Studies on water quality of aquaculture farms with an emphasis on *Flavobacterium psychrophilum* and UV treatment

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Abstract

Flavobacterium psychrophilum, the causative agent of Coldwater Disease, affects salmonid fish in aquaculture operations worldwide and causes skin lesions which if left untreated results in spinal deformities, spiral swimming and eventual death. Currently, there are no available vaccines for Coldwater Disease. The use of antimicrobials is limited to reduce the potential development of antibacterial resistance in bacteria. This study examined the association between *F. psychrophilum* and water quality parameters (specifically dissolved oxygen and nitrite) to determine what associations would be best to develop alternative management techniques. In addition, this study investigated the impact of ultraviolet disinfection on planktonic bacteria concentrations and biofilm development in the treatment of land-based aquaculture effluent.

The study locations were two commercial partial recirculation rainbow trout *Oncorhynchus mykiss* (Walbaum) aquaculture facilities located in Coldwater, ON and New Dundee, ON. At the Coldwater Fishery, water samples were collected monthly from March 2013 to October 2013 from 6 sampling locations and water quality parameters and planktonic bacterial densities were measured. At Lyndon Fish Hatchery, in New Dundee, samples were collected from June 2014 to August 2014 from 4 sampling locations and to measure water quality parameters, planktonic bacterial densities, biofilm bacterial densities and UV efficacy on planktonic bacterial load. A field study of biofilm growth was investigated by suspending glass slides on either side of a UV reactor connected to the effluent flow.

Results indicated that the abundance *F. psychrophilum* did not demonstrate a clear association with water quality parameters. The water quality parameters that had the strongest correlations with *F. psychrophilum* at Coldwater Fishery were PO_4, pH and NH_3. The water quality parameters that had the strongest correlations with *F. psychrophilum* at the Lyndon Fish hatchery were NO_2^-, TKN and Temperature. The concentration of biofilm heterotrophic bacteria and *F. psychrophilum* remained similar at the UV influent and UV effluent areas despite reductions in the planktonic bacterial density.

Lay Summary

The mission statement of Lakehead University's Department of Biology is "Faculty and students in the Department of Biology are bound together by a common interest in explaining the diversity of life, the fit between form and function, and the distribution and abundance of organisms." The current study focuses on *Flavobacterium psychrophilum*, which is a bacterial fish pathogen causing Coldwater Disease in salmonid fish. This study contributes to one of the central research themes outlined in the mission statement, the relationship between life forms and their environmental functions. The study advances our understanding of the association between *F. psychrophilum* and water quality parameters in land-based fish farms. Understanding this relationship may provide a valuable tool in helping control the spread of this disease. In addition, the study advances our understanding of the effect of ultraviolet irradiation on the growth and development of biofilm in land-based fish farms. Two major research questions were investigated. 1. What association exists between *F. psychrophilum* and water quality parameters? 2. Are surviving bacteria able to form a biofilm in ultraviolet irradiated water? When the farms were examined separately there were some meaningful relationships between water quality and *F. psychrophilum*. The water quality parameters that had the strongest correlations with *F. psychrophilum* at Coldwater Fishery were PO_4, pH and NH_3. The water quality parameters that had the strongest correlations with *F. psychrophilum* at the Lyndon Fish hatchery were NO_2^-, TKN and temperature. Furthermore, the study demonstrated that biofilm growth in ultraviolet-treated water was not significantly lower than non-treated water. UV treatment reduced planktonic densities of heterotrophic bacteria and *F. psychrophilum*, however, the concentration of attached heterotrophic bacteria and *F. psychrophilum* was not different between the UV influent and UV effluent areas.

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List of Abbreviations

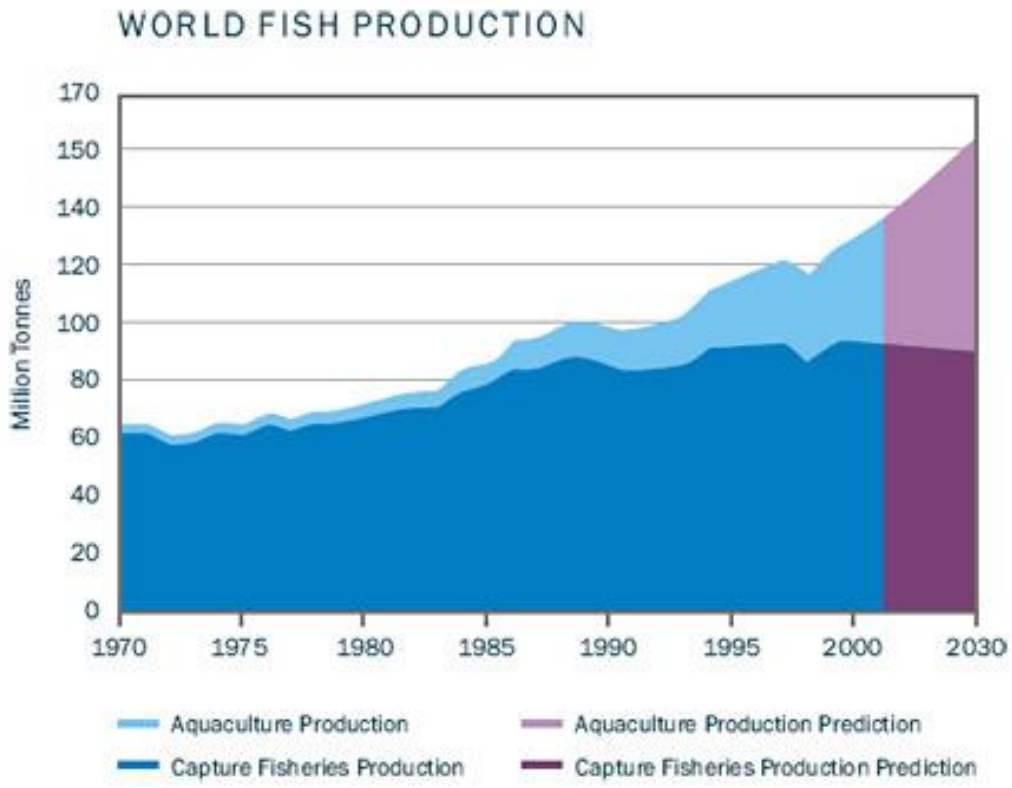
ANOVA	One-Way Analysis of Variance
ATCC	American Type Culture Collection
BOD	Biological Oxygen Demand
CAWT	Centre for Alternative Waster Treatment
CFU	Colony Forming Units
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
FTS	Flow-Through Systems
MDPE	medium density polyethylene
NH_3	Ammonia
NO_2^-	Nitrite
NO_3^-	Nitrate
OMAFRA	Ontario Ministry of Agriculture, Food and Rural Affairs
PO_4	Dissolved Phosphate
PVC	Polyvinyl Chloride
RAS	Recirculating Aquaculture Systems
TKN	Total Kjeldahl Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
uPVC	Unplasticised Polyvinyl Chloride
UV	Ultraviolet
UVT	Ultraviolet transmittance
VIF	variance inflation factor

Chapter 1 Literature Review

1.1 Introduction

Aquaculture is defined as the farming of fish, mollusks, crustaceans and aquatic plants. More specifically, it implies the managed production of fish with "some form of intervention in the rearing process to enhance production" (Fisheries, F. A. O. Aquaculture Department, 2012). Different techniques and forms of aquaculture have been known and used for centuries. The earliest records of aquaculture arise from China; although its exact origins are unknown (Rabanal, 1988). Presently, there are roughly 600 species farmed in aquaculture in 190 countries (Fisheries, F. A. O. Aquaculture Department, 2012). During the last five decades, 1961-2009, world fish supply had an average growth rate of 3.2 percent per year, reaching nearly 148 million tonnes in 2010 (Fisheries, F. A. O. Aquaculture Department, 2012). Aquaculture, the fastest growing food producing industry in the world, provides 3 billion people with roughly 20 percent of their animal protein intake (Fisheries, F. A. O. Aquaculture Department, 2012). From 2006 to 2010 world capture fisheries remained relatively stable around 90 million tonnes, while there was continual growth in farmed production from 47.3 million tonnes in 2006 to 59.9 million tonnes in 2010 (see Figure 1) (Fisheries, F. A. O. Aquaculture Department, 2012). By 2020, the United Nations Food and Agriculture Organization predicts the contribution of aquaculture to global supply will surpass that of capture fisheries; illustrating the potential for production.

Figure 1: Graph illustrating aquaculture and capture fisheries production from 1970-2010 with predicted growth until 2030 (http://www.aquaculture.ca/files/opportunity-expansion.php)



As with livestock farming, aquaculture has many impacts on the environment. In order to continue meet the increasing demand for aquaculture production, facilities will need to become more intensive without compromising environmental conditions on and off the facility.

1.2 Aquaculture in Ontario

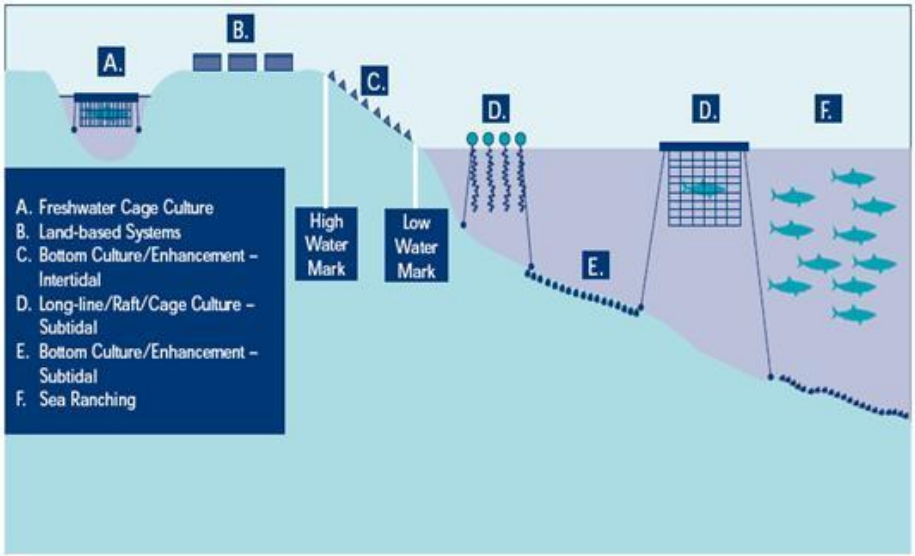
There are 37 species of finfish permitted for aquaculture production in Ontario (Statistics Canada, 2010), however, the industry is dominated by the production of rainbow trout (*Oncorhynschu mykiss*), representing 97% of the fish produced (Canadian Aquaculture Systems Inc., 2009). Other species cultivated that account for a small fraction of the total production include Artic charr (*Salvelinus alpinus*), Nile tilapia (*Oreochromis niloticus)* and brook trout (*Salvelinus fontinalis*) (Statistics Canada, 2010).

Aquaculture facilities within Ontario differ in their output objectives. For example, not all sites are producing fish for direct human consumption. Some sites raise fish for the stocking of Ontario lakes and rivers. It is imperative to ecosystem health that such farms are providing healthy fish into the natural system to help reduce potential negative impacts on wild populations. The Ontario Ministry of Natural Resources has ten hatcheries for the stocking of salmonids in the Great Lakes Watershed (Good, Thornburn and Stevenson, 2008).

1.3 Production Systems

There are three main systems of finfish aquaculture: open, semi-closed and closed (Figure 2). An open system is one in which the farmed species are in direct contact with the environment and farmed at densities typically found in nature (see F in Figure 2). In a semi-closed system the species are also in direct contact with the environment, but at densities exceeding those found in nature (see C-D in Figure 2 and Figure 3) (Flimlin, Buttner, and Webster, 2008).

Figure 2: Schematic of Aquaculture Production Systems (http://www.dfo-mpo.gc.ca/aquaculture/lib-bib/nasapi-inpasa/nasapi-inpasa-eng.htm)



In Canada the only large-scale commercially operating salmon fisheries are net-pen systems (a semi-closed system) as shown in Figure 3 (Ayer and Tyedmers, 2009).

Figure 3: Images of semi-closed, net pen systems (right image http://www.aquaculture.ca/files/opportunity-expansion.php, left image http://www.dfo-mpo.gc.ca/aquaculture/RD2007/rdfreshwater-dulcaquicole_02-eng.htm)

The third type of production system, closed, is one in which the species farmed is not in contact with the natural environment (B in Figure 2 and Figure 4). Instead, the fish are reared in an environment that can be closely monitored and manipulated.

1.3.1 Land-Based Production Systems

Land-based aquaculture production systems provide fish farmers' greater control over fish rearing conditions and environmental impact. For example, land-based facilities provide the advantage of the ability to capture and treat effluent leaving the farm whereas this is not possible within open or semi-closed systems (Snow et al., 2012). In Ontario, water used for land-based systems is typically accessed from either ground or surface sources, but can also be accessed from municipal sources depending on the quantities utilized (Moccia and Bevan, 2005). Closed systems can also be broken down further into two sub categories – flow through systems and recirculating systems.

Figure 4: Photographs from Coldwater Fishery of indoor raceway (left) and indoor tanks (right).


1.3.2 Flow-Through Systems

Flow-through systems (FTS) require high amounts of water which get pumped into the system and are discharged after a single cycle through the system. FTS create an environment where tank water contains relatively low levels of organic matter and bacterial numbers (Attramadal et al., 2012a). The main drawback of a FTS is the high volume of water needed to function which places great demand on local water resources (Snow et al., 2012). Due to the large volume of water movement throughout a FTS, effluent is characterized as high volume with "very dilute waste" (Tello et al., 2010). Despite this dilution, effluent from flow-through facilities can still cause negative environmental impacts on the receiving environments if left untreated (Snow et al., 2012).

1.3.3 Recirculating Systems

Recirculating aquaculture systems (RAS's) require small amounts of input water as water is treated after each cycle and reused. Solids must be removed from the system prior to reuse, generating effluent characterized as low volumes of concentrated waste (Tello et al., 2010). Water is treated to remove contaminants such as pathogens from fish excretions and uneaten food. Inefficient removal of wastes and pathogens leads to a reduction of water quality and fish health as pollutants accumulate within the system (Bartoli et al., 2007). The increased retention of water within a RAS has led to several defining features; such as, the

stabilization of the microbial community, increased ability to control water quality parameters, and reduced possibilities of pathogenic intruders from intake water (Attramadal et al., 2012a). Attramadal et al. (2012a) has proposed that the stabilization of the microbial community within a RAS is attributed to either the retention time of water within the system or due to the relatively stable organic content of the water, whereas the organic content of the water in a FTS varies and fluctuates with the intake water. RAS's, in theory, provide the operator of a farm greater control over the rearing environment, thus providing the farmed species optimal rearing conditions year round (Rurangwa and Verdegem, 2015). This, however, comes with a drawback: RAS's require constant monitoring and have high operational costs (Badiola et al., 2012).

1.4 Water Quality

Land-based aquaculture facilities provide farmers with the ability to monitor, capture, and treat effluent prior to release into the environment or re-use by the facility (Bartoli et al., 2007). Wastewater treatment's principle concern is removing pathogens and excess nutrients that cause a decline in water quality. Wastewater treatment is of utmost importance in RAS's because it provides farmers a means to prevent the buildup of pathogens and waste; therefore decreasing the prevalence of infection and disease. In addition, treatment allows for a reduction in the transmission of diseases within an aquaculture facility (Crane and Hyatt, 2011). Table 1 describes the range of values for commonly measured water quality parameters that are required for the growth and survival of rainbow trout. The range of growth is the optimal rearing conditions required for growth and maintenance. The range of survival described in Table 1 indicates the thresholds beyond which rainbow trout can't live, according to Molony (2001), but not all research conducted on the tolerance of rainbow trout for variation in water quality is consistent. Davidson et al. (2014) conducted a controlled study to establish a chronic nitrate nitrogen threshold for juvenile rainbow trout by comparing the effects of high nitrate (80-100 mg/L) and low nitrate (20-40mg/L) on fish health.

They found that the growth rates between the two treatments were not significantly different (Davidson et al., 2014). The authors did observe side swimming behavior in both treatments. Side swimming is a primary health concern for cultured rainbow trout. Rainbow trout in the high nitrate treatment had a significantly greater percentage exhibiting side swimming behavior (Davidson et al., 2014). Davidson et al. (2014) recommend 75mg/L nitrate as the upper limit for rainbow trout culture.

Table 1: Summary of the requirements for successful of water quality parameters growth and survival of rainbow trout (Molony 2001).

Parameter	Range for Growth	Range for survival
Temperature (°C)	10-22	<26.5
Salinity ($^{g}/_{kg}$)		0-30
pH	7.0-8.0	6.0-9.0
Dissolved Oxygen (mg L^{-1})	7.0	>5.0
Ammonia (NH_3-N mg L^{-1})	<0.0125	<1.8
Nitrite (NO_2-N mg L^{-1})	<0.000012	<0.23
Nitrate (NO_3-N mg L^{-1})	<5.7*	<57*

*** (Westin, 1974.)**

1.5 Bacteria

Bacteria are ubiquitous in aquatic environments. Rearing fish in densities up to 1000 times greater than that found under natural conditions (Pulkkinen et al., 2010) allows for the buildup of bacteria (Moriarty, 1997). The carrying capacity for heterotrophic bacteria is determined by the supply of organic matter and increases with increased fish density due to associated higher concentrations of organic matter from fish feed and waste (Blancheton et al., 2013). The main entry points of bacteria into a land-based facility are live feed, intake water, and with the introduction of new fish (Attramadal et al., 2012a; Blancheton et al., 2013; Rurangwa and Verdegem, 2015). There are a variety of routes a pathogen can enter

its host: through the gills, skin, or stomach (Nematollahi et al., 2003a). When examining bacterial infections in fish, it is important to understand the interactions between the fish, the pathogen and the environment (Austin and Austin, 1999). In addition, understanding of the spatial and temporal dynamics of bacteria within aquaculture facilities is vital for system management (Rurangwa and Verdegem, 2015).

1.5.1 *Flavobacterium psychrophilum*

Flavobacterium psychrophilum is the causative agent of cold water disease (CWD). CWD primarily affects rainbow trout and mortalities range from 10-70% (Nematollahi et al., 2003a). *F. psychrophilum* was initially restricted to North America; however, infections have been reported in several countries throughout the world, and from a range of salmonid and non-salmonid species (Orieux et al., 2011). Young fish, fry and fingerlings are the most susceptible to this disease, and as such CWD is also known as rainbow trout fry syndrome (RTFS). The pathogenicity of *F. psychrophilum* infections is only partially elucidated and there is limited information about the factors determining virulence and the events leading to infection (Nematollahi, et al., 2003b). The disease is most prevalent at temperatures under 16^{o}C (Starliper, 2011). The infection is characterized by one or more of the following physical characteristics; skin lesions on the peduncle and caudal area which if left untreated results in exposure of underlying muscle tissue and skeletal process, lethargic appearance, swimming high in the water column, spiral swimming and eventual spinal deformities (Cipriano and Holt, 2005; Starliper, 2011; Boyacioglu and Akar 2012). Early detection and treatment are vital in limiting the effects of *F. psychrophilum*, as horizontal transmission occurs between infected fish, carrier fish and healthy fish (Starliper, 2011; Long et al., 2014). There is no vaccine available for *F. psychrophilum* (Orieux et al., 2011; Long et al., 2014). Treatment is typically administered orally using florfenicol (Boyacioglu and Akar 2012). There are 4 antibiotic drugs approved for the use in salmonid farming in Canada: florfenicol, sulfadimethoxine plus ormetoprim, oxytetracycline hydrochloride and trimethoprim plus sulfadizazine powder (Health Canada, 2010).

A major concern however is the development of antibiotic resistance among the target and non-target organisms (Defoirdt, Sorgeloos and Bossier, 2011), and thus the use of antibiotics is restricted (Fisheries and Oceans Canada, 2011). Despite the availability and widespread use of control methods for *F. psychrophilum*, it continues to be a major problem in aquaculture (Oplinger and Wagner, 2013).

Barnes and Brown (2011) review *F. psychrophilum* pathogenesis and suggest that increased nutrient concentrations (specifically, levels of nitrite as high as 5 mg/L) enhance *F. psychrophilum* infection by playing a role in the attachment of *F. psychrophilum* to gill arches. Soltani and Burke (1995) examined the response of *F. psychrophilum* to fluctuating environmental conditions in temperature, pH and salinity. They concluded that there was a decline in growth of *F. psychrophilum* when temperatures were above 20°C, salinity was kept above 10 g/kg, and pH values were beyond 6-8 (Soltani and Burke, 1995). Oplinger and Wager (2013) investigated the use of osmotic and thermal shocks and rapid pH changes to control *F. psychrophilum*. The results indicated that sudden increases in temperature (≥55°C) might be able to kill *F. psychrophilum* however osmotic shock and changes in pH were ineffective in killing *F. psychrophilum* (Oplinger and Wager, 2013).

Strepparava et al. (2014) quantified *F. psychrophilum* from water samples from 22 Swiss farms and examined changes in water temperature, dissolved oxygen, pH and conductibility. This was a preliminary study assessing the use of quantitative real time PCR as a possible technique for quantifying *F. psychrophilum* in natural conditions. The authors noted this study "was neither planned nor powered to allow drawing any conclusions or making any interpretations about the disease distribution" (Strepparava et al., 2014). The results of this study indicated no clear correlation between the four environmental parameters and *F. psychrophilum*, but the authors did note that changes in two or more parameters seemed to correlate with the detection of *F. psychrophilum* (Strepparava et al., 2014). The authors did not put forward an explanation.

A comparison between *F. psychrophilum* density and environmental parameters has not been examined fully (Strepparava et al., 2014). Decostere et al. (1999) investigated the influence of water quality and temperature on the adhesion of

Flavobacterium columnare. They reported that elevated organic loads (2 g/L mixture of food residues and faeces collected from the bottom of a fish tank) increased the incidence of disease. In addition, they noted that high levels of nitrite (5 mg/L) enhanced the adhesion of *F. columnare* to fish tissue (Decostere et al., 1999).

Garcia et al. (2000) conducted infection trials of *F. psychrophilum* at two fish farms using fish from the same stock. The results failed to produce the same mortality rate at each location, and during one trial there was no effect at one of the locations (Garcia et al., 2000). The main difference between the two farms was the source of supplied water, one farm was fed spring water from an especially calcareous region and the other was receiving treated tap water, which the authors suggest as a possible explanation for the different results (Garcia et al., 2000).

1.6 Biofilm Development

Biofilms develop and form at the water/solid interface. The typical growth regime of biofilm development is a cyclic succession which begins with planktonic bacteria attaching to a surface, followed by the proliferation of bacteria, and lastly maturation and dispersal (Sundell and Wiklund, 2011; Wietz et al., 2009). Biofilm formation is a cyclic succession of attachment and detachment of micro flora present in the water column and biofilm. Sessile cells of biofilms differ from their planktonic cells in a number of ways due to the response of the microorganisms during biofilm formation (King et al., 2001). There are modifications in growth rate, cellular enzyme activity, and cell wall composition noted in some bacteria (King et al., 2001). In aquaculture, biofilms can form on many of the components of the system including tank walls, piping and treatment devices (Rios-Castillo et al., 2011). Kerr et al. (1998) compared biofilm bacterial density and heterotrophic bacterial diversity on three different pipe materials in a controlled lab study. The pipe material they examined was cast iron, medium density polyethylene (MDPE), and unplasticised polyvinyl chloride (uPVC) (Kerr et al., 1998). They reported that MDPE and uPVC pipe supported a reduced biofilm in relation to the cast iron pipe,

and suggested the pitted surface of the iron may support greater numbers of bacteria (Kerr et al., 1998). Wietz et al. (2009) demonstrated significant differences between bacterial communities forming on glass slides and on the fiberglass tank walls in seawater aquaculture using denaturing gradient gel electrophoresis community profiling. They suggested the variations were likely due differences in surface chemistry between the two substrates favouring the growth of different populations (Wietz et al., 2009).

Biofilms, composed of various micro floras present in the water (Rios-Castillo et al., 2011), are capable of providing a "protective matrix that allow specific species to colonize and survive in an otherwise unfavourable environment" (Bourne et al., 2006). Thus, within biofilms, it has been reported that bacteria are protected from agents added to the water, such as chemicals or antibiotics (Costerton, 1995). Pathogenic bacteria can enter the biofilm and be protected against treatment agents (Karunasagar et al., 1996). In addition, pathogenic bacteria incorporated within the biofilm can be periodically released into the water column and cause recurrent infections in fish (Rios-Castillo et al., 2011).

Karunasagar et al. (1996) reported that physical removal of biofilms is the best defense against removing unwanted bacterial populations from the biofilm. King et al. (2008) studied the response of biofilms to various sanitizers on different material in recirculating aquaria. The effectiveness of water, an alkaline cleanser, sodium hypochlorite, and peracetic acid were evaluated on Buna-N rubber, polyvinyl chloride (PVC), chlorinated PVC, glass, fiberglass and stainless steel (King et al., 2008). They determined that the type of material had no significant effect on the effectiveness of the sanitizers (King et al., 2008). Furthermore, they concluded that none of the sanitizers tested in the study were effective at biofilm removal when used independently, and suggested future research is needed in developing ways to prevent the introduction of pathogen into an aquaculture facility to limit the need for sanitation methods (King et al., 2008).

Schwartz et al. (2003) examined biofilm formation within a drinking water distribution system to study the influence of ultraviolet disinfection versus chemical disinfection (chlorine dioxide) on biofilm formation. They excluded the influence of

pipe material (hardened polyethylene, polyvinyl chloride, steel and copper) on bacterial density (Schwartz et al., 2003). Schwartz et al. (2003) reported bacteria were able to regenerate and increase density more effectively in ultraviolet treated water than water treated with chlorine dioxide. They suggested this may be owing to some bacteria's ability to repair DNA damages caused by ultraviolet irradiation, but unable to repair after chloride dioxide (Schwartz et al., 2003).

1.7 Ultraviolet Disinfection

Ultraviolet (UV) disinfection is a physical disinfection method (Attramadal et al., 2012b) that provides treatment without harmful byproducts (Mamane et al., 2010; Liberti, Notarnicola and Petruzzelli, 2002; Litved, Hektien and Efraimsen, 1995). UV irradiation is a widely used water disinfection technique as it is able to provide a safe alternative to chemical disinfectants when treating wastewater (Bullock et al, 1997; Liberti, Notarnicola and Petruzzelli 2002). UV irradiation disinfects the incoming water by inactivating microorganisms. These microorganisms are inactivated by UV irradiation damaging their DNA, which prevents them from replicating (Summerfelt, 2003). The efficacy of UV disinfection is dependent on the UV dose (Gullian et al., 2012). UV dose (expressed in mJ/cm^2) is calculated by multiplying the UV intensity (in mW/cm^2) by the exposure time (in seconds) (Gullian et al., 2012). The dose recommended for aquaculture is $30mJ/cm^2$ (Sharrer et al., 2005; Liltved et al., 1995). Several studies have evaluated UV performance in recirculating aquaculture facilities (Mamane et al., 2010; Sharrer et al., 2005; Zhu et al., 2002). These articles concluded that successful treatment of water in aquaculture facility prior to reuse relied on the amount of suspended particles in the water and associated transmittance, and that flow rates were adequate in meeting exposure times required for disinfection (Mamane et al., 2010; Sharrer et al., 2005; Zhu et al., 2002). Thus there are several factors that affect UV performance such as fouling, dissolved organics and inorganics, clumping of microorganisms, turbidity, UV transmittance and power outages (Harley et al., 2008).

Pozos et al. (2004) compared the concentration of heterotrophic bacteria in a biofilm in a model potable water distribution systems receiving UV irradiated influent to that of control with no disinfectant. They determined that at a dose of 106mJ/cm^2 was insufficient to significantly lower the concentration of heterotrophic bacteria in the biofilm receiving UV irradiated influent (Pozos et al., 2004). They also examined the community of each biofilm using DNA fingerprinting, and determined that the communities were nearly indiscernible from each other for one of the trials (Pozos et al., 2004). The explanation offered by Pozos et al. (2004) for the similarities between the two communities was bacteria being shielded by particles. Differences in the two communities would have been expected if only UV resistant bacteria were surviving and attaching to the biofilm receiving UV irradiated water. Particles shield indiscriminately and therefore allowing for the two communities in each treatment to remain indiscernible from each other

1.7.1 Collimated Beam Testing

Collimated beam testing is designed to assess the susceptibility of bacteria to UV irradiation in a controlled manner (Gehr, 2007). A typical collimated beam apparatus is shown in Figure 5. Collimated beam tests provide dose response data that serve as the basis for establishing the UV dose for a particular species. Sensitivity of specific microbes to UV light is measured by exposure to a fixed intensity over varying exposure times. There are several features of the collimated beam apparatus and design that need to be taken into account when determining the delivered dose described by Equation 1:

Equation 1: UV Dose (Pirnie, Linden, and Malley, 2006)

$$D_{CB} = E_s P_f (1 - R) \frac{L\,(1-10^{-A_{254}\,d})}{(d+L)A_{254}\,d\ln(10)}\,t,$$

where D_{CB} is UV dose (mJ/cm^2), E_s is the average UV intensity (mW/cm^2), P_f is the Petri Factor (unitless), R is reflectance at the air-water interface at 254nm

(unitless), L is distance from lamp centerline to suspension surface (cm), d is depth of suspension (cm), A_{254} is UV absorbance at 254nm (unitless), and t is time exposed. The average UV intensity is measured by averaging the irradiance before and after each test using a radiometer (Pirnie, Linden, and Malley, 2006). The Petri Factor is a measurement of the uniformity of intensity and is a ratio that is equal to the average intensity measured across the surface area of a petri dish divided by the intensity at the center of a petri dish (Pirnie, Linden, and Malley, 2006). A spectrophotometer is used to measure the UV absorbance at 254nm (Pirnie, Linden, and Malley, 2006). The distance from the lamp to the surface of the suspension and the depth of the suspension are determined to account for the divergence of the UV light as it passes through the suspension (Pirnie, Linden, and Malley, 2006). The reflectance at the air-water interface is accounted for using Fresnel's Law (Pirnie, Linden, and Malley, 2006).

Hedrick et al. (2000) conducted collimated beam tests on *F. psychrophilum* examining 3 different doses (42, 126 and 252 mJ/cm^2) and one control. They determined that a dose of 42 mJ/cm^2 was ineffective at inactivating *F. psychrophilum* while doses of 126 and 252 mJ/cm^2 were effective doses (Hedrick et al., 2000).

1.7.2 Limitations of UV

The effectiveness of UV irradiation is highly dependent on the quality of water being disinfected (Harley, et al., 2008). UV transmittance (UVT) is the measurement of the amount of UV light transmitted through the water (Sharrer et al., 2005), and is an important factor in the disinfection of water. When the UV light is absorbed by the water itself or contaminants in the water it is no longer available to eliminate microorganisms (Sharrer et al., 2005). The presence and abundance of suspended solids in the incoming water has the ability to shield or protect microorganisms from inactivation (Gullian et al., 2012). The higher the UVT the more the light is able to penetrate the water. In addition to the water itself and characteristics of the water such as turbidity, there are other factors that limit the effectiveness of UV

disinfection. Some microorganisms have the ability to repair the damage caused by UV irradiation (Bohrerova and Linden, 2007). In addition, biofouling, or the buildup of algal or bacterial growth on the UV lamps, can significantly limit the effectiveness of UV (Bullock et al., 1997).

1.8 Knowledge Gap

There is a limited amount of published research examining the relationship between water quality parameters and *F. psychrophilum* growth.

Land-based farms may have the ability to control, to some extent, water quality parameters. The association between *F. psychrophilum* and different water quality parameters in land-based farms is unknown. Studies on the relationship between water quality parameters and bacterial growth could help determine whether an association between *F. psychrophilum* and water quality parameters could be taken advantage of to help control the spread of *F. psychrophilum*.

There is no published research examining the effect of effluent UV irradiation on biofilm development in an aquaculture setting. Biofilms form on many different parts within an aquaculture facility and with an increasing use of UV as a means of disinfection (Summerfelt, 2003) it is important to determine how UV irradiation of process water affects the growth and development of biofilms. Furthermore, *F. psychrophilum* is capable of adhering and forming biofilms (Decostere et al., 1999) but there is no information about the survival of *F. psychrophilum* after UV treatment and the subsequent development of biofilm in UV-treated water.

1.9 Specific Aims and research rationale

The work described in this thesis was part of a larger project funded by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). The project, titled "Innovative approaches to water treatment for land-based fish farms for recirculation and discharge", focused on four wastewater treatment technologies (an iron/aluminum oxide phosphorus sorption media, an ultrafiltration membrane,

a fixed film biofilter and UV disinfection) for use in land-based aquaculture.

As mentioned above, diseases are the major constraint on aquaculture production worldwide (Moriarty, 1997). Despite the availability and widespread use of control methods for *F. psychrophilum*, it continues to be a recurrent problem in the Ontario aquaculture industry. This suggests that research on control methods is required to mitigate the spread of *F. psychrphilum*. Elucidating what, if any, association exists between water quality parameters and *F. psychrophilum* densities could shed light on an effective means to prevent its proliferation.

UV disinfection is becoming a widely used treatment method in the aquaculture industry due to its ability to inactivate microorganisms without producing harmful by-products (Summerfelt, 2003). The second aim of this thesis was to determine the efficacy of UV treatment in eliminating *F. psychrophilum* from an aquaculture facility and examine how UV disinfection affects biofilm development. The potential of biofilms to harbor detrimental bacteria in aquaculture facilities highlights the importance to restrict the load of potential unfavorable microorganisms in the water column and on surfaces (Wietz et al., 2009), stressing the need to study management techniques for biofilm control.

This project attempts to determine the relationship between planktonic *F. psychrophilum* and water quality parameters, and to examine the ability of UV to inactivate *F. psychrophilum* under both laboratory and field conditions.

To accomplish the above the study was divided into 4 objectives:

1. Investigate the relationship between planktonic *F. psychrophilum* and water quality parameters in land-based aquaculture facilities.
2. Determine the UV inactivation of planktonic forms of *F. psychrophilum* under field conditions.
3. Determine the UV inactivation of attached (biofilm) forms of *F. psychrophilum* under field conditions.
4. Generate a UV dose response for a pure culture of *F. psychrophilum* by performing a standardized collimated beam test in the laboratory.

Hypothesis Tested

The hypotheses tested were:

1. If planktonic *F. psychrophilum* has an association to water quality then abundance will depend on nutrient loads. We expect elevated levels of nitrite and dissolved oxygen will coincide with an increase in planktonic *F. psychrophilum* abundance.
2. If planktonic bacteria survive UV irradiation then the biofilm in the inflow and outflow of treated effluents will have similar bacterial densities.

The following part of the thesis is divided into four chapters. Chapter 2 describes the methodology used in this study. Chapter 3 focuses on *F. psychrophilum* and total heterotrophic bacteria abundance in relation to water quality parameters. Chapter 4 evaluates UV irradiation as a method for the control for planktonic and attached *F. psychrophilum*. Lastly, Chapter 5 is a summary.

Chapter 2 Methodology

2.1 Aquaculture Production and Facility Design

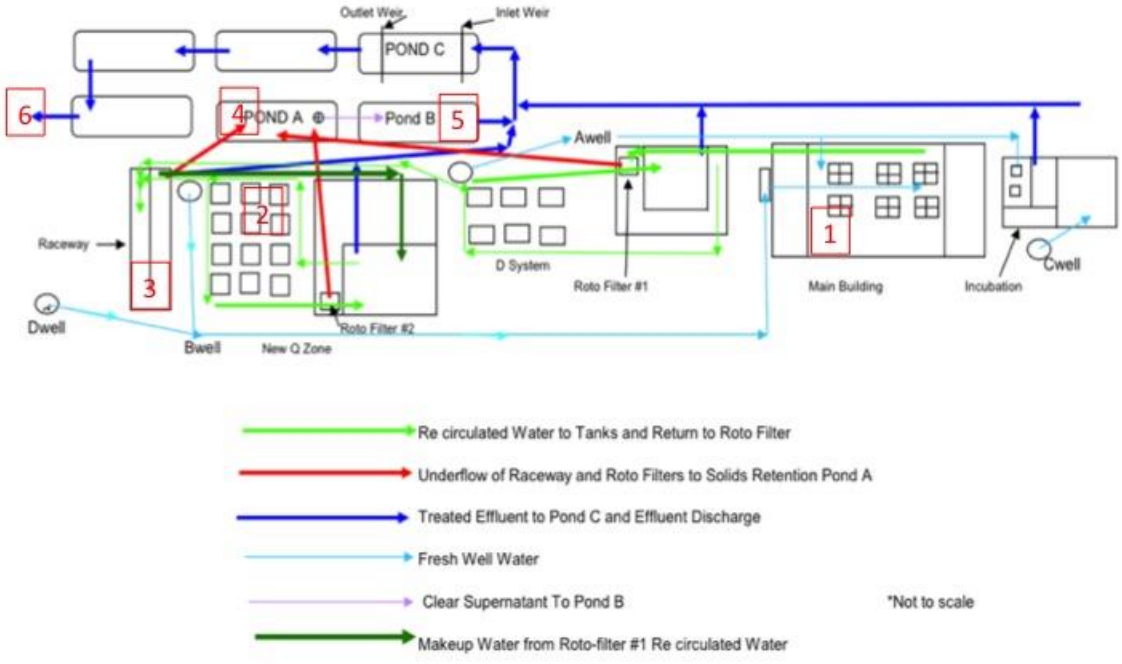
All field measurements described in this thesis were conducted at two aquaculture facilities: Coldwater Fishery, Coldwater ON and Lyndon Fish Hatchery, New Dundee, ON. These facilities are both partial reuse systems and both had had previous confirmed *F. psychrophilum* infections.

2.1.1 Coldwater Fishery

The Coldwater Hatchery is a commercial partial recirculation aquaculture facility located in Coldwater, ON, farming rainbow trout, *Oncorhynchus mykiss* (Walbaum). The farm draws between 1.8 and 1.9 million litres of water per day from four wells throughout its property. This water is not treated, heated nor cooled prior to entry into the system. The farm design houses an indoor raceway and a series of outdoor tanks that are partial re-use, with roto filters and treatment ponds as their main method of wastewater treatment prior to release. Within this system 100% fresh water is used in the early rearing sector. After this, water moves into the advanced rearing system loop and is reused 3 times after solids are removed each time. After solid are removed a small amount of new water is added to the loop mainly for oxygen injection (Figure 5).

Figure 5 shows the sampling locations: well water (1), an outdoor tank (2), the indoor raceway (3), the concentrated drum filter effluent (4), the process water (5) and the treated water leaving the farm (6). Table 2 outlines each sampling site and provides a brief description of each site.

Figure 5: Coldwater Facility Design showing sampling locations (not to scale)



Sampling location indicated are well water (1), an outdoor tank (2), the indoor raceway (3), the concentrated drum filter effluent (4), the process water (5) and the treated water leaving the farm (6).

Table 2: Coldwater sampling locations with brief description of site water

Site 1 Well water	Site 2 Outdoor Tank	Site 3 Indoor Raceway	Site 4 Concentrated Drum Filter Effluent	Site 5 Process Water	Site 6 Treated Effluent
100% freshwater from well and oxygenated	TD* = 4.16m x 4.16m x 1.21m FR* = 150-350 l/m Water is reused after solids removal and a bio-filter along with a small amount of fresh water (for oxygen injection). Fingerlings	TD=26.97m x 1.83m x .91m FR = 75m^3/hour Water is reused for the third time after solids removal and bio-filter Fingerlings	Solids that have come from the drum filters is deposited in this pond	Water that is moving from Pond A to pond B after settling.	Water that is leaving the farm.

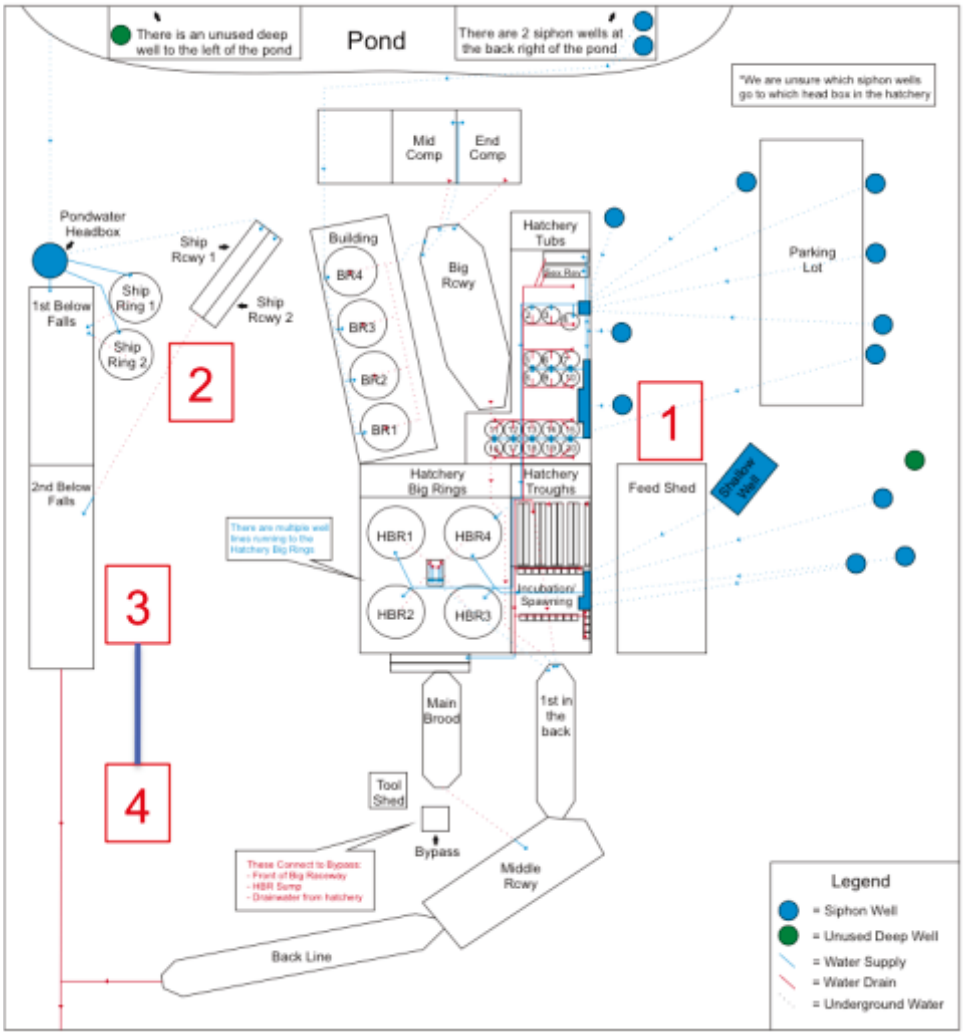
*TD = Tank dimension; FR= Flow rate

2.1.2 Lyndon Fish Hatchery

The Lyndon Fish Hatchery is a commercial aquaculture facility located in New Dundee, ON, farming rainbow trout, *Oncorhynchus mykiss* (Walbaum). The farm draws between 7.4 and 7.5 million litres of water per day from 8 wells and a surface pond. Approximately 2.8 million litres are from the surface pond and 4.6 million litres are from spring-fed wells. The fingerlings (3-4.5 months old) are raised in water from the spring-fed wells only. The farm raises approximately 2 million fingerlings annually. The spots indicated in Figure 6 are locations from where samples were collected: early rearing egg tray (1), an outdoor tank (2), UV influent (3), and UV effluent (4). Table 3 outlines each sampling site and provides a brief

description of each site.

Figure 6: Lyndon Hatchery Facility Design showing locations of sample collection (not to scale).



Sampling location indicated are early rearing egg tray (1), an outdoor tank (2), UV influent (3), and UV effluent (4).

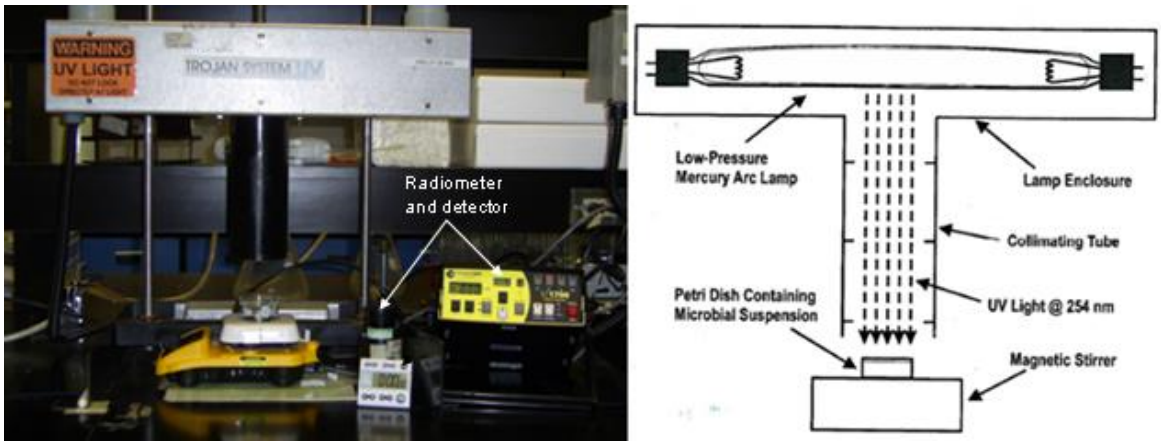
Table 3: Lyndon Fish Hatchery sampling locations with brief description of site water

Site 1 Egg Tray	Site 2 Outdoor Tank	Site 3 UV Influent	Site 4 UV Effluent
100% freshwater from well water.	Tank receives water from outdoor pond. Fish in this tank are deemed slaughter fish (at their last spawning). Fish range in age between 2.5 to 8 years of age.	Lyndon farm effluent pre UV treatment	Lyndon farm effluent post UV treatment

2.1.3 Collimated Beam Apparatus

The collimated beam apparatus (Figure 7) was an in-house designed fixture within the Trojan Technologies Micro Lab consisting of a 20 Watt low pressure UV lamp which emits UV light at a wavelength of 254nm. The UV lamp is centered in an aluminum housing directly over a collimating tube. The distance from the lamp to the end of the collimating tube is 26.65cm and the distance from the end of the collimating tube to the samples surface is 7.30 cm. A 60 x 35 mm glass petri dish containing a 3 x 10 mm stir bar is used to hold the microbial suspension, which is centered on a magnetic stirrer.

Figure 7: Collimated beam apparatus at Trojan Technologies Microbiology Laboratory left and diagram of assembly right.



2.1.3.1 Collimated Beam Trials

American Type Culture Collection (ATCC) certified pure strain #49418 of *F. psychrophilum* was exposed to five different UV doses (25, 50, 75, 100 and 125 mJ/cm^2) and also one control in duplicate in order to determine the inactivation of *F. psychrophilum* (See Equation 2). These doses were selected based on the findings by Hedrick et al. (2000). The results showed inactivation of *F. psychrophilum,* therefore another set of tests was conducted to determine the threshold of UV dose required to inactivate *F. psychrophilum*. The UV doses selected for the second set of tests were 1, 2, 3, 4 and 5 mJ/cm^2 and one control in order to determine the threshold of inactivation of *F. psychrophilum.* Pirnie, Linden, and Malley (2006) suggest duplicate irradiations and duplicate plating for each dose. The UV intensity was measured with an IL 1700 radiometer and the Petri Factor was determined. The UV Transmittance of the *F. psychrophilum* working stock was measured using a Real Tech RealUVT 254nm P200 photometer. Log inactivation, log *I*, was calculated according to Equation 2:

Equation 2: Log inactivation (Pirnie, Linden, and Malley, 2006)

$$\log I = \log\left(\frac{N_0}{N}\right),$$

where N_0 is the initial concentration of microorganisms (cfu/mL), and N is the concentration of microorganism after exposure to UV light (cfu/mL).

Cytophaga agar was used to enumerate *F. psychrophilum* in duplicate after each UV dose. The spread plate technique was used and plates were incubated at 15°C for 7 days.

2.1.4 UV Irradiation System

An open channel UV system, TrojanUV3000 Package Treatment Plant pilot system, equipped with two 64" 80-Watt G64Y5L germicidal UV lamps was installed at the Lyndon Fish Hatchery (Figure 8, see Appendix for details of the apparatus). Trojan Technologies (the equipment supplier) estimated the reduction equivalent dose delivery from the UV system while in clean condition with new lamps, a nominal flow rate of 40 USGPM and the prevalent site UVT (typically 95%UVT) to be approximately 60 mJ/cm^2 based upon prior work using MS2 bacteriophage as a challenge organism (Hijnen et al., 2006).

The compartment on either side of the UV reactor where the slides were immersed had the following dimensions 14'' depth, 8.5'' width, and a length of 14''.

Figure 8: Photograph of the installed UV lamp at Lyndon Hatchery.

2.2 Water Analyses

2.2.1 Bacterial Analyses

Aseptic procedures were used throughout the sampling of this study to collect and analyze bacteria. Water samples were collected in 250ml sterile bottles and plated within 24hr.

Serial dilutions using 1ml of sample water and 9ml of sterilized distilled water were made and then standard duplicate spread plating techniques were used.

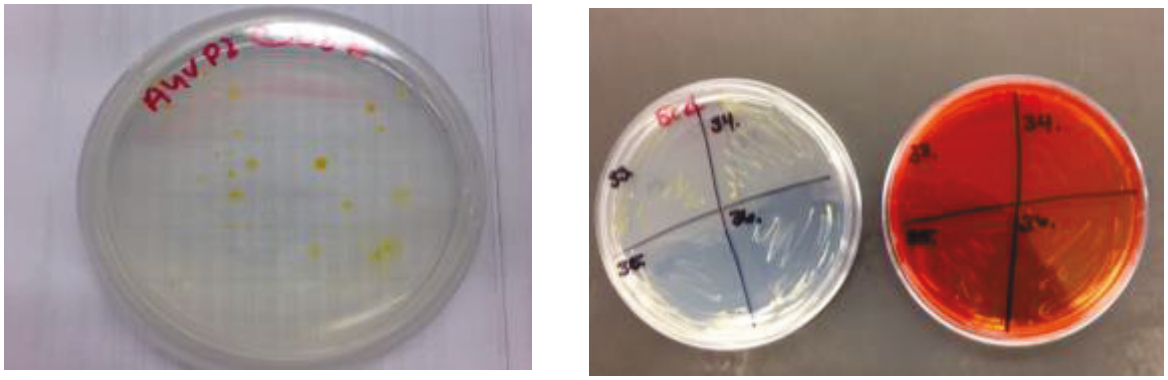
2.2.2 Heterotrophic Bacteria

Bacteria were isolated using R2A agar medium (Difco). R2A agar is a low-nutrient medium used for viable culturable bacterial count and isolation of bacteria from aquatic environment (Starliper, 2008). The medium is composed of each 0.5 grams of yeast extract, meat peptone, casamino acids, glucose and starch, 0.3 grams of each di-potassium hydrogen phosphate, sodium pyruvate and 0.05 gram of magnesium sulphate and 15 grams of agar per litre. The plates were incubated at 25 ^{o}C for 72 hrs. Plates containing 30-300 colonies were counted.

2.2.3 *Flavobacterium psychrophilum*

Flavobacterium spp. was enumerated on cytophaga agar (Hardy Diagnostics). This is a low nutrient media designed for the cultivation and maintenance of Cytophaga-Flavobacterium-Bacteriodes group. The medium is composed of 0.5 grams of casein peptone, yeast extract, and 0.2 grams of beef extract and sodium acetate and 15 grams of agar per litre. Standard spread plating technique was used. The plates were incubated at 15 ^{o}C for 7 days. On cytophaga agar *F. psychrophilum* colonies appear as bright yellow colonies with thin spreading margins (Barnes and Brown, 2011) (Figure 9, left).

Figure 9: Photograph of growth on cytophaga agar (left) and streaks on selective cytophaga agar (middle) and Congo red cytophaga agar (right).



Presumptive colonies typical of *F. psychrophilum* were then streaked onto two media for confirmation, a commonly used method (Figure 9, right). Selective cytophaga agar and congo red cytophaga agar were used as a positive and negative test, respectively. Selective cytophaga media was prepared by adding 5 µg/mL of neomycin and 5 units/mL of polymyxin B (Madsen, Møller, Dalsgaard, 2005). Congo red cytophaga media was prepared by incorporating 100 µg/mL congo red into the media (Crump and Kay, 2008). Plates were incubated at 15 ^{o}C for 4 days (Crump and Kay, 2008).

2.2.4 Water Quality Parameters

Dissolved oxygen, pH and water temperature were measured *in situ.*

Dissolved oxygen and pH were measured using a hydro lab (VWR symphony) and water temperature was measured using a thermometer. Samples were collected in Sterile 1 L sample bottles at each site and sent to the Centre for Alternative Wastewater Treatment (CAWT) (Lindsay, ON) for analysis of conductivity, alkalinity, total Kjeldahl nitrogen (TKN), ammonia (NH_3), nitrite (NO_2^-), nitrate ($N0_3^-$), total phosphorus (TP), dissolved phosphate as phosphorus (PO_4), chemical oxygen demand (COD), biochemical oxygen demand -5 Day (BOD), and total suspended solids (TSS). Table 4 outlines the analytical methods used for each of the water quality parameters tested.

Table 4 Analytical Methods used by the Center for Alternative Wastewater Treatment for analysis of water quality parameters.

Water quality parameter	Analytical Methods
Conductivity	Samples were analyzed using YSI 3100 Conductivity meter, based on the method outlined in APHA 1998 (SM 2510 B).
Alkalinity	Samples were analyzed by utilizing a potentiometric titration. This involves titrating a known volume of sample with sulphuric acid at a specific concentration of 0.2N
NH_3	Samples were analyzed using the Salicylate (colorimetric) Method by HACH for DR-2800 (Method: 10031).
NO_3^-	Samples were analyzed by anion chromatography using a Dionex Ion Chromatograph (model DX120, anion AS14 analytical column)
NO_2^-	Samples were analyzed by anion chromatography using a Dionex Ion Chromatograph (model DX120, anion AS14 analytical column)
TKN	Samples were analyzed using the Salicylate (colorimetric) Method by HACH for DR-2800 (Method: 10031).
TP	Samples were analyzed by a colorimetric method using HACH colorimeter (DR-2800: Method 8190 Ascorbic Acid with Acid Persulfate Digestion). The phosphates present in organic and inorganic forms are converted to reactive phosphates by Acid Persulfate digestion. The reactive phosphates are then reacts with the ascorbic acid giving an intense blue colour. The results are measured at 880 nm.
PO_4	Samples were analyzed using one of two methods. Sample analysis was performed analyzed on a Dionex ion chromatrograph (model DX120, anion AS14 analytical column). The second was a colorimetric method utilizing the HACH DR-2800 colorimeter. In this method (HACH Method 8048), orthophosphate reacts with molybdate in an acid medium to produce a mixed phosphate/moybdate complex. Ascorbic acid then reduces the complex giving an intense molybdenum blue colour. The results are measured at 880 nm.
COD	HACH reactor Method designed for DR-2800 (Methdo-8000). The sample is heated for two hours with potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion to a green chromic ion, which is measured with a HACH colorimeter.
BOD	BOD method outlined in APHA 1998 (SM 5210 B). The method consists of filling 500ml bottles with sample to overflowing, air-tight

	sealing of the bottles and incubating them at 20 degrees Celsius for a 5 day period. The BOD represents the consumption of oxygen within the sample over this 5 day period as determined by assessing the difference in oxygen concentration from the initial DO concentration and the final DO concentration.
TSS	Standard Method 2540. The suspended particles are trapped by a filter of specified pore size and weighed when a consistent dried state is reached.

2.3 Biofilm Analyses

The development of the biofilm was studied using a PVC rig (Figure 10). The rig contained standard microscope slides (2.5cm x 7.5cm). Glass is commonly used because it is an inert surface and it provides for easy biofilm removal (Hallam et al., 2001; Wietz et al., 2003; Bourne et al., 2006). Once removed from the rig, the slides were placed in 250 ml sample bottles with site water for transport from site to lab. The biofilm was scraped off from one side of each slide with a sterile razor blade into 9 ml of sterile water and the slide was rinsed with 1 mL of sterile water (Lam and Lei, 1999). Serial dilutions were then made and standard duplicate spread plating was performed for heterotrophic and F. *psychrophilum* bacterial counts.

Figure 10: Photograph of the PVC rig holding slides.

*** twist ties were used to keep slides separated.**

2.4 Sampling Protocol

2.4.1 Coldwater Fisheries

Water samples were taken monthly from March 2013 to October 2013 and bacterial counts and water quality parameters were analyzed.

2.4.2 Lyndon Fisheries

The study occurred over a 2-month period and was divided into 2 sampling periods with analysis occurring on day 1, 3, 7, 16, 21 and 24 for sample period 1 and on days 1, 3, 10, 17, 26 and 34 for sample period 2. The sampling was divided into two sampling periods as a result of the biofilm sampling regime; a new set of slides was immersed for the second sampling period. The sample schedule was revised for sample period 2, as the CAWT receiving the samples required a longer duration between sampling days to analyze water quality parameters, which consequently resulted in a longer duration for sample period 2. The sampling schedule is outlined in Table 5.

Table 5: Observation days for sample period 1 and 2 at Lyndon Fish Hatchery.

Sample Period 1	Sample Period 2
Day 0 – June 16, 2014	Day 0 – July 10, 2014
Day 1 – June 17, 2014	Day 1 – July 11, 2014
Day 3 – June 19, 2014	Day 3 – July 14, 2014
Day 7 – June 23, 2014	Day 10 – July 21, 2014
Day 16 – July 2, 2014	Day 17 – July 28, 2014
Day 21 – July 7, 2014	Day 26 – August 5, 2014
Day 24 – July 10, 2014	Day 34 – August 13, 2014

For biofilm analysis in the early rearing egg tray and outdoor rearing tank, 2 slides from each location were removed from the sampling assembly for analysis. For biofilm analysis of UV influent and UV effluent 4 slides were removed. Slides were removed from each arm of the sampler each sampling day (Figure 11). Due to the design of the rig, slides had to be taken out from the top down. In addition, a

random removal of slides was thought to create a non-homogenous water flow in the chamber. During the removal of slides, they were clipped with a sterile clip on one of the edges that was being held in the sampler. This clip was used to identify and label the sides of the slide. Slide 1 and 2 had the side closest to the center scraped, and slides 3 and 4 had the side closest to the outer tank scraped. This was done in order to determine if the growth of the biofilm was impacted by differences that may exist between the flow at the surface facing the side of the chamber and at the surface facing the inside of the chamber.

Figure 11: Diagram of the placement of slides within each side compartment within the UV reactor.

Slide 1 Slide 2 UV Effluent Slide 3 Slide 4	← ← Water flow ← ←	Slide 1 Slide 2 UV Influent Slide 3 Slide 4

Chapter 3 *F. psychrophilum* and total heterotrophic bacteria abundance and water quality parameters

3.1 Introduction

In this section the abundance of planktonic *F. psychrophilum* and total heterotrophic bacterial counts were analyzed along with DO, pH, conductivity, alkalinity, TKN, NH, NO_2^-, $N0_3^-$, TP, PO_4, COD, BOD, and TSS at Coldwater Fishery and Lyndon Hatchery.

3.2 Data Analyses

Colony forming units were calculated according to Equation 3:

Equation 3: Colony forming Units

$$\frac{CFU}{mL} = \frac{AC \; x \; D}{DV},$$

where *AC* is the average of the raw data counts (CFU), *DV* is the volume of the drop plated (mL), and *D* is $1/10^{-k}$ where, k is the integer for 10-fold dilutions (no units).

Data was analyzed using R (R Project for Statistical Computing http://www.r-project.org). One-way analysis of variance (ANOVA) was performed on all water quality parameters and bacterial densities with respect to sampling periods, days of observation and site. ANOVA was performed to determine if there were spatial or temporal variations in the parameters tested at each site within each farm. A Levene's test was used to assess homogeneity of variances and a Shapiro Wilk test to assess normality (Abbink et al., 2012). When assumptions passed, differences between groups were calculated by Bonferroni post-hoc test. When the normality assumption was violated Kruskal Wallis ANOVA was used (Abbink et al., 2012). When data violated homogeneity of variances a Welch's ANOVA was used (Jan and Shieh, 2013). Bacterial numbers were log transformed in order to meet assumptions.

The relationships between each water quality parameter and bacterial growth were examined by Pearson product correlations (Zhang et al., 2011

Multiple linear regressions were used to examine the relationship between planktonic hetertrophic bacteria and *F. psychrophlium* using all the water quality parameters. The assumptions of linearity, independence of errors, homoscedasticy, unusual points and normality of residuals were tested. In order to select the best subset of variables forward selection was used. The process of eliminating covariates was accomplished by examining the variance inflation factor (VIF) with a threshold value of 3 (Zuur et al., 2010). Whittingham et al. (2006) outline the shortcomings of using stepwise multiple regression. Forward selection was chosen as the technique to help best explain bacterial densities based on the observed water quality parameters. The method of forward selection is commonly used in studies where there are a large number of different predictors and when the underlying ecology of the organism is unknown (Whittingham et al., 2006).

3.3 Results

3.3.1 Measured Parameter Values

3.3.1.1 Coldwater Fishery Water Quality Parameters

Over the study period DO concentrations ranged from 1.20-13.96 mg/L. The highest concentration was observed at site 5 (process water) on September 30, 2013. The lowest concentration was observed at site 4 (concentrated drum filter effluent) on June 25, 2013. DO concentrations varied significantly between sites; site 4 had significantly lower DO concentration than sites 1 and 6 (Table 6).

pH ranged from 6.8-8.2. The lowest pH was observed at site 4, on April 2, 2013. The highest pH was observed at site 3, water from the indoor raceway on October 30, 2013. One way ANOVA showed significance variation between sites. Post hoc test revealed that sites 1 (well water) and 3 had significantly higher pH from site 4 (Table 6).

Over the entire study period COD concentrations varied from <3.0-539 mg/L. During two observed days the concentration of COD was below the detectable limit of <3.0mg/L at the well site (site 1) on September 30 and October 30, 2013. The

highest concentration was observed at the concentrated drum filter effluent (site 4) on May 21, 2013. COD did not significantly vary between sites.

Over the entire sampling period TSS values varied from <3-630 mg/L. Over the entire sample period site 1, and site 6 (treated effluent) had TSS levels below the detectable limit of <3.0mg/L. The highest values were observed TSS at site 4. TSS varied significantly between sites, with post hoc test revealing site 4 was significantly higher than site 1 and 6. TSS values did not significantly vary between observed days.

TKN values varied from <0.21 – 41 mg/L over the duration of the sampling. During three observed days the concentration of TKN was below the detectable limit at site 1 in the months of April, May and October. TKN varied significantly between sites. Post hoc test revealed that site 4 had significantly higher values than site 1 (Table 4). TKN did not vary significantly between sampling dates.

Over the entire study period NO_3^- values ranged from <0.20 – 1.108 mg/L. NO_3^- concentrations were consistently below the detectable limit at site 1 during the study period. The highest concentration was observed at site 6 on July 13, 2013. NO_3^- varied significantly between sites but not over the observed sampling dates. Post hoc analysis revealed that site 1 was significantly lower than all other sites sampled (Table 6).

Over the entire study period NO_2^- values ranged from <0.006 -0.109 mg/L. NO_2^- concentrations at site 1 were below the detectable limit of <0.006 mg/L. Welch's ANOVA results showed significant variation between sites. Post hoc analysis revealed that site 1 was significantly lower than all other sites.

NH_3 values ranged from <0.02-8.88 mg/L over the sampling periods. NH_3 concentrations at site 1 were intermittently (June 25, and September 13) below the detectable limit of <0.002 mg/L. The highest concentrations were observed at site 4. NH_3 varied significantly between sites. Post hoc test revealed that sites 3 and 4 had significantly higher concentrations that site 1 (Table 6).

TP values ranged from <0.006 – 18.59 mg/l over the sampling period. TP concentrations were consistently below the detectable limit at site 1 during the

study period. The highest concentrations were observed at site 4 on May 21, 2013. TP did not vary significantly between sampling sites.

PO_4 varied from <0.003- 1.816 mg/L over the sampled periods. On several observed days PO_4 was below the detection limit of <0.003 mg/L for site 1. The highest concentration was observed at site 4. PO_4 varied significantly between sites (p<0.05). Post hoc test revealed that site 4 was significantly higher than site 1.

Over the study period temperature ranged from 7-18 °C. Temperature did not significantly vary between sample sites. Conductivity values varied from 827-1015 µS/cm. Conductivity did not vary significantly between sampling sites. Over the entire study period alkalinity concentrations varied from 205 -288mg/L as $CaCo_3$. Alkalinity did not vary significantly between sampling sites. The water quality parameters did not vary significantly over the days sampled (See Appendix).

Table 6: Mean (±standard deviations)) for water quality parameters measured at six different sites at Coldwater Fishery. AN=one way ANOVA, KW = Kruskal Wallis, WE = Welch's ANOVA. A post-hoc test Bonferroni was used for one way ANOVA, Tukey was used for Kruskal Wallis and Games-Howell for Welch's ANOVA. Letters within each row sharing the same letter are not significantly different (p>0.05).

Parameter	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	p Value
DO mg/L	10.6±0.71^{a}	9.83±0.84^{ab}	10.3±0.67^{ab}	5.81±2.77^{b}	10.5±1.1^{ab}	12.5±2.1^{a}	p=0.0067^{KW}
Temperature °C	9±0	11±4.5	11±5	11±3	11±1	11±1.5	p=0.8554^{WE}
COD mg/L	4.2±3.5	9.2±3.3	12.2±9.9	192.6±177.6	27.6 ±36.3	11.2±7.3	p=0.1136^{WE}
TP mg/L	0.03±0.00^{a}	0.13±0.04^{ab}	0.29±0.43^{ab}	4.67±6.41^{b}	0.17±0.08^{ab}	0.10±0.03^{ab}	p=0.00024^{KW}
Conductivity µS	905.7±15.1	891.4±37.0	920.9±20.4	942.3±51.4	923.3±5.2	907.0±8.5	p=0.1679^{KW}

pH	7.9±0.2[a]	7.7±0.1[ab]	7.7±0.3[a]	7.3±0.2[b]	7.6±0.0[ab]	7.7±0.1[ab]	p= 0.0125[AN]
TKN mg/L	0.20±0.15[a]	1.14±0.50[ab]	1.80±1.51[ab]	13.9±13.4[b]	1.74±1.13[ab]	1.07±0.62[ab]	p=0.01874[WE]
NH_3 mg/L	0.02±0.008[a]	0.62±0.23[ab]	1.20±0.56[b]	3.12±2.60[b]	0.57±0.49[ab]	0.57±0.37[ab]	p=0.00040[KW]
NO_2^- mg/L	0.03±0.00[a]	0.03±0.02[a]	0.01±0.01[ab]	0.02±0.01[ab]	0.02±0.01[ab]	0.07±0.05[b]	p=0.01086[WE]
NO_3^- mg/L	0.01±0.00[a]	0.23±0.15[b]	0.13±0.06[b]	0.13±0.23[b]	0.10±0.03[b]	0.74±0.53[b]	p=0.00069[WE]
TSS mg/L	1.50±0.00[a]	2.29±1.35[ab]	3.14±3.38[ab]	151.3±225.5[b]	4.35±3.60[ab]	1.50±0.00[a]	p=0.0030[KW]
Alkalinity mg/L	233.7±12.8	236.1±6.3	247.3±9.1	255.3±20.9	242.3±12.6	229.0±5.7	p=0.1238[KW]
PO_4 mg/L	0.002±0.0[a]	0.06±0.03[ab]	0.19±0.28[ab]	0.904±0.77[b]	0.05±0.02[ab]	0.05±0.03[ab]	p=0.0024[KW]
BOD mg/L	1.66±0.29[a]	4.8±3.34[ab]	9.21±8.15[ab]	51.03±61.67[b]	22.2±26.5[b]	8.2±0.61[ab]	p=0.0056[KW]

3.3.1.2 Lyndon Fish Hatchery Water Quality Parameters

Water temperature varied from 10-23°C. The lowest temperature was recorded during both sample periods (June 23 – July 11) at site 1 (10°C). The maximum temperature was recorded during sampling period 1 at site 3, the UV influent water (23°C). One-way ANOVA results showed a significant variation in water temperature between sites during each sampling period. Post hoc analysis revealed that site 1 (egg tray water) was significantly lower than the other three sites for both sampling periods.

DO varied from 4.85-12.61mg/L. The lowest concentrations were observed at site 1. DO concentrations varied significantly between sites for both sample periods. During sample period 1 post hoc analysis revealed that site 1 was significantly lower than the other three sites (Table 7). During sample period 2 site 1 was significantly lower than sites 3 and 4, the influent and effluent for the UV system, respectively (Table 8).

pH varied from 6.87-8.27 over the sampling periods. The lowest pH was observed during sample period 1 at site 1 (6.87). The highest pH was observed during sample period 2 at site 4 (8.27). It varied significantly between sites during both sampling periods. Post hoc analyses revealed that site 1 was significantly lower than the other three sites during sample period 1 (Table 7). Post hoc analysis

revealed that site 1 was significantly lower than site 4 during sample period 2 (Table 8).

Conductivity values varied from 422-693 µS. The highest conductivity values were observed in site 1. Conductivity varied significantly between sites, post hoc analysis revealed that site 1 had significantly higher conductivity than sites 3 and 4 during sample period 1 (Table 7). During sample period 2 conductivity was significantly higher at site 1 than the other 3 sites (Table 8).

Over the entire study period turbidity values ranged from 0.01 – 7.66 (NTU) and varied significantly between sites. Turbidity did not vary significantly between sites during sample periods 1 (Table 7). Turbidity varied significantly between sites during sample period 2 post hoc analysis revealed that site 1 had significantly lower values than site 2 and site 3 (Table 8).

Over the entire study period COD concentrations varied from <3.0-24.46 mg/L. During one observed day the concentration of COD was below the detectable limit of <3.0mg/L at both site 1 and site 4 on August 5, 2014. The highest concentration was observed at site 3 on July 28, 2014. COD varied significantly between sites during the first sampling period. Post hoc test showed that site 1 was significantly lower than the other 3 sites (Table 7).

Over the entire sampling period TSS values varied from 3-9mg/L. Over the entire sample period the site 1 had TSS levels below the detectable limit of <3.0mg/L. In addition, the site 4 was below the detectable limit on all days sampled except for on July 28, 2014 when the observed value was 9.0mg/L. TSS varied significantly between sites during the first sampling period. Post hoc analysis revealed that sites 1 and 4 were significantly lower than sites 2 and 3 (Table 7).

Alkalinity values ranged from 180-317mg/L over the duration of the sampling. Alkalinity varied significantly between sites for both sampling periods. Post hoc analysis revealed that site 1 was significantly higher than the other three sites for both sampling period 1 and 2 (Table 7 and 8).

TKN values varied from 0.105 – 1.120mg/L over the duration of the sampling. One way ANOVA results showed a significant variation between sites for both

sampling period 1 and 2. For both sampling periods, post hoc analysis revealed that site 1 was significantly lower than the other 3 sites (Table 7 and 8).

Over the entire study period NO_2^- values ranged from <0.006-0.067 mg/L. NO_2^- concentrations at site 1 were below the detectable limit of <0.006 mg/L. NO_2^-varied significantly between sites for both sampling period 1 and 2. Post hoc analysis revealed that site 1 was significantly lower than the other 3 sites (Table 7 and 8).

Over the entire study period NO_3^- values ranged from 2.47 – 5.98 mg/L. NO_3^- varied significantly between sites for both sampling periods. Post hoc analyses revealed that site 1 was significantly higher than the other three sites for both sample periods.

NH_3 values ranged from <0.02-0.31 mg/L over the sampling periods. NH_3 concentrations at site 1 were intermittently (June 23, July 2,7,10,21 and August 13) below the detectable limit of <0.002 mg/L. One-way ANOVA results showed a significant variation between sites for sampling period 1. Post hoc analysis revealed that site 1 was significantly lower from the other 3 sites (Table 7).

Over the sample period TP ranged from <0.02 - 0.14 mg/L. For the majority of the study, site 1 had TP levels below the detectable limit of <0.02 mg/L. TP levels did not vary significantly between sites.

$P0_4$ varied from <0.003 - 0.064mg/L over the sampled periods. On several observed days $P0_4$ was below the detection limit of <0.003 mg/L for all sites (June 17, July 21, August 5, and August 13). Over the entire study $P0_4$ was not detected at site 1. $P0_4$ values did not vary significantly between sites.

The water quality parameters did not vary significantly over the days sampled or between the sampling periods (See Table 34 in Appendix).

Table 7: Sample Period 1 mean (± standard deviations) for water quality parameters measured at 4 different sites at Lyndon Fish Hatchery. AN=one way ANOVA, KW = Kruskal Wallis, WE = Welch's ANOVA. A post-hoc test Bonferroni was used for one way ANOVA, Tukey was used for Kruskal Wallis and Games-Howell for Welch's ANOVA. Letters within each row sharing the same letter are not significantly different (P>0.05).

Site	Site 1	Site 2	Site 3	Site 4	P value
Temperature °C	11.27±1.68[a]	18.50±1.97[b]	18.67±2.34[b]	18.50±1.97[b]	p= 0.000583[AN]
DO mg/L	7.24±1.39[a]	9.87±1.02[b]	9.99±1.42[b]	10.01±0.64[b]	p=0.00219[AN]
pH	7.30±0.38[a]	7.54± 0.08[b]	7.82±0.13[b]	7.85±0.11[b]	p<0.001[AN]
Conductivity µS	676.09±14.81[a]	572.67±50.76[ab]	568.67±49.64[b]	565.67±51.48[b]	p=0.003142[KW]
Turbidity	0.81±0.96	2.16 ±1.74	2.27±1.66	2.08±2.03	p=0.4518[KW]
COD mg/L	4.9 ±2.05[a]	13.63±2.80[b]	16.42±3.45[b]	12.80±4.81[b]	p= 0.00489[AN]
TSS mg/L	1.50±0.00[a]	3.85±3.05[b]	4.83±1.74[b]	1.50±0.00[a]	p= 0.006182[WE]
Alkalinity mg/L	269.36±9.57[a]	240.67±24.30[b]	239.50±26.82[b]	240.00±23.56[b]	p=0.006803[KW]
TKN mg/L	0.27±0.09[a]	0.80±0.09[b]	0.85±0.19[b]	0.72±0.09[b]	p<0.001[WE]
$N0_2$ mg/L	0.00±0.00[a]	0.05 ±0.02[b]	0.05±0.01[b]	0.05±0.01[b]	p<0.001 [WE]
$N0_3$ mg/L	5.70±0.26[a]	4.6±0.65[b]	4.50±0.73[b]	4.58±0.80[b]	p= 0.003932[KW]
NH_3 mg/L	0.03±0.05[a]	0.16 ±0.04[b]	0.13±0.06[b]	0.12±0.04[b]	p<0.001[WE]
TP mg/L	0.02±0.02	0.05±0.02	0.05±0.02	0.03±0.01	p= 0.801[AN]
$P0_4$ mg/L	0.00±0.00	0.00±0.00	0.01±0.01	0.01±0.01	p= 0.136[KW]

Table 8: Sample Period 2 mean (± standard deviations) for water quality parameters measured at 4 different sites at Lyndon Fish Hatchery. AN=one way ANOVA, KW = Kruskal Wallis, WE = Welch's ANOVA. A post-hoc test Bonferroni was used for one way ANOVA, Tukey was used for Kruskal Wallis and Games-Howell for Welch's ANOVA. Letters within each row sharing the same letter are not significantly different (P>0.05).

Site	Site 1	Site 2	Site 3	Site 4	P value
Temperature °C	10.80±0.41[a]	17.00±0.63[b]	17.00±0.63[b]	17.00±0.63[b]	p= 0.00181[KW]
DO mg/L	7.68±0.61[a]	8.54±0.51[ab]	9.12±1.28[b]	9.46±1.06[b]	p= 0.00337[KW]
pH	7.38±0.37[a]	7.71±0.21[ab]	7.82±0.34[ab]	7.94±0.21[b]	p= 0.00239[AN]
Conductivity µS	677.33±10.89[a]	581.00±64.28[b]	579.33±65.77[b]	566.8±72.29[b]	p= 0.00309[KW]
Turbidity NTU	0.57±0.44 [a]	1.88±1.54 [b]	1.70±1.28 [b]	2.22±2.69[ab]	p= 0.02613[KW]
COD mg/L	4.90±1.83	13.88±6.52	12.87±7.64	10.43±6.77	p= 0.212[AN]
TSS mg/L	1.50±0.00	3.23±2.76	3.80±2.60	2.75±3.06	p= 0.1522[KW]
Alkalinity mg/L	266.33±3.72[a]	235.33±27.18[b]	235.50±27.89[b]	256.5±52.48[b]	p= 0.04185[KW]
TKN mg/L	0.32±0.05[a]	0.73±0.17[b]	0.70±0.18[b]	0.74±0.23[b]	p= 0.00307[AN]
$N0_2$ mg/L	0.00±0.00[a]	0.05±0.00[b]	0.04±0.00[b]	0.04±0.00[b]	p<0.001 [WE]
$N0_3$ mg/L	5.59±0.32[a]	4.36±0.90[b]	4.42±1.00[b]	4.36±0.96[b]	p= 0.00437[KW]
NH_3 mg/L	0.04±0.07	0.15±0.08	0.11±0.07	0.13±0.06	p= 0.1265[WE]
TP mg/L	0.01±0.00	0.05±0.05	0.04±0.04	0.04±0.04	p= 0.458[KW]
$P0_4$ mg/L	0.00 ±0.00	0.02±0.02	0.01 ±0.02	0.01±0.03	p= 0.2209[KW]

3.3.1.3 Coldwater Fishery Bacterial Analyses

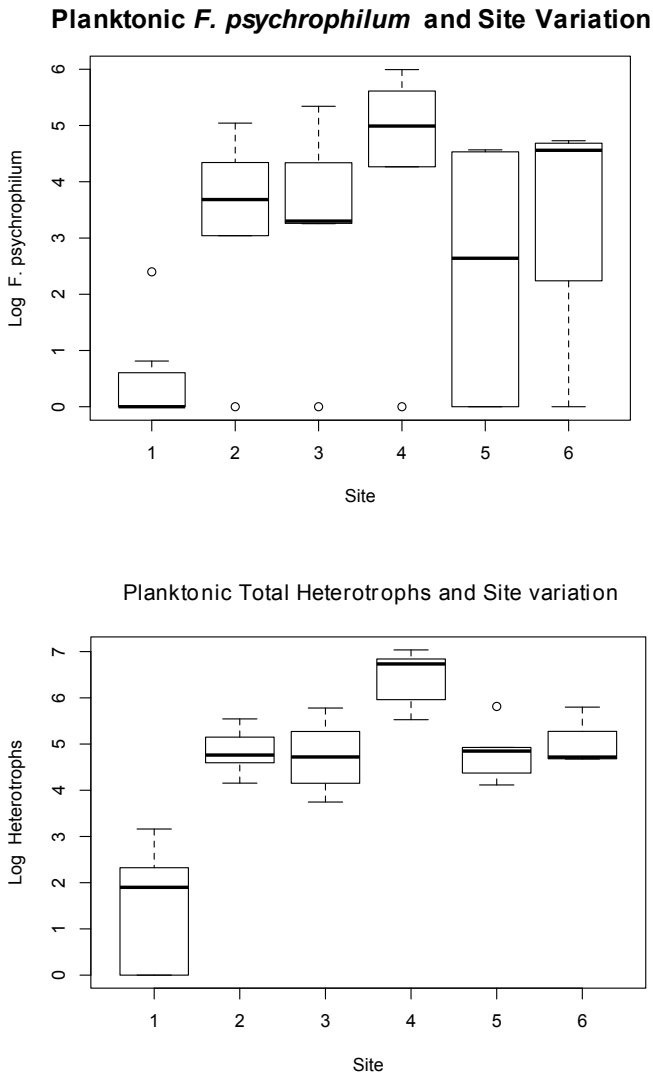
Over the entire study period planktonic *F. psychrophilum* concentrations varied from undetectable to 9.85 x 10^5 CFU/ml. The highest concentrations of *F. psychrophilum* was observed at site 4. *F. psychrophilum* showed significant variation between the observed sites. Post hoc analysis showed site 1 was significantly lower than site 4 (Table 9 and Figure 12).

Over the study period planktonic heterotrophic bacteria varied from undetectable to 1.09 x 10^7 CFU/ml. Planktonic heterotrophic bacteria showed significant variation between sites. Post hoc analysis showed that site 1 was significantly lower than the other 5 sites (Table 9 and Figure 12).

Table 9: Mean (± standard deviations) of heterotrophic bacteria and *Flavobacterium psychrophilum* at Coldwater Fishery. AN=one way ANOVA, KW = Kruskal Wallis, A post-hoc test Bonferroni was used for one way ANOVA, and Tukey was used for Kruskal Wallis. Letters within each row sharing the same letter are not significantly different (P>0.05).

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	P value
Heterotrophic Bacteria CFU/mL	$2.86 \times 10^2 \pm 5.28 \times 10^{2a}$	$9.52 \times 10^4 \pm 1.21 \times 10^{5b}$	$1.88 \times 10^5 \pm 2.72 \times 10^{5b}$	$4.29 \times 10^6 \pm 4.02 \times 10^{6b}$	$2.01 \times 10^5 \pm 3.01 \times 10^{5b}$	$3.43 \times 10^5 \pm 4.06 \times 10^{5b}$	$<0.001^{AN}$
F. psychrophilum CFU/mL	$3.70 \times 10^1 \pm 9.40 \times 10^{1a}$	$2.10 \times 10^4 \pm 4.00 \times 10^{4ab}$	$4.06 \times 10^4 \pm 8.15 \times 10^{4ab}$	$2.34 \times 10^5 \pm 3.61 \times 10^{5b}$	$2.88 \times 10^2 \pm 4.52 \times 10^{2ab}$	$2.68 \times 10^4 \pm 3.78 \times 10^{4ab}$	0.0127^{KW}

Figure 12: Boxplot of significant variation among planktonic *Flavobacterium psychrophilum* and sites at Coldwater Fishery (top). Boxplot of significant variation among planktonic heterotrophic bacteria and sites at Coldwater Fishery (bottom). Boxplots show median values (solid horizontal line), 50th percentile values (box outline), 90th percentile values (whiskers) and outlier values (open circles).



3.3.1.4 Lyndon Bacteria Analyses

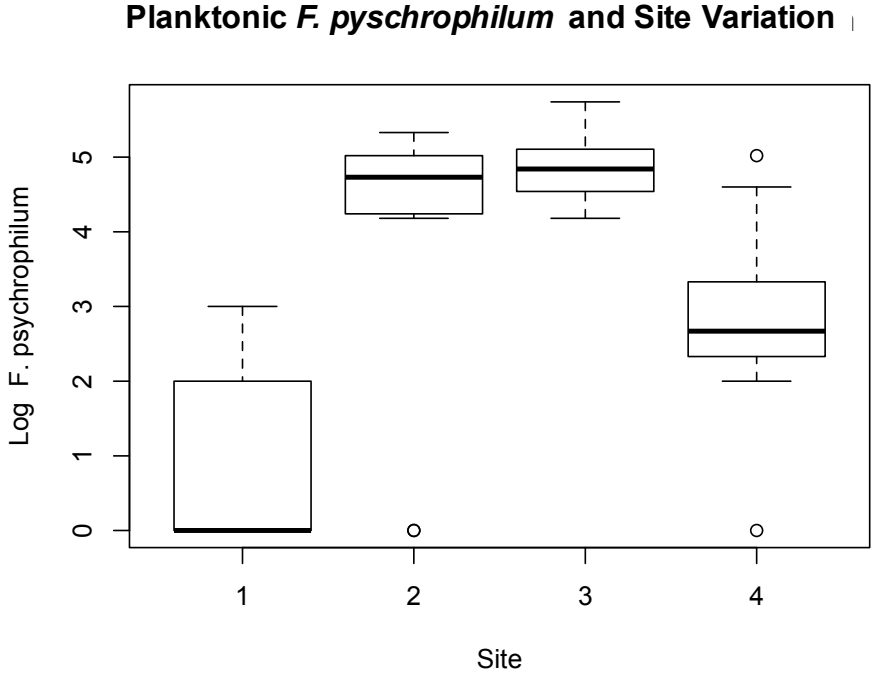
Planktonic *F. psychrophilum* varied from undetectable to 5.50 x10^5 CFU/ml. Planktonic *F. psychrophilum* was not present at site 1 on numerous occasions over the study period. The highest concentration of planktonic *F. psychrophilum* was observed at Site 3 in June 17, 2014. Over the entire study period planktonic *F. psychrophilum* showed significant variation between the observed sites (Table 10). Post hoc analysis revealed that site 1 and 4 had significantly lower concentrations than sites 2 and 3 for both sampling periods (Table 10 and Figure 13).

Over the study period planktonic heterotrophic bacteria varied from 5.00 x 10^2 CFU/ml to 1.48 x 10^7 CFU/ml. The lowest value was observed at Site 1 on July 11, 2014. The highest value was observed Site 3 on June 17, 2014. Planktonic heterotrophic bacteria showed significant variation between sampling sites during the first sampling period. Post hoc analysis revealed that site 1 and 4 had significantly lower concentrations than sites 2 and 3 for both sampling periods (Table 10 and Figure 13). Planktonic heterotrophic bacteria did not significantly vary among sites during the second sampling period (Table 10).

Table 10: Means (± standard deviations) of Heterotrophic Bacteria and *Flavobacterium psychrophilum* at Lyndon Fish Hatchery. AN=one way ANOVA, KW = Kruskal Wallis. A post-hoc test Bonferroni was used for one way ANOVA, and Tukey was used for Kruskal Wallis. Letters within each row sharing the same letter are not significantly different (P>0.05).

	Sample Period 1				
	Site 1	Site 2	Site 3	Site 4	P value
Heterotrophic Bacteria	4.39 x 10^4 ± 8.61 x 10^4 [a]	2.07 x 10^6 ± 1.16 x 10^6 [b]	4.09 x 10^6 ± 5.46 x 10^6 [b]	8.83 x 10^4 ± 1.92 x 10^5 [a]	0.001114[KW]
F. psychrophilum	1.50 x 10^2 ± 3.20 x 10^2 [a]	7.75 x 10^4 ± 7.41 x 10^4 [b]	1.78 x 10^5 ± 1.87 x 10^5 [b]	1.90 x 10^4 ± 4.22 x 10^4 [a]	0.003334[KW]
	Sample Period 2				
Heterotrophic Bacteria	7.49 x 10^4 ± 1.1 x 10^5	2.66 x 10^6 ± 2.54 x 10^6	1.23 x 10^6 ± 1.11 x 10^6	2.28 x 10^5 ± 4.79 x 10^5	0.701[AN]
F. psychrophilum	1.00 x 10^2 ± 2.00 x 10^2 [a]	5.92 x 10^4 ± 5.38 x 10^4 [b]	4.75 x 10^4 ± 2.36 x 10^4 [b]	6.94 x 10^3 ± 1.62 x 10^4 [c]	0.005097[KW]

Figure 13: Boxplot of significant variation among planktonic *Flavobacterium psychrophilum* and sites (top) and boxplot of at planktonic heterotrophic bacteria and sites at Lyndon (bottom). Boxplots show median values (solid horizontal line), 50th percentile values (box outline), 90th percentile values (whiskers) and outlier values (open



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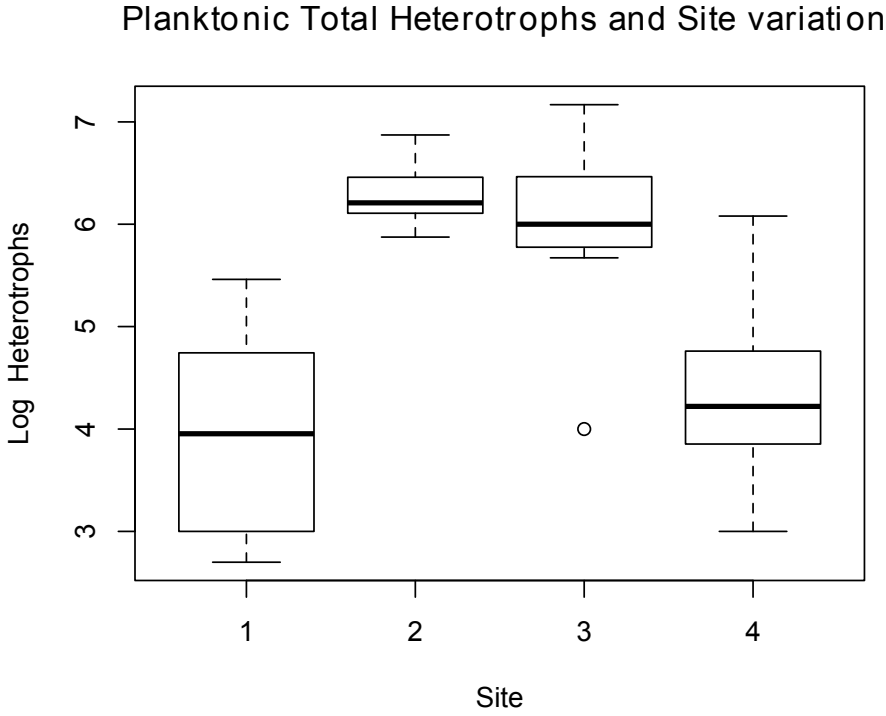


3.3.2 Correlation

The Lyndon Fish Hatchery and Coldwater Fishery data was analyzed for each location separately.

The relationship between water quality parameters and bacteria counts at the Coldwater Fishery was investigated by analyzing data from all sites. The relationship between water quality parameters and bacteria counts at the Lyndon Fish hatchery was investigated by analyzing data from 3 sites: early rearing egg tray, outdoor tank and UV influent sites. The UV effluent site was not included in the data analysis, as the bacterial counts were influenced by the UV disinfection and the focus of this section is on determining an association between water quality parameters and *F. psychrophilum*. The data was analyzed by computing a Pearson product-moment correlation for both *F. psychrophilum* and heterotrophic bacteria counts and each water quality parameter. The results are displayed in the charts below.

3.3.2.1 Coldwater Fishery

According to the Pearson correlation test the water quality parameter that showed the highest correlation with planktonic heterotrophic bacteria was pH ($r=-0.60$, $p<0.001$) (Table 11). Total heterotrophic bacteria showed moderate positive correlations with alkalinity ($r=0.39$, $p<0.05$), TKN ($r=0.40$, $p<0.05$), PO_4, ($r=0.43$, $p<0.05$), COD ($r=0.38$, $p<0.05$) and BOD ($r=0.35$ $p<0.1$) (Table 11). Total heterotrophic bacteria showed moderate negative correlation with DO ($r=-0.45$, $p<0.01$). Total heterotrophic bacteria showed a small positive correlation with water temperature ($r=0.29$, $p<0.1$), conductivity ($r=0.18$), NO_2^- ($r=0.24$), NO_3^- ($r=0.19$), TP ($r=0.27$) and TSS ($r=0.25$) (Table 11).

Table 11: Summary of Pearson correlation (r value) of heterotrophic bacteria and water quality parameters at Coldwater Fishery. Significance "*" = p<0.1, "" = p<0.05, "***" = p<0.01, "****"= p<0.001.**

Parameter	r value
DO	r= -0.45***
Temperature	r=0.29*
pH	r= -0.60****
Conductivity	r= 0.18
Alkalinity	r= 0.39**
TKN	r= 0.37**
NH_3	r=0.40**
NO_2^-	r= 0.24
NO_3^-	r= 0.19
TP	r= 0.27
PO_4	r=0.43**
COD	r= 0.38**
BOD	r= 0.35*
TSS	r= 0.25

According to the Pearson correlation test the water quality parameters that showed the highest correlation with planktonic *F. psychrophilum* were pH (r=-0.52, p<0.01) and PO_4 (r=0.58, p<0.01) (Table 12). *F. psychrophlium* showed moderate positive correlations with alkalinity (r=0.30, p<0.1), and NH_3 (r=0.39, p<0.05). *F. psychrophlium* showed moderate negative correlation with DO (r=-0.30) (Table 10). *F. psychrophlium* showed small positive correlations with conductivity (r=0.16), TKN (r=0.25), NO_3^- (r=0.10), COD (r=0.20) and TSS (r=0.138).

Table 12: Summary of Pearson correlation (r value) of *Flavobacterium psychrophilum* and water quality parameters at Coldwater Fishery. Significance "*" = p<0.1, "" = p<0.05, "***" = p<0.01, "****"= p<0.001.**

Parameter	r value
DO	r= -0.30
Temperature	r= -0.04
pH	r= -0.52***
Conductivity	r= 0.16
Alkalinity	r= 0.31*
TKN	r= 0.25
NH_3	r= 0.39**
NO_{2^-}	r=0.042
NO_{3^-}	r= 0.10
TP	r= 0.21
PO_4	r=0.58 ***
COD	r=0.20
BOD	r=-0.007
TSS	r=0.138

3.3.2.2 Lyndon Fish Hatchery

According to the Pearson correlation test the water quality parameters that showed highest correlation with planktonic heterotrophic bacteria were NO_2^- (r=0.81, p<0.001), temperature (r=0.76, p<0.001), and TKN (r=0.72, p<0.001) (Table 13). Total heterotrophic bacteria showed strong positive correlation with NH_3 (r=0.68, p<0.001) (Table 13). Total heterotrophic bacteria showed strong negative correlations with conductivity (r=-0.60, p<0.001), alkalinity (r=-0.56, p<0.001) and NO_3^- (r=-0.63, p<0.01) (Table 13). Total heterotrophic bacteria showed moderate positive correlations with DO (r=0.47, p<0.01), pH (r=0.46, p<0.01), turbidity (r=0.36, p<0.05), TP (r=0.33, p<0.05) and TSS (r=0.42, p<0.01) (Table 13). Total heterotrophic bacteria showed a small positive correlation with PO_4 (r= 0.22) (Table 13). Total heterotrophic bacteria showed a small negative correlation with BOD (r=-0.17) (Table 13).

Table 13: Summary of Pearson correlation (r value) of heterotrophic bacteria and water quality parameters at Lyndon Fish Hatchery. Significance "*" = p<0.1, "" = p<0.05, "***" = p<0.01, "****"= p<0.001.**

Parameter	r value
DO	r= 0.47***
Temperature	r=0.76****
pH	r= 0.46***
Turbidity	r= 0.36**
Conductivity	r= -0.60****
Alkalinity	r= -0.56****
TKN	r= 0.72****
NH_3	r= 0.68****
NO_2^-	r= 0.81****
NO_3^-	r= -0.63***
TP	r= 0.33**
PO_4	r= 0.22
COD	r= 0.07
BOD	r=-0.17
TSS	r= 0.42***

According to the Pearson correlation test the water quality parameters that showed the highest correlation with planktonic *F. psychrophilum* were water temperature (r=68, p<0.001), TKN (r=0.70, p<0.001) and NO_2^- (r=0.81, p<0.001) (Table 14). *F. psychrophlium* showed strong positive correlations with DO (r=0.62, p<0.001), and COD (r=0.57, p<0.001) (Table 14). *F. psychrophlium* showed a strong negative correlation with conductivity (r=-0.60, p<0.001), alkalinity (r=-0.48, p<0.01), NO_3^- (r=-0.52, p<0.001) (Table 14). *F. psychrophlium* showed moderate positive correlations with pH (r=0.48, p<0.01), turbidity (r=0.36, p<0.05), TP (r=0.45, p<0.01), PO_4 (r=0.27, p<0.1) and TSS (r=0.47, p<0.01) (Table 14)

Table 14: Summary of Pearson correlation (r value) of *Flavobacterium psychrophilum* and water quality parameters at Lyndon Fish Hatchery. Significance "*" = p<0.1, "" = p<0.05, "***" = p<0.01, "****"= p<0.001.**

Parameter	r value
DO	r= 0.62****
Temperature	r=0.68****
pH	r= 0.48***
Turbidity	r=0.36**
Conductivity	r= -0.60****
Alkalinity	r= -0.48***
TKN	r= 0.70****
NH_3	r= 0.53****
NO_2^-	r= 0.81****
NO_3^-	r= -0.52****
TP	r= 0.45***
PO_4	r= 0.27*
COD	r= 0.57****
BOD	r=0.06
TSS	r= 0.47***

3.3.3 Multiple Linear Regression

3.3.3.1 Coldwater Fishery

A multiple regression was run to predict planktonic *F. psychrophilum* from water quality parameters. After forward selection the predictor included in the model was pH. It predicted planktonic *F. psychrophilum* $F(1, 14) = 6.842$, $p<0.05$, $adj.R^2=0.280$.

Table 15: Summary of multiple regression analysis for planktonic *Flavobacterium psychrophilum* and water quality parameters at Coldwater Fishery. *p<0.05; B= unstandardized regression coefficient; SE_B = Standard error of the coefficient; β = standardized coefficient.

Variable	B	SE_B	β
Intercept	44.854	16.309	
pH	-5.583	2.134	-0.573*

A multiple regression was run to predict planktonic heterotrophic bacteria from water quality parameters. After forward selection the predictors included in the model were pH, PO_4, date and NH_3. pH, PO_4, date and NH_3 predicted planktonic heterotrophic bacteria $F(4, 11) = 20.235$, $p<0.0005$, $adj.R^2=0.837$.

Table 16: Summary of multiple regression analysis for planktonic heterotrophic bacteria and water quality parameters at Coldwater Fishery. *p<0.05; B= unstandardized regression coefficient; SE_B = Standard error of the coefficient; β = standardized coefficient.

Variable	B	SE_B	β
Intercept	109.499	13.246	
pH	-14.042	1.741	-1.404*
PO_4	-7.091	2.130	-1.663*
Date	0.601	0.230	0.288*
NH_3	0.864	0.389	1.032*

3.3.3.2 Lyndon Hatchery

A multiple regression was run to predict planktonic *F. psychrophilum* from water quality parameters. After forward selection the predictors included in the model were NO_2^-, and TSS. NO_2^-, and TSS predicted planktonic *F. psychrophilum* $F(2, 50) = 31.850$, $p<0.0005$, $adj.R^2=0.543$.

Table 17: Summary of multiple regression analysis for planktonic *Flavobacterium psychrophilum* and water quality parameters at Lyndon Fish Hatchery. *p<0.05; B= unstandardized regression coefficient; SE_B = Standard error of the coefficient; β = standardized coefficient.

Variable	B	SE_B	β
Intercept	0.279	0.382	
NO_2^-	60.554	9.376	0.643*
TSS	0.207	0.093	0.223*

A multiple regression was run to predict planktonic heterotrophic bacteria from water quality parameters. After forward selection the predictors included in the summary were NH_3, BOD, temperature, site, NO_3^-, PO_4, and TSS. NH_3, BOD, temperature, site, NO_3^-, PO_4, and TSS predicted planktonic heterotrophic bacteria $F(7, 45) = 18.638$, $p<0.0005$, $adj.R^2=0.704$.

Table 18: Summary of multiple regression analysis for planktonic heterotrophic bacteria and water quality parameters at Lyndon Fish Hatchery. *p<0.05; B= unstandardized regression coefficient; SE_B = Standard error of the coefficient; β = standardized coefficient.

Variable	B	SE_B	β
Intercept	9.215	1.531	
NH_3	5.939	2.096	0.355*
BOD	-0.642	0.135	-0.398*
Temperature	0.209	0.55	0.584*
Site	-0.646	0.138	-0.600*
NO_3^-	-0.804	0.234	-0.560*
PO_4	-47.823	12.711	-0.557*
TSS	0.143	0.067	0.255*

3.4 Discussion

The water quality parameters that had the strongest correlations with *F. psychrophilum* at Coldwater Fishery were PO_4 (r=58, p<0.01, Table 12), pH (r=-0.52, p<0.01, Table 12) and NH_3 (r=0.39, p<0.05, Table 12). The water quality parameters that had the strongest correlations with *F. psychrophilum* at Lyndon Fish hatchery were NO_2^- (r=0.81, p<0.001, Table 14), TKN (r=0.70, p<0.001, Table 14) and Temperature (r=0.68, p<0.001, Table 14). When examining both farms there are some distinct differences between the water quality parameters, some of these differences may be due to the differences in the water makeup of the intake water.

Dissolved Oxygen (DO) concentrations at Coldwater Fishery are the highest at the intake water, due to the use of an oxygenator prior to entry into their farm. Lyndon Fish Hatchery does not use an oxygenator prior to entry into their system and their intake water had the lowest concentrations. It was expected to see an association between bacterial concentrations and DO. Bell et al. (1982) demonstrated that viable heterotrophic bacteria counts in two Canadian rivers had significant positive correlations with DO. Lyndon Fish Hatchery and Coldwater Fishery both revealed significant moderate correlations with DO and heterotrophic bacteria (r=0.47, p<0.01 and r=-0.45, p<0.01, respectively). At Lyndon *F. psychrophilum* and DO showed a significant positive correlation (r=0.62, p<0.001, Table 13), and Coldwater showed a negative correlation (r=-0.30, Table 11). These

differing correlations are most likely due to the use of an oxygenator at Coldwater Fishery causing elevated DO values across the farm. The results of the Lyndon Fish Hatchery more closely match the results of the Bell et al. (1982) study because the dissolved oxygen concentrations more closely resemble a natural system. Strepparava et al. (2014) quantified *F. psychrophilum* in water and fish tissue. They attempted to shed light on *F. psychrophilum* in the environment. Strepparava et al. (2014) could not show any clear correlation between the presence of *F. psychrophilum* and DO. The DO levels at the farm studied in Strepparava et al. (2014) ranged from 7-10mg/L, which is similar to the ranges observed at both farms (Tables 6-8). The author noted that the limited amount of outbreaks (4) during the period of study restricted the use of analysis to adequately quantify the importance of each factor (Strepparava et al., 2014).

Nitrogen levels at each of the farms differed. TKN and NH_3 values had a much larger range at Coldwater Fisheries than at Lyndon and the concentrations of NO_3^- did not overlap between the farms. NO_2^- concentrations between the farms were comparable. NO_2^- had the strongest correlation with *F. psychrophilum* at the Lyndon Fish Hatchery ($r=0.81$, $p<0.001$, Table 14); however, it had one of the weakest correlations at the Coldwater Fishery ($r=0.042$, Table 12). Decostere et al. (1999) investigated the influence of water quality and temperature on the adhesion of *Flavobacterium columnare*. They reported that elevated organic loads increased the disease. Moreover, that high levels of NO_2^- enhanced the adhesion of *F. columnare* to fish tissue. The levels manipulated in the Decostere et al. (1999) study were not comparable to levels observed at either farm. The authors noted the presence of an influence of NO_2^- but were unable to determine the mechanism of how it affects *F. columnare* (Decostere et al., 1999). The relationships between NO_2^- and *F. psychrophilum* may be differing at the two farms due to differing strains of *F. psychrophilum.* Nematollahi et al. (2003a) studied the adhesion high and low virulence strains of *F. psychrophilum* under differing environmental conditions. At varying temperatures and with added organic matter and nitrite they noted that the highly virulent strain of *F. psychrophilum* was more influenced than the low virulent strain by environmental factors (Nematollahi et al., 2003a). The dependence of *F.*

psychrophilum with NO_2^- observed at Lyndon and the lack of dependence observed at Coldwater could therefore be the result of different strains present at each farm. Another possible explanation for the dissimilar associations observed the two farms may be to the increased levels of nitrite causing an increase in fish shedding of *F. psychrophilum* resulting in the correlation at the Lyndon Fish Hatchery.

NO_3^- had a weak positive correlation with *F. psychrophilum* at Coldwater Fishery ($r=0.10$, $p>0.1$, Table 12) and a moderately negative correlation at Lyndon Fish Hatchery ($r=-0.52$, $p<0.001$, Table 14). NO_3^- concentrations were the highest at the Lyndon intake and lowest at the Coldwater intake, which could have resulted in the differing associations between *F. psychrophilum* and NO_3^- at each of the farms. Another possible explanation for the dissimilar associations observed the two farms may be to the distinct concentrations observed at each farm. It hasn't been previously reported that a relationship exists between NO_3^- and *F. psychrophilum*.

Lyndon's intake water was the most acidic of the sampled locations and Coldwater Fisheries intake water was the most basic of the sampled locations. pH ranged from 6.80-8.20 at both locations over the study periods. Soltani and Burke (1995) examined *F. psychrophilum* response to different doses of pH, specifically pH 4 -10. The results of their study concluded that *F. psychrophilum* grew well in a pH range of 6-8 (Soltani and Burke, 1995). At Coldwater Fishery pH was moderately negatively correlated with *F. psychrophilum* and at Lyndon it was moderately positively correlated. In addition, Strepparava et al. (2014) examined pH and found no clear correlation to *F. psychrophilum*. Therefore, the lack of a clear association at both farms is understandable due to the wide growth range of *F. psychrophilum*.

The water quality parameters showed no significant variation over time at each farm. This is an expected result for closed, controlled systems such as land-based aquaculture, where processes are designed to avoid fluctuation in water quality. The time span over which sampling occurred wasn't large enough to capture a change in water quality parameters. At each farm the water quality parameters varied significantly as a function of sampling site (Table 6-8).

Garcia et al. (2000) conducted infection trials of *F. psychrophilum* at two fish farms using fish from the same stock. The results failed to produce the same

mortality rate at each location (Garcia et al., 2000). The main difference between the two farms in this study was the source of supplied water (Garcia et al., 2000). This helps support the observation of the dissimilar associations between the farms in my study; because as mentioned above there were many differences in composition of the source water from each farm. Therefore, the role that source water plays in *F. psychrophilum* abundance is possibly a determining factor in the spread of *F. psychrophilum*.

Another possible explanation for the dissimilar associations observed in the two farms may be due to *F. psychrophilum* acclimatizing to the environmental parameters at each farm.

Water quality parameters did not provide an indicator for predicting the abundance of *F. psychrophilum* within the two aquaculture facilities. When examining the facilities separately, the hypothesis was supported at the Lyndon Fish Hatchery. Elevated levels of NO_2^- and DO coincided with increased concentrations of *F. psychrophilum*. Overall, the results did not support the hypothesis because similar associations were not observed at each farm.

Chapter 4 Evaluation of UV irradiation to control planktonic and attached *F. psychrophilum* and heterotrophic bacteria

4.1 Collimated Beam Trials

4.1.1 Introduction

The initial irradiation doses tested in this trial were based on published literature (Hedrick et al., 2000). Hedrick et al. (2000) reported that a UV dose of 126 mJ/cm^2 was able to inactivate *F. psychrophilum,* and that a dose of 42 mJ/cm^2 was unable to effectively inactivate *F. psychrophlium*. Following the first trial, with doses of 0, 25, 50, 75, 100 and 125 mJ/cm^2, it was discovered that the sensitivity of *F. psychrophlium* was significantly higher than expected and therefore *F. psychrophlium* was exposed to doses 1, 2, 3, 4 and 5mJ/cm^2 in the next trial.

4.1.2 Data Analysis

The UV dose delivered to the sample was calculated using equation 1.

The log inactivation (log I) was calculated using equation 2.

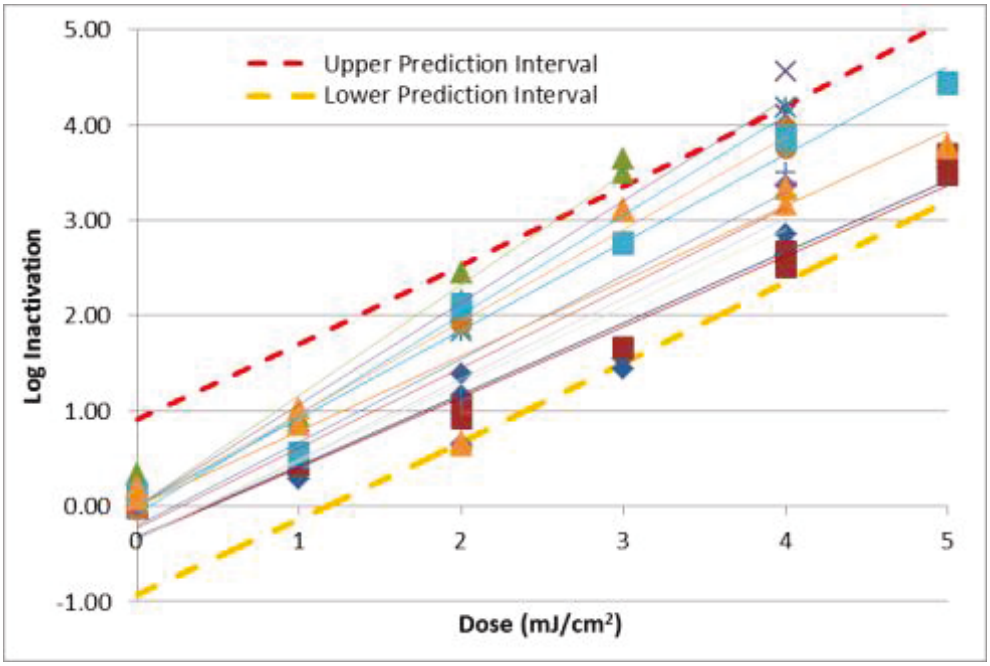
Linear regression analysis was performed on the log geometric mean of the enumerated *F. psychrophilum* values obtained after each delivered dose. Regression analysis of measured log inactivation for the range of UV doses produces the dose-response curve (Figure 14) (Pirnie, Linden, and Malley, 2006).

4.1.3 Results

The results from the individual collimated beam trials will be presented separately, as these tests were performed at Trojan Technologies. Figure 14 shows the results of the 12 collimated beam tests, plotted with the upper and lower prediction interval and the best fit linear trendline for each of the collimated beam tests. The UV dose required to achieve a 3.5 log reduction of *F. psychrophilum* was

determined to be 5 mJ/cm^2. *F. psychrophilum* was determined to have a range of sensitivity, as was demonstrated by the differences in each of the results of the collimated beam test (Figure 14).

Figure 14: Dose-response curve for *Flavobacterium psychrophilum* from the 12 collimated beam tests with the upper and lower prediction intervals and the best fit linear trendline for each of the collimated beam tests.



4.2 Field Study

4.2.1 Introduction

In this section the efficacy of UV irradiation as a disinfection method for both planktonic and attached *F. psychrophilum* was evaluated. The following objectives were set out to test this hypothesis:

1) Measure and compare planktonic total heterotrophic bacteria densities before and after UV treatment for bacterial removal efficiencies.
2) Measure and compare planktonic *F. psychrophilum* densities before and after UV treatment for bacterial removal efficiencies.
3) Measure and compare total heterotrophic biofilm bacteria densities before

and after UV treatment for bacterial removal efficiencies.

4) Measure and compare biofilm *F. psychrophilum* densities before and after UV treatment for bacterial removal efficiencies

4.2.2 Data Analyses

Colony forming units for planktonic samples were calculated using equation 1

Colony forming units (CFU) per cm^2 for biofilm slides were calculated according to equation 4:

Equation 4: Colony Forming Unit

$$\frac{\mathbf{CFU}}{\mathbf{cm^2}} = \frac{AC}{DV}\ x\ D\ x\ VS\ x\ \frac{1}{SA},$$

where *AC* is the average of the raw data counts (CFU), *DV* is the volume of the drop plated (mL), and *D* is the $1/10^{-k}$ where k is the integer for 10-fold dilutions (no units)., *VS* is the volume of the liquid the coupon was scraped into (mL), and *SA* is the scraped surface of the coupon (cm^2)

Removal efficiencies of the UV treatment was calculated as the percentage difference between the bacterial values measured from the influent and effluent water samples, defined by equation 5:

Equation 5: Bacterial Removal Efficiency (Gullian et al., 2012; Sharrer et al., 2005)

$$Bacterial\ Removal\ (\%) = \frac{Value_{\ influent} - Value_{\ effluent}}{Value_{\ influent}}\ x\ 100.$$

log_{10} reductions of bacteria achieved by UV treatments were based on the percent removal values calculated above, using equation 6:

Equation 6: Log_{10} Reductions Gullian et al., 2012; Sharrer et al., 2005)

$$logG_{10} = -log_{10}\left(1 - \frac{\%\ removal}{100}\right).$$

Data was analyzed using R (R Project for Statistical Computing http://www.r-project.org). The values for bacterial density were $\log_{10}$ transformed. A Shapiro-Wilk test was performed on each water quality data set to determine if that data demonstrated a normal distribution and homogeneity of variances was determined by Levene's test (Zhang et al., 2011, Abbink et al., 2012). A student two-sample t-test was performed to compare the differences in water quality parameters before and after UV treatment for each sampling period for data sets that were normally distributed and a two-sample Wilcoxon signed-rank test was performed on data sets that were not normally distributed (Sharrer et al., 2007). Significant difference between slides of each group was calculated by one-way analysis of variance (ANOVA), when conditions passed assumptions differences between groups were calculated by Bonferroni post-hoc test to rule out spatial differences between the slides within the chamber. When the data was found to be not normal a Kruskal Wallis (KW) test was performed followed by a Tukey post hoc test (Abbink et al., 2012). Repeated measures ANOVA (rmANOVA) was performed using SPSS. Due to the variation between the sampling days of each sampling period, the sampling periods were subdivided into 3 phases; early (days 1-5), middle (days 6-20) and late (21-34) and were analyzed using rmANOVA. The 3 phases represent the biofilm growth regime. rmANOVA was performed the attached bacterial concentrations, phase and sample period for *F. psychrophilum*, heterotrophic bacteria, and colony pigmentation diversity. When Mauchly's test of sphericity was violated, the Greenhouse-Geisser corrected values were reported. Colony pigmentation diversity was calculated using a Shannon-wiener index to examine a general trend in population changes.

4.2.3 Results

4.2.3.1 Water quality parameters

No significant differences ($p>0.05$) existed between influent and effluent levels for water quality parameters during the study period (Table 19).

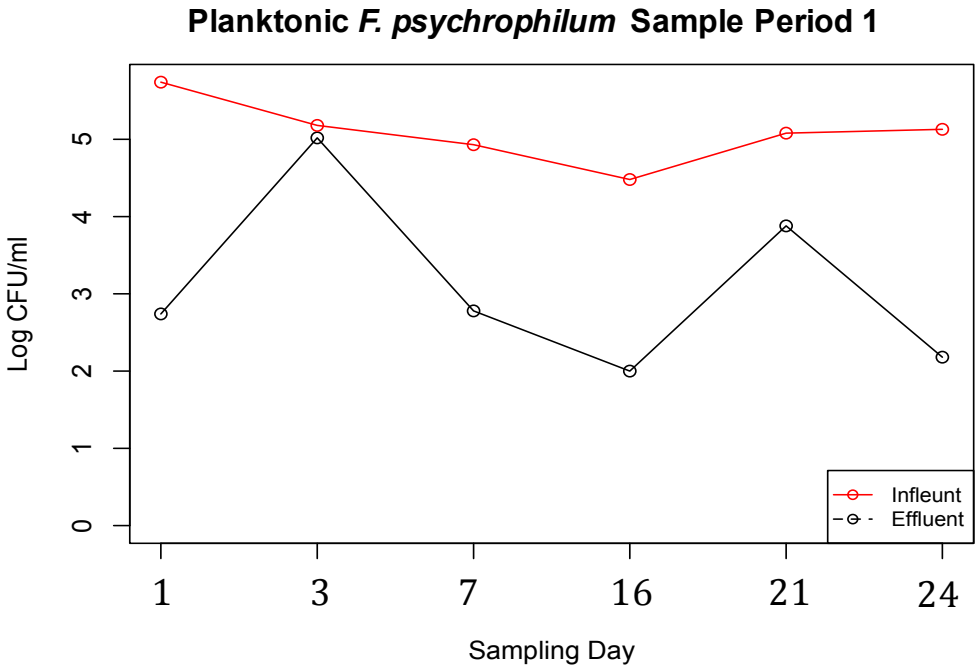
Table 19: Composition of the water in the UV influent and UV effluent in sample period 1 and 2 (mean ± SD) and the results of t-tests or a two sample Wilcoxon signed rank test (P-value).
[a] Analyzed using two sample Student's t-test.
[b] analyzed using a two-sample Wilcoxon signed-rank test

Parameters						
	Sample period 1			Sample Period 2		
	UV Influent	UV Effluent	P value	UV Influent	UV Effluent	P value
DO	9.99 ± 1.42	10.01± 0.64	0.9724[a]	9.12 ± 1.28	9.46 ± 1.06	0.5845[a]
Temperature	18.67 ± 2.34	18.50± 1.97	1[b]	17.00 ± 0.63	17.00 ± 0.63	1[b]
pH	7.82 ± 0.13	7.85 ± 0.11	0.7102[a]	7.82 ± 0.34	7.94 ± 0.21	0.457[a]
Conductivity	568.67 ± 49.64	565.67 ± 51.48	0.3991[b]	579.33 ± 65.77	566.83 ± 72.29	0.4604[a]
Turbidity	2.27 ± 1.66	2.08 ± 2.03	0.6037[a]	1.70 ± 1.28	2.22 ± 2.69	0.4649[b]
COD	16.42 ± 3.45	12.80 ± 4.81	0.1676[a]	12.87 ± 7.64	10.43 ± 6.77	0.5717[a]
TSS	4.83 ± 1.74	1.50 ± 0.00	0.0797[b]	3.80 ± 2.60	2.75± 3.06	0.0797[b]
Alkalinity	239.50 ± 26.82	240.00 ± 23.56	0.9357[b]	235.50 ± 27.89	256.50 ± 52.48	0.936[b]
TKN	0.85 ± 0.19	0.72 ± 0.09	0.2071[a]	0.70 ± 0.18	0.74 ± 0.23	0.7667[a]
NO_2^-	0.05 ± 0.01	0.05 ± 0.01	0.8263[a]	0.04 ± 0.00	0.04± 0.00	0.739[a]
NO_3^-	4.50 ± 0.73	4.58 ± 0.80	0.6868[b]	4.42 ± 1.00	4.36 ± 0.96	0.8364[a]
NH_3	0.13 ± 0.06	0.12 ± 0.04	0.5969[a]	0.11 ± 0.07	0.13 ± 0.06	0.5645[a]
TP	0.05 ± 0.02	0.03 ± 0.01	0.1808[a]	0.04 ± 0.04	0.04 ± 0.04	0.07193[b]
PO_4	0.01 ± 0.01	0.01 ± 0.01	0.3452[b]	0.01 ± 0.02	0.01 ± 0.03	0.8637[b]

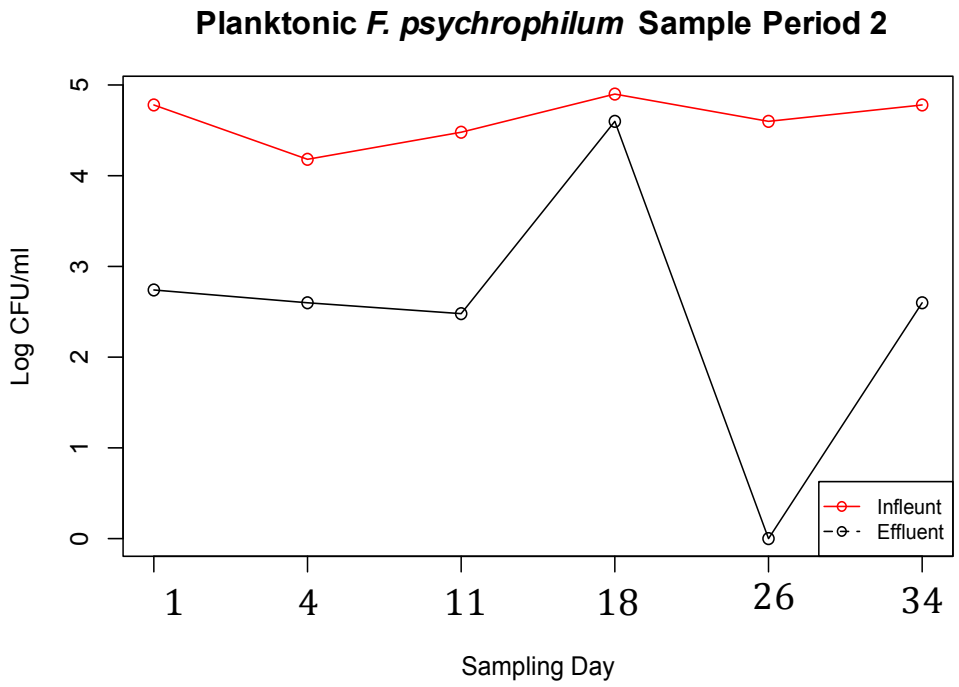
4.2.3.2 Planktonic Bacteria

Planktonic *F. psychrophilum* ranged from 5 x10^4 to 5.50 x 10^5 CFU/ml in the UV influent over the entire study period. The highest value was observed in the first sampling period on June 17, 2014 and the lowest value was observed in the second sampling period on July 14, 2014. In the UV effluent water *F. psychrophium* ranged from undetectable to 1.05 x 10^5 CFU/ml. *F. psychrophilum* was undetectable in the UV effluent on August 5, 2014. *F. psychrophilum* levels in the UV effluent were consistently lower than the levels in the UV influent (Figure 15). Planktonic concentrations of *F. psychrophlium* remained relatively stable in the UV influent water over each sample period (Figure 15). The densities in the UV effluent water had intermittent spikes of higher densities. One-way ANOVA, however, showed there was no significant variation over the sampling days for either the UV influent or the UV effluent densities of *F. psychrophilum* for either sampling period.

Figure 15: Planktonic *Flavobacterium psychrophilum* in UV influent and UV effluent for sampling period 1 (top) and sample period 2 (bottom).



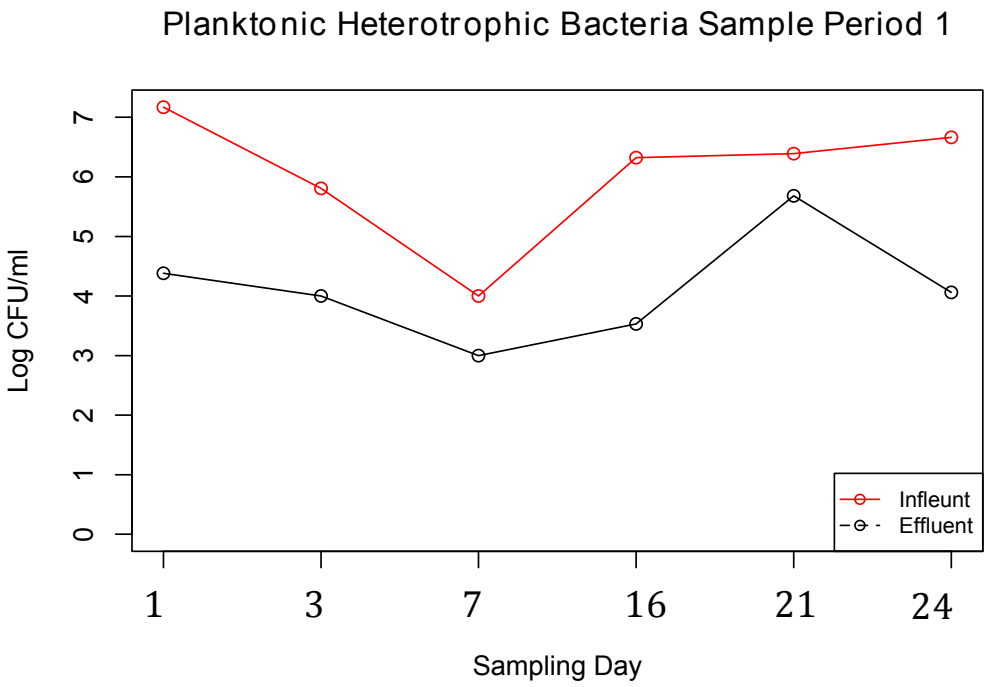
*** There were power outages on day 2, 9 and 20 during the sampling period.**



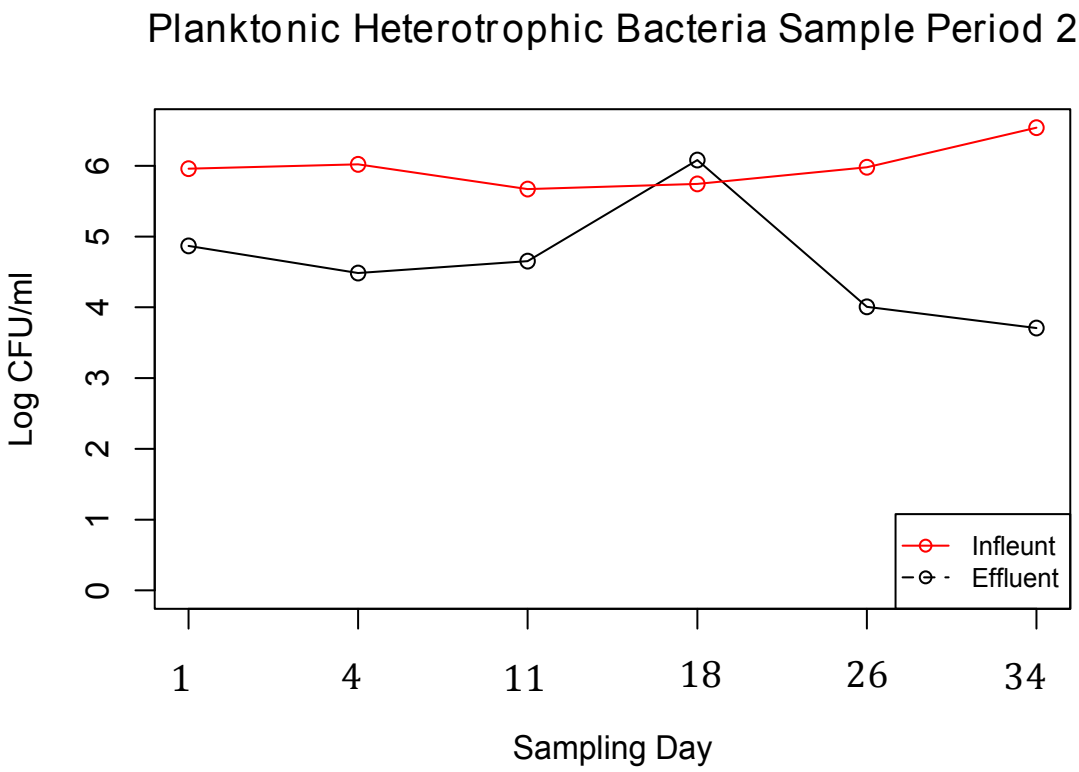
*** There was power outage on day 25 of the sampling period.**

Planktonic heterotrophic bacteria ranged from 1.00×10^4 to 1.48×10^7 CFU/ml in the UV influent over the study period. The lowest value was observed on June 23, 2014 and the highest value was observed on June 17, 2014. Planktonic heterotrophic bacteria in the UV effluent ranged from 1.00×10^3 to 1.21×10^6 CFU/ml over the study period. The lowest value was observed on June 23, 2014 and the highest value was observed on July 28 2014. One-way ANOVA showed there was no significant variation over the sampling days for UV influent or UV effluent concentrations of heterotrophic bacteria for either sampling period.

Figure 16: Planktonic heterotrophic bacteria from UV influent and UV effluent for sample period 1 (top) and sample period 2 (bottom).



*** There were power outages on day 2, 9 and 20 during the sampling period.**



*** There was power outage on day 25 of the sampling period.**

UV Influent and UV effluent levels were significantly ($p<0.05$) different from each other for planktonic heterotrophic bacteria and *F. psycrhophilum* (Table 20) for both sampling periods. The boxplots below show the significant variation for sampling period 1 (Figure 17) and for sampling period 2 (Figure 18).

Table 20: Bacterial concentrations of heterotrophic bacteria and *Flavobacterium psychrophlium* in the UV influent and UV effluent for Sample period 1 and 2 (mean ± SD) and results of t-test (P-value).
[a] Analyzed using two sample Student's t-test.
[b] analyzed using a two-sample Wilcoxon signed-rank test.

Parameters						
	Sample period 1			Sample Period 2		
	UV Influent	UV Effluent	P value	UV Influent	UV Effluent	P value
Heterotrophic Bacteria	$4.09 \times 10^6 \pm 5.46 \times 10^6$	$8.83 \times 10^4 \pm 1.92 \times 10^5$	0.0078[a]	$1.23 \times 10^6 \pm 1.11 \times 10^6$	$2.28 \times 10^5 \pm 4.79 \times 10^5$	0.0085[a]
F. psychrophilum	$1.78 \times 10^5 \pm 1.87 \times 10^5$	$1.90 \times 10^4 \pm 4.22 \times 10^4$	0.0065[a]	$4.75 \times 10^4 \pm 2.36 \times 10^4$	$6.94 \times 10^3 \pm 1.62 \times 10^4$	0.01575[b]

Figure 17: Sample Period 1 planktonic heterotrophic bacteria concentrations in the UV influent and UV effluent (left) and planktonic *Flavobacterium psychrophilum* concentrations in the UV influent and UV effluent (right). Boxplots show median values (solid horizontal line), 50th percentile values (box outline), 90th percentile values (whiskers) and outlier values (open circles) Site 1 is influent, and Site 2 is effluent.

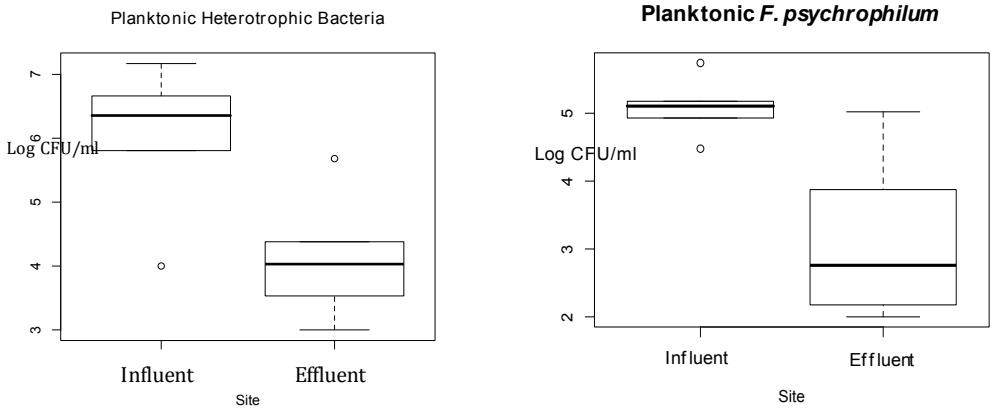
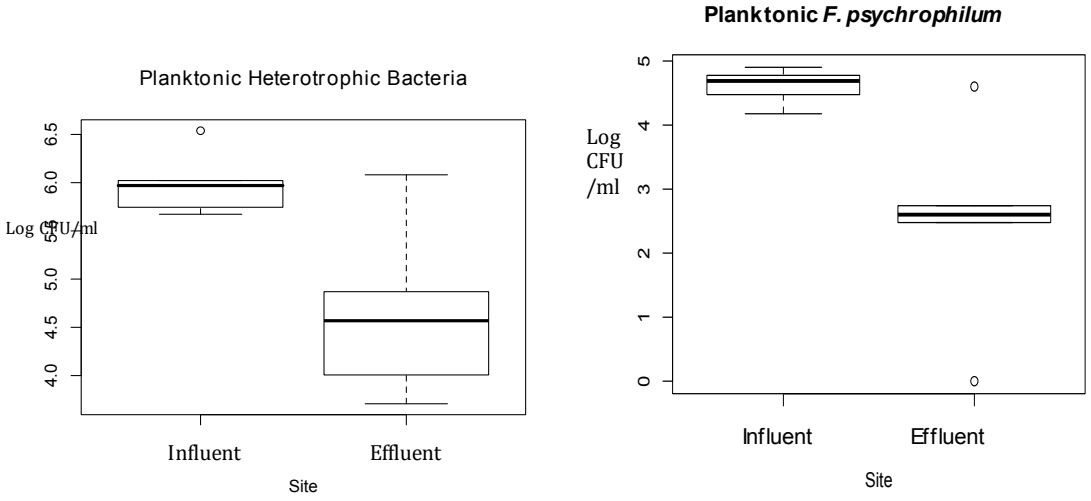


Figure 18: Sample period 2 planktonic heterotrophic bacteria concentrations in the UV influent and UV effluent (left) and planktonic *Flavobacterium psychrophilum* concentrations in the UV influent and UV effluent (right).Boxplots show median values (solid horizontal line), 50th percentile values (box outline), 90th percentile values (whiskers) and outlier values (open circles). Site 1 is influent, and Site 2 is effluent.



During the study period removal efficiencies of *F. psychrophilum* was 87.99 ± 0.24% (Table 21). On several sampling dates 99% removal efficiency was achieved. The lowest removal efficiency occurred on June 19, 2014 (30%). On one occasion, August 5, 2014, there was no detection of *F. psychrophilum* in the UV effluent.

Table 21: Planktonic *Flavobacterium psychrophilum* over the study period mean (± standard deviations) with removal efficiency and log reduction.

Date of Collection	UV Influent	UV Effluent	Removal Efficiency	LOG_{10} Reduction
June 17, 2014	$5.50 \times 10^5 \pm 7.07 \times 10^4$	$5.50 \times 10^2 \pm 3.54 \times 10^2$	99.9%	3
June 19. 2014	$1.50 \times 10^5 \pm 2.12 \times 10^5$	$1.05 \times 10^5 \pm 4.95 \times 10^4$	30%	0.15
June 23, 2014	$8.50 \times 10^4 \pm 7.07 \times 10^3$	$6.00 \times 10^2 \pm 8.49 \times 10^2$	99.29%	2.15
July 2, 2014	$3.00 \times 10^4 \pm 1.41 \times 10^4$	$1.00 \times 10^2 \pm 1.41 \times 10^2$	99.67%	2.48
July 7, 2014	$1.20 \times 10^5 \pm 4.24 \times 10)$	$7.50 \times 10^3 \pm 7.07 \times 10^2$	93.75%	1.20
July 10, 2014	$1.35 \times 10^5 \pm 1.20 \times 10^5$	$1.50 \times 10^2 \pm 7.07 \times 10^1$	99.89%	2.95
July 11, 2014	$6.00 \times 10^4 \pm 7.07 \times 10^4$	$5.50 \times 10^2 \pm 2.12 \times 10^2$	99.083%	2.04
July 14, 2014	$1.50 \times 10^4 \pm 7.07 \times 10^3$	$4.00 \times 10^2 \pm 0$	97.33%	1.578
July 21, 2014	$3.00 \times 10^4 \pm 2.83 \times 10^4$	$3.00 \times 10^2 \pm 0$	99%	2
July 28, 2014	$8.00 \times 10^4 \pm 5.66 \times 10^4$	$4.00 \times 10^4 \pm 0$	50%	0.30
August 5, 2014	$4.00 \times 10^4 \pm 1.41 \times 10^4$	$0.00 \times 10^0 \pm 0$	100%	
August 13, 2014	$6.00 \times 10^4 \pm 4.24 \times 10^4$	$4.00 \times 10^2 \pm 2.83 \times 10^2$	99.33%	2.18

During the study period removal efficiencies of heterotrophic bacteria was 77.45 ± 0.62% (Table 22). On several sampling dates 99% removal efficiency was achieved. The lowest removal efficiency occurred on July 28, 2014 (-117.12%) on this date the heterotrophic bacteria levels were higher in the UV effluent than in the UV influent.

Table 22: Planktonic heterotrophic bacteria over the study period mean (± standard deviation) with removal efficiency and log reduction.

Date of Collection	UV Influent	UV Effluent	Removal Efficiency	LOG_{10} Reduction
June 17, 2014	$1.48x\ 10^7 \pm 1.91 \times 10^6$	$2.40 \times 10^4 \pm 4.95 \times 10^3$	99.84%	2.79
June 19. 2014	$6.40 \times 10^5 \pm 0$	$1.00 \times 10^4 \pm 0$	98.44%	1.80
June 23, 2014	$1.00 \times 10^4 \pm 0$	1.00×10^3 (0)	90%	1
July 2, 2014	$2.10 \times 10^6 \pm 2.33 \times 10^5$	$3.40 \times 10^3 \pm 1.41 \times 10^2$	99.84%	2.79
July 7, 2014	$2.46 \times 10^6 \pm 9.19 \times 10^4$	$4.80 \times 10^5 \pm 1.27 \times 10^5$	80.49%	0.71
July 10, 2014	$4.60 \times 10^6 \pm 8.48 \times 10^4$	$1.15 \times 10^4 \pm 2.26 \times 10^3$	99.75%	2.60
July 11, 2014	$9.10 \times 10^5 \pm 2.83 \times 10^4$	$7.40 \times 10^4 \pm 1.27 \times 10^4$	91.87%	1.09
July 14, 2014	$1.05 \times 10^6 \pm 1.13 \times 10^5$	$3.05 \times 10^4 \pm 7.07 \times 10^2$	97.09%	1.54
July 21, 2014	$4.70 \times 10^5 \pm 1.41 \times 10^4$	$4.50 \times 10^4 \pm 2.83 \times 10^3$	90.43%	1.02
July 28, 2014	$5.55 \times 10^5 \pm 7.07 \times 10^3$	$1.21 \times 10^6 \pm 4.88 \times 10^5$	-117.12%	-0.34
August 5, 2014	$9.55 \times 10^5 \pm 4.74 \times 10^5$	$1.02 \times 10^4 \pm 3.46 \times 10^3$	98.94%	1.97
August 13, 2014	$3.45 \times 10^6 \pm 2.12 \times 10^5$	$5.10E \times 10^3 \pm 1.70 \times 10^3$	99.85%	2.83

Removal efficiencies of *F. psychrophilum* and heterotrophic bacteria between the first sampling period and the second sampling period were not significantly different ($p=0.7981$; $p=0.3762$).

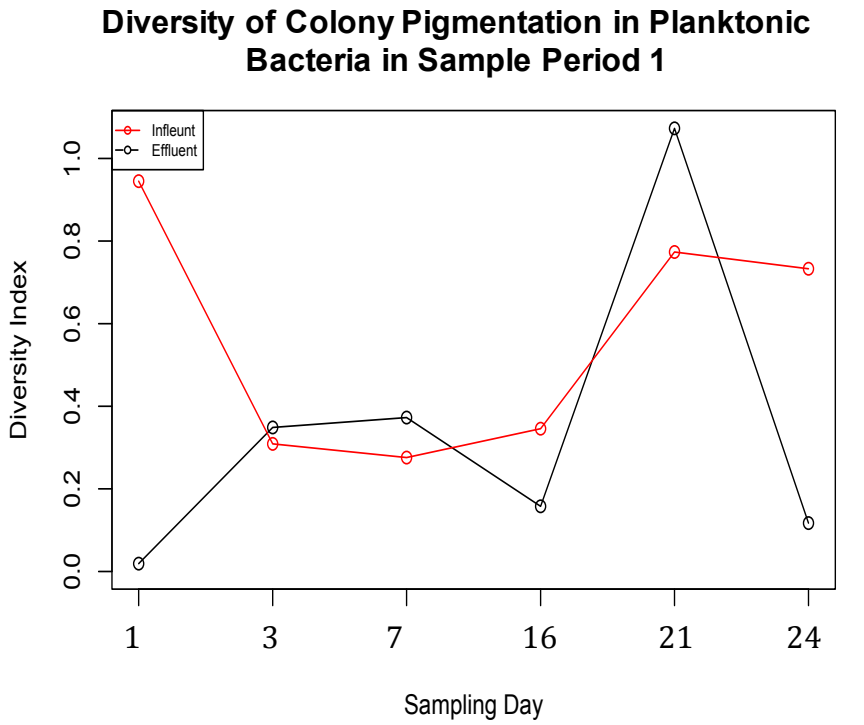
Table 23: Mean (± standard deviations) of bacterial counts, removal efficiency and log reduction by UV between the first and second sampling period.

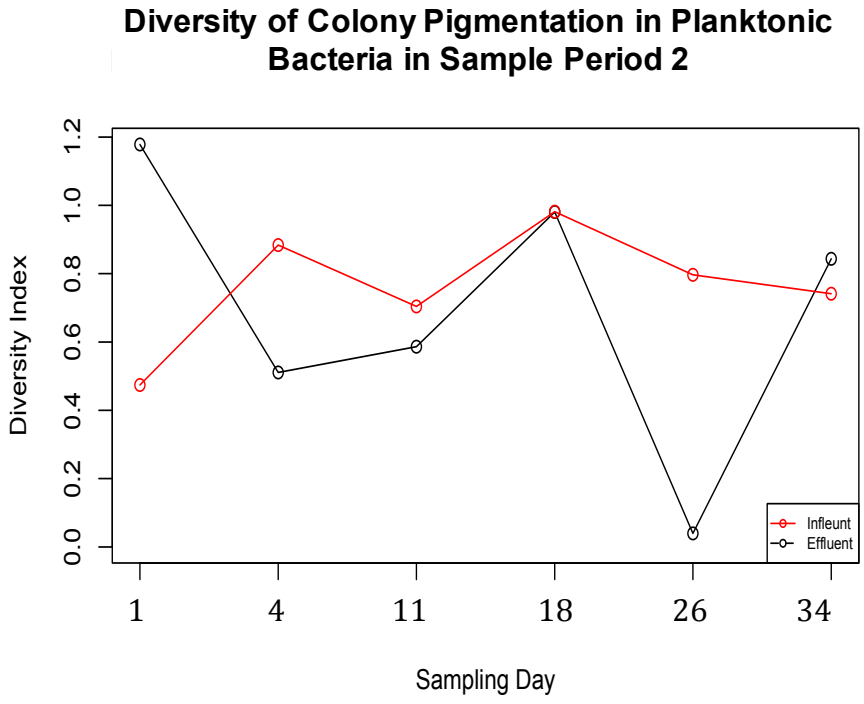
	SP	UV Influent (CFU/mL)	UV Effluent (CFU/mL)	Removal (%)	Log_{10} Reduction
F. psychrophilum	1	$9.77 \times 10^7 \pm 9.48 \times 10^6$	$7.09 \times 10^6 \pm 6.37 \times 10^6$	87.08 ±0.28	1.98±1.12
F. psychrophilum	2	$8.54 \times 10^6 \pm 6.39 \times 10^6$	$1.13 \times 10^7 \pm 9.66 \times 10^6$	90.79 ±0.20	1.84±0.89
Heterotrophic Bacteria	1	$2.55 \times 10^8 \pm 2.13 \times 10^8$	$1.61 \times 10^8 \pm 1.62 \times 10^8$	94.73 ±0.08	1.95±0.93
Heterotrophic Bacteria	2	$2.03 \times 10^8 \pm 1.17 \times 10^8$	$4.63 \times 10^8 \pm 3.85 \times 10^8$	60.18 ±1.35	0.87 ±1.06

In order to investigate the number of different heterotrophic bacterial groups which established in the UV influent and UV effluent, the number of distinct colony types detected on R2A plates were recorded according to the colour and morphology (Kerr et al., 1998). The diversity of Planktonic colony pigmentation in planktonic bacteria ranged from 0.28-0.94 in the UV influent for sample period one, with an average of 0.56 (0.28). The lowest diversity was observed on June 23, 214 (day 7), and the highest on June 17, 2014 (day1) (Figure 19). Planktonic colony pigmentation diversity ranged from 0.019-1.07 in the UV effluent for sample period one with an average of 0.35 (0.38). The lowest diversity was observed on June 17, 2014 (day 1) and the highest was observed on July 7, 2014 (day 21) (Figure 19).

Planktonic colony pigmentation diversity ranged from 0.47-0.98 in the UV influent for sample period two with an average of 0.76 (0.17). The lowest diversity was observed on July 11, 2014 (day 1), and the highest on July 28, 2014 (day 18) (Figure 19). Planktonic colony pigmentation diversity ranged from 0.040-1.18 in the UV effluent for sample period one with an average of 0.69 (0.40). The lowest diversity was observed on August 5, 2014 (day 26) and the highest was observed on July 11, 2014 (day 1). (See Appendix for area graphs which offer a visual depiction of the diversity of colony pigmentation in the planktonic bacteria from the UV influent and UV effluent).

Figure 19: Diversity of Colony Pigmentation in Planktonic bacteria over sampling days for UV influent and UV effluent for sample period 1 (top) and sample period 2 (bottom).





One-way analysis of variance showed that planktonic colony pigmentation diversity did not vary significantly over the days sampled for either the UV influent or UV effluent (see Table in appendix).

Planktonic colony pigmentation did not vary significantly between the UV influent and UV effluent during either sample period 1 or sample period 2 (Table 24).

Table 24: Bacterial concentrations planktonic colony pigmentation in the UV influent and UV effluent for Sample period 1 and 2 (mean ± SD) and results of t-test (P-value).
[a] Analyzed using two sample Student's t-test.
[b] analyzed using a two-sample Wilcoxon signed-rank test.

Parameters						
	Sample period 1			Sample Period 2		
	UV Influent	UV Effluent	P value	UV Influent	UV Effluent	P value
Colony Pigmentation	0.56 ± 0.28	0.35 ± 0.38	0.296[a]	0.76 ± 0.17	0.69 ± 0.30	0.6934[a]

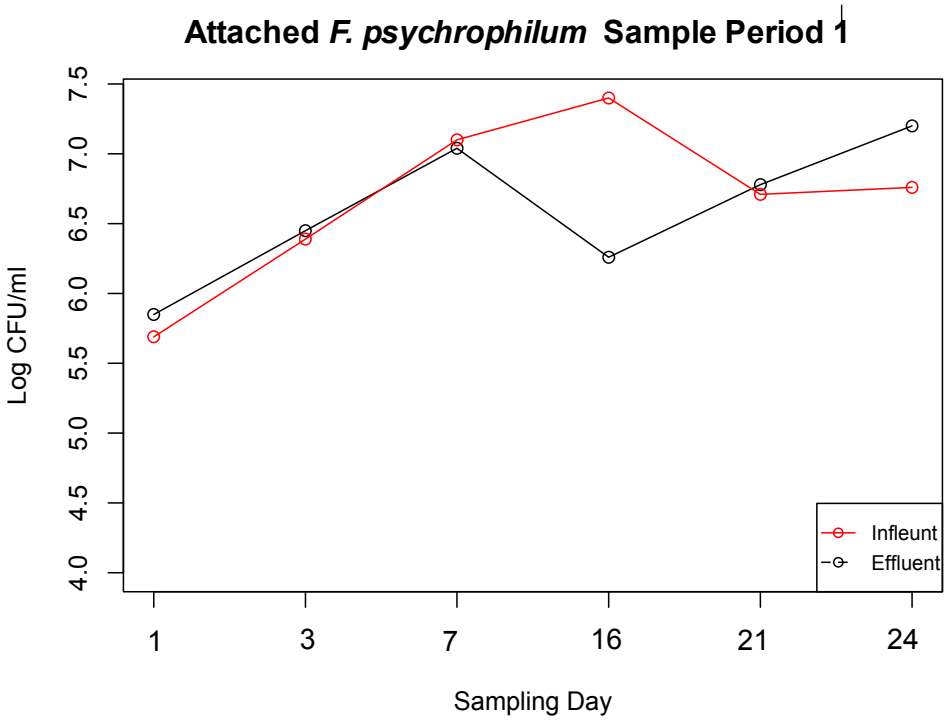
4.2.3.3 Attached Bacteria

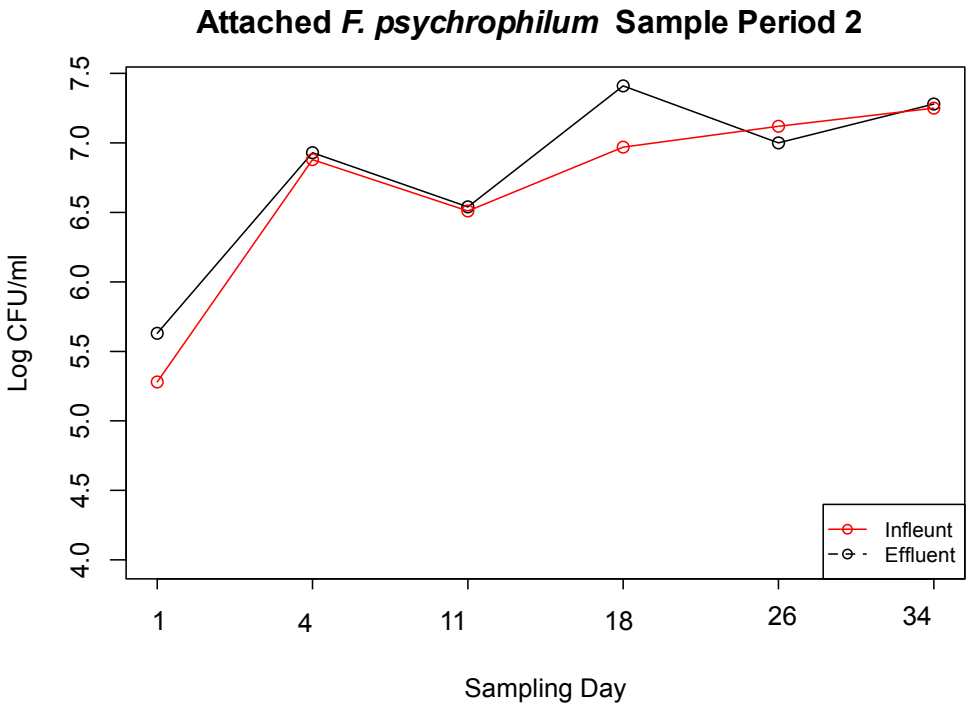
One-way ANOVA showed no significant difference ($p>0.05$) between attached heterotrophic bacteria or *F. psychrophilum* in the UV influent or UV effluent for either sampling period (Figure 24 and Table Appendix).

Attached *F. psychrophilum* ranged from 1.89×10^5 to 2.49×10^7 CFU/cm^2 in the UV influent over the study period (Figure 20). The lowest concentration was observed on July 11, 2014 (day 1) in the second sampling period. The highest concentration was observed on July 16, 2014 (day 16) in the first sampling period. Attached *F. psychrophilum* ranged from 4.29×10^5 to 2.60×10^7 CFU/cm^2 in the UV effluent over the study period. The lowest concentration was observed on July 11, 2014 (day 1) in the second sampling period. The highest concentration was observed on July 28, 2014 (day 18) during the second sampling period. One-way analysis of variance showed that attached *F. psychrophilum* did not vary significantly over the sampling days for sample period 1. Attached *F. psychrophilum* in the UV

influent reached near significant ($p<0.1$) difference over sampling days during sample period 2, but did not vary significantly over sampling days in the UV effluent.

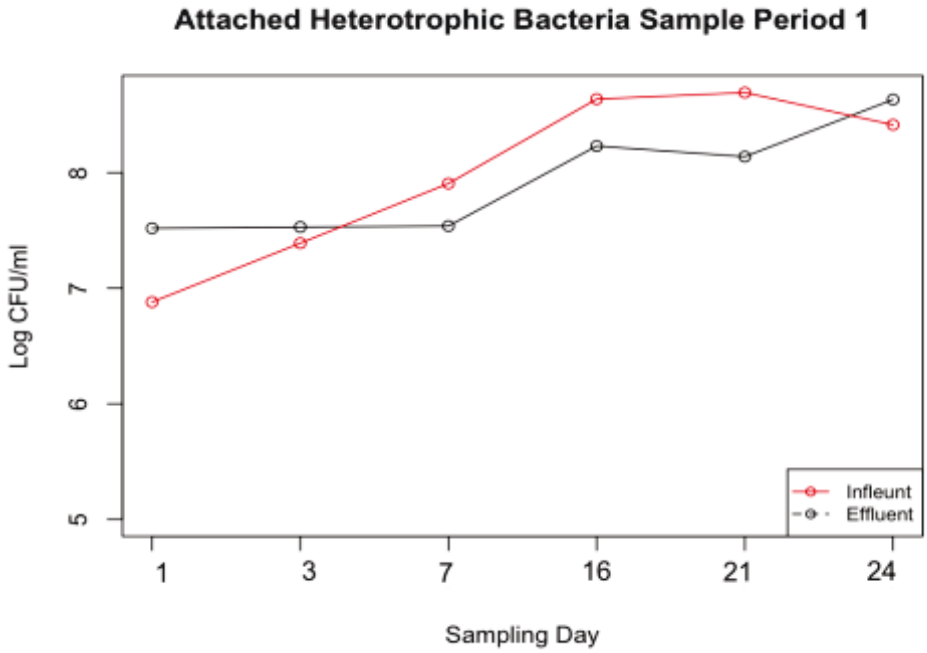
Figure 20: Attached *Flavobacterium* psychrophilum from UV influent and UV effluent for sample period 1 (top) and sample period 2 (bottom).

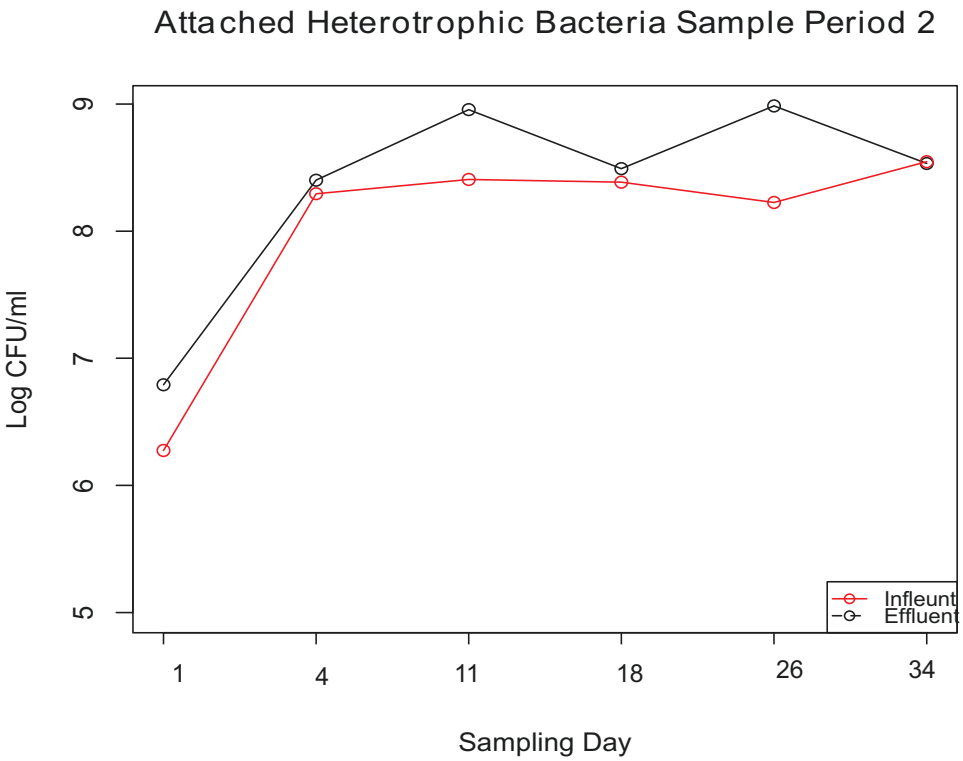




Attached heterotrophic bacteria ranged from 1.88 x 10^6 to 4.94 x 10^8 CFU/cm^2 in the UV influent during the study period (Figure 21). The lowest concentration was observed on July 11, 2014 (day 1) during the second sampling period. The highest concentration was observed on July 10 (day 21) during the first sampling period. Attached heterotrophic bacteria ranged from 6.19 x 10^6 to 9.66 x 10^8 CFU/cm^2 in the UV effluent during the study period. The lowest concentration was observed on July 11, 2014 (day 1) in the second sampling period. The highest concentration was observed on August 5, 2014 (day 26) during the second sampling period. One-way analysis of variance showed significant variation ($p<0.05$) for attached heterotrophic bacteria in the UV influent and UV effluent during sampling period 1. Post hoc tests revealed that days 1,3, and 7 where significantly different from days 16, 21, and 24. One-way ANOVA results showed a significant variation in attached heterotrophic over the sampling days in the UV effluent ($p<0.01$). Post hoc analysis revealed that day 1 varied significantly from all other days.

Figure 21: Attached heterotrophic bacteria from UV influent and UV effluent for sample period 1 (top) and sample period 2 (bottom)





UV influent and UV effluent levels were not significantly ($p>0.05$) different from each other for attached heterotrophic bacteria and *F. psycrhophilum* (Table 25) for both sampling periods.

Table 25: Sample period 1 and 2 (mean ± SD, n= 5 n= 6, respectively) and results of t-test (P-value).
[a] Analyzed using t-test.
[b] analyzed using a two-sample Wilcoxon signed-rank test.

Parameters						
	Sample period 1			Sample Period 2		
	UV Influent	UV Effluent	P value	UV Influent	UV Effluent	P value
Heterotrophic Bacteria	2.55×10^8 (2.13×10^8)	1.61×10^8 (1.62×10^8)	0.8197[a]	2.03×10^8 (1.17×10^8)	4.63×10^8 (3.85×10^8)	0.1797[b]
F. psychrophilum	9.77×10^7 (9.48×10^6)	7.09×10^6 (6.37×10^6)	0.7935[a]	8.54×10^6 (6.39×10^6)	1.13×10^7 (9.66×10^6)	0.5887[b]

According to the rmANOVA results attached *F. psychrophilum* reached near significant ($p<0.1$) difference between the phases ($F_{2,12}=3.632$, $p<0.1$, Table 26) but did not vary significantly between sites or between sampling periods. Interaction results of rmANOVA between sites, phase and sampling periods resulted in significant variation of attached *F. psychrophilum.*

According the results of the rmANOVA attached heterotrophic bacteria varied significantly between phases ($F_{2,12}=4.839$, $p<0.05$, Table 26). The post hoc analysis indicated significant variation for attached heterotrophic bacteria for phase 1 and phase 3. Interaction results of rmANOVA between sites and sampling period, and site, phase and sampling periods resulted in significant variation (Table 26).

Table 26: Results of repeated measnures ANOVA for attached *Flavobacterium psychrophilum* and heterotrophic bacteria. The resultant F and p values were obtained after a Greenhouse-Geisser correction.

	F	p		F	p
F. psychrophilum			Heterotrophic Bacteria		
Sites	0.385	0.688	Sites	2.747	0.123
Phase*	3.632	0.058	Phase	4.839	0.029
Sampling Periods	0.928	0.354	Sampling Periods	1.384	0.262
Sites x Phase	1.660	0.231	Sites x Phase	0.242	0.788
Sites x Sampling period	5.634	0.035	Sites x Sampling period	27.025	0.000
Phase x Sampling period	0.557	0.587	Phase x Sampling period	0.149	0.864
Site x Phase x Sampling period	14.394	0.001	Site x Phase x Sampling period	6.564	0.012

***3 phases; early (days 1-5), middle (days 6-20) and late (21-34)**

During the study period the differences in *F. psychrophilum* biofilm growth in the UV influent and UV effluent of attached *F. psychrophilum* was -40.35 ± 0.86% (Table 27). The lowest difference between UV influent and UV effluent occurred on July 28, 2014 (-178.57%). The highest difference between UV influent and UV effluent that was achieved for *F. psychrophilum* was 92.71%. On 8 occasions the biofilm in the UV effluent contained more *F. psychrophilum* than the UV influent.

Table 27: Attached differences between UV influent and UV effluent of *Flavobacterium physprophilum*

Date of Collection	UV Influent	UV Effluent	Biofilm Differences	LOG_{10} Reduction
June 17, 2014	$4.85x\ 10^5$ (4.13×10^5)	7.13×10^5 (6.15×10^5)	-46.98%	-0.17
June 23, 2014	1.25×10^7 (1.05×10^7)	1.09×10^7 (3.76×10^6)	12.77%	0.06
July 2, 2014	2.49×10^7 (2.35×10^7)	1.81×10^6 (1.65×10^6)	92.71%	1.14
July 7, 2014	5.16×10^6 (1.53×10^6)	6.07×10^6 (4.74×10^6)	-17.67%	-0.07
July 10, 2014	5.78×10^6 (5.47×10^6)	1.59×10^7 (1.40×10^7)	-175.77%	-0.44
July 11, 2014	1.89×10^5 (1.84×10^5)	4.29×10^5 (3.33×10^5)	-127.56%	-0.36
July 14, 2014	7.67×10^6 (2.48×10^6)	8.51×10^6 (7.29×10^6)	-11.04%	-0.05
July 21, 2014	3.20×10^6 (8.55×10^5)	3.43×10^6 (3.43×10^6)	-7.29%	-0.03
July 28, 2014	9.33×10^6 (5,74 $\times 10^6$)	2.60×10^7 (1.71×10^7)	-178.57%	-0.44
August 5, 2014	1.33×10^7 (6.33×10^6)	1.01×10^7 (5.34×10^6)	24.12%	0.12
August 13, 2014	1.76×10^7 (1.17×10^7)	1.91×10^7 (1.39×10^7)	-8.71%	-0.04

During the study period the difference between the UV influent and the UV effluent of attached heterotrophic bacteria was -110.92 ± 1.83% (Table 28). The lowest difference between UV influent and UV effluent was recorded on August 5, 2014 (-474.33%). The highest difference between UV influent and UV effluent that was achieved for heterotrophic bacteria was 72.10%. On 7 occasions the biofilm in the UV effluent contained more heterotrophic bacteria than the UV influent.

Table 28: Attached differences between UV influent and UV effluent of heterotrophic bacteria.

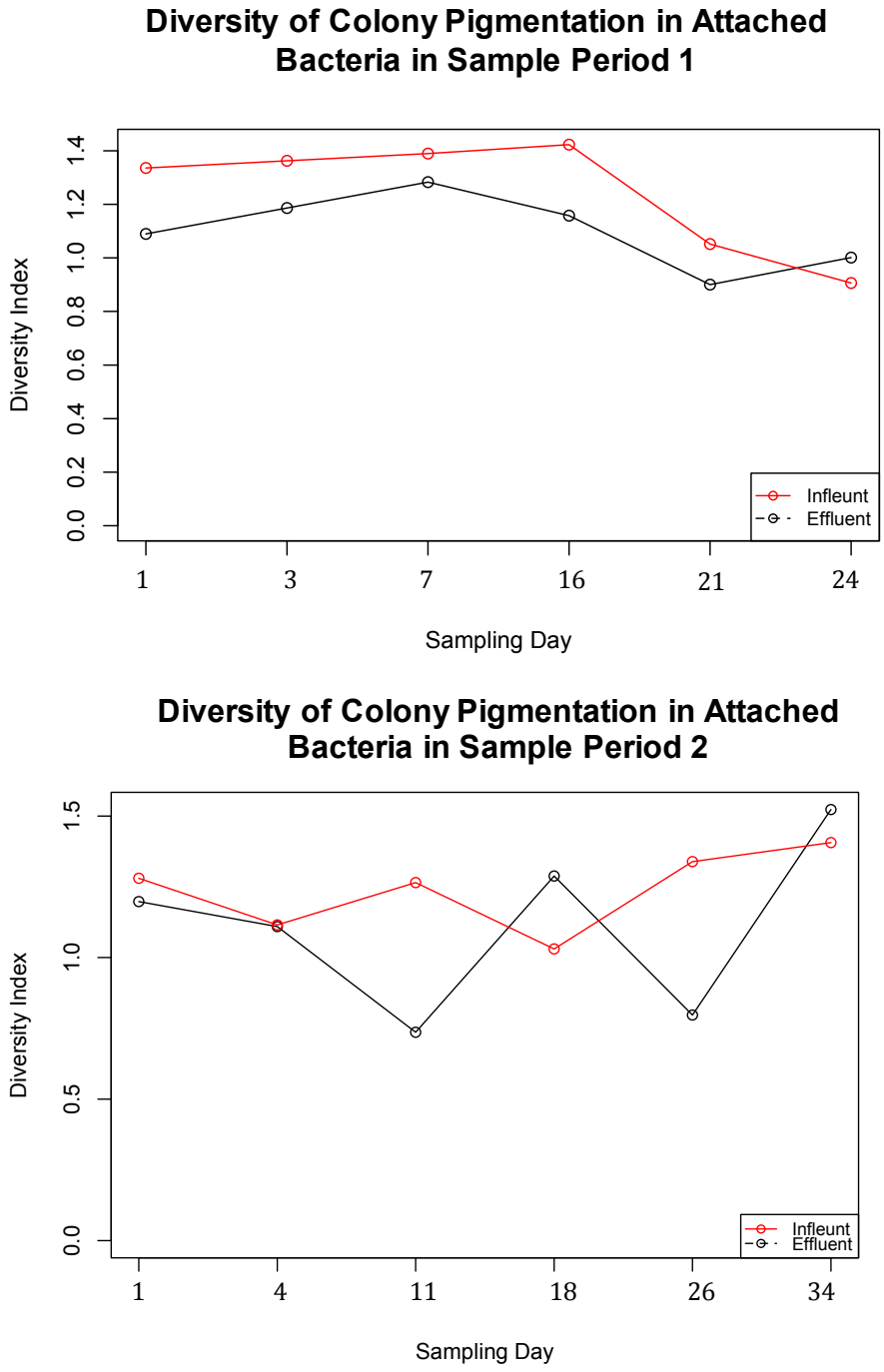
Date of Collection	UV Influent	UV Effluent	Biofilm Differences	LOG_{10} Reduction
June 17, 2014	7.60×10^6 (9.02×10^5)	3.30×10^7 (8.79×10^6)	-334.74%	-0.64
June 23, 2014	8.07×10^7 (1.27×10^7)	3.46×10^7 (5.82×10^6)	57.20%	0.37
July 2, 2014	4.34×10^8 (1.07×10^8)	1.70×10^8 (2.17×10^7)	60.78%	0.41
July 7, 2014	4.94×10^8 (8.65×10^7)	1.38×10^8 (1.09×10^7)	72.10%	0.55
July 10, 2014	2.60×10^8 (7.39×10^7)	4.30×10^8 (1.20×10^8)	-65.44%	-0.22
July 11, 2014	1.88×10^6 (1.29×10^5)	6.19×10^6 (1.80×10^6)	-229.22%	-0.52
July 14, 2014	1.97×10^8 (1.00×10^7)	2.52×10^8 (2.29×10^7)	-27.74%	-0.11
July 21, 2014	2.55×10^8 (2.13×10^7)	9.04×10^8 (1.38×10^8)	-254.23%	-0.55
July 28, 2014	2.43×10^8 (4.73×10^7)	3.10×10^8 (3.92×10^8)	-27.63%	-0.11
August 5, 2014	1.68×10^8 (1.32×10^7)	9.66×10^8 (1.44×10^8)	-474.33%	-0.76
August 13, 2014	3.52×10^8 (7.96×10^7)	3.41×10^8 (8.16×10^7)	3.18%	0.01

The diversity of colony pigmentation diversity in attached bacteria ranged from 0.91-1.43 in the UV influent for sample period one, with an average of 1.22 (0.23). The lowest diversity was observed on July 10, 2014 (day 24), and the highest on July 2, 2014 (day 16) (Figure 22). Attached colony pigmentation diversity ranged from 1.03-1.41 in the UV effluent for sample period one with an average of 1.24 (0.14). The lowest diversity was observed on July 28, 2014 (day 18) and the highest was observed on August 13, 2014 (day 34) (Figure 22).

Attached colony pigmentation diversity ranged from 0.90-1.28 in the UV influent for sample period two with an average of 1.09 (0.15). The lowest diversity was observed on July 7, 2014 (day 21), and the highest on June 17, 2014 (day 1) (Figure 22). Attached colony pigmentation diversity ranged from 0.74-1.52 in the UV effluent for sample period one with an average of 1.11 (0.30). The lowest

diversity was observed on July 21, 2014 (day 11) and the highest was observed on August 13, 2014 (day 34).

Figure 22: Attached colony pigmentation diversity over sampling days for UV influent and UV effluent for sample period 1 (top) and sample period 2 (bottom).



UV influent and UV effluent colony pigmentation diversity was not significantly ($p>0.05$) different from each other for attached heterotrophic bacteria and *F. psycrhophilum* for both sampling periods (Table 29) (See Appendix for area graphs which offer a visual depiction of the diversity of colony pigmentation in the attached bacteria from the UV influent and UV effluent).

Table 29: Bacterial concentrations attached colony pigmentation in the UV influent and UV effluent for Sample period 1 and 2 (mean ± SD) and results of t-test (P-value).
[a] Analyzed using two sample Student's t-test.
[b] analyzed using a two-sample Wilcoxon signed-rank test.

Parameters						
	Sample period 1			Sample Period 2		
	UV Influent	UV Effluent	P value	Influent	UV Effluent	P value
Colony Pigmentation	1.22±0.23	1.09±0.15	0.3049[a]	1.24±0.14	1.11±0.30	0.3644[a]

4.2.4 Discussion

In this trial, *F. psychrophilum,* was demonstrated to be more sensitive to UV than was previously reported (Hedrick et al., 2000). Potential factors that may have affected the test results are differences in: apparatus setup, column dimensions, UV lamp type and output, intensity measurement, shutter type and operation, petri dish specifications, sample volume and depth of liquid, mixing condition, laboratory setting, water temperature, and types of microbial organism (strains, age, and assay methods to quantify inactivation) (Kuo et al., 2003). It is difficult to determine which factor could be responsible for the discrepancy between the two results. Further investigation is required to determine more accurately a reproducible UV dose for *F. psychrophilum*.

As expected, planktonic concentrations of both heterotrophic bacteria and *Flavobacterium psychrophilum* were significantly lowered in the UV effluent relative to the UV influent water. The concentration of attached heterotrophic bacteria and *F. psychrophilum* was not significantly affected by UV treatment. The results did support the hypothesis that planktonic bacterial loads would be reduced but attached bacterial densities would remain similar.

During the sample period power outages occurred on several days (see Table 30). During these times both the lamp and the pump were off, except for June 25 when the lamp was off and the pump remained powered. The water remained in the unit (and the slides submerged), and the slides in the UV effluent chamber were exposed to untreated water. Only one power outage coincided with a sampling date: the power was off from the evening before sampling on July 28, 2014 (day 18) and remained off until the morning prior to sampling. The largest concentrations for both planktonic *F. psychrophilum* and heterotrophic bacteria were observed in samples collected that day. In addition, on that day the UV effluent concentration of planktonic heterotrophic bacteria was measured to be higher than the UV influent level (Figure 16). Therefore, the power outages may be responsible for the decreased efficacy in reducing levels of both planktonic heterotrophic bacteria and planktonic *F. psychrophilum*.

Table 30: Days and times of power outage
*** pump was still running**

Sample Period 1	Sample period 2
June 18, 2014 (day2) - 2:25-3:03pm June 25, 2014 (day 9)– 1:30-2:45pm* July 6, 2014 (day 20)– 2:45-4:00pm (and power flickered on/off all night)	July 28, 2014 (day 18)– 8:40pm -8:15am

Environmental changes, such as UV irradiation, can be stressful for bacteria. Irie and Parsek (2008) stated "to protect themselves from such types of stress, bacteria may form biofilms, a lifestyle that is characteristically more stress-resistant". The study did not examine *F. psychrophilum* or UV irradiation specifically, but intercellular signaling in multiple species. A study by Pérez-Pascual et al. (2010) studied the spreading of *F. psychrophilum* in response to different nutrient concentrations. These authors speculated that *F. psychrophlium* is able to alternate between a free-living and attached state and this response was "to

enhance the survival of bacteria in nutrient stressed environments" (Pérez-Pascual et al., 2010). The authors only examined stress related to nutrient concentrations, however. Similarly, the observed biofilm in the UV effluent may be a response of the surviving bacteria to enhance survival due to stress caused by the UV irradiation.

UV disinfection is an effective method for the inactivation of bacterial fish pathogens in "good quality water" (Liltved et al., 1995). The presence of particles in water may provide protection to bacteria (Liltved et al., 1995). The particle size and the particle-microbe association affect the effectiveness of UV (Gullian et al., 2012). Therefore the quality of water entering a UV unit has a strong impact on UV efficacy and the subsequent penetration level of the UV (Guerrero-Beltr and Barbosa, 2004). A study by Sharrer et al. (2005) applied 6 doses of UV irradiation on a recirculating aquaculture facility and found that the UV dose required to inactivate 100% heterotrophic bacteria was in excess of 1800mWs/cm^2. The TSS levels at the Lyndon Fish Hatchery were similar to those from the Sharrer et al. (2005) study (3.5 ± 0.4mg/L), even at these low concentrations, they hypothesized it was sufficient enough to reduce UV effectiveness due to bacteria embedded in or shielded by particles. The possibility of bacteria being shielded by particles is supported by the colony diversity data. The colony diversity was not significantly different in the UV effluent. One would expect the diversity to be different in the UV effluent, if only UV resistant bacteria were to survive (Pozos et al., 2004).

Several authors have noted the difficulty of inactivating 100% bacteria in a given system due to the bacteria embedded in particulate matter (Sharrer et al., 2005; Liltved et al., 1995). Therefore, when total inactivation of bacteria is not achieved it leads to the growth and development of biofilms that are not significantly different in treated and untreated water. In this study we did not achieve 100% inactivation of bacteria and therefore the biofilm in the UV influent and UV effluent remained similar.

It is important to state that during sample period 1, there was an infestation of black fly larvae attached to the slides. The combination of pumped flow of water and the smooth surfaces of the glass slides provided suitable conditions for the

proliferation of black fly larva. Black fly larvae inhabit fast flowing water and prefer attachment on smooth surfaces (Donahue and Schindler, 1998). Larva diet consists of unselectively filter feeding on bacteria (Kurtak, 1978). The amount of larvae on the slides was not quantified, however, Donahue and Schindler (1998) investigated the emigration and colonization responses of blackfly larvae to ultraviolet radiation and found that blackfly larvae respond to changes in UV exposure . Thus, this suggests that blackfly larvae attachment in the UV influent could have been greater than the UV effluent as blackflies were exposed to the UV dose may not initially attach downstream. Thus, the biofilm in the UV influent may be understated due to more grazing of bacteria than in UV effluent, during sampling period 1. The results of the present study, however, revealed that there was no significant difference between the sampling periods (Table 26).

Chapter 5 Conclusions

As the intensity of aquaculture continues to grow it is imperative that pathogen control techniques are optimized to control the spread of pathogens and limit mortality. *Flavobacterium psychrophilum* can cause mortalities ranging from 10-70% (Nematollahi et al., 2003a) and thus alternative pathogen techniques need to be explored to reduce the prevalence of *F. psychrophilum* in Ontario land-based fish farms. A relationship between water quality parameters and bacterial growth could help determine whether an association between *F. psychrophilum* and water quality parameters could be taken advantage of to help control the spread of *F. psychrophilum*.

This study attempted to fill a knowledge gap in finding a relationship between *Flavobacterium psychrophilum* and the environment. The results outlined in Chapter 3 led to the following conclusion. The abundance *F. psychrophilum* did not demonstrate a clear association with water quality parameters consistent between each farm. And therefore, the results did not support the hypothesis that *F. psychrophilum* abundance will depend on dissolved oxygen and nitrite concentrations. The results however did provide site-specific associations. The water quality parameters that had the strongest correlations with *F. psychrophilum* at Coldwater Fishery were PO_4 (r=58), pH (r=-0.52) and NH_3 (r=0.39). The water quality parameters that had the strongest correlations with *F. psychrophilum* at Lyndon Fish hatchery were NO_2^- (r=0.81), TKN (r=0.70) and Temperature (r=0.68). Future research is needed in several areas. Most notably, a lab study conducted on *F. psychrophilum* and nutrient concentrations could provide useful information of growth of *F. psychrophilum* under controlled conditions. Moreover, the determination of the specific *F. psychrophilum* strains at each farm would provide greater insight into the understanding of possible relationships between water quality parameters and *F. psychrophilum* observed at each of the farms. In addition, a long-term study on the same farms may provide more insight into farm specific relationships between water quality parameters and *F. psychrophilum* that could

provide specific management strategies. And finally, a long-term study examining several fish farms would provide a larger data set and a possible understanding into the relationships of *F. psychrophilum* and water quality parameters.

This study attempted to fill a knowledge gap in the understanding of biofilm development in UV treated aquaculture effluent. The results outlined in Chapter 4 led to the following conclusion. As expected, planktonic concentrations of both heterotrophic bacteria and *F. psychrophilum* were significantly reduced by ultraviolet irradiation. The concentration of attached heterotrophic bacteria and *F. psychrophilum* remained similar in the UV influent and UV effluent. The results supported the hypothesis because the bacterial concentrations in the biofilm at the UV effluent and UV influent area remained similar.

References Cited

Abbink, W., Garcia, A. B., Roques, J. A., Partridge, G. J., Kloet, K., & Schneider, O. (2012). The effect of temperature and pH on the growth and physiological response of juvenile yellowtail kingfish Seriola lalandi in recirculating aquaculture systems. *Aquaculture*, *330*, 130-135.

Attramadal, K. J., Salvesen, I., Xue, R., Øie, G., Størseth, T. R., Vadstein, O., & Olsen, Y. (2012a). Recirculation as a possible microbial control strategy in the production of marine larvae. *Aquacultural engineering*, *46*, 27-39.

Attramadal, K. J., Øie, G., Størseth, T. R., Alver, M. O., Vadstein, O., & Olsen, Y. (2012b). The effects of moderate ozonation or high intensity UV-irradiation on the microbial environment in RAS for marine larvae. *Aquaculture*, *330*, 121-129.

Austin, B., & Austin, D. A. (1999). *Bacterial fish pathogens: disease of farmed and wild fish*. Springer Science & Business Media.

Ayer, N. W., & Tyedmers, P. H. (2009). Assessing alternative aquaculture technologies: life cycle assessment of salmonid culture systems in Canada. *Journal of Cleaner Production*, *17*(3), 362-373.

Badiola, M., Mendiola, D., & Bostock, J. (2012). Recirculating Aquaculture Systems (RAS) analysis: Main issues on management and future challenges. *Aquacultural Engineering*, *51*, 26-35.

Barnes, M. E., & Brown, M. L. (2011). A review of Flavobacterium psychrophilum biology, clinical signs, and bacterial cold water disease prevention and treatment. *Open Fish Science Journal*, *4*, 40-48.

Bartoli, M., Nizzoli, D., Longhi, D., Laini, A., & Viaroli, P. (2007). Impact of a trout farm on the water quality of an Apennine creek from daily budgets of nutrients. *Chemistry and Ecology*, *23*(1), 1-11.

Bell, C. R., Holder-Franklin, M. A., & Franklin, M. (1982). Correlations between predominant heterotrophic bacteria and physicochemical water quality parameters in two Canadian rivers. *Applied and environmental microbiology*, *43*(2), 269-283.

Blancheton, J. P., Attramadal, K. J. K., Michaud, L., d'Orbcastel, E. R., & Vadstein, O. (2013). Insight into bacterial population in aquaculture systems and its implication. *Aquacultural engineering*, *53*, 30-39.

Bohrerova, Z., & Linden, K. G. (2007). Standardizing photoreactivation: comparison of DNA photorepair rate in Escherichia coli using four different fluorescent lamps. *Water research*, *41*(12), 2832-2838.
Bourne, D. G., Høj, L., Webster, N. S., Swan, J., & Hall, M. R. (2006). Biofilm development within a larval rearing tank of the tropical rock lobster, Panulirus ornatus. *Aquaculture*, *260*(1), 27-38.

Boyacioglu, M., & Akar, F. (2012). Isolation of Flavobacterium psychrophilum causing rainbow trout fry syndrome and determination of an effective antibacterial treatment in rainbow trout (Oncorhynchus mykiss) fry. *Kafkas Univ Vet Fak*, *18*, 197-203.

Bullock, G. L., Summerfelt, S. T., Noble, A. C., Weber, A. L., Durant, M. D., & Hankins, J. A. (1997). Ozonation of a recirculating rainbow trout culture system I. Effects on bacterial gill disease and heterotrophic bacteria. *Aquaculture*, *158*(1), 43-55.

Canadian Aquaculture Systems Inc. (2009). Strategy for sustainable aquaculture development in ontario. www.ontarioaquaculture.com/strategy_for_sustainable_aquaculture_development_in_ontario_2009.pdf

Cipriano, R. C., & Holt, R. A. (2005). *Flavobacterium psychrophilum, cause of bacterial cold-water disease and rainbow trout fry syndrome*. US Department of the Interior, US Geological Survey, National Fish Health Research Laboratory.

Costerton, J. W. (1995). Overview of microbial biofilms. *Journal of industrial microbiology*, *15*(3), 137-140.

Crane, M., & Hyatt, A. (2011). Viruses of fish: an overview of significant pathogens. *Viruses*, *3*(11), 2025-2046.

Crump, E. M., & Kay, W. W. (2008). Congo red inhibition as a convenient diagnostic for Flavobacterium psychrophilum. *Journal of fish diseases*, *31*(7), 553-557.

Davidson, J., Good, C., Welsh, C., & Summerfelt, S. T. (2014). Comparing the effects of high vs. low nitrate on the health, performance, and welfare of juvenile rainbow trout Oncorhynchus mykiss within water recirculating aquaculture systems. *Aquacultural Engineering*, *59*, 30-40.

Decostere, A., Haesebrouck, F., Turnbull, J. F., & Charlier, G. (1999). Influence of water quality and temperature on adhesion of high and low virulence Flavobacterium columnare strains to isolated gill arches. *Journal of Fish Diseases*, *22*(1), 1-11.

Defoirdt, T., Sorgeloos, P., & Bossier, P. (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Current opinion in microbiology*, *14*(3), 251-258.

Donahue, W. F., & Schindler, D. W. (1998). Diel emigration and colonization responses of blackfly larvae (Diptera: Simuliidae) to ultraviolet radiation. *Freshwater Biology*, *40*(2), 357-365.

Fisheries and Oceans Canada. (2011). Therapeutant Use. http://www.dfo-mpo.gc.ca/fm-gp/sustainable-durable/aquaculture/pro-thr-eng.htm

Flimlin, G., Buttner, J., & Webster, D. (2008). Aquaculture systems for the Northeast. *University of Maryland, Northeastern Regional Aquaculture Center, College Park, MD. NRAC Publication*, (104-2008).

Fisheries, F. A. O. Aquaculture Department (2012) The state of world fisheries and aquaculture. *Food and Agriculture Organization of the United Nations, Rome*, http://www.fao.org/docrep/016/i2727e/i2727e.pdf

Garcia, C., Pozet, F., & Michel, C. (2000). Standardization of experimental infection with Flavobacterium psychrophilum, the agent of rainbow trout Oncorhynchus mykiss fry syndrome. *Diseases of aquatic organisms*, *42*(3), 191-197.

Gehr, R. (2007). Collimated beam tests: their limitations for assessing wastewater disinfectability by UV, and a proposal for an additional evaluation parameter. *Journal of Environmental Engineering and Science*, *6*(3), 265-270.

Good, C. M., Thorburn, M. A., & Stevenson, R. M. (2008). Factors associated with the incidence of bacterial gill disease in salmonid lots reared in Ontario, Canada government hatcheries. *Preventive veterinary medicine*, *83*(3), 297-307.

Gullian, M., Espinosa-Faller, F. J., Núñez, A., & López-Barahona, N. (2012). Effect of turbidity on the ultraviolet disinfection performance in recirculating aquaculture systems with low water exchange. *Aquaculture Research*, *43*(4), 595-606.

Guerrero-Beltr, J. A., & Barbosa-C, G. V. (2004). Advantages and limitations on processing foods by UV light. *Food science and technology international*, *10*(3), 137-147.

Hallam, N. B., West, J. R., Forster, C. F., & Simms, J. (2001). The potential for biofilm growth in water distribution systems. *Water Research*, *35*(17), 4063-4071.

Harley, S., Schuba, B., & Carkal, D. (2008). Ultraviolet disinfection of private water supplies for household or agricultural use. *Agriculture and Agri-Food Canada*, *125*(11), 1-12.

Health Canada. (2010). List of veterinary drugs that are authorized for sale by health Canada for use in food-producing aquatic animals. http://www.hc-sc.gc.ca/dhp-mps/vet/legislation/pol/aquaculture_anim-eng.php

Hedrick, R. P., McDowell, T. S., Marty, G. D., Mukkatira, K., Antonio, D. B., Andree, K. B., ... & Clancy, T. (2000). Ultraviolet irradiation inactivates the waterborne infective stages of Myxobolus cerebralis: a treatment for hatchery water supplies. *Diseases of aquatic organisms*, *42*(1), 53-59.

Hijnen, W. A. M., Beerendonk, E. F., & Medema, G. J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo) cysts in water: a review. *Water research*, *40*(1), 3-22.

Irie, Y., & Parsek, M. R. (2008). Quorum sensing and microbial biofilms. In *Bacterial biofilms* (pp. 67-84). Springer Berlin Heidelberg.

Jan, S. L., & Shieh, G. (2014). Sample size determinations for Welch's test in one-way heteroscedastic ANOVA. *British Journal of Mathematical and Statistical Psychology*, *67*(1), 72-93.

Karunasagar, I., Otta, S. K., & Karunasagar, I. (1996). Biofilm formation by Vibrio harveyi on surfaces. *Aquaculture*, *140*(3), 241-245.

Kerr, C. J., Osborn, K. S., Robson, G. D., & Handley, P. S. (1998). The relationship between pipe material and biofilm formation in a laboratory model system. *Journal of applied microbiology*, *85*(S1).

King, R. K., Pierson, M. D., Smith, S. A., Boardman, G. D., & Coale, C. W. (2001). The presence of bacterial pathogens in biofilms of recirculating aquaculture systems and their response to various sanitizers.

King, R. K., Flick Jr, G. J., Smith, S. A., Pierson, M. D., Boardman, G. D., & Coale Jr, C. W. (2008). Response of bacterial biofilms in recirculating aquaculture systems to various sanitizers. *Journal of Applied Aquaculture*, *20*(2), 79-92.

Kurtak, D. C. (1978). Efficiency of filter feeding of black fly larvae (Diptera: Simuliidae). *Canadian Journal of Zoology*, *56*(7), 1608-1623.

Kuo, J., Chen, C. L., & Nellor, M. (2003). Standardized collimated beam testing protocol for water/wastewater ultraviolet disinfection. *Journal of Environmental Engineering*, *129*(8), 774-779.

Lam, P. K. S., & Lei, A. (1999). Colonization of periphytic algae on artificial substrates in a tropical stream. *Diatom Research*, *14*(2), 307-322.

Liberti, L., Notarnicola, M., & Petruzzelli, D. (2003). Advanced treatment for municipal wastewater reuse in agriculture. UV disinfection: parasite removal and by-product formation. *Desalination*, *152*(1), 315-324.

Liltved, H., Hektoen, H., & Efraimsen, H. (1995). Inactivation of bacterial and viral fish pathogens by ozonation or UV irradiation in water of different salinity. *Aquacultural Engineering*, *14*(2), 107-122.

Long, A., Call, D. R., & Cain, K. D. (2014). Investigation of the link between broodstock infection, vertical transmission, and prevalence of Flavobacterium psychrophilum in eggs and progeny of rainbow trout and coho salmon. *Journal of aquatic animal health*, *26*(2), 66-77.

Madsen, L., Møller, J. D., & Dalsgaard, I. (2005). Flavobacterium psychrophilum in rainbow trout, Oncorhynchus mykiss (Walbaum), hatcheries: studies on broodstock, eggs, fry and environment. *Journal of Fish Diseases*, *28*(1), 39-47.

Mamane, H., Colorni, A., Bar, I., Ori, I., & Mozes, N. (2010). The use of an open channel, low pressure UV reactor for water treatment in low head recirculating aquaculture systems (LH-RAS). *Aquacultural engineering*, *42*(3), 103-111.

Moccia, R.D. and Bevan, D.J. (2005). Environmental issues concerning water use and wastewater impacts of land-based aquaculture facilities in Ontario. http://www.aps.uoguelph.ca/aquacentre/files/research-publications/OSAWG%20Report%201%20Land-Based%20Issues%20(Sep2005).pdf

Molony, B. (2001). *Environmental requirements and tolerances of rainbow trout (Oncorhynchus mykiss) and brown trout (Salmo trutta) with special reference to Western Australia: a review*. Fisheries Research Division.

Montgomery, D. C., Peck, E. A., & Vining, G. G. (2012). *Introduction to linear regression analysis*. John Wiley & Sons.

Moriarty, D. J. (1997). The role of microorganisms in aquaculture ponds. *Aquaculture*, *151*(1), 333-349.

Nematollahi, A., Decostere, A., Pasmans, F., Ducatelle, R., & Haesebrouck, F. (2003a). Adhesion of high and low virulence Flavobacterium psychrophilum strains to isolated gill arches of rainbow trout Oncorhynchus mykiss. *Diseases of aquatic organisms*, *55*(2), 101-107.

Nematollahi, A., Decostere, A., Pasmans, F., & Haesebrouck, F. (2003b). Flavobacterium psychrophilum infections in salmonid fish. *Journal of fish diseases*, *26*(10), 563-574.

Oplinger, R. W., & Wagner, E. J. (2013). Control of Flavobacterium psychrophilum: tests of erythromycin, streptomycin, osmotic and thermal shocks, and rapid pH change. *Journal of aquatic animal health*, *25*(1), 1-8.

Orieux, N., Bourdineaud, J. P., Douet, D. G., Daniel, P., & Le Henaff, M. (2011). Quantification of Flavobacterium psychrophilum in rainbow trout, Oncorhynchus mykiss (Walbaum), tissues by qPCR. *Journal of fish diseases*, *34*(11), 811-821.

Pérez-Pascual, D., Menéndez, A., Fernández, L., Méndez, J., Reimundo, P., Navais, R., & Guijarro, J. A. (2010). Spreading versus biomass production by colonies of the fish pathogen Flavobacterium psychrophilum: role of the nutrient concentration. *International Microbiology*, *12*(4), 207-214.

Pirnie, M., Linden, K. G., & Malley, J. P. J. (2006). Ultraviolet disinfection guidance manual for the final long term 2 enhanced surface water treatment rule. *US Environmental Protection Agency*, 1-436.

Pozos, N., Scow, K., Wuertz, S., & Darby, J. (2004). UV disinfection in a model distribution system:: biofilm growth and microbial community. *Water Research*, *38*(13), 3083-3091.

Pulkkinen, K., Suomalainen, L. R., Read, A. F., Ebert, D., Rintamäki, P., & Valtonen, E. T. (2010). Intensive fish farming and the evolution of pathogen virulence: the case of columnaris disease in Finland. *Proceedings of the Royal Society of London B: Biological Sciences*, *277*(1681), 593-600.

Rabanal, H. R. (1988). History of aquaculture. http://www.fao.org/docrep/field/009/ag158e/AG158E02.htm

Ríos-Castillo, A. G., Thompson, K. D., & Adams, A.(2011) Adherence and biofilm formation of Flavobacterium psychrophilum in the presence of aquarium or loch water. http://web.abo.fi/konferens/flavobacterium2012/pdf/Thompson2.pdf

Rurangwa, E., & Verdegem, M. C. (2015). Microorganisms in recirculating aquaculture systems and their management. *Reviews in Aquaculture*, *7*(2), 117-130..

Schwartz, T., Hoffmann, S., & Obst, U. (2003). Formation of natural biofilms during chlorine dioxide and uv disinfection in a public drinking water distribution system. *Journal of Applied Microbiology*, *95*(3), 591-601.

Sharrer, M. J., Summerfelt, S. T., Bullock, G. L., Gleason, L. E., & Taeuber, J. (2005). Inactivation of bacteria using ultraviolet irradiation in a recirculating salmonid culture system. *Aquacultural Engineering*, *33*(2), 135-149.

Sharrer, M. J., & Summerfelt, S. T. (2007). Ozonation followed by ultraviolet irradiation provides effective bacteria inactivation in a freshwater recirculating system. *Aquacultural Engineering*, *37*(2), 180-191.

Soltani, M., and C. M. Burke. "Responses of fish-pathogenic Cytophaga/Flexibacter-like bacteria (CFLB) to environmental conditions." *Bulletin of the European Association of Fish Pathologists (United Kingdom)* (1995).

Snow, A., Anderson, B., & Wootton, B. (2012). Flow-through land-based aquaculture wastewater and its treatment in subsurface flow constructed wetlands. *Environmental Reviews*, *20*(1), 54-69.

Starliper, C. E. (2008). General and specialized media routinely employed for primary isolation of bacterial pathogens of fishes. *Journal of wildlife diseases*, *44*(1), 121-132.

Starliper, C. E. (2011). Bacterial coldwater disease of fishes caused by Flavobacterium psychrophilum. *Journal of Advanced Research*, *2*(2), 97-108.

Statistics Canada. (2010). Aquaculture Statistics

Strepparava, N., Wahli, T., Segner, H., & Petrini, O. (2014). Detection and quantification of Flavobacterium psychrophilum in water and fish tissue samples by quantitative real time PCR. *BMC microbiology*, *14*(1), 1.

Summerfelt, S. T. (2003). Ozonation and UV irradiation—an introduction and examples of current applications. *Aquacultural engineering*, *28*(1), 21-36.

Sundell, K., & Wiklund, T. (2011). Effect of biofilm formation on antimicrobial tolerance of Flavobacterium psychrophilum. *Journal of fish diseases*, *34*(5), 373-383.

Tello, A., Corner, R. A., & Telfer, T. C. (2010). How do land-based salmonid farms affect stream ecology?. *Environmental Pollution*, *158*(5), 1147-1158..

Westin, D. T. (1974). Nitrate and nitrite toxicity to salmonoid fishes. *The Progressive Fish-Culturist*, *36*(2), 86-89.

Whittingham, M. J., Stephens, P. A., Bradbury, R. B., & Freckleton, R. P. (2006). Why do we still use stepwise modelling in ecology and behaviour?. *Journal of animal ecology*, *75*(5), 1182-1189.

Wietz, M., Hall, M. R., & Høj, L. (2009). Effects of seawater ozonation on biofilm development in aquaculture tanks. *Systematic and Applied Microbiology*, *32*(4), 266-277.

Zhang, S. Y., Li, G., Wu, H. B., Liu, X. G., Yao, Y. H., Tao, L., & Liu, H. (2011). An integrated recirculating aquaculture system (RAS) for land-based fish farming: The effects on water quality and fish production. *aquacultural Engineering*, *45*(3), 93-102.

Zhu, S., Saucier, B., Durfey, J., & Chen, S. (2002, June). Evaluation of UV disinfection performance in recirculating systems. In *Proceedings of the 3 rd. International Conference on Recirculating Aquaculture.[np]. 2002.*

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, *1*(1), 3-14.

Appendix

Figure 23: A cross-section view of the TojanUV3000 Package Treatment Plant pilot system installed at Lyndon Fish Hatchery.

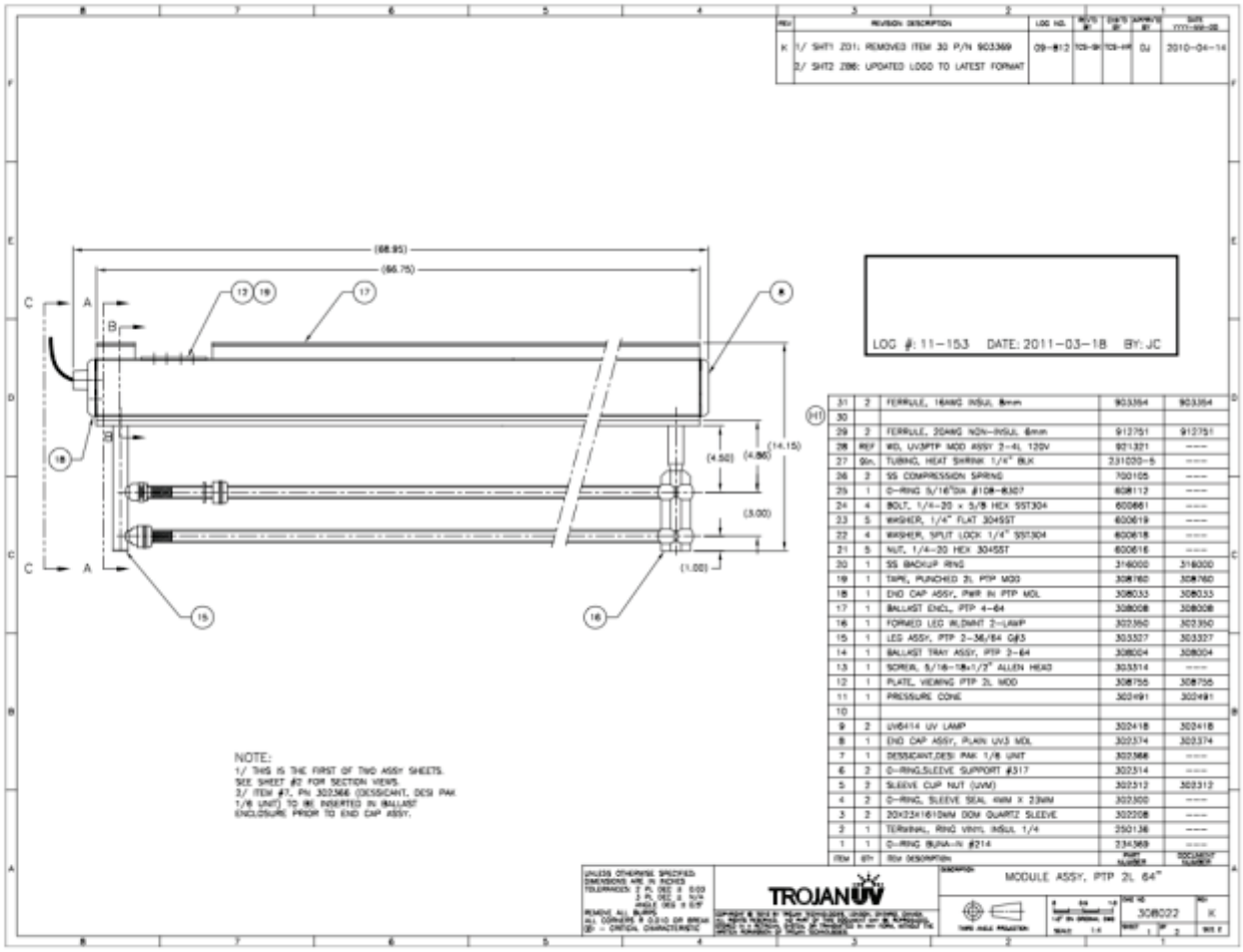


Table 31: p value for variation of water quality parameters measured over days sampled at Coldwater Fishery. AN=one way ANOVA, KW = Kruskal Wallis, WE = Welch's ANOVA. A post-hoc test Bonferroni was used for one way ANOVA, Tukey was used for Kruskal Wallis and Games -Howell for Welch's ANOVA. Letters within each row sharing the same letter are not significantly different (P>0.05).

Parameter	P value
Temperature °C	p= 0.2858^{WE}
DO mg/L	p= 0.3149^{KW}
pH	p=0.0537^{AN}
Conductivity µS	p=0.143^{KW}
COD mg/L	p= 0.4241^{W}
TSS mg/L	p= 0.8647^{KW}

Alkalinity mg/L	p=0.6049[KW]
TKN mg/L	p=0.5991[KW]
$N0_2$ mg/L	P=0.439[WE]
$N0_3$ mg/L	p= 0.2805[KW]
NH_3 mg/L	p=0.8607[KW]
TP mg/L	p= 0.8536[KW]
$P0_4$ mg/L	p= 0.136[KW]

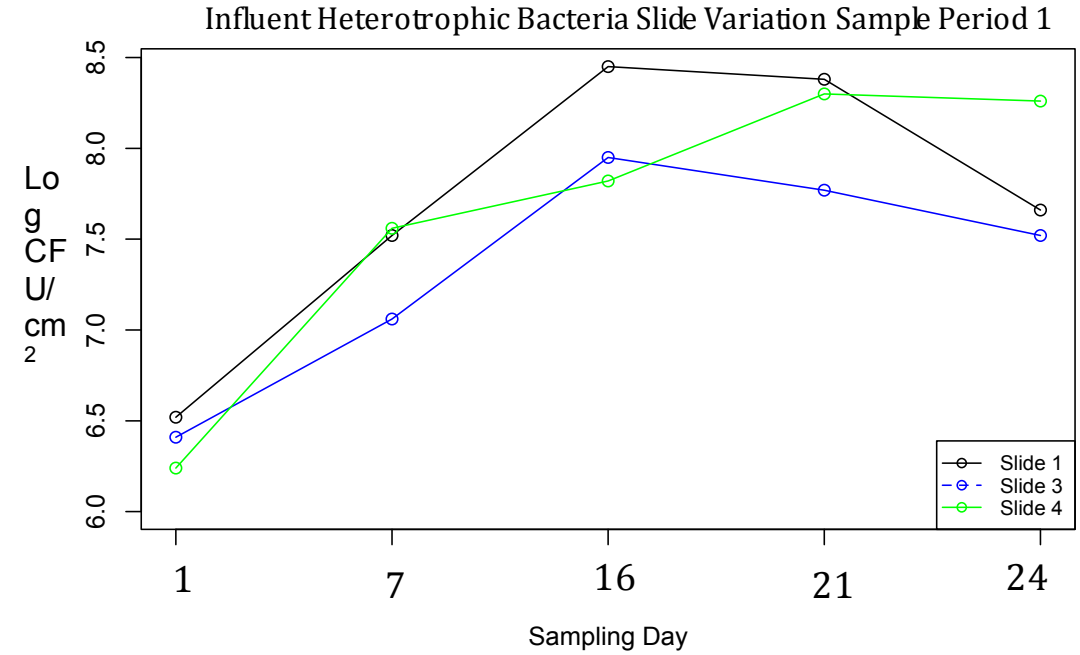
Table 32: p value for variation of water quality parameters measured over days sampled at Lyndon Fish Hatchery. AN=one way ANOVA, KW = Kruskal Wallis, WE = Welch's ANOVA. A post-hoc test Bonferroni was used for one way ANOVA, Tukey was used for Kruskal Wallis and Games-Howell for Welch's ANOVA. Letters within each row sharing the same letter are not significantly different (P>0.05).

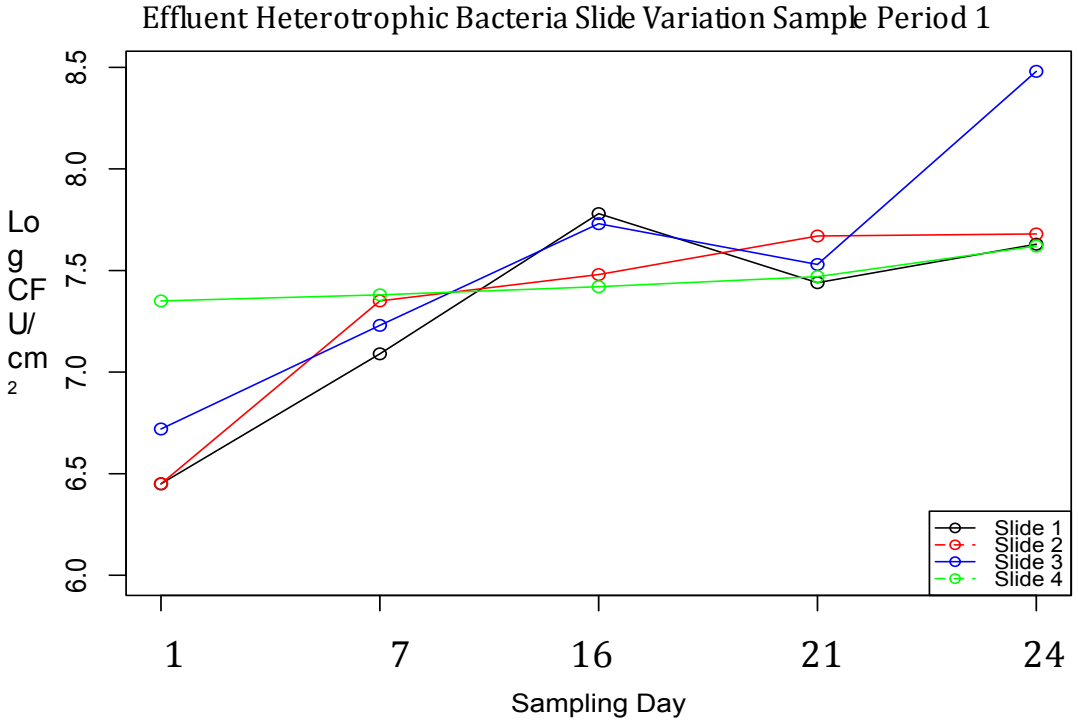
Parameter	Sample Period 1 P value	Sample Period 2 P value	Sampling Periods 1 and 2 P value
Temperature °C	p= 0.1882[KW]	p=0.2449[KW]	p=0.3403 [KW]
DO mg/L	p= 0.1302[KW]	p= 0.4649[WE]	p=0.574 [WE]
pH	p=0.346[KW]	p=0.0537[AN]	p=0.3212 [KW]
Conductivity µS	p=0.2615[KW]	p=0.143[KW]	p=0.7007 [KW]
COD mg/L	p= 0.0508[KW]	p=0.12 [KW]	p=0.8023 [KW]
TSS mg/L	p= 0.6386[KW]	p=0.08453 [KW]	p=0.8661 [KW]
Alkalinity mg/L	p=0.4290[KW]	p=0.07954 [KW]	p=0.2205 [KW]
TKN mg/L	p=0.617[KW]	p=0.815 [AN]	p=0.695 [AN]
$N0_2$ mg/L	P=0.694[KW]	p=0.867 [KW]	p=0.2161 [KW]
$N0_3$ mg/L	p= 0.2003[KW]	p=0.1417 [KW]	p=0.1427 [KW]
NH_3 mg/L	p=0.2882[KW]	p=0.7 [AN]	p=0.6215 [KW]
TP mg/L	p= 0.05787[WE]	p=0.5165 [KW]	p=0.3681 [KW]
$P0_4$ mg/L	p= 0.8188[KW]	p=0.1781 [KW]	p=0.5114 [KW]

Table 33: One-way ANOVA of slide variation over sampling days ay Lyndon Fish Hatchery

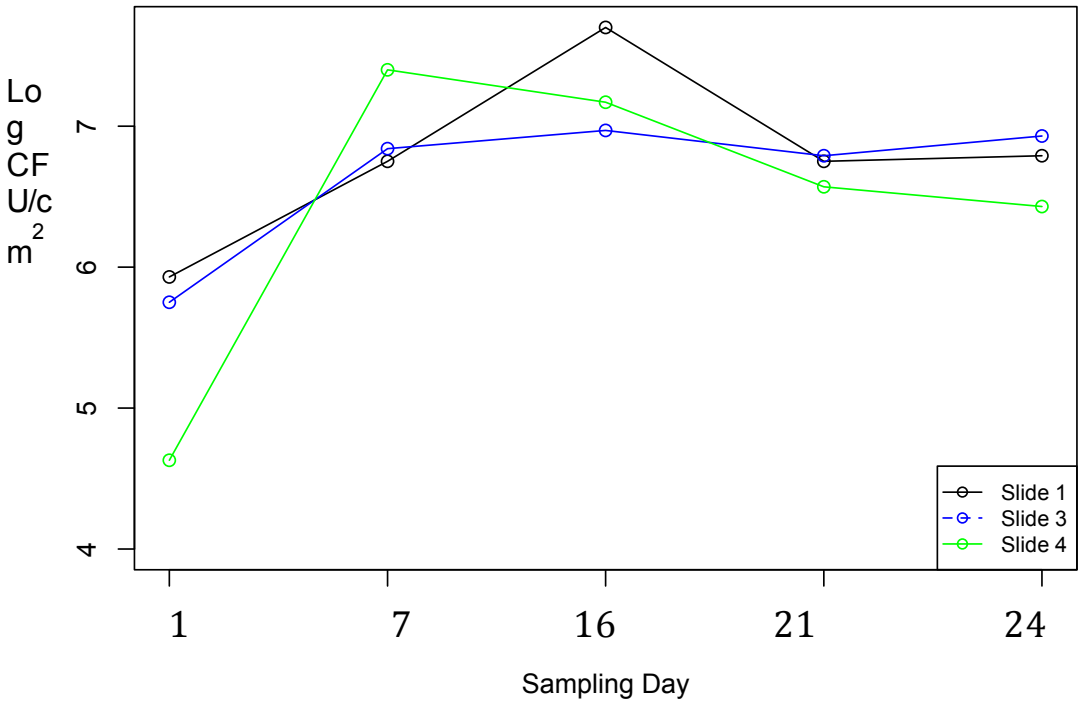
		Sample Period 1		Sample Period 2	
Influent		F	p	F	p
	F. psychrophilum	0.385	0.688	0.848	0.484
	Heterotrophic bacteria	0.329	0.726	0.025	0.995
Effluent					
	F. psychrophilum	0.361	0.782	0.138	0.986
	Heterotrophic bacteria	0.375	0.772	0.137	0.937

Figure 24: Line graphs showing no significant variation between the slides sampled for *F. psychrophilum* and heterotrophic bacteria in the UV influent and UV effluent for sample periods 1 and 2.





Influent *F. psychrophilum* Slide Variation Sample Period 1



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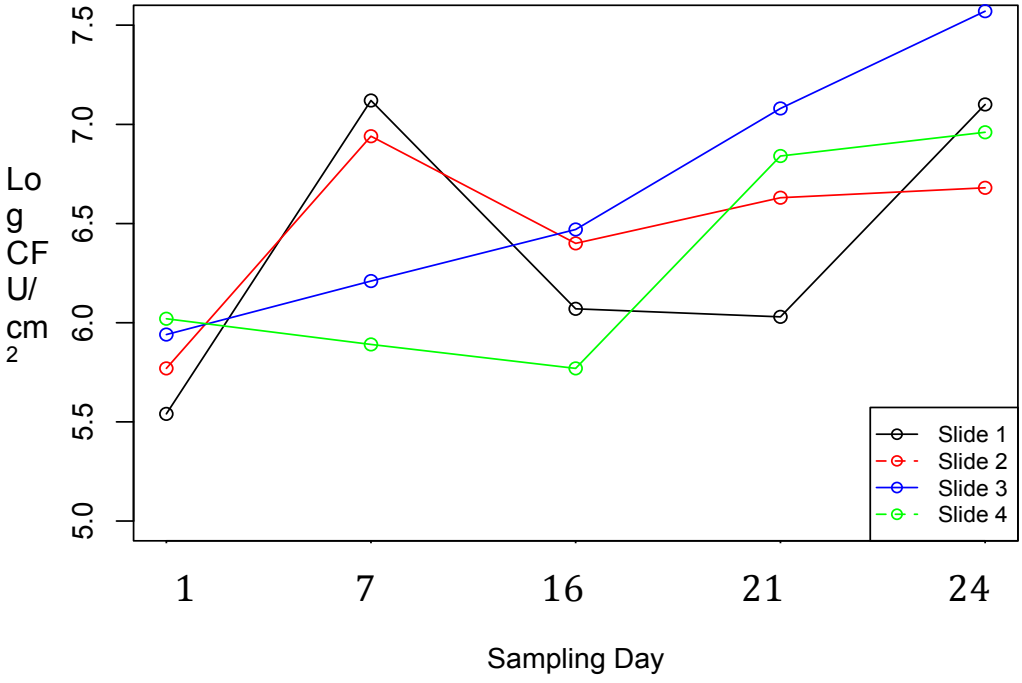
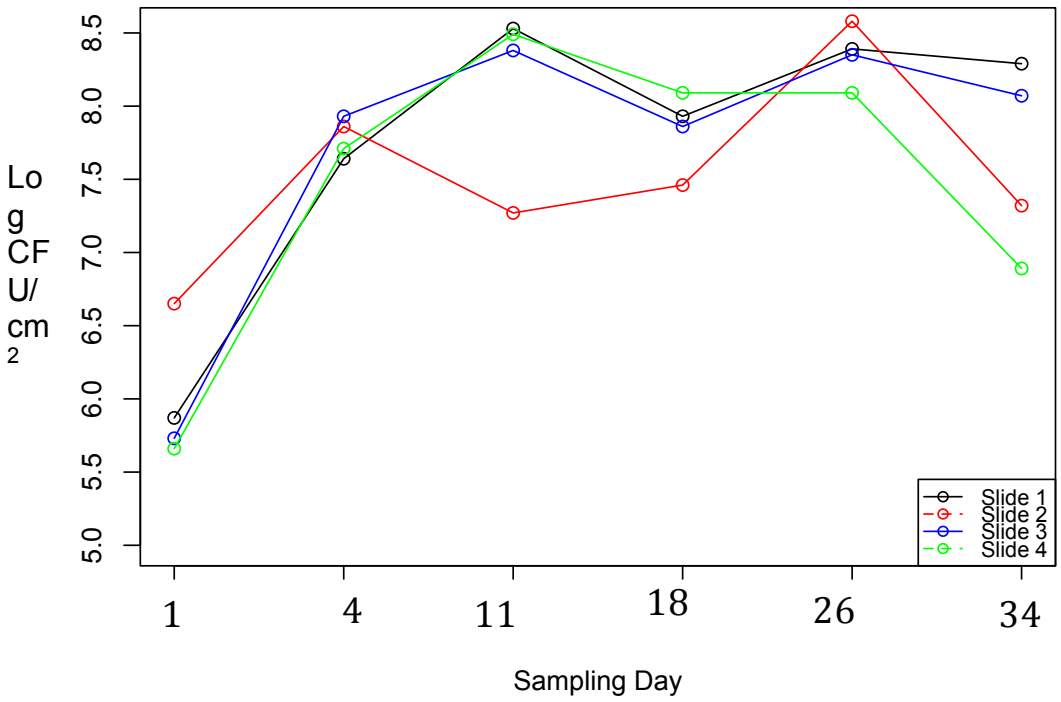
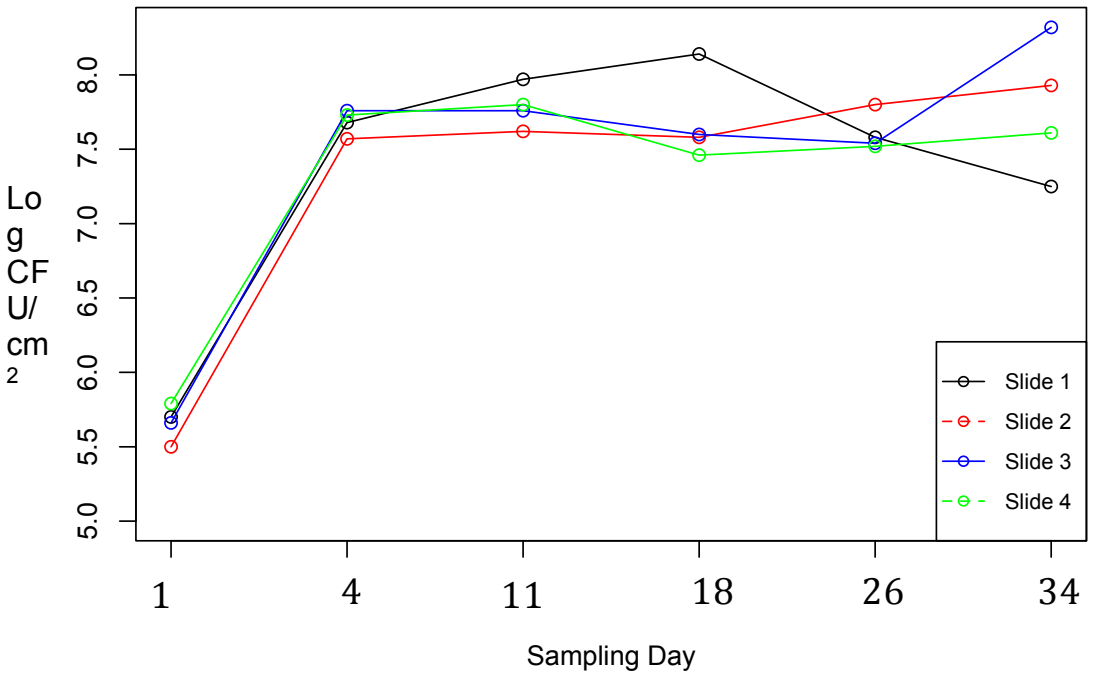
Effluent *F. psychrophilum* Slide Variation Sample Period 1



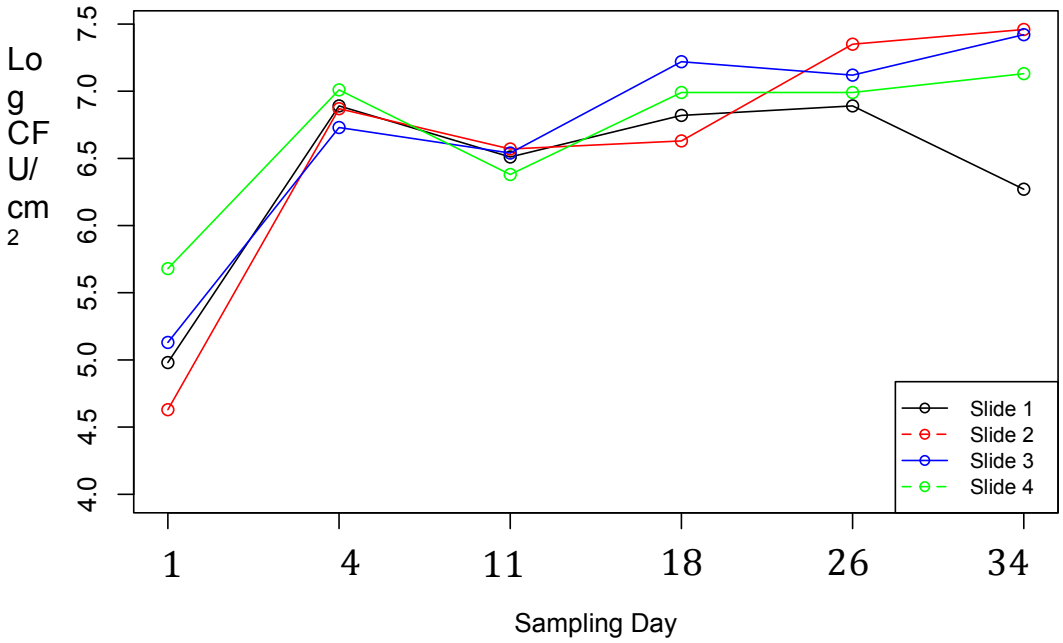
Effluent Heterotrophic Bacteria Slide Variation Sample Period 2



Influent Heterotrophic Bacteria Slide Variation Sample Period 2



Influent *F. psychrophilum* Slide Variation Sample Period 2



Effluent *F. psychrophilum* Slide Variation Sample Period 2

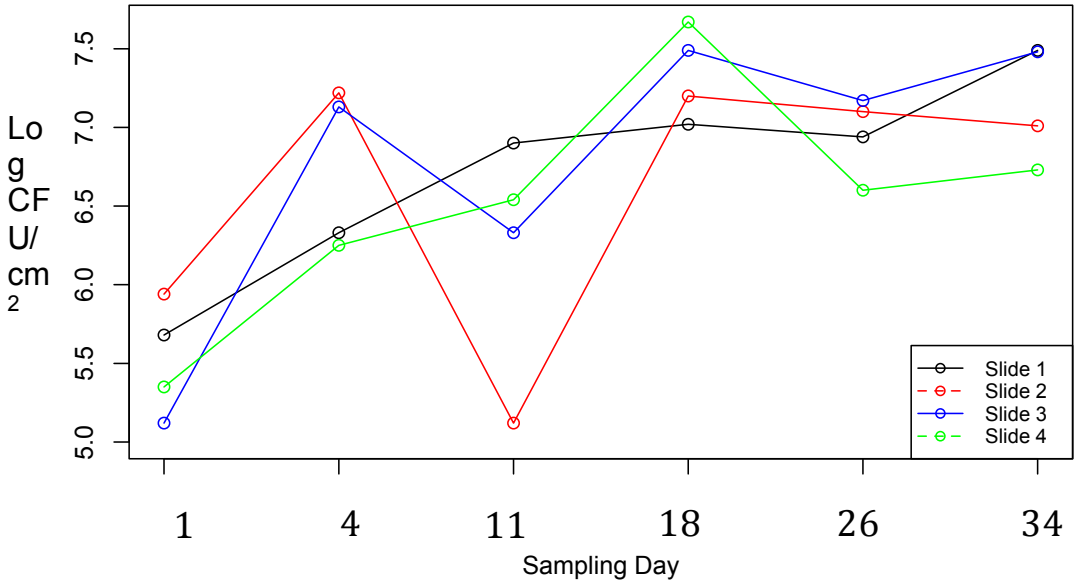
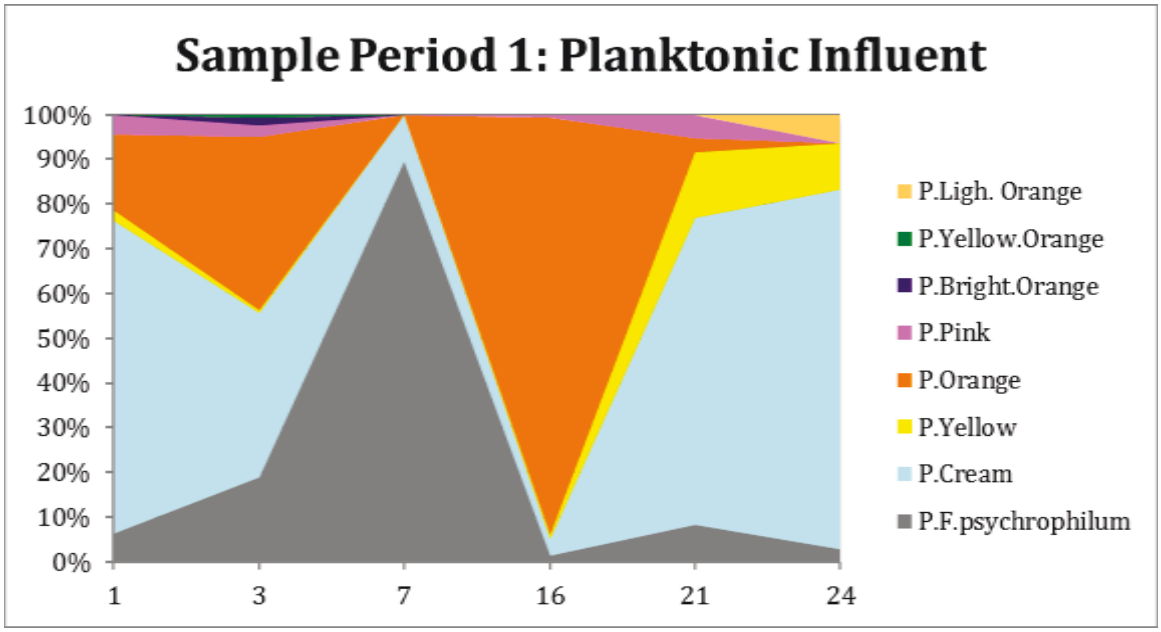


Table 34: One-way ANOVA of colony pigmentation diversity over sampling days.

	Sample Period 1	Sample Period 2

		F	p	F	p
Influent					
	Planktonic	0.143	0.725	0.456	0.537
	Attached	3.802	0.146	1.189	0.336
Effluent					
	Planktonic	0.566	0.494	0.403	0.56
	Attached	1.759	0.277	0.32	0.602

Figure 25: Variation in average planktonic colony pigmentation diversity for sample period 1 influent (top) and effluent (bottom)



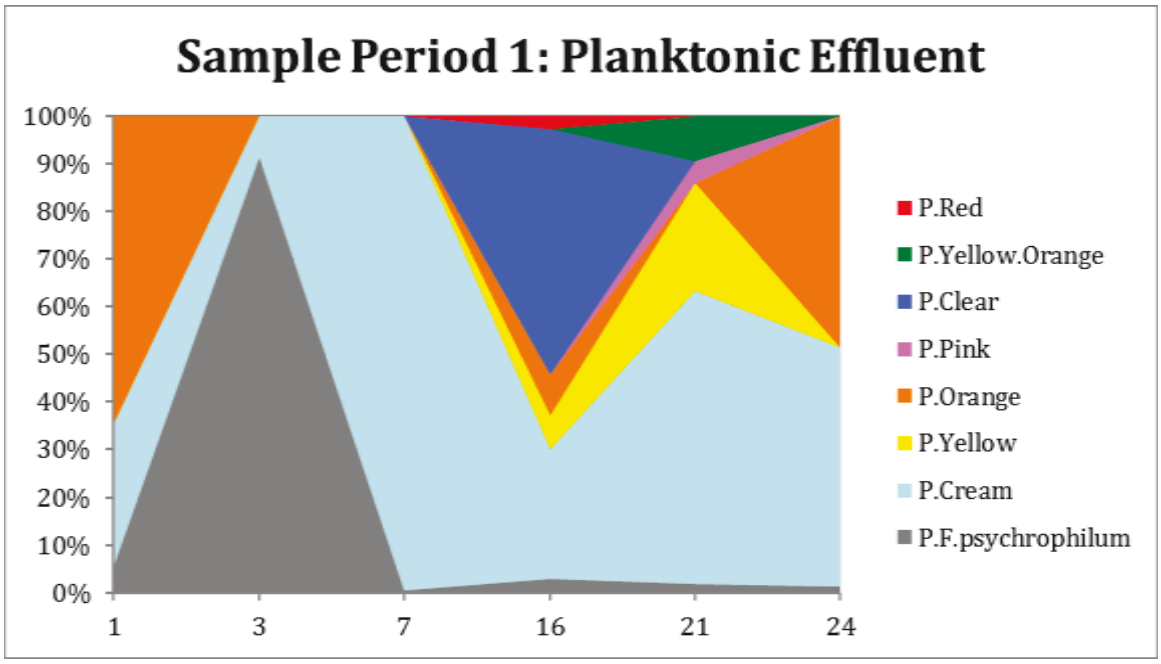
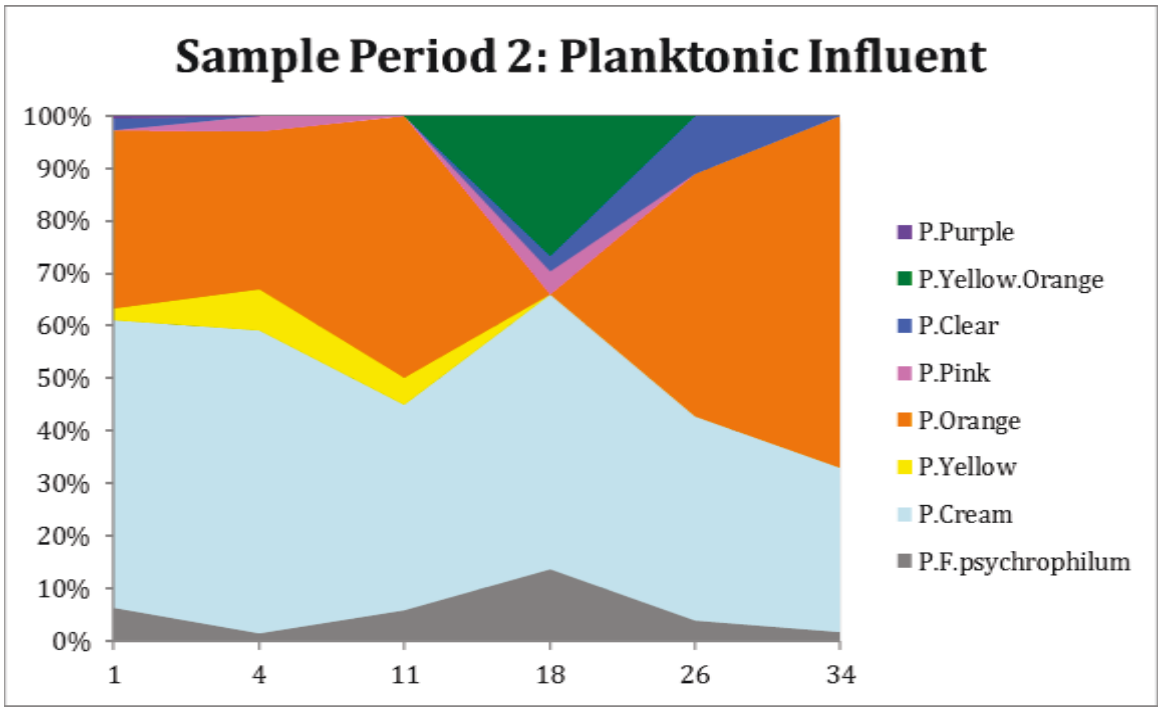


Figure 26: Variation in average planktonic colony pigmentation diversity for sample period 2 influent (top) and effluent (bottom)



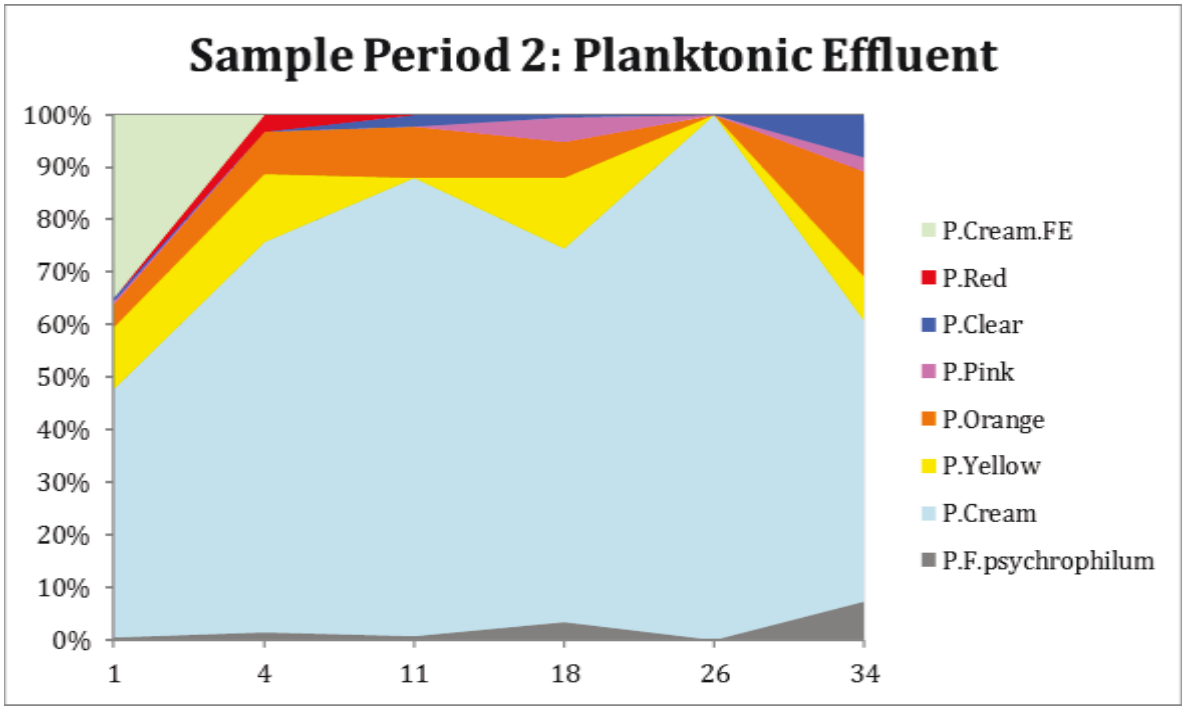
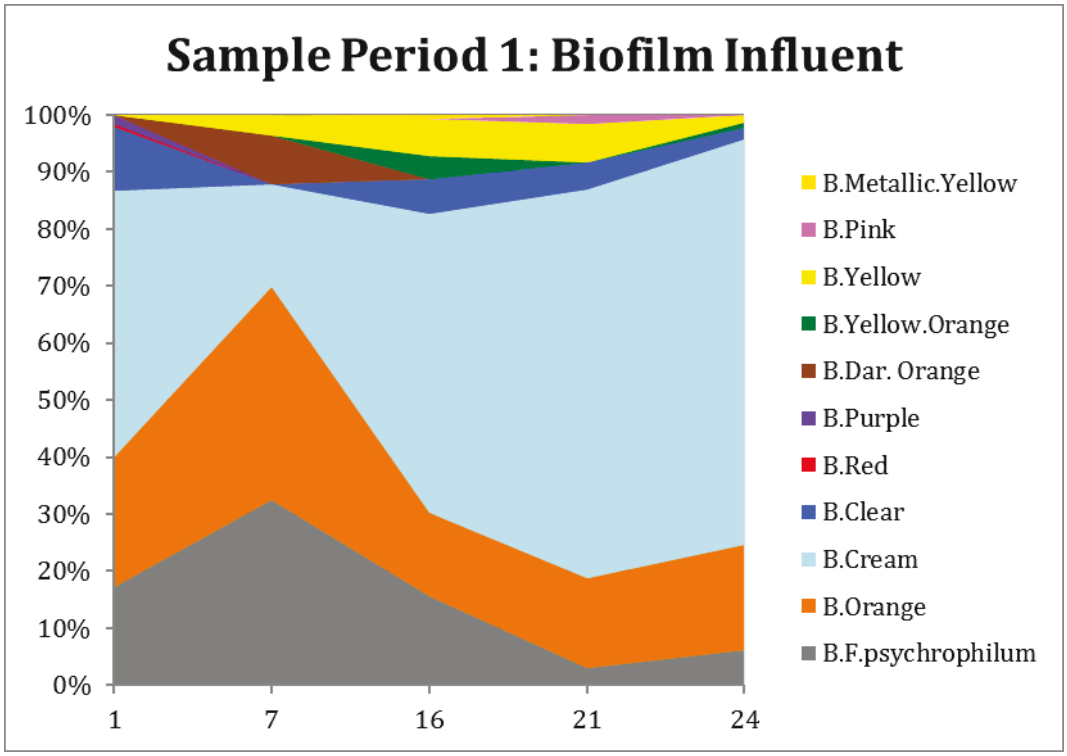


Figure 27: Variation in average attached colony pigmentation diversity for sample period 1 influent (top) and effluent (bottom).



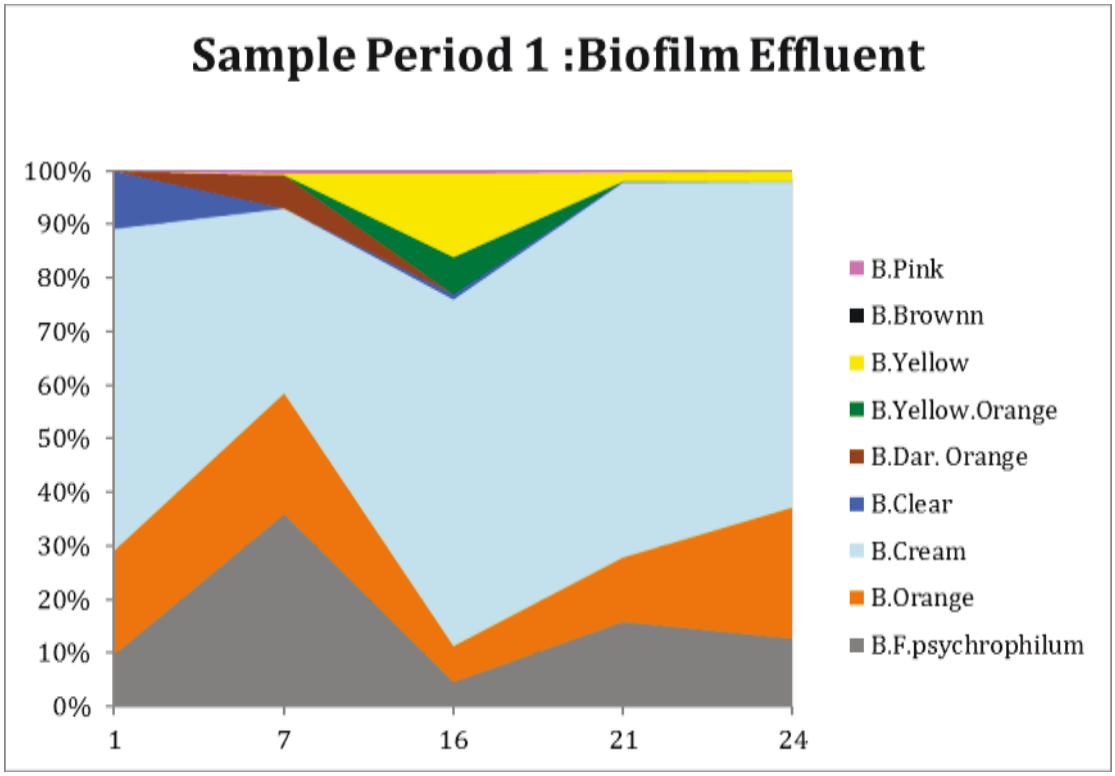


Figure 28: Variation in average attached colony pigmentation diversity for sample period 2 influent (top) and effluent (bottom).