due to measurements taken on April 6, 2004 and June 2, 2004, which are at the two ends of the seasonal range. Dissolved oxygen typically has an inverse relationship with temperature: as temperature decreases, DO increases. In the winter, DO levels ranged from 13.7 to 15.3 mg/L. Summer DO levels were significantly lower, averaging 8.4 mg/L. Although temperature and DO are inversely related, other factors influence the DO concentration in surface waters and thus there is some variability in the data.

The pH of the water samples was approximately neutral for all seasons, however, summer levels were slightly elevated compared to the other seasons (see Table 4.7). The average pH for the summer in the Beaman Pond subbasin was 13% greater than the fall, 7% greater than the winter, and 10% greater than the summer. These percent differences also show that there was a small difference in pH. The highest levels of conductivity were found during the winter. The average winter R-values were 12% greater than the fall, 13% greater than the spring, and 8% greater than the summer. The higher level of conductivity during the winter could be an indication of road salt entering the water body from runoff.

Table 4.7. Beaman Pond levels of pH and conductivity by season.

	pH			Conductivity (μS)		
Season	Maximum	Average	Minimum	Maximum	Average	Minimum
Fall*	7.14	6.62	6.28	785	616	447
Winter	7.36	7.14	6.95	805	699	601
Spring*	6.98	6.88	6.65	784	607	387
Summer	7.78	7.65	7.51	568	549	530

* Data based on two sampling events per season

4.2.4.3 Particulate Matter

The highest level for turbidity was during the spring (4.84 ntu). The average amount of turbidity was 57% greater than the winter, 46% greater than the spring, and 24% greater than the summer. Particle counts were the highest during the spring samplings (4154/mL). The percent difference was not as high for particle counts where the differences between seasons averaged 8% – 21%. The two parameters typically have similar trends, however, slight variations in the nature of the measurements can cause different trends.

4.2.4.4 Indicator Organism

The levels of fecal coliforms and enterococci varied by a season. The highest levels were observed during the fall of 2003 where fecal coliform levels were 1364 cfu/100 mL and enterococci levels were 1636 cfu/100 mL. The lowest microbial levels were observed during the winter with a fecal coliform level of 6 cfu/100 mL and 25 cfu/100 mL for enterococci. Table 4.8 shows all maximum, average, and minimum levels for the two indicator organisms.

The highest average concentration for both indicator organisms was found during the fall sampling season. The fecal coliform and enterococci concentrations in the fall were 45% – 94% and 37% – 83% greater than the other seasons, respectively. The high concentrations of microbiological organisms are more likely a result of the presence of the horses at the farm rather than a seasonal affect.

Table 4.8. Beaman Pond indicator organism concentrations by season.

	Fecal Coliforms (#/100 mL)			Enterococci (#/100 mL)		
Season	Maximum	Average	Minimum	Maximum	Average	Minimum
Fall*	1364	320	15	1636	355	29
Winter	42	20	6	85	59	25
Spring*	283	64	11	1000	223	32
Summer	283	176	69	103	71	38

* Data based on two sampling events per season

4.3 University of Massachusetts Data

In conjunction with the University of Massachusetts in Amherst, several other parameters were analyzed to further characterize the water quality, and in particular determine the source of microbial inputs to the waters. The three parameters measured at the UMass laboratory were *Rhodococcus coprophilus,* sorbitol-fermenting *Bifidobacteria*, and coliphages. *Rhodococcus coprophilus* is an indicator of grazing animal contamination. Sorbitol-fermenting *Bifidobacteria* is an indicator of human fecal or domestic waste water pollution. Lastly, F-specific RNA coliphages are indicators of sewage and fecal pollution, but can be further classified as originating from humans or non-human animals by genotyping or serotyping.

4.3.1 UMass Data – Malagasco Brook

R. coprophilus, sorbitol-fermenting *Bifidobacteria*, and F+RNA coliphages were analyzed for the Malagasco Brook subbasin for all sampling dates except November 10, 2004. On two occasions, December 9, 2003 and August 11, 2004, samples were not taken at MB6 due to low or no flow. *R. coprophilus* was found to be less than 20 cfu/100 mL at all sites on October 21, 2003 and December 9, 2003. On May 4, 2004, all sites were <20 cfu/100 mL, except MB3 and MB5 which had concentrations of 13 cfu/100 mL. On August 11, sites MB1-MB3 were below 20 cfu/100mL while MB4 and MB5 each had 13 cfu/100mL. The threshold level for indicating grazing animals contamination is 50 cfu/100 mL. Therefore, contamination from grazing animals is not suspected in the Malagasco Brook subbasin.

Levels of *Bifidobacteria* were below detection limits (<2 cfu/100 mL) for all sites on December 12, 2003 and May 4, 2004. Site MB4 had a level of 4 cfu/100 mL on the August 11, 2004 sampling date, while all other sites were <2 cfu/100 mL. On October 21, 2003 detectable concentrations were observed at four sites, MB1-MB4. The *Bifidobacteria* level at site MB4 was 46 cfu/100 mL, followed by decreasing levels of 16 cfu/100 mL at MB3, 11 cfu/100 mL at MB2, and 4 cfu/100 mL at the most downstream site, MB1.This data indicates that contamination from human sources may have occurred. Higher levels were found at MB3 and MB4 which were located within the condominiums.

F+RNA coliphages were less than 1 cfu/100 mL for all sites for both the October 21, 2003 and December 9, 2003 sampling dates. On the May 5, 2004 sampling date, the most upstream sites, MB6 and MB5, had levels less than 1 cfu/100 mL. Concentrations ranging from 0.5-1.0 cfu/100 mL were measured at sites MB4-MB1. There was no sample taken at MB6 on August 11, 2004. Sites MB5 and MB4 had levels of 0.5 cfu/100 mL. The next site, MB3, had 8.5 cfu/100 mL then decreased to less than 1.0 cfu/100 mL for the rest of the downstream sites. The peak in F+RNA coliphage concentration at site MB3 in combination of the elevated levels of *Bifidobacteria* concentrations further support the possibility of contamination from septic systems.

4.3.2 UMass Data – Beaman Pond

R. coprophilus, sorbitol-fermenting *Bifidobacteria*, and F+RNA coliphages were measured by UMass for all sites on all sampling dates with the exception of site HF3 on September 12, 2004 and October 26, 2004, when there was little or no flow at the time of sampling. The highest level of *R. coprophilus* was detected on November 13, 2003. The most upstream site was less than 20 cfu/100 mL, followed by a concentration of 320 cfu/100 mL at HF2 and then a decrease to 53 cfu/100 mL at HF1. The highest levels of *R. coprophilus* was observed at HF2 on four of the six sampling events. *R. coprophilus* was not detectable at HF2 (<20 cfu/100 mL), on September 13, 2004. The *R. coprophilus* concentration exceeded the threshold level of 50 cfu/100mL four times. Three of these times occurred at HF2, and one at HF1, downstream of HF1. This indicates that there was

contamination from a grazing animal. Based on the location of HF2, contamination from the horses at the farm downstream of the site is suspected.

Bifidobacteria levels of less than 2 cfu/100 mL occurred at all three sampling sites on three occasions, November 13, 2003, June 2, 2004, and October 26, 2004. The highest level was observed on January 12, 2004 at HF3 (8 cfu/100 mL). The *Bifidobacteria* level on September 13, 2004 at HF2 was 6 cfu/100 mL and a level of 5 cfu/100 mL was observed on April 6, 2004 at HF1. All levels of F+RNA coliphages were less than 1.0 cfu/100 mL at all sites except on June 2, 2004 where the level was measured at 0.5 cfu/100 mL. The higher concentrations of *Bifidobacteria* could be an indication of human contamination such as from septic systems; however, elevated levels of F+RNA coliphages were only observed once out of the 16 samples tested. Since these two concentrations were not observed with similar patterns it is hypothesized that human contamination is not present in the Beaman Pond subbasin.

4.4 Statistical Analysis

Several statistical analyses were performed of the data measured in the field and laboratory. All analyses were performed using Microsoft Excel data analysis tools. Correlation analyses were performed to identify relationships among the individual water quality parameters that were measured. ANOVA analysis was completed to determine differences between water quality at different sites and differences between seasons.

4.4.1 Correlation Analysis

Correlation analysis was used to determine relationships between water quality parameters. Three analyses were performed: correlation between the water quality parameters in the Malagasco Brook subbasin, correlation between the water quality parameters within the Beaman Pond subbasin, and correlation between the water quality parameters for the combined data from both subbasins. The correlation analysis performed by Microsoft Excel provides an R-value, which shows how well two factors are correlated. A correlation coefficient table is used to determine the confidence level of the data (see Appendix C). This table shows the R-value which must be met or exceeded

in order for a statistically significant correlation to exist. The R-value is dependant on the number of data points, n, which are evaluated. A P-value of 0.05, or a 95% confidence level, was used to determine statistically significant correlations for this research.

4.4.1.1 Analysis of Malagasco Brook

Fourteen water quality parameters were analyzed for the Malagasco Brook subbasin. There were 28 data points for each parameter (five sampling dates with six sites per date, except MB6 was not sampled on two dates due to no flow). Based on the correlation coefficient table, an R-value of 0.375 is needed for a statistically significant correlation with a P-value of 0.05, or a 95% confidence level. Table 4.4 presents the correlation data where the statistically significant correlations are shown in bold. Out of 90 possible correlations, 41 were found to be 95% statistically significant. The highest R-values (greater than or equal to 0.99) were found for the relationship between TOC and UV_{254}, DOC and UV_{254}, and DOC and TOC. These relationships are expected to be significant as they are all measures of organic matter. Also, the organic matter in Malagasco Brook is predominantly dissolved as the TOC and DOC concentrations were very similar. Therefore, these parameters should be highly correlated. There was also a strong correlation between particle counts and turbidity, which may be expected as both are measures of solids in the brook. Numerous other correlations were observed. For example, correlations were observed between DO and temperature (–0.460), TOC and DO (–0.408), temperature and pH (0.508), fecal coliforms and temperature (0.733), enterococci and temperature (0.605), and fecal coliforms and enterococci (0.818).

Table 4.9. Malagasco Brook Correlation Analysis

	Temp	*DO*	*Conduct.*	*pH*	*UV_{254}*	*TOC*	*DOC*	*turbidity*	*particles*	*FC*	*Enterococci*	*Rhodo*	*Bifido*	*Phage*
Temp	1													
DO	**-0.460**	1												
Conduct.	0.372	0.317	1											
pH	**0.508**	**0.426**	**0.649**	1										
UV_{254}	-0.279	-0.331	**-0.576**	**-0.460**	1									
TOC	-0.267	**-0.408**	**-0.617**	**-0.500**	**0.990**	1								
DOC	-0.308	-0.373	**-0.615**	**-0.508**	**0.991**	**0.998**	1							
turbidity	**0.615**	-0.315	-0.015	0.276	-0.136	-0.121	-0.173	1						
particles	**0.595**	-0.317	0.027	0.221	-0.162	-0.155	-0.206	**0.930**	1					
FC	**0.733**	-0.249	0.242	**0.417**	**-0.445**	**-0.432**	**-0.462**	**0.727**	**0.726**	1				
Enterococci	**0.605**	-0.177	0.274	**0.381**	**-0.407**	**-0.399**	**-0.423**	**0.682**	**0.608**	**0.818**	1			
Rhodo	**0.420**	-0.320	-0.042	-0.016	-0.037	-0.046	-0.067	0.210	0.303	0.214	0.314	1		
Bifido	0.000	0.082	-0.095	0.257	**0.415**	**0.397**	**0.420**	-0.204	-0.262	-0.157	-0.077	-0.126	1	
Phage	**0.380**	-0.080	0.074	0.200	-0.327	-0.316	-0.337	**0.684**	**0.562**	**0.463**	**0.693**	-0.030	-0.122	1

The inverse correlation between DO and temperature is expected based on changing saturation levels of dissolved oxygen with temperature. Several correlations were observed between particulate counts and microbiological concentrations. Microorganisms can attach to solids found in water, which may be problematic from a treatment perspective as solids can shield microorganisms from disinfection. Fecal coliforms had a statistically significant correlation with every other water quality parameter except for DO and conductivity. The same was true for enterococci. These observations are important because they indicate relationships between the parameters, which help determine which tests are critical for water quality analysis and which may be excluded in favor of other tests.

4.4.1.2 Analysis of Beaman Pond

Considering data points from the Beaman Pond subbasin, there were 16 data points for each water quality parameter. From the correlation coefficient table, an R-value of 0.497 was necessary in order for the relationship to have a correlation with a significance of P = 0.05 (95% confidence). Out of 90 possible correlations between parameters, 10 were found to be statistically significant at the 95% level. Table 4.5 shows the R-value for all correlations in Beaman Pond. Both DOC and TOC were inversely correlated with DO. This may be due to a high organic loading which causes an oxygen demand in the water. Both particle counts and turbidity were correlated to TOC, a relationship that may be observed when organic matter is largely particulate in nature rather than dissolved. An inverse correlation was observed for temperature and DO, with an R-value of –0.755. Positive correlations were observed for temperature and DOC (0.646), turbidity and particles (0.765), fecal coliforms and enterococci (0.833), fecal coliforms and *R. coprophilus* (0.897), and enterococci and *R. coprophilus* (0.744). These last correlations were also found to have a greater confidence level at 99% confidence.

Table 4.10. Beaman Pond Correlation Analysis.

	Temp	*DO*	*Conduct.*	*pH*	*UV_{254}*	*TOC*	*DOC*	*turbidity*	*particles*	*FC*	*Enterococci*	*Rhodo*	*Bifido*	*Phage*
Temp	1													
DO	**-0.755**	1												
Conduct.	0.018	0.130	1											
pH	0.067	0.328	-0.281	1										
UV_{254}	0.180	-0.223	-0.330	0.056	1									
TOC	0.432	**-0.587**	-0.158	-0.262	-0.010	1								
DOC	**0.646**	**-0.531**	-0.290	0.291	0.477	0.256	1							
turbidity	0.154	-0.378	-0.094	-0.365	-0.225	**0.901**	-0.166	1						
particles	0.116	-0.321	-0.167	-0.407	-0.203	**0.576**	-0.318	**0.765**	1					
FC	0.267	-0.174	0.132	-0.309	0.216	0.043	0.288	-0.122	-0.121	1				
Enterococci	0.104	-0.117	-0.087	-0.387	0.241	-0.012	0.088	-0.068	0.017	**0.833**	1			
Rhodo	0.062	-0.041	0.089	-0.405	0.343	-0.126	0.212	-0.255	-0.229	**0.897**	**0.744**	1		
Bifido	-0.264	0.247	-0.108	0.227	0.071	0.074	-0.118	0.105	0.066	-0.158	-0.180	-0.180	1	
Phage	0.243	-0.008	0.286	0.006	-0.148	-0.182	-0.137	-0.114	0.093	-0.058	-0.081	-0.102	-0.121	1

There were several correlations that were expected with the Beaman Pond data that were not found as statistically significant. One of these was the correlation between TOC and DOC. These two parameters may not be related when there is variation between the particulate and dissolved fractions of the organic matter at different sites. The positive correlation in particulate matter and microbiological organisms that was observed in Malagasco Brook was not observed in the Beaman Pond subbasin. However, fecal coliforms and enterococci had a strong correlation meeting a 99% statistical significance.

4.4.2.3 Analysis of Both Subbasins

Data collected from both subbasins included 44 data points for each water quality parameter. Thirty two statistically significant correlations were identified out of the possible 90. Based on 44 data points, an R-value of 0.298 was needed to meet a 95% statistical significance. Many of the same correlations for water quality indicators on the analysis on both sites were found to be the same as the analyses on each individual sites. For instance, a statistically significant correlation was observed for all three analyses for temperature and DO, turbidity and particles, and fecal coliforms and enterococci. Table 4.11 identifies all R-values for the analysis on both sites.

Table 4.11. Combined Subbasins Correlation Analysis.

	Temp	*DO*	*Conduct.*	*pH*	*UV_{254}*	*TOC*	*DOC*	*turbidity*	*particles*	*FC*	*Enterococci*	*Rhodo*	*Bifido*	*Phage*
Temp	1													
DO	**-0.505**	1												
Conduct.	0.185	0.218	1											
pH	**0.403**	**0.413**	**0.477**	1										
UV_{254}	-0.229	-0.291	**-0.767**	**-0.529**	1									
TOC	-0.214	**-0.349**	**-0.778**	**-0.556**	**0.994**	1								
DOC	-0.241	**-0.319**	**-0.782**	**-0.552**	**0.995**	**0.998**	1							
turbidity	**0.328**	-0.258	0.122	0.016	-0.167	-0.125	-0.180	1						
particles	**0.494**	**-0.326**	-0.116	0.037	-0.023	-0.007	-0.050	**0.639**	1					
FC	**0.631**	-0.249	-0.044	0.191	-0.181	-0.172	-0.186	0.247	**0.608**	1				
Enterococci	**0.508**	-0.174	0.002	0.176	-0.205	-0.201	-0.212	0.256	**0.522**	**0.820**	1			
Rhodo	0.027	0.017	**0.356**	-0.106	-0.252	-0.263	-0.257	-0.162	-0.162	0.236	0.232	1		
Bifido	-0.016	0.070	-0.175	0.165	**0.400**	**0.387**	**0.401**	-0.084	-0.177	-0.114	-0.061	-0.102	1	
Phage	**0.341**	-0.097	-0.152	0.082	-0.094	-0.085	-0.097	0.264	**0.532**	**0.454**	**0.645**	-0.086	-0.084	1

The correlations observed for both sites was very similar to the correlations found just for Malagasco Brook. Correlations that differed from both of the previous analyses included correlations between DO and DOC, DO and particle counts, and *R. coprophilus* and conductivity. These correlations would not have been hypothesized to be significant since these correlations were not found in the other two analyses, with the exceptions of DO and DOC which was observed in the Beaman Pond analysis.

4.4.2 ANOVA Analysis

ANOVA analysis was performed to determine differences between sites and differences between seasons. For the former test, the null hypothesis was that the means of the data for each constituent in a subbasin were the same at every site. For the later test, the null hypothesis was that the means of the data for each constituent were the same for every season. The null hypothesis was rejected if the P-value was 0.05 or less, meeting a confidence level of 95%.

4.4.2.1 Site Analysis for Malagasco Brook

Five sites in the Malagasco Brook subbasin (MB1 – MB5) each had five data points and the last site (MB6) had three data points, totaling 28 data points for analysis. Data analyzed by the University of Massachusetts for Malagasco Brook had 22 data points, where four data points were taken at each of sites MB1 – MB5, and two points at MB6.

Three water quality parameters in the Malagasco Brook subbasin were found to be statistically different by site. The parameters were dissolved oxygen (P-value = 1.01×10^{-5}), conductivity (P-value = 0.002), and pH (P-value = 6.21×10^{-4}). All other water quality measures were not statistically different by site at the 95% confidence level. A statistical difference in pH was not expected for Malagasco Brook; however, the distance between each site does allow differences in makeup of surrounding materials and land use which can have a significant affect on the pH of a water sample. The DO of the samples would be assumed to be statistically different at each site because of the different characteristics at each site. Approximately half of the sites were located within heavily wooded areas where the amount of foliage in the water could cause the water to have a high oxygen demand, while the weir near MB3 could increase DO concentrations. However, it was also hypothesized that the differences in organic matter would be statistically different for the same reasons the DO was expected to be different, and this result was not found.

4.4.2.2 Site Analysis for Beaman Pond

The Beaman Pond subbasin had six data points for sites HF1 and HF2, and four data points for the last site, HF3, for a total of 16 data points for ANOVA analyses. In the Beaman Pond subbasin, based on a P-value less than or equal to 0.05, two of the water quality parameters were different by site. Turbidity varied by site with a P-value of 0.017 and particle counts with a P-value of 0.002. The two particulate matter indicators differing by site would be assumed since each site was very different. Site HF1 was surrounded by a sparse wooded area, site HF2 was near bushes but also had a stock pile of refuse plant material near it, and HF3 was in a swampy area. With the difference in turbidity and particle counts, differences in TOC and DOC might also be observed. This difference was not made between the sites.

4.4.2.3 Seasonal Analysis

The seasonal analysis was based on all data points for both Malagasco Brook and Beaman Pond subbasins divided into seasons. Nine data points were analyzed for spring, seven during summer, 17 during the fall, and 11 in the winter, totaling 44 data points. There were fewer data points for the constituents analyzed by the University of

Massachusetts. Six less data points were analyzed for the fall, totaling 34 data points for seasonal analysis.

Table 4.12 shows the eight water quality parameters that were statistically different based on seasonal analysis. Statistical differences in DO and temperature would be expected for seasonal analysis due to the obvious temperature differences in New England weather and the inverse correlation between DO and temperature. Particle counts varied by season, while turbidity did not. This result may demonstrate the sensitivity of particle count data compared to turbidity, which is an aggregate measurement. Both TOC and UV_{254} were statistically different by season; however DOC was not. All three of these measurements may not be necessary in a watershed sampling plan. Lastly, fecal coliforms and enterococci both differed by season. Typically during warmer temperatures, microbiological organisms can live and grow more readily than during colder temperatures when the organisms die due to harsh living conditions.

Table 4.12. Statistically different water quality parameters by season.

Water Quality Parameter	P-Value
Temperature	3.14×10^{-12}
DO	7.7×10^{-4}
pH	0.017
UV_{254}	0.01
TOC	0.007
Particle Count	0.002
Fecal Coliforms	2.05×10^{-4}
Enterococci	0.008

4.5 Summary

Within the Malagasco Brook subbasin, the nursery had the largest affect on the water quality at sites MB5 and MB6. At these sites, high levels of organic carbon were detected. In relationship to the elevated levels of plant material, a demand on oxygen was detected in these same sites lowering DO levels. Elevated levels of microbiological indicators were also found in the more downstream sites, the highest levels observed at MB3 and MB4. The indicators found at higher concentrations were fecal coliforms,

enterococci, sorbitol-fermenting *Bifidobacteria*, and F+RNA coliphages. These are all indicators of contamination by human origin.

The water quality indicators measured in the Beaman Pond subbasin showed high levels of conductivity at HF1 and HF3. These levels are most likely due to leachate from septic systems entering the water body. Microbiological indicators such as fecal coliforms, enterococci, and *R. coprophilus* were found at high concentrations at HF2. This is a clear indication that animal waste was a source of the contamination at the site. However, the highest levels of these indicators were only detected once at this site so the contamination from the horses was not a reoccurring problem.

Many of the observations made for analyses by season were typical of the seasonal changes and affects that occur because of that particular season. For instance, temperature was the most typical seasonal change in the two subbasins. Based on seasonal temperatures within the New England area, warmer water temperature were detected during the summer and spring, where colder temperatures were found during the winter and fall. Based on those temperatures, DO concentrations were observed with the opposite levels.

High concentrations of organic carbon were also found during the fall in the Malagasco Brook subbasin. This is typical of high amounts of plant foliage falling in and around water bodies. Eventually the leaves and plants enter the water, adding organic material to the water source. However, conversely higher levels of organic carbon were found during the summer in the Beaman Pond subbasin. This is attributed to the fact that there is increased outside activity during this season which can lead to a higher impact on water quality due to people in and around the water body. Generally, when high concentrations of organics were observed, high levels of particulate matter would also be detected.

Several of the correlations and results are typical of naturally occurring relationships. For example, the inverse correlation between DO and temperature is a well-known and commonly observed relationship. Similarly, a correlation between turbidity and particle

counts is expected since they are both measurements on the amount of solids within the water sample. However, there were correlations and trends that may indicate site-specific problems with water quality.

Chapter 5

Conclusions & Recommendations

Conclusions and recommendation were made based on the culmination of the water quality parameters analyses on the two watershed subbasins.

5.1 Conclusions

The following sections provide the conclusions that were drawn from the analyses of the water quality parameters. The first section describes the conclusions drawn from the data on the two subbasins, followed by the conclusions from the statistical analyses, and lastly the conclusions based on the type of land use within the two subbasins.

5.1.1 Malagasco Brook

The water quality indicators that were found to be most significant in the Malagasco Brook subbasin were organic carbon loading and human sources of microbiological contamination. Organic carbon was a significant contribution to the brook in this subbasin due to the activities of the nearby nursery, in addition to the location of the sites within wooded areas. Other water quality parameters were likely affected by the organic matter. Dissolved oxygen typically decreased with higher organic carbon loadings due to the demand for oxygen during decomposition of the materials. Particulate matter also increased with increasing levels of organic carbon. Higher levels of conductivity were also present due to the increased amounts of solids and ions in the water. This could be a result of runoff from the adjacent roadways washing road salt into the water body.

Microorganisms were generally higher when particulate matter was elevated; microorganisms can adhere to the solid particles. The highest concentrations of microorganisms were found at sites MB4 and MB5. These sites were located within the condominiums which is an indication of human contamination due to septic systems. This area was more highly developed so animal contamination would be minimal.

5.1.2 Beaman Pond

The Beaman Pond subbasin was affected by both human and animal contamination. Increased concentrations of *R. coprophilus* as well as fecal coliforms and enterococci levels were observed once at HF2. *R. coprophilus* is an indicator of grazing animal contamination which would indicate contamination from the horses at the upstream farm. However, this increased concentration of microbiological indicators was only observed once so the problem was not a reoccurring result of the horses on the farm. During the other sampling dates (including the one time when the horses were relocated off of the property) high concentrations of microbial contamination were found at HF1 and HF3. These two locations were located behind residences that were connected to septic systems.

Other indicators in the Beaman Pond subbasin that were found to be significant included high levels of conductivity and organic carbon. The high levels of conductivity were found most often at HF1, which can be an indication of contamination from a waste. Excessive nitrates and other ions increase a water bodies ability to carry a current. The higher levels of organic carbon were also typical of seasonal patterns as well as increased concentrations at HF2 from organic material entering from the stockpile of refuse waste at the site.

5.1.3 Statistical Analyses

The correlations found at both sites included: temperature and DO, DO and TOC, turbidity and particle counts, and fecal coliform and enterococci. These relationships were expected due to previous researched relationships and assumptions about the measurement the parameter indicated. For example, several studies have shown the relationship of temperature and dissolved oxygen. With changing temperatures, the saturation level of DO also changes. Total organic carbon and DOC did not have a significant correlation in the Beaman Pond subbasin. This was possible due to differences in the amounts of organic carbon in the dissolved and particulate state.

ANOVA analyses were performed to determine differences by site and by season. Statistical differences were found by site in Malagasco Brook for pH, conductivity, and DO. However, a statistical difference in organic carbon, which can affect DO levels, was not found. Since organic carbon was observed in such different concentrations at the sites, a difference might have been expected. In Beaman Pond, only the particulate matter measurements varied by site. Both TOC and UV_{254} were found to be statistically significant by season, however, DOC was not. Other parameters that varied by season included temperature, DO, pH, particle counts, fecal coliforms, and enterococci.

5.1.4 Land Use

The sites sampled in Malagasco Brook were found in either wooded areas or along a developed street. Based on these two land uses, the water quality indicators were consistent with hypothesized results. In the wooded areas, higher levels of organic carbon were detected due to the abundance of plant materials. The more developed areas showed increased levels in contamination based on human sources. These included not only contamination by means of microbiological contamination but also contamination from human uses. For example, higher levels of conductivity are a product of runoff from paved surfaces which wash road salt from the winter season into adjacent surface water bodies.

Beaman Pond sites were all located along a developed road. With the exception of the two acre farm, most lots were used for single family homes. The farm had the greatest impact on the site directly downstream. The other two sites were directly behind single family houses with septic systems. The contamination at these sites was likely from septic systems.

5.2 Recommendations

Based on the analyses on the two subbasins, several observations were made on the sources and types of contamination on the surface waters. Several measurements can be made on water to determine the water quality but feasibility, cost, and time may prevent analysis on all parameters. It is recommended that only seven to eight measurements be

performed when analyzing surface water for contamination, instead of the 14 that were sampled in this thesis. Measurements of temperature, DO, conductivity, pH, DOC, turbidity or particle counts, and fecal coliforms. These parameters are mainly chosen because they are quick, simple, and inexpensive indicators to measure water quality. Temperature, DO, conductivity, and pH are all measurements that can be taken in the field. Scientific tools are available that can measure all of these parameters. Although UV_{254} was highly correlated to TOC and DOC, absolute values of UV_{254} are related not only to the concentration of organic matter but also to the composition of those organics. As UV_{254} is only a surrogate for organic carbon concentrations, the more conclusive measurement of DOC is recommended.

Turbidity or particle counts can be analyzed. Both had the same trend for all analyses, so the basis of the deciding factor would be whether an actual number was needed or if a general measurement of the amount of solids was needed. Fecal coliforms are recommended for analysis over enterococci because it is an easier, faster analysis, and both produce a similar results. Enterococcus enumeration requires more preparatory work for analysis as well as very specific time frames for analyses. All analyses performed by UMass would not be recommended for routine use when evaluating the water quality of a surface water. All three tests are time consuming, expensive, and require personnel that are knowledgeable in the methodology needed for the tests. The only time these tests would be recommended are if inconclusive results have been drawn from the previously discussed analyses.

In addition to the parameters measured in this thesis, alkalinity and inorganic carbon may be useful for monitoring. These two measurements may determine in stream changes in water quality, as opposed to effects from land uses outside the stream.

In the past, DCR has requested that the horses on the farm in the Beaman Pond subbasin be removed. The horses were removed for a short period of time and then moved back onto the site. Removal of the horses would reduce the amount of contamination coming from the farm; however, since it was not found to be a reoccurring problem, removal may

not be necessary. In addition, the septic systems behind the houses should be checked for possible leaking.

In the future it is recommended that more sampling dates be analyzed. For many of the analyses only one seasonal measurement was observed. In addition it would also be recommended that other types of areas with different land uses be analyzed. Impact from several other land uses, such as agricultural, industrial, and commercial uses, could affect the quality of water greatly.

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Appendix A

Experiment Results

Sample: Malagasco Brook

Site	Date	Field measurements			Lab measurements - physical/chemical						Lab measurements - microbiological				
		°C	mg/L	ηS		cm-1	mg/L	mg/L	ntu	#/mL	cfu/100 mL	cfu/100 mL	cfu/100 mL	cfu/100 mL	cfu/100 mL
		Temp	DO	Conduct.	pH	UV254	TOC	DOC	turbidity	particles	FC	Enterococci	Rhodo	Bifido	Phage
MB 1	10/21/03	8.7	11.0	297	6.83	1.344	26.38	25.72	1.16	1318	16	27	<20	4	<1
MB 2	10/21/03	8.3	10.8	142	6.99	2.077	39.45	39.49	1.46	1967	25	64	<20	11	<1
MB 3	10/21/03	8.6	9.0	144	6.96	2.152	42.10	41.29	1.70	3023	75	83	<20	16	<1
MB 4	10/21/03	8.9	9.7	129	6.95	2.398	44.77	44.15	1.71	3187	31	129	<20	46	<1
MB 5	10/21/03	9.2	3.4	106	6.35	2.817	51.86	50.13	2.69	3756	20	19	<20	<4	<1
MB 6	10/21/03	7.5	2.3	58	6.09	2.261	50.84	46.89	3.67	5632	19	1	<20	<4	<1
MB 1	12/09/03	0.8	15.2	304	6.83	0.799	15.28	15.24	1.65	4449	2	7	<20	<2	<1
MB 2	12/09/03	-0.1	14.2	161	6.73	1.150	20.91	20.79	2.26	4697	4	10	<20	<2	<1
MB 3	12/09/03	0.1	12.6	158	6.53	1.187	22.79	21.59	2.77	6076	18	13	<20	<2	<1
MB 4	12/09/03	0.0	12.8	142	6.32	1.358	25.80	24.75	2.04	4836	46	12	<20	<2	<1
MB 5	12/09/03	0.2	8.2	95	5.58	1.690	31.93	32.62	1.48	3541	9	3	<20	<2	<1
MB 6	12/09/03														
MB 1	05/04/04	9.5	10.8	226	7.04	0.338	8.62	8.37	1.96	3851	175	85	<20	<2	1.0
MB 2	05/04/04	9.9	10.9	153	6.94	0.486	10.56	10.00	2.21	4061	213	115	<20	<2	1.5
MB 3	05/04/04	10.0	10.2	161	6.76	0.427	10.56	10.28	2.30	4675	205	123	13	<2	1.0
MB 4	05/04/04	10.5	10.3	167	6.71	0.543	11.76	11.17	2.42	4258	233	115	<20	<2	0.5
MB 5	05/04/04	11.4	5.4	74	6.26	1.118	23.12	20.87	3.82	4788	394	1061	13	<2	<1
MB 6	05/04/04	11.2	4.1	38	5.95	0.797	19.36	18.39	1.22	3936	734	31	<20	<2	<1
MB 1	08/11/04	13.9	9.2	554	6.95	0.046	1.41	1.49	0.26	1814	152	45	<20	<2	<1
MB 2	08/11/04	18.9	7.8	215	7.33	0.405	9.67	8.31	5.99	9741	2459	1240	<20	<2	<1
MB 3	08/11/04	18.2	7.2	217	6.94	0.301	8.00	6.16	8.27	11618	1832	3233	<20	<2	8.5
MB 4	08/11/04	18.1	7.6	341	7.29	0.433	9.26	8.89	2.59	6048	1664	2758	13	4	0.5
MB 5	08/11/04	18.6	2.9	110	6.29	2.625	47.87	44.43	5.10	10917	568	77	13	<2	0.5
MB 6	08/11/04														
MB 1	11/10/04	1.7	12.9	172	6.62	1.112	20.54	20.76	0.90	1604	11	27	<20	<2	<1
MB 2	11/10/04	0.8	13.0	79	6.51	1.463	28.58	28.51	1.23	1746	8	29	<20	<2	<1
MB 3	11/10/04	1.2	11.9	79	6.21	1.579	29.81	29.54	1.01	2523	4	36	<20	<2	<1
MB 4	11/10/04	1.3	12.3	71	6.09	1.771	33.46	33.29	1.14	2370	26	41	0	<2	<1
MB 5	11/10/04	2.1	5.3	56	5.31	2.013	39.49	39.24	0.83	2225	3	1	0	<2	<1
MB 6	11/10/04	1.5	2.3	30	5.60	1.591	36.07	35.85	1.00	3012	2	17	0	<2	<1

Data suspect due to improper incubation

Sample: Horse Farm

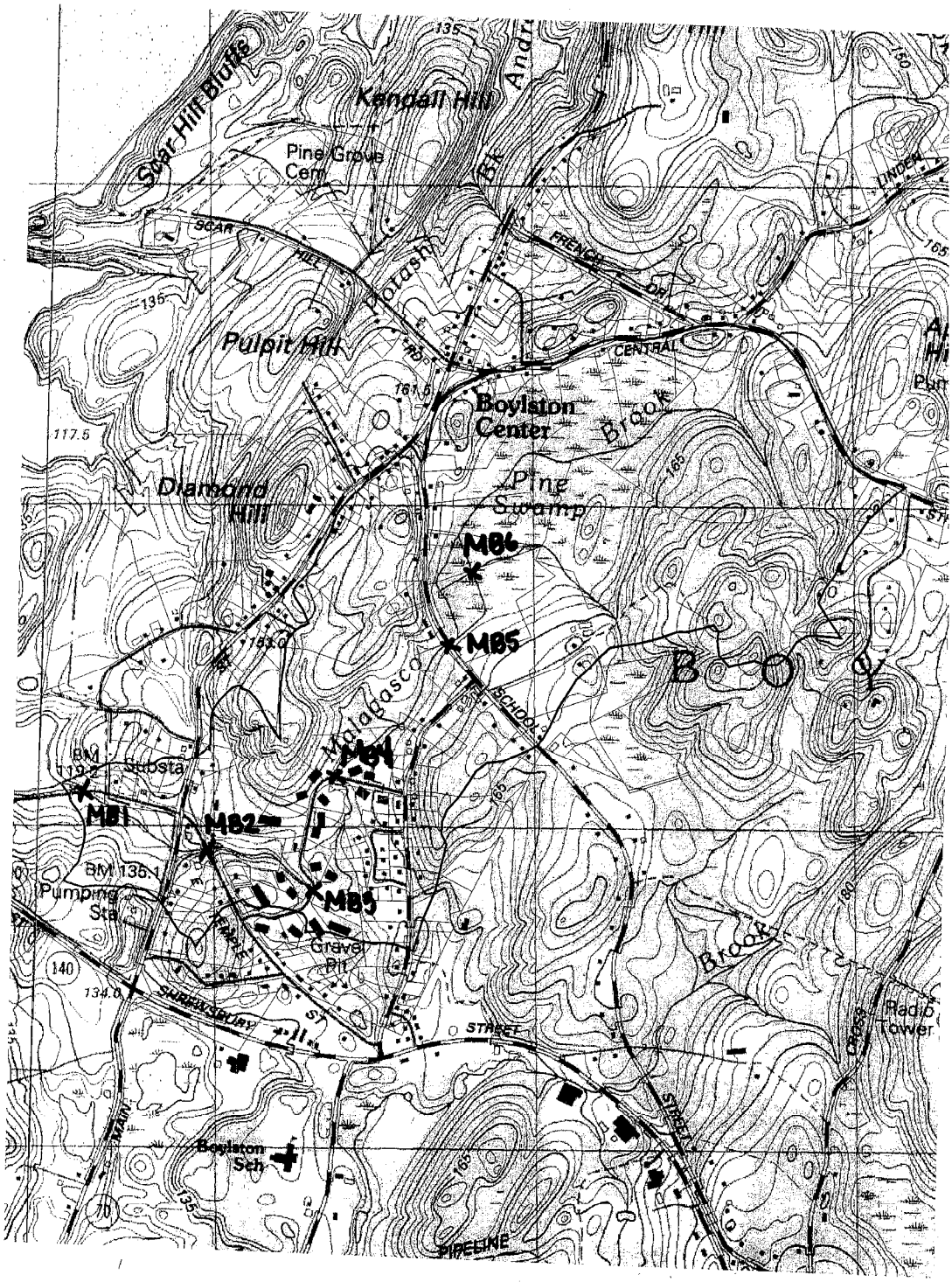
Site	Date	Field measurements			Lab measurements - physical/chemical						Lab measurements - microbiological				
		Temp	DO	Conduct.	pH	UV254	TOC	DOC	turbidity	particles	FC	Enterococci	Rhodo	Bifido	Phage
		°C	mg/L	mS		cm-1	mg/L	mg/L	ntu	#/mL	cfu/100 mL	cfu/100 mL	cfu/100 mL	cfu/100 mL	cfu/100 mL
HF 1	11/13/03	9.7	7.99	785	6.33	0.048	2.14	2.10	0.90	2843	15	29	53	<2	<1
HF 2	11/13/03	10.6	9.03	712	6.32	0.058	2.22	1.97	1.34	3137	1364	1636	320	<2	<1
HF 3	11/13/03	10.5	6.83	678	6.28	0.027	6.11	1.31	17.75	6881	67	33	<20	<2	<1
HF 1	01/12/04	2.0	14.30	805	7.36	0.021	0.94	0.98	0.75	1372	6	25	13	<2	<1
HF 2	01/12/04	4.0	15.30	690	7.11	0.023	1.00	0.89	0.93	4558	13	85	27	<2	<1
HF 3	01/12/04	1.0	13.70	601	6.95	0.017	2.11	0.81	4.60	3923	42	68	<20	8	<1
HF 1	04/06/04	4.0	10.32	600	6.98	0.080	1.63	1.55	0.73	3229	11	32	53	5	<1
HF 2	04/06/04	6.2	10.50	489	6.94	0.083	1.81	1.57	2.23	3570	28	52	67	<2	<1
HF 3	04/06/04	7.4	9.09	386.7	6.81	0.051	2.34	1.36	4.85	4901	57	1000	<20	<2	<1
HF 1	06/02/04	11.6	7.81	784	6.92	0.028	1.63	1.52	0.64	2198	92	59	<20	<2	<1
HF 2	06/02/04	12.3	9.76	762	6.95	0.030	1.44	1.30	1.49	4331	78	79	13	<2	0.5
HF 3	06/02/04	9.8	7.01	621	6.65	0.023	2.26	1.04	5.68	6697	115	118	13	<2	<1
HF 1	09/13/04	15.6	8.40	530	7.78	0.059	2.83	2.89	0.52	2075	283	103	13	<3	<1
HF 2	09/13/04	14.7	8.37	568	7.51	0.052	3.61	2.24	6.84	4757	69	38	<20	6	<1
HF 3	09/13/04														
HF 1	10/26/04	6.8	8.63	445.6	7.14	0.028	2.48	2.38	2.83	3764	95	30	27	<2	<1
HF 2	10/26/04	8.6	10.29	461	7.02	0.033	1.80	1.64	1.40	2457	61	48	93	<2	<1
HF 3	10/26/04														

Appendix B

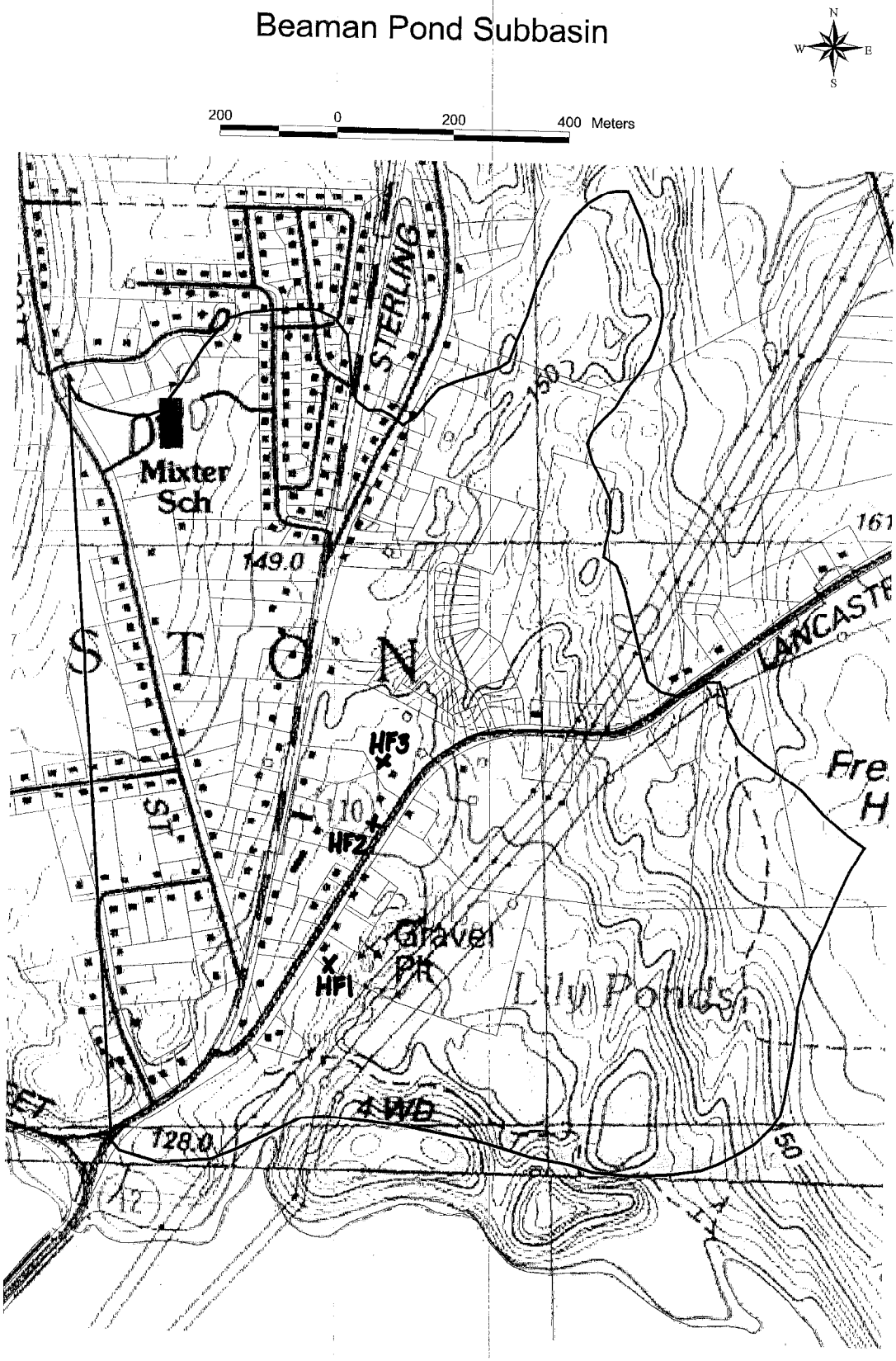
Site Locus Maps

Malagasco Brook Subbasin

200 0 200 400 600 800 Meters



Beaman Pond Subbasin



Appendix C

Statistical Correlation Table

	α				
n	0.20	0.10	0.05	0.02	0.01
3	0.951	0.988	0.997	1.000	1.000
4	0.800	0.900	0.950	0.980	0.990
5	0.687	0.805	0.878	0.934	0.959
6	0.608	0.729	0.811	0.882	0.917
7	0.551	0.669	0.754	0.833	0.875
8	0.507	0.621	0.707	0.789	0.834
9	0.472	0.582	0.666	0.751	0.798
10	0.443	0.549	0.632	0.715	0.765
11	0.419	0.521	0.602	0.685	0.735
12	0.398	0.497	0.576	0.658	0.708
13	0.380	0.476	0.553	0.634	0.684
14	0.365	0.458	0.532	0.612	0.661
15	0.351	0.441	0.514	0.592	0.641
16	0.338	0.426	0.497	0.574	0.623
17	0.327	0.412	0.482	0.558	0.606
18	0.317	0.400	0.468	0.543	0.590
19	0.308	0.389	0.456	0.529	0.575
20	0.299	0.378	0.444	0.516	0.561
25	0.265	0.337	0.396	0.462	0.505
30	0.241	0.306	0.361	0.423	0.463
35	0.222	0.283	0.334	0.392	0.430
40	0.207	0.264	0.312	0.367	0.403
45	0.195	0.248	0.294	0.346	0.380
50	0.184	0.235	0.279	0.328	0.361
100	0.129	0.166	0.197	0.233	0.257
200	0.091	0.116	0.138	0.163	0.180

Adapted from online source:

Pennsylvania State University. 2004. Correlation, Regression, and Outlier Points. World Wide Web: accessed: Feb. 11, 2004. <http://www.mne.psu.edu/me82/Learning/Stat_2/stat_2.html>.

Appendix D

ANOVA Analyses

Malagasco Brook Subbasin

Anova: Single Factor

Temperature

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MB1	5	34.6	6.92	30.812
MB2	5	37.85	7.57	59.557
MB3	5	38.1	7.62	54.082
MB4	5	38.8	7.76	54.418
MB5	5	41.5	8.3	55.14
MB6	3	20.2	6.733333	23.96333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7.040458	5	1.408092	0.029116	0.999526	2.661274
Within Groups	1063.963	22	48.36194			
Total	1071.003	27				

Anova: Single Factor

DO

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MB1	5	59.13	11.826	5.31183
MB2	5	56.69	11.338	5.98232
MB3	5	50.82	10.164	4.65023
MB4	5	52.7	10.54	4.28755
MB5	5	25.14	5.028	4.35517
MB6	3	8.66	2.886667	1.068133

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	276.8511	5	55.37022	12.12269	1.01E-05	2.661274
Within Groups	100.4847	22	4.567485			
Total	377.3358	27				

Anova: Single Factor

Conductivity

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MB1	5	1552.8	310.56	21476.38
MB2	5	750.3	150.06	2356.918
MB3	5	759.6	151.92	2428.292
MB4	5	849.7	169.94	10403.52
MB5	5	441.4	88.28	515.787
MB6	3	125.9	41.96667	207.6233

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	182170.4	5	36434.09	5.374521	0.002235	2.661274
Within Groups	149138.8	22	6779.038			
Total	331309.3	27				

Anova: Single Factor

pH

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MB1	5	34.27	6.854	0.02493
MB2	5	34.5	6.9	0.0939
MB3	5	33.4	6.68	0.09895
MB4	5	33.36	6.672	0.23082
MB5	5	29.79	5.958	0.22947
MB6	3	17.64	5.88	0.0637

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.325606	5	0.865121	6.702398	0.000621	2.661274
Within Groups	2.83968	22	0.129076			
Total	7.165286	27				

Anova: Single Factor

UV_{254}

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MB1	5	3.6394	0.72788	0.287311
MB2	5	5.58145	1.11629	0.486777
MB3	5	5.64575	1.12915	0.607476
MB4	5	6.50235	1.30047	0.688619
MB5	5	10.2634	2.05268	0.479551
MB6	3	4.64975	1.549917	0.537038

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.970817	5	0.994163	1.940173	0.128216	2.661274
Within Groups	11.27301	22	0.51241			
Total	16.24383	27				

Anova: Single Factor

TOC

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MB1	5	72.227	14.4454	96.05624
MB2	5	109.173	21.8346	157.9197
MB3	5	113.263	22.6526	197.5726
MB4	5	125.043	25.0086	221.4937
MB5	5	194.27	38.854	136.5873
MB6	3	106.265	35.42167	247.9842

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1905.516	5	381.1033	2.245094	0.085721	2.661274
Within Groups	3734.486	22	169.7494			
Total	5640.003	27				

Anova: Single Factor

DOC

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MB1	5	71.577	14.3154	93.03494
MB2	5	107.098	21.4196	169.8827
MB3	5	108.859	21.7718	204.202
MB4	5	122.241	24.4482	221.1813
MB5	5	187.29	37.458	127.733
MB6	3	101.13	33.71	206.4972

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1692.013	5	338.4027	2.024638	0.114629	2.661274
Within Groups	3677.13	22	167.1423			
Total	5369.143	27				

Anova: Single Factor

Turbidity

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MB1	5	5.923	1.1846	0.435983
MB2	5	13.13	2.626	3.741355
MB3	5	16.0425	3.2085	8.426455
MB4	5	9.89	1.978	0.336107
MB5	5	13.9025	2.7805	2.994483
MB6	3	5.89	1.963333	2.196633

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	12.88658	5	2.577316	0.832237	0.540696	2.661274
Within Groups	68.1308	22	3.096855			
Total	81.01738	27				

Anova: Single Factor

Particles

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MB1	5	13036.82	2607.364	2059753
MB2	5	22212.08	4442.416	10419407
MB3	5	27914.89	5582.978	13351787
MB4	5	20697.87	4139.573	2045511
MB5	5	25227.22	5045.443	11604326
MB6	3	12580.23	4193.408	1766027

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	25540332	5	5108066	0.696029	0.632034	2.661274
Within Groups	1.61E+08	22	7338872			
Total	1.87E+08	27				

Anova: Single Factor

FC

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MB1	5	355.74	71.148	7171.176
MB2	5	2710.143	542.0287	1155985
MB3	5	2134.61	426.922	623271.9
MB4	5	1999.517	399.9033	506853.5
MB5	5	994.03	198.806	70171.31
MB6	3	755.59	251.8633	174676.5

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	732568.4	5	146513.7	0.328802	0.890126	2.661274
Within Groups	9803166	22	445598.4			
Total	10535734	27				

Anova: Single Factor

EC

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MB1	5	190.9167	38.18333	855.3008
MB2	5	1458.15	291.63	282784
MB3	5	3488.783	697.7567	2010898
MB4	5	3055.15	611.03	1442303
MB5	5	1161.623	232.3247	215345.7
MB6	3	49.00333	16.33444	215.4922

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1846165	5	369232.9	0.513823	0.762902	2.661274
Within Groups	15809173	22	718598.8			
Total	17655338	27				

Anova: Single Factor

Rhodo

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MB1	4	0	0	0
MB2	4	0	0	0
MB3	4	13	3.25	42.25
MB4	4	13	3.25	42.25
MB5	4	26	6.5	56.33333
MB6	2	0	0	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	130.5909	5	26.11818	0.989091	0.45477	2.85241
Within Groups	422.5	16	26.40625			
Total	553.0909	21				

Anova: Single Factor

Bifido

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MB1	4	4	1	4
MB2	4	11	2.75	30.25
MB3	4	16	4	64
MB4	4	50	12.5	502.3333
MB5	4	0	0	0
MB6	2	0	0	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	425.0227	5	85.00455	0.754862	0.594823	2.85241
Within Groups	1801.75	16	112.6094			
Total	2226.773	21				

Anova: Single Factor

Phage

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MB1	4	1	0.25	0.25
MB2	4	1.5	0.375	0.5625
MB3	4	9.5	2.375	16.89583
MB4	4	1	0.25	0.083333
MB5	4	0.5	0.125	0.0625
MB6	2	0	0	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	15.40341	5	3.080682	0.92025	0.493004	2.85241
Within Groups	53.5625	16	3.347656			
Total	68.96591	21				

Beaman Pond Brook Subbasin

Anova: Single Factor

Temp

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
HF1	6	49.65	8.275	25.24775
HF2	6	56.3	9.383333	15.50667
HF3	4	28.7	7.175	18.70917

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11.89401	2	5.947005	0.297465	0.747621	3.805567
Within Groups	259.8996	13	19.99228			
Total	271.7936	15				

Anova: Single Factor

DO

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
HF1	6	57.45	9.575	6.15635
HF2	6	63.25	10.54167	6.063817
HF3	4	36.63	9.1575	10.22263

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.237835	2	2.618918	0.370997	0.697121	3.805567
Within Groups	91.76871	13	7.059131			
Total	97.00654	15				

Anova: Single Factor

Cond

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
HF1	6	3949.6	658.2667	23695.07
HF2	6	3682	613.6667	15686.67
HF3	4	2286.7	571.675	16271.22

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	18367.74	2	9183.868	0.485875	0.625895	3.805567
Within Groups	245722.3	13	18901.72			
Total	264090.1	15				

Anova: Single Factor

pH

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
HF1	6	42.51	7.085	0.23399
HF2	6	41.85	6.975	0.14747
HF3	4	26.69	6.6725	0.083492

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.419719	2	0.209859	1.264345	0.31496	3.805567
Within Groups	2.157775	13	0.165983			
Total	2.577494	15				

Anova: Single Factor

UV_{254}

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
HF1	6	0.26395	0.043992	0.000513
HF2	6	0.2785	0.046417	0.000505
HF3	4	0.11715	0.029288	0.000225

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000778	2	0.000389	0.877141	0.439202	3.805567
Within Groups	0.005763	13	0.000443			
Total	0.00654	15				

Anova: Single Factor

TOC

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
HF1	6	11.65	1.941667	0.46512
HF2	6	11.88	1.98	0.805412
HF3	4	12.82	3.205	3.757302

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.64826	2	2.32413	1.714294	0.218389	3.805567
Within Groups	17.62457	13	1.355736			
Total	22.27283	15				

Anova: Single Factor

DOC

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
HF1	6	11.4065	1.901083	0.471024
HF2	6	9.608	1.601333	0.229674
HF3	4	4.5165	1.129125	0.067133

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.430513	2	0.715257	2.509748	0.119758	3.805567
Within Groups	3.704889	13	0.284991			
Total	5.135402	15				

Anova: Single Factor

Turb

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
HF1	6	6.3685	1.061417	0.766513
HF2	6	14.2175	2.369583	4.964631
HF3	4	32.87	8.2175	40.59848

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	131.9619	2	65.98096	5.701202	0.016685	3.805567
Within Groups	150.4511	13	11.57317			
Total	282.4131	15				

Anova: Single Factor

Particles

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
HF1	6	15480.45	2580.075	750406.1
HF2	6	22809.91	3801.651	813756.8
HF3	4	22402.17	5600.543	2048617

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	21896410	2	10948205	10.19045	0.002177	3.805567
Within Groups	13966666	13	1074359			
Total	35863075	15				

Anova: Single Factor

FC

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
HF1	6	502	83.66667	11191.87
HF2	6	1613	268.8333	288477.4
HF3	4	281	70.25	995.5833

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	136568.1	2	68284.04	0.59127	0.567846	3.805567
Within Groups	1501333	13	115487.1			
Total	1637901	15				

Anova: Single Factor

EC

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
HF1	6	278	46.33333	919.8667
HF2	6	1938	323	414088
HF3	4	1219	304.75	216048.9

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	272893.4	2	136446.7	0.651372	0.537534	3.805567
Within Groups	2723186	13	209475.9			
Total	2996079	15				

Anova: Single Factor

Rhodo

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
HF1	6	159	26.5	494.3
HF2	6	520	86.66667	14273.87
HF3	4	13	3.25	42.25

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	19393.42	2	9696.708	1.704222	0.220137	3.805567
Within Groups	73967.58	13	5689.814			
Total	93361	15				

Anova: Single Factor

Bifido

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
HF1	6	5	0.833333	4.166667
HF2	6	6	1	6
HF3	4	8	2	16

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.604167	2	1.802083	0.237036	0.792299	3.805567
Within Groups	98.83333	13	7.602564			
Total	102.4375	15				

Anova: Single Factor

Phage

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
HF1	6	0	0	0
HF2	6	0.5	0.083333	0.041667
HF3	4	0	0	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.026042	2	0.013021	0.8125	0.46506	3.805567
Within Groups	0.208333	13	0.016026			
Total	0.234375	15				

Combined Data

Anova: Single Factor

Temperature

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	113.75	9.479167	5.962481
Summer	7	117.95	16.85	4.2925
Fall	17	105.85	6.226471	14.28597
Winter	8	8.05	1.00625	1.941741

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1018.354	3	339.4513	40.71258	3.14E-12	2.838746
Within Groups	333.5101	40	8.337752			
Total	1351.864	43				

Anova: Single Factor

DO

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	106.12	8.843333	5.16077
Summer	7	51.56	7.365714	4.280929
Fall	17	146.54	8.62	12.3974
Winter	8	106.25	13.28125	5.22907

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	163.3197	3	54.43991	6.860387	0.000776	2.838746
Within Groups	317.4159	40	7.935398			
Total	480.7356	43				

Anova: Single Factor

Cond

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	4461.8	371.8167	73269.24
Summer	7	2535.2	362.1714	35674.01
Fall	17	4444.8	261.4588	65203.44
Winter	8	2956.2	369.525	80780.61

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	120252.3	3	40084.11	0.60994	0.612489	2.838746
Within Groups	2628725	40	65718.12			
Total	2748977	43				

Anova: Single Factor

pH

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	80.91	6.7425	0.10662
Summer	7	50.09	7.155714	0.233662
Fall	17	109.6	6.447059	0.261785
Winter	8	53.41	6.67625	0.301027

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.553379	3	0.851126	3.837992	0.016621	2.838746
Within Groups	8.870537	40	0.221763			
Total	11.42392	43				

Anova: Single Factor

UV_{254}

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	4.00445	0.333704	0.126848
Summer	7	3.9215	0.560214	0.857431
Fall	17	22.77005	1.339415	0.915359
Winter	8	6.2457	0.780713	0.456862

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7.892154	3	2.630718	4.315535	0.009969	2.838746
Within Groups	24.3837	40	0.609593			
Total	32.27586	43				

Anova: Single Factor

TOC

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	95.091	7.92425	55.70119
Summer	7	82.654	11.80771	263.658
Fall	17	458.088	26.94635	320.1258
Winter	8	120.758	15.09475	151.2534

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2886.321	3	962.107	4.594892	0.007427	2.838746
Within Groups	8375.448	40	209.3862			
Total	11261.77	43				

Anova: Single Factor

DOC

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	87.406	7.283833	49.94358
Summer	7	74.403	10.629	230.7889
Fall	17	444.25	26.13235	315.272
Winter	8	117.667	14.70838	154.1358

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2863.393	3	954.4643	4.738315	0.006394	2.838746
Within Groups	8057.416	40	201.4354			
Total	10920.81	43				

Anova: Single Factor

Turbidity

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	29.528	2.460667	2.461416
Summer	7	29.558	4.222571	9.854763
Fall	17	42.684	2.510824	16.04304
Winter	8	16.464	2.058	1.496253

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	20.81335	3	6.937785	0.785336	0.509193	2.838746
Within Groups	353.3666	40	8.834165			
Total	374.18	43				

Anova: Single Factor

Particles

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	50494.12	4207.843	1170838
Summer	7	46969.48	6709.925	16780272
Fall	17	51446.53	3026.266	2009327
Winter	8	33451.5	4181.437	1837847

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	67386487	3	22462162	5.666003	0.002482	2.838746
Within Groups	1.59E+08	40	3964376			
Total	2.26E+08	43				

Anova: Single Factor

FC

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	2335.96	194.6633	40390.65
Summer	7	7027	1003.857	924712.5
Fall	17	1842.58	108.3871	105463.7
Winter	8	140.09	17.51125	288.0554

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4785465	3	1595155	8.305927	0.000205	2.838746
Within Groups	7682008	40	192050.2			
Total	12467473	43				

Anova: Single Factor

EC

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	2867.81	238.9842	137848.3
Summer	7	7494.847	1070.692	1932050
Fall	17	2251.15	132.4206	151079.5
Winter	8	224.82	28.1025	951.5028

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5268309	3	1756103	4.522377	0.008014	2.838746
Within Groups	15532566	40	388314.1			
Total	20800875	43				

Anova: Single Factor

Rhodo

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	172	14.33333	500.7879
Summer	7	39	5.571429	48.28571
Fall	11	493	44.81818	9249.164
Winter	8	40	5	99.71429

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	10411.25	3	3470.415	1.192004	0.327415	2.882601
Within Groups	98988.02	34	2911.412			
Total	109399.3	37				

Anova: Single Factor

Bifido

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	5	0.416667	2.083333
Summer	7	10	1.428571	6.285714
Fall	11	77	7	197
Winter	8	8	1	8

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	300.2112	3	100.0704	1.630568	0.200481	2.882601
Within Groups	2086.631	34	61.3715			
Total	2386.842	37				

Anova: Single Factor

Phage

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Spring	12	4.5	0.375	0.278409
Summer	7	9.5	1.357143	9.97619
Fall	11	0	0	0
Winter	8	0	0	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	9.422462	3	3.140821	1.697211	0.186033	2.882601
Within Groups	62.91964	34	1.850578			
Total	72.34211	37				