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Effectiveness of aquatic phytoremediation of nutrients via watercress (*Nasturtium officinale*), basil (*Ocimum basilicum*), dill (*Anethum graveolens*) and lettuce (*Lactuca sativa*) from effluent of a flow-through aquaculture operation

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Effectiveness of Aquatic Phytoremediation of Nutrients via
Watercress (*Nasturtium officinale*), Basil (*Ocimum basilicum*),
Dill (*Anethum graveolens*) and Lettuce (*Lactuca sativa*) from
Effluent of a Flow-Through Aquaculture Operation

Derek J. Dyer

Thesis submitted to the
College of Engineering and Mineral Resources at
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ABSTRACT

EFFECTIVENESS OF AQUATIC PHYTOREMEDIATION OF NUTRIENTS VIA WATERCRESS (*Nasturtium officinale*), BASIL (*Ocimum basilicum*), DILL (*Anethum graveolens*) AND LETTUCE (*Lactuca sativa*) FROM EFFLUENT OF A FLOW-THROUGH AQUACULTURE OPERATION

Derek J. Dyer

Effluent from the aquaculture industry is a source of nutrient loading upon water bodies nationwide. Aquaponics, the simultaneous cultivation of fish and plants, has the potential to effectively reduce nutrient concentrations through phytoremediation, with the added benefit of producing a valuable crop. The purpose of this preliminary research was to determine if watercress (*Nasturtium officinale*) could be used to reduce nutrients from aquaculture effluent in sufficient concentrations to be functional as a remediation technique. Additionally, basil (*Ocimum basilicum*), dill (*Anethum graveolens*) and lettuce (*Lactuca sativa*) were correspondingly examined. Conditions which affected plant growth versus nutrient remediation were also to be established. These conditions include the effects of water velocity and plant density as they pertain to nutrient uptake and plant growth. Tentatively, the lowest water velocity and highest plant density should provide the greatest potential for uptake of nutrients via the plants in the system.

Effluent from an existing aquaculture operation, rearing brook trout (*Salvelinus fontinalis*), was distributed to an aquatic phytoremediation system consisting of twelve experimental channels used for the cultivation of the plants. The dimensions for each channel were 243 cm long, 30 cm wide and 15 cm deep. Experimental treatments, varying by plant density and water velocity conditions, were implemented throughout the system. Plant densities of 0.04, 0.09 and 0.16 plants/cm² and water velocities of 0.61, 0.30 and 0.061 cm/s were alternatively combined to create nine experimental treatments. Effluent samples were taken, from the aquaculture operation and the end of each channel, every three weeks throughout three consecutive studies conducted over a total period of thirteen months. Water samples were analyzed in the laboratory for ammonia, nitrate, nitrite and phosphate.

No statistically significant differences existed between the experimental treatments throughout each study of the aquaponics research. Some operational conditions were established through notable trends within the results. The low velocity low density treatment was most effective at the removal of nitrate and phosphate via watercress. The low velocity high density treatment was most effective at the removal of phosphate via watercress. The low velocity high density treatment was most effective at the removal of ammonia via lettuce. The watercress cultivated in the high velocity high density treatment resulted in greater plant biomass and height. The average total percent nutrient removals achieved via the plants did not exceed 11.2%. Significant reductions throughout the aquaponic system may not have occurred due to initially low nutrient concentrations, insufficient plant biomass and/or densities, inadequate light intensity or environmental temperatures, or short contact times between constituents and plants.

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CHAPTER 1. INTRODUCTION

1.1 Problem Statement

Nutrients that enter receiving waters via aquaculture effluents have the potential to be the cause of degradation to the quality of water and health of aquatic life. Nutrients discharged into the aquatic environment can greatly accelerate a natural water process known as eutrophication. Eutrophication is a condition in an aquatic ecosystem where high nutrient concentrations stimulate the growth of algal blooms, phytoplankton or other aquatic plants (USEPA, 2006). The stimulated growth of these plants can lead to significant water impairments such as depleted oxygen levels within an aquatic ecosystem (Chapra, 1997). The nutrients responsible for excessive eutrophication consist of an assortment of chemical compounds derived from nitrogen and phosphorus. These nutrients may be abundant in the effluent discharge of aquaculture operations.

Increased eutrophication from nutrient enrichment due to human activities is one of the leading problems facing some estuaries in the mid-Atlantic (USEPA, 2006). Specifically, the aquatic ecosystems of the Chesapeake Bay watershed have become degraded by eutrophication (CBF, 2006). Nitrogen and phosphorus have entered the watershed originating from sewage discharge, aquaculture effluent, and storm-water and agricultural runoff (CBF, 2006). The nutrients from these sources are in many cases transported to the Chesapeake Bay watershed via streams that begin in West Virginia.

In order to prevent or reduce nutrient enrichment of water bodies nationwide, the United States Environmental Protection Agency (USEPA) established a plan in 1998 known as the National Strategy for the Development of Regional Nutrient Criteria (USEPA, 1998). This policy would ultimately compel existing facilities, responsible for

the mass loading of nutrients in local water bodies, to develop techniques to safely, practically and inexpensively remediate nutrient constituents.

In addition to the Environmental Protection Agency, organizations such as the Natural Resource Conservation Service (NRCS) and Agricultural Research Service (ARS), both part of the United States Department of Agriculture (USDA), aid in the management of nutrients nationwide. When considering the Chesapeake Bay along, many organizations such as the Chesapeake Bay Foundation (CBF) are committed to the remediation of nutrient constituents in the watershed.

Presently, flow-through aquaculture systems do not have a viable strategy to remove dissolved nutrients from effluent before discharge into receiving water bodies. Due to increased growth of aquaculture nationwide, additional remediation methods, beyond simple settling of solid constituents of nitrogen and phosphorus from aquaculture effluent, will eventually be needed to help prevent against eutrophication of watersheds and aquatic ecosystems. Phytoremediation and more specifically the practice of aquaponics are two newly emerging techniques which may aid in the nitrogen and phosphorus remediation efforts.

1.2 Objectives

The objectives of this aquaponics research project were (1) to determine if watercress (*Nasturtium officinale* R. BR.) can be cultivated and utilized, via aquatic phytoremediation, to reduce nutrients from aquaculture effluent in sufficient quantities to be useful as an effective treatment or remediation technique, and (2) to determine operational and environmental conditions which affect nutrient removal and plant

growth. Limited research was also conducted using dill (*Anethum graveolens*), lettuce (*Lactuca sativa*) and basil (*Ocimum basilicum*) in the same type of experiments.

To meet these two objectives, water quality characterization based on nutrient concentrations was conducted at an operational aquaculture facility located at West Virginia University's Reymann Memorial Farm in Wardensville, West Virginia. Specifically, the nutrients in the forms of ammonia, nitrate, nitrite and phosphate were analyzed. The influent and effluent nutrient concentration of water samples were compared and contrasted from multiple treatment segments of an aquatic phytoremediation system to quantify if removal of the constituents had occurred.

CHAPTER 2. BACKGROUND

2.1 Aquaculture

2.1.1 Overview

Aquaculture is an agricultural industry employed for recreational and food production purposes both nationally and locally. A multitude of aquatic creatures, including fish, crustaceans and mollusks are raised for food. In West Virginia, an abundance of fresh water resources are available for the continual growth of aquaculture. In addition, West Virginia's close proximity to eastern markets adds to the State's opportunity for expansion in this area. Due to the abundance of cool fresh spring and groundwater sources, various species of Salmonids, such as rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*), are cultivated in West Virginia. In 2005, trout rearing aquaculture operations nationwide produced nearly 61,676,000 lbs (28,034,545 kg) of fish valued at \$69.1 million for the year (Linstedt, 2006).

2.1.2 Fish Water Quality Criteria

Water quality criteria relevant to the rearing of Salmonids, such as brook trout (*Salvelinus fontinalis*), are as follows in *Table 2.1.2.1*:

Table 2.1.2.1 – Water Quality Criteria for Salmonid Rearing

Parameter	Upper Continuous Exposure Limits / Tolerance Ranges	Reference
Ammonia Nitrogen	0.0125 mg/L	Soderberg, 1995
Carbon Dioxide (CO₂)	0 to 10.0 mg/L	Meade, 1989
Nitrate (NO₃⁻)	0 to 3.0 mg/L	Meade, 1989
Nitrite (NO₂⁻)	0.03 to 0.06 mg/L	IDEQ, 1998
pH	6.5 to 8.0	Meade, 1989
Phosphate (PO₄³⁻)	0.01 to 3.00 mg/L	IDEQ, 1998
Temperature	0.5 to 25.5 °C	Piper, 1982
Total Alkalinity (CaCO₃)	10 to 400 mg/L	Meade, 1989
Total Suspended Solids (TSS)	80.00 mg/L	IDEQ, 1998

2.1.2.1 Brook Trout

Brook trout (*Salvelinus fontinalis*) are a species of fish residing in the salmon family, Salmonidae, and order Salmoniformes. Brook trout are native to streams, lakes, springs and ponds primarily in eastern North America, though they have been introduced to habitats all over the continent. They reach a maximum recorded length of 86 cm (33 in) and a maximum recorded weight of 9.4 kg (21 lb). They also can reach an age of at least seven years. For production in aquaculture, brook trout prefer an aquatic environment of cool and clear water with temperatures ranging from 7 to 19 °C (Soderberg, 1995). They also have certain tolerances and limits to the amounts or concentrations of multiple chemical constituents and physical parameters associated with water quality criteria.

2.1.3 Water Resources

One of the most important factors in an aquaponics operation, for both aquaculture and plant cultivation, is the availability of water resources. For an aquaponics or other types of phytoremediation operations, the source of water includes both surface water and groundwater. Surface water and groundwater result from the complex processes of the hydrologic cycle such as evapotranspiration, precipitation, evaporation and infiltration.

2.1.3.1 Groundwater

Groundwater accounts for most of the water on the planet, excluding the oceans and the ice caps (Wurbs & James, 2002). Most groundwater is saline in composition, due to the higher dissolved mineral concentrations of groundwater, resulting from intimate contact with rocks and soils in the ground, as well as the length and time allotted for dissolution (Snoeyink & Jenkins, 1980). The subsurface of soil consists of two zones. The vadose zone, or zone of aeration, is the region consisting of soil pores containing both air and water (Fetter, 2001). The zone of saturation lies below the vadose zone, separated by a border known as the capillary fringe (Fetter, 2001). The saturation zone consists of soil pores filled and surrounded entirely by water (Fetter, 2001). This is the region of groundwater flow. A schematic of these subsurface zones is presented in *Figure 2.1.3.1.1*:

Figure 2.1.3.1.1 – Soil Subsurface Zones

Groundwater will ultimately flow, discharge or seep into surface waters such as a spring, pond, lake, stream, river or ocean (Fetter, 2001).

2.1.3.2 Surface Water

Surface water accounts for all springs, ponds, lakes, streams, rivers, bays and oceans on the planet. Surface waters, such as a stream, derive from precipitation overland flow or groundwater flow that has seeped into the stream bed (Fetter, 2001). The groundwater input to surface water is termed base-flow, while the total flow of surface water is termed runoff (Fetter, 2001). Surface water often originates from runoff collected in rock basins, which often consist of granite or other similar material (Snoeyink & Jenkins, 1980). These rock basin materials constitutes for the minute amount of dissolved minerals frequently found in surface waters (Snoeyink & Jenkins, 1980). Surface water deriving from rock basins other than granite, are widely used as public water supplies or for agricultural industries (Snoeyink & Jenkins, 1980).

Springs provide a high quality of water that can be useful in aquaponic operations. Springs contain many minerals which are beneficial to plants grown hydroponically. The state of West Virginia has 1,193 documented springs (McColloch, 1986) These springs have an average discharge ranging from 0.38-37.4 m³/min (100-9870 gal/min).

2.1.4 Water Composition

The composition of water sources in the environment includes an assortment of varying constituents. When considering an aquaculture or hydroponic operation, the water constituents include ammonia, carbon dioxide, nitrate, nitrite, phosphate, and the total amount of suspended solids. Other factors of aquaculture and hydroponic water are the pH, temperature, and total alkalinity.

2.1.4.1 Ammonia

Ammonia is often the product of anaerobic degradation of proteinaceous substances (Rittmann & McCarty, 2001). Ammonia exists in two chemical forms in natural waters: (1) ammonium ion (NH₄⁺) and (2) unionized ammonia gas (NH₃) (Chapra, 1997). Ammonium is usually innocuous at the concentration levels encountered in most natural waters, while unionized ammonia is toxic to aquatic receptors (Chapra, 1997). At high ammonia nitrogen concentrations, the dominant form of ammonia is dependent upon primarily the pH, though water salinity and temperature can also have a minor affect (Rittmann & McCarty, 2001). Additionally, the pK_A of an ammonium ion is 9.2. The normal equilibrium expression between these two species is as follows in *Equation 2.1.4.1.1*:

Equation 2.1.4.1.1 – Ammonia Equilibrium Expression (Rittmann & McCarty, 2001).

2.1.4.2 Carbon Dioxide

Carbon dioxide (CO₂) is absorbed by plants for the use in various metabolic processes. Carbon dioxide is also a key compound to the carbonate system of natural bodies of water. The carbonate system can have a great effect on the pH of a water system and thus the presence of carbon dioxide can additionally have pH effects (Snoeyink & Jenkins, 1980).

2.1.4.3 Nitrate

Nitrate (NO₃⁻) is in part produced through the bacterially-mediated oxidation of nitrite (NO₂⁻) in most natural systems, though it can also be formed through chemical oxidation-reduction reactions (Colt, 2001). Nitrate is generally not considered toxic to aquatic organisms, though the chemical compound can be particularly dangerous to certain aquatic and human receptors at high concentrations (Chapra, 1997). Nitrate is also an important nutrient in plant cultivation (Rittmann & McCarty, 2001). The equilibrium expression for the oxidation of nitrite is as follows in *Equation 2.1.4.3.1*:

Equation 2.1.4.3.1 – Nitrate Equilibrium Expression (Rittmann & McCarty, 2001).

2.1.4.4 Nitrite

Nitrite (NO₂⁻) is the product of bacterially-mediated oxidation of ammonium (NH₄⁺) (Colt, 2001). Depending upon the sensitivity of the organism, nitrite concentrations above 0.1 mg/L can be fatally toxic to aquatic receptors (Rittmann & McCarty, 2001). Nitrite is, however, typically further oxidized to nitrate (NO₃⁻) by bacteria in natural waters. The equilibrium expression for the oxidation of nitrite is as follows in *Equation 2.1.4.4.1*:

Equation 2.1.4.4.1 – Nitrite Equilibrium Expression (Rittmann & McCarty, 2001).

2.1.4.5 pH

The pH of natural waters can affect multiple chemical systems such as the carbonate and nitrogen systems. The pH can also affect the salinity, alkalinity and acidity of the water. The pH of water is proportional and related to the amount of hydrogen ions (H^+), or hydrated protons, in a water system. The pH is furthermore expressed as follows in *Equation 2.1.4.5.1*:

$$pH = -\log_{10}[H^+]$$

Equation 2.1.4.5.1 – pH of Natural Water (Snoeyink & Jenkins, 1980).

In *Equation 2.1.4.5.1*, $[H^+]$ is equal to the molar concentration of hydrogen (Snoeyink & Jenkins, 1980).

2.1.4.6 Phosphate

Phosphate (PO_4^{3-}) is the oxidized form of phosphorus in natural waters. Phosphate is an important nutrient requirement for the growth of all living protoplasm, including plants (Snoeyink & Jenkins, 1980).

2.1.4.7 Temperature

The temperature of natural waters can affect many different aquatic processes. These processes include chemical systems such as the carbonate and nitrogen systems, as well as, biological systems such as microbial or protoplasmic communities. The temperature can also affect the salinity, alkalinity, acidity and pH of natural waters. Aquaculture operations and plant cultivation can also be affected by the temperature. The saturation concentration of oxygen (O_2) dissolved in water is also greatly affected by the

temperature. Fundamentally, oxygen saturation levels decrease with increasing temperature in fresh and natural waters (Chapra, 1997).

2.1.4.8 Total Alkalinity

Alkalinity is a measure of the capacity of water to neutralize a strong acid, a protonated compound that readily donates a proton (H^+) to a water molecule (H_2O) (Snoeyink & Jenkins, 1980). In natural waters, alkalinity is directly related to the carbonate system. Thus, alkalinity can be formulated as follows in *Equation 2.1.4.8.1*:

$$Alkalinity = [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+]$$

Equation 2.1.4.8.1 – Total Alkalinity Expression (Chapra, 1997).

2.1.4.9 Total Suspended Solids

The total suspended solids are in simple terms particulate matter floating within a water body. Total suspended solids in natural waters have two primary origins. These origins are from the drainage basin of a water system and from photosynthetic processes (Chapra, 1997). Particles from these two sources are formally identified as allochthonous and autochthonous solids, respectively (Chapra, 1997). These suspended solids differ in several ways such as their organic carbon content, unit density and particle size. Each of these parameters affects the transport and settling of the particles. Organic compounds and opportunistic pathogens can also be absorbed onto these particles and subsequently carried with them through a water system (Chapra, 1997).

2.2 Aquaculture Effluent Constituents

2.2.1 Origins & Sources

In aquaculture operations, uneaten feed for the fish is the one source of wastes and constituents in the effluent water (Meade, 1989). This feed coupled with fish fecal

matter constitutes the major sources of dissolved and suspended solids in the water. The dissolved and suspended wastes account for the organic and nutrient constituents found in the water. Additionally, extraneous constituents found in aquaculture effluent can originate from the source waters used in the operation. Since many aquaculture operations exist on rural sites, such as farms, agricultural constituents have the potential to enter the source waters. Agricultural areas can contribute high concentrations of nutrient rich fertilizers, pesticides, herbicides and specialized synthetic organic chemicals to both surface water and groundwater sources (Fetter, 2001).

2.2.2 Nutrients

Nutrients are chemicals significantly required for the production of biomass, or growth, of most living plants, animals and microorganisms (Rittmann & McCarty, 2001). Elemental nutrients comprise the physical structure of most living cells (Rittmann & McCarty, 2001). Elemental nutrients are of great concern, however, in high concentrations in the environment. When forming and breaking from more complex chemical compounds, nutrient constituents affect aquatic organisms, human receptors and natural water systems. The two common nutrient constituents in aquaculture wastewaters which have the greatest potential to effect an aquatic environment are nitrogen (N) and phosphorus (P).

2.2.2.1 Nitrogen

Nitrogen (N) exists in three major chemical forms in the environment, including aquaculture wastewater. These forms, which constitute fractions of the nitrogen cycle in nature, are as ammonium (NH_4^+) or ammonia gas (NH_3), nitrate (NO_3^-) and nitrite (NO_2^-)

(Snoeyink & Jenkins, 1980). Other forms include organic nitrogen in the environment and nitrogen gas (N_2) in the atmosphere (Snoeyink & Jenkins, 1980).

The nitrogen cycle is comprised of microbially and chemically catalyzed oxidation and reduction reactions, or “redox” reactions (Snoeyink & Jenkins, 1980). These reactions are directionally designated by the terms and phrases nitrification, nitrate reduction, denitrification and nitrogen fixation. First, nitrification reactions include the oxidation of ammonium to nitrite and then to nitrate (Chapra, 1997). Microbes responsible for the oxidation of nitrite to nitrate are called nitrobacters, while the microbes responsible for the oxidation of ammonium to nitrite are called nitrosomonas (Chapra, 1997). Next, nitrate reduction reactions consist of the reduction of nitrate to nitrite and then to ammonium (Snoeyink & Jenkins, 1980). Then, denitrification reactions include the reduction of nitrate to nitrogen gas (Chapra, 1997). Finally, nitrogen fixation reactions consist of the reduction of nitrogen gas to ammonium (Snoeyink & Jenkins, 1980). The incorporation or release of ammonium into organic chemical forms of nitrogen is also achieved through processes called amination and ammonification, respectively (Snoeyink & Jenkins, 1980). These reactions are the only nonredox reactions involved in nitrogen transformation in the entire nitrogen cycle (Snoeyink & Jenkins, 1980). A basic schematic representation of the nitrogen cycle is as follows in *Figure 2.2.2.1.1*:

Figure 2.2.2.1.1 – The Nitrogen Cycle

The reactions involved in the nitrogen cycle, particularly nitrification, consume abundant amounts of oxygen (O_2) and can add to the depletion of oxygen levels in a water system (Chapra, 1997). If conditions in the water become anaerobic, without oxygen, denitrification can vastly occur and convert large quantities of free nitrogen gas into the system (Chapra, 1997). The free nitrogen gas and nitrate can then be readily utilized by nitrogen-fixing algae and bacteria (Chapra, 1997). This can subsequently lead to an eutrophication process in a water system. Eutrophication can lead to correlated problems such as depleted oxygen levels in an aquatic ecosystem.

Elevated nitrogen concentrations can cause harmful effects and/or disease in fish. One such disease, brown-blood disease, is a clinical condition in fish known as methemoglobinemia (Klontz, 1991). This disease occurs in fish when water contains sufficient concentrations of nitrite, usually greater than 0.55 mg/L, to oxidize ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) (Klontz, 1991). Nitrite enters the bloodstream through the gills,

oxidizes any ferrous iron, and turns the blood to a chocolate-brown color (Brunson *et al.*, 2006). Hemoglobin, which transports oxygen in the blood, combines with nitrite to form methemoglobin, which is incapable of oxygen transport (Brunson *et al.*, 2006). Brown blood cannot carry sufficient amounts of oxygen, and affected fish can suffocate despite adequate oxygen concentration in the water (Brunson *et al.*, 2006). This accounts for the gasping behavior often observed in fish with brown blood disease, even when oxygen levels are abundant (Brunson *et al.*, 2006). Brown-blood disease can greatly affect many species of fish such as trout and catfish (Brunson *et al.*, 2006).

Elevated nitrate concentrations can also have harmful effects on certain human receptors. The presence of sufficiently abundant levels of nitrate in drinking water can cause methemoglobinemia, or “blue baby syndrome” (Chapra, 1997). This condition is similar to the brown-blood disease of fish. Methemoglobinemia reduces the oxygen concentration of the blood stream in infants and children. This disease primarily affects infants less than six month of age, but can impact children up to the age of six (Chapra, 1997). Levels of nitrate above 10 mg/L in drinking water are believed to lead to this condition (Chapra, 1997). Normally, fish farm effluent do not contain nitrate concentrations above the level required for the syndrome to occur.

Ammonia existing in the form of unionized ammonia gas can be severely toxic to fish and other similar aquatic organisms (Chapra, 1997). Exposure to unionized ammonia gas can cause fish to develop predispositions to disease (Soderberg, 1995). To be toxic to most aquatic receptors, specifically fish, unionized ammonia gas must be present at a concentration above 0.0125 mg/L (Meade, 1985). Ammonia existing as the ammonium ion on the other hand is relatively nontoxic to aquatic receptors (Soderberg, 1995).

Ammonium, however, can lead to other problems in fish chronically exposed to the ion. Ammonium ions can affect fish, by forcing the fish's gills to develop excessive protective tissue which decreases the epithelial surface area available for gas exchange (Soderberg, 1995). This gill distortion consequently reduces the amount of oxygen the gill membrane can absorb (Soderberg, 1995). The pH of a water system is the governing factor between the two ammonia forms (Chapra, 1997).

Sources of nitrogen in aquaculture wastewater are fish feed and fish excreted wastes. Nitrogen sources excreted by fish consist of unused dietary protein, sloughed intestinal cells and amino acids absorbed, in amounts greater than a fish can digest and metabolize (Ramseyer & Garling, 1997). Roughly, 52-95% of nitrogen from feed is excreted by fish as nitrogenous wastes in the forms dissolved ammonia gas and nitrogen-containing suspended biosolids, while the remaining percentage is actually retained (Gatlin & Hardy, 2002). Of the excreted nitrogen, roughly 75-90% is in the form of ammonia gas excreted by the gills (Gatlin & Hardy, 2002). The remaining percentage exists in the suspended biosolids form (Ramseyer & Garling, 1997). These percentages make the removal of nitrogen from aquaculture wastewaters particularly difficult, since a very small percentage is in the settling suspended solid form.

2.2.2.2 Phosphorus

Phosphorus (P) is chemical element beneficial to plant life. Phosphorus in natural waters exists in five conventional forms. The first form exists as soluble reactive phosphorus (Chapra, 1997). This form of phosphorus, also called orthophosphate, or soluble inorganic phosphorus, is readily available and useful to plants (Chapra, 1997). Orthophosphates consist of the compounds H_2PO_4^- , HPO_4^{2-} and PO_4^{3-} (Chapra, 1997).

The dominant form of orthophosphate is determined by the pH of the water (Snoeyink & Jenkins, 1980). The second form is particulate organic phosphorus, which mainly consists of living plants, animals and microorganisms (Chapra, 1997). The third form exists as non-particulate organic phosphorus, which primarily are dissolved, and colloidal organic compounds containing phosphorus (Chapra, 1997). The fourth form subsists as particulate inorganic phosphorus, which consists of phosphate minerals, sorbed orthophosphates and phosphates complexed with solid matter (Chapra, 1997). The fifth form exists as non-particulate inorganic phosphorus (Chapra, 1997). This group includes condensed phosphates, such as those once common in household detergents.

Phosphorus can be the cause of negative effects on the environment in a manner similar to that of nitrogen. Just as nitrogen stimulates eutrophication in a water system, phosphorus is a limiting nutrient used by plants to thrive and grow. Elevated concentration of phosphorus, notably phosphate, in water can intensify the eutrophication process such as the excessive growth of aquatic plants and algal blooms. Correlated problems in the system, such as depleted oxygen levels, could then occur.

Sources of phosphorus in aquaculture wastewater are principally from fish feed. The phosphorus availability is dependent upon the type and consistency of the feed used in the cultivation. Generally, only 32% of phosphorus from the feed is retained by the fish (Ramseyer & Garling, 1997). The remaining 68% of phosphorus is excreted in dissolved and suspended solid forms (Ramseyer & Garling, 1997). The excreted phosphorus ranges from 10-40% in the dissolved form and 60-90% in the suspended solid form (Ramseyer & Garling, 1997). Phosphorus can be more easily controlled and

removed from wastewaters than nitrogen, since a larger percentage of phosphorus in aquaculture waste exists in solid forms.

2.2.3 Eutrophication

Eutrophication, or nutrient enrichment, is an undesirable condition in an aquatic ecosystem only when excessive concentrations of nutrients create unwanted growth of algae in slow-flowing or still freshwater (USEPA, 2006). Algae are generally identified as microscopic unicellular plants which use limiting nutrients, such as nitrate and phosphate, in their metabolism (Prescott, 2002). However, certain types of algae, designated as blue-green algae, are actually eukaryotic photosynthetic bacteria (Prescott, 2002). These blue-green algae, which also use nitrate and phosphate throughout their metabolic processes, are additionally termed cyanobacteria (Prescott, 2002).

The algal blooms caused by eutrophication can lead to or directly have harmful effects on an aquatic ecosystem. Some types of harmful algal blooms can produce chemicals which are toxic to other aquatic organisms (USEPA, 2006). Other algal blooms can vastly cloud the surface of the water and block sunlight, causing benthic plants and other photosynthetic organisms to eventually die and decay (USEPA, 2006). These benthic plants are useful to the aquatic ecosystem since they provide food and shelter for many aquatic organisms, as well as spawning and nursery habitat (USEPA, 2006). Thus, the death of these plants can cause other benthic organisms to die and decompose. Additionally, as the growth of the algae in the water exceeds the available nutrient concentrations, the algae bloom itself will begin to die and decay.

When the algae and other aquatic organisms die and decompose, the growth of heterotrophic bacteria is stimulated (USEPA, 2006). The heterotrophic bacteria use the

decaying organic material and the dissolved oxygen in the water in their respective metabolic processes (Chapra, 1997). The dissolved oxygen, specifically, is used as an electron acceptor in the bacteria's metabolic pathways to create energy (Prescott, 2002). This phenomenon is thus responsible for the majority of dissolved oxygen depletion within an aquatic ecosystem affected by excessive eutrophication.

The depletion of oxygen levels can severely affect aquatic organisms, such as fish, living in an aquatic ecosystem (Klontz, 1991). Without appropriate levels of oxygen, aquatic organisms can essentially suffocate and expire (Klontz, 1991). This detrimental effect, known as hypoxia, has the potential to completely annihilate organisms which require aerobic conditions in an aquatic ecosystem (Soderberg, 1995). A schematic flowchart of the process of eutrophication is presented in *Figure 2.3.2.1*:

Figure 2.3.2.1 – Eutrophication Flowchart

2.3 Aquaponics

2.3.1 Overview

Aquaponics is a vernacular term used to describe the technique of simultaneous fish and plant cultivation or a fusion of aquaculture and hydroponics. The key to this process is that the waste of one part is a benefit to the other. Aquaculture operations are a source for nutrients which limit and regulate plant growth. Essentially, the effluent from an aquaculture operation acts as a substrate and a water resource for hydroponic plant production. Plants produced in this manner are beneficial two ways. First, the plants remediate the nutrients from the aquaculture effluent. Second, the certain plants have an added value of being a marketable cash crop. When considering both nutrient recovery and the cultivation of a valuable byproduct, aquaponics has potential as a remediation technique.

2.4 Regulations

2.4.1 Federal

The wastewater of most aquaponic operations eventually discharges into receiving surface waters and/or recharges groundwater. This is particularly true for the aquaculture division of aquaponic operations. These discharges are regulated throughout the United States by the federal Clean Water Act (CWA) (LaGrega, 2001). This regulation, which underwent major amendments in 1972, 1977 and 1987, was established to restore and protect the integrity of national waters (LaGrega, 2001). This regulation also established the National Pollutant Discharge Elimination System (NPDES) program to provide for the issuance of permits for point source discharges to surface water

(LaGrega, 2001). The Safe Drinking Water Act (SDWA) also helps to establish regulations for discharges into surface waters.

These federal regulations are primarily enforced by the United States Environmental Protection Agency (USEPA). In order to prevent or reduce nutrient enrichment of water bodies nationwide, the USEPA established a plan in 1998 known as the National Strategy for the Development of Regional Nutrient Criteria (USEPA, 1998). The principle goal of this strategy is to stimulate granted research by a wide and diverse range of organizations and individuals (USEPA, 1998). This goal will help improve the federal and statewide understanding of the causal variables of nutrient loading, as well as proper remediation responses necessary to reduce or eliminate the nutrient contamination. Another goal of this strategy is the development of numerical nutrient standards, according to water body type and ecoregion, for the states to use in their water quality protection and remediation programs (USEPA, 1998). This policy would thus compel existing facilities responsible for the mass loading of nutrients in local water bodies to undertake the development of techniques to safely, practically and inexpensively remediate such constituents.

In addition to the Environmental Protection Agency, organizations such as the National Resource Conservation Service (NRCS) and Agricultural Research Service (ARS), both part of the United States Department of Agriculture (USDA), aid in the management of nutrients nationwide. Through these services, advancements and progress in the remediation of nutrient constituents and the conservation of the national environment have been accomplished.

The USEPA and the USDA have worked closely with other federal agencies, academia, industry associations, state governments, citizen groups, environmental groups and other stakeholders in an effort to develop national regulations for nutrients (USEPA, 2000). The Office of Water in the USEPA has been exclusively focused on new efforts to aid in the reduction of nutrient loading from commercial, agricultural and industrial operations nationwide (USEPA, 2000). Among these efforts, a new motion exists to specifically develop constituent controls in the form of nationally applicable discharge principles for commercial and public aquaculture operations (USEPA, 2000). These principles are commonly known as effluent limitation guidelines and standards. This motion was built upon the technical expertise of nationally-recognized leaders in aquaculture field, such as the Federal Joint Subcommittee on Aquaculture (JSA) and the Aquaculture Effluents Task Force (AETF) (USEPA, 2000).

Through this new rule, the USEPA is now setting standards for the discharge of wastewater from concentrated aquatic animal production facilities, known as aquaculture fish farms (USEPA, 2006). This rule targets facilities that produce at least 100,000 lbs (44,761 kg) of fish annually in both flow-through and recirculating systems that discharge wastewater at least thirty days out of the year (USEPA, 2006). The rule requires these facilities to carry out a multitude of responsibilities (USEPA, 2006). The facilities must regularly maintain the production and wastewater treatment systems, as well as, keep records on the number and weights of the animals, amounts of feed and frequency of all management activities (USEPA, 2006). They must also minimize the discharge of solids, such as uneaten feed, settled solids and animal carcasses, into local water bodies (USEPA, 2006). These regulations for flow-through aquaculture systems

basically require the removal of solid waste prior to effluent discharge. Most of the responsibilities are covered in the “best management practice plan” that the facilities must develop, maintain and certify through the National Permit Discharge Elimination System (NPDES) permitting program (USEPA, 2006). This plan details how a facility will meet the nutrient requirements for their operation.

2.4.2 State

In the state of West Virginia, wastewater discharges into surface water and groundwater are regulated by the West Virginia Department of Environmental Protection (WVDEP), specifically the office of Environmental Enforcement (EE). This office complies with federal regulations such as the Water Pollution Control Act and the Groundwater Protection Act, both of which help to control nutrient loading into receiving bodies of water (WVDEP, 2006).

In addition to the NPDES program and TMDL development, another water quality improvement program is being implemented to further eliminate the discharge of constituents into the nation’s water as is mandated in the Clean Water Act (Skousen, 2006). As another level of review and protection in the NPDES program, the Clean Water Act also directed that each state develop an anti-degradation policy to further protect water quality (Skousen, 2006). In 2001, West Virginia finalized the development of an anti-degradation policy which gives for levels of protection for the State’s waters (Skousen, 2006). These levels of protections are known as Tiers 1, 2, 2.5, and 3 (Skousen, 2006). Tier 3 protects waters which cannot be degraded under any circumstances (Skousen, 2006). Tier 2.5 protects waters which abundant biological and aquatic life which requires immediate special concern due to the level of quality

(Skousen, 2006). Tier 2 protects waters which require only standard protection, since the level of quality is equal to or above acceptable standards (Skousen, 2006). Tier 1 protects waters with existing uses which require continuous maintenance to preserve quality standards (Skousen, 2006).

2.5 Phytoremediation

2.5.1 Definition

Phytoremediation is a relatively new environmental technology that focuses on the treatment of environmental problems using plants. As early as 1967, interests in the use of plants to treat pollution were developing from investigations into the ability of plants to uptake and translocate constituents from the soil (LaGrega, 2001). Since 1995, the actual applicability of phytoremediation processes has attracted increased attention from research groups, industries, and government agencies (LaGrega, 2001).

Many different species of plants and their associated biota can, directly or indirectly, be used to extract, absorb, degrade, or detoxify various types of constituents from the environment. Furthermore, some plants can be used to contain or immobilize constituents from the environment. The types of constituents that can be physically and chemically attenuated by the phytoremediation process include metals, pesticides, volatiles, solvents, oils and organics (LaGrega, 2001).

Additionally, the terms *in-situ* and *ex-situ* have new denotations when used in the field of phytoremediation. *In-situ* treatment refers to plant processes that take place below the ground or water surface, specifically around or within the root zone of a plant. Accordingly, *ex-situ* treatment refers to plant processes that take place above the ground

or water surface, particularly within or around the stem and leaves of a plant (LaGrega, 2001).

2.5.2 Environments

The two primary environments in which phytoremediation can take place are terrestrial and aquatic. Terrestrial phytoremediation deals with the remediation of contaminated soil and groundwater. Aquatic phytoremediation deals with the remediation of contaminated surface water, such as streams or lagoons.

2.5.3 Mechanisms

Six mechanisms are implemented by the phytoremediation process of a plant to remediate constituents from the environment. These mechanisms, or phytoremediation techniques, are phytoextraction, phytotransformation, phytovolatilization, phytostimulation, phytostabilization and rhizofiltration. The first four mechanisms are reactions, which occur inside and outside the plant to destroy or extract constituents from the environment (Rittmann & McCarty, 2001). The final two mechanisms are reactions which occur outside the plant to sequester constituents in the environment (Rittmann & McCarty, 2001). Through these mechanisms, targeted constituents can be affected in many ways to remove, reduce or retard the abundance and transport of constituents. The distinctions among these mechanisms in a phytoremediation operation, however, are not clear. Identical forms of phytoremediation can lead the same constituents through different mechanisms and treatment results. For example, rhizosphere peroxidases can catalyze oxidation reactions that lead either to phytodegradation or phytostabilization (Rittmann & McCarty, 2001).

A schematic representation of all of the phytoremediation mechanisms is presented in *Figure 2.5.3.0*:

Figure 2.5.3.0 – Phytoremediation Mechanisms Schematic

2.5.3.1 Phytoextraction

Phytoextraction (1), or phytoaccumulation, is the physical uptake, translocation and concentration of constituents from the environment into the plant biomass (LaGrega, 2001). This mechanism is primarily used in the treatment of constituents from soil or groundwater, specifically the saturated, or vadose, zone of the soil subsurface (LaGrega, 2001). Additionally, phytoextraction can be used in the removal of constituents from free-flowing or non-flowing surface water. Moderately hydrophobic constituents are thus the most susceptible to phytoextraction (Rittmann & McCarty, 2001). This is due to the fact that hydrophobic constituents are not chemically bonded strongly to water. Also, phytoextraction usually leads to the phytotransformation or phytovolatilization of

removed constituents. This mechanism was exploited in the aquaponics research objectives.

As mentioned before, the constituents in the environment are extracted or absorbed through the roots or leaves of a plant. The constituents are then transported and stored throughout the root, leaf and stem matter of a plant. Most phytoextraction occurs through biological hyper-accumulation, where the plants naturally remove the constituents from the environment without assistance. Plants which remove constituents in this manner are termed hyperaccumulators (Aboulroos, 2006). Induced hyper-accumulation occurs when certain constituents require an added treatment before they can be removed. In this case, a conditioning fluid containing a chelator is added to the environment. This fluid increases the solubility and mobility of constituents to allow plants to more effectively remove the targeted constituents. An example of this type of chelating agent is ethylenediaminetetraacetic acid (EDTA), which forms coordination compounds with most monovalent, divalent, trivalent and tetravalent metal ions. The form of EDTA salt utilized is a function of the ionic strength of the constituents.

2.5.3.2 Other Mechanisms

The other mechanisms of phytoremediation are as follows: phytotransformation (2), or phytodegradation, is the biochemical modification of environmental constituents into benign products as a direct result of the metabolic processes of a plant (Rittmann & McCarty, 2001), phytovolatilization (3) is the enhancement of the volatilization process in which the volatile constituents within a plant are released into the atmosphere via evapotranspiration (Rittmann & McCarty, 2001), phytostimulation (4) is the biological remediation and degradation of constituents in the environment by microorganisms

within the rhizosphere through the aid of plants (LaGrega, 2001)., phytostabilization (5) is the reduction or hindrance of the mobility of constituents in the environment through the interference of plants. A schematic representation of phytostabilization is previously presented in *Figure 2.5.3.0*. The benefit of this mechanism is that it limits the bioavailability of harmful constituents to humans and other receptors (Rittmann & McCarty, 2001), and rhizofiltration (6) is the filtering of constituents from water through the use of plants and their roots (Rittmann & McCarty, 2001).

2.5.4 Applications

To remediate constituents successfully using phytoremediation, the following factors need to be considered: the type and amount of constituents of concern, existing environmental conditions, and potential risks to human health and other receptors. The variety and number of plants used in phytoremediation are also important. Consequently, different plant species are appropriate for the remediation of different types of constituents (LaGrega, 2001). Environmental conditions that affect a phytoremediation process include: the homogeneity of the soil or water present, as well as the size and depth of the contaminated area (USEPA, 2001). Phytoremediation has also been found to be most applicable for the treatment of vadose zone soils (LaGrega, 2001). Phytoremediation may also be suitable for the remediation of saturated zone soils and groundwater, depending upon the depth of plant and tree roots utilized in the process (LaGrega, 2001). Additionally, the phytoremediation technique can be applied to a hydroponic system.

Phytoremediation has been used to at least partially to remediate constituents such as heavy metals, pesticides, solvents, volatiles, explosives, oils and organics (LaGrega,

2001). Phytoremediation can also be applied to recover nutrients such as nitrogen and phosphorus from a hydroponic or terrestrial environment.

2.5.5 Design

Plants that have been found to be most useful in phytoremediation typically have abundant biomass, high tolerance for concentrated constituents, produce supportive exudates, have long growing seasons, germinate quickly and have roots that extend to the depth of the contamination (LaGrega, 2001). The use of native species to a contaminated environmental site is preferential to using nonnative species due to their proven ability to establish in the specific ecosystem of the site (LaGrega, 2001). Additionally, invasive species may place native species at risk due to the increased competition for natural resources.

To help ensure the success of a phytoremediation project design, bench or pilot-scale studies may be required before field-scale phytoremediation efforts are implemented (LaGrega, 2001). Design factors of typical phytoremediation projects consist of the topography, available space for planting, growth rates of plants, planting depth, plant root penetration and constituent uptake rates of plants (LaGrega, 2001). Physical and chemical characteristics of soil or water, such as porosity and pH, can also impact the design of a phytoremediation system.

The success of phytoremediation can be determined in two ways: through examining constituent reductions in the remediation system or environment or assessing the accumulation of constituents, such as nutrients, in plant tissues. Both methods must be benchmarked against a controlled condition (e.g. identical plants grown on a non-

contaminated site or a contaminated site void of the same plant growth), which ensures that constituent removal was a consequence of phytoremediation.

To measure the success level of a designed phytoremediation project, a monitoring plan is necessary. The complexity and urgency of a monitoring plan will depend on a number of factors, including the site-specific risk posed by the presence of constituents (LaGrega, 2001). Monitoring conducted before, during and after the span of a remediation project can indicate any need for design modifications, thus allowing the project to evolve and improve.

2.5.6 Limitations

There are several limitations to phytoremediation, the most notable of which is the potential to introduce a new exposure pathway to harmful constituents. The presence of hazardous constituents in plants could cause them to become bio-available in the human food chain and thus closer to human receptors (LaGrega, 2001). Such pathways should be evaluated to estimate any absorbed dosages by humans. These dosages can then be compared to thresholds, such as the “no observed adverse effect level” (NOAEL), to conclude if any unacceptable circumstances could result (LaGrega, 2001).

Another notable limitation is that phytoremediation often takes many years to clean up a contaminated site to required and acceptable levels (USEPA, 2001). This is true even if a phytoremediation project is conducted at an ideally selected contamination site with highly favorable characteristics and environmental conditions. If the constituent concentrations at a site are immediately harmful to humans and other receptors, a more aggressive remediation alternative may be required (LaGrega, 2001).

2.5.7 Advantages

Phytoremediation is advantageous because it uses natural plant processes in the remediation of constituents. The process generally requires less equipment and labor than other similar remediation methods which consequently lowers the total project expense (USEPA, 2001). Other similar remediation methods include ion exchange, reverse osmosis, or electro-dialysis (Adler *et al.*, 2000). Since plants do most of the work, employees can avoid contact with hazardous constituents and the site can be remediated without removing polluted soil or pumping polluted groundwater (USEPA, 2001). Additionally, trees and plants used in phytoremediation can make a contaminated site more aesthetically appealing.

2.5.8 Case Studies

There are many case studies available that deal with subject of phytoremediation and/or aquaponics. Internationally, phytoremediation related research has grown and evolved due to increased interest by environmentally conscious groups. The following are a few representative studies related to the aspects of phytoremediation that have been previously mentioned and described.

Adler *et al.* conducted an aquaponics study in which they investigated the removal of phosphorus from aquaculture effluent (Adler *et.al.*, 2000). The plant used to recover the phosphorus was lettuce (*Lactuca sativa*) (Adler *et.al.*, 2000). The aquaculture effluent originated from the recirculating system at The Conservation Fund's Freshwater Institute in Shepherdstown, West Virginia (Adler *et.al.*, 2000). The wastewater from the system was filtered through a micro-screen. The aquaculture operation only reared rainbow trout (*Oncorhynchus mykiss*) (Adler *et.al.*, 2000). The bulk of the aquaculture

effluent contained nitrate and phosphorus concentrations at 25 mg/L and 0.7 mg/L, respectively (Adler *et.al.*, 2000). Other essential nutrients were added in excess to the system in order to make phosphorus the most limiting nutrient and thereby maximizing phosphorus removal (Adler *et.al.*, 2000). These excess nutrients included compounds of iron, manganese, molybdenum, and potassium (Adler *et.al.*, 2000). The pH of the system was steadily 7.2 (Adler *et.al.*, 2000). The plants were grown hydroponically using thin-film technology, also known as the Nutrient Film Technique (NFT) (Adler *et.al.*, 2000). Thin-film technology is a hydroponic crop production system in which plants are cultivated in water that flows continuously as a thin-film over the plant roots (Adler *et.al.*, 2000). The plants were cultivated via this technology in long 21.9 m (71.8 ft) troughs in which the aquaculture effluent flowed from end to the other (Adler *et.al.*, 2000). These troughs could hold approximately 126 lettuce plants (Adler *et.al.*, 2000). The hydroponic system removed phosphorus to an average concentration of 0.3 mg/L (Adler *et.al.*, 2000). The reduction in phosphorus concentration was thus equal to 0.4 mg/L. This constitutes that the hydroponic system removes an average of 57% of phosphorus from the aquaculture effluent. Adler et al. concluded that nutrients can be removed to very low levels by plants if the targeted nutrients are equally limited or balanced within the phytoremediation system (Adler *et.al.*, 2000).

Johnson, in conjunction with the North Carolina Division of Environmental Management, conducted nutrient concentration analysis on aquaculture effluent sampled above and below three ponds constructed below a trout rearing farm (Johnson, 1995). The ponds were constructed for watercress (*Nasturtium officinale*) cultivation and production (Johnson, 1995). The effluent from the aquaculture operation had a flowrate

of approximately 55 gal/min (0.2 m³/min) which was slightly less than 10% of the total effluent discharge (Johnson, 1995). The aquaculture operation stocked a total of about 25,000 lbs (11,364 kg) of fish (Johnson, 1995). The initial concentration of nutrients in the aquaculture effluent was not reported. However, the results from the nutrient concentration analysis indicated that ammonia and phosphorus was reduced by 74% and 58%, respectively (Johnson, 1995). They concluded that the quality of watercress grown in trout aquaculture effluent in Western North Carolina is superior to watercress grown by other methods (Johnson, 1995).

Rakocy et al. conducted multiple cultivation experiments in which thirty different kinds of plants, specifically vegetables, were raised in integrated fish and plant recirculating farming systems (Rakocy *et al.*, 1992). A wide range of systems were implemented which utilized different cultivation environments such as greenhouses in the northern United States to outdoor systems in the subtropics of the Virgin Islands (Rakocy *et al.*, 1992). Crops which were studied the most intensively included lettuce (*Lactuca sativa*) and tomatoes (*Solanum lycopersicum*) (Rakocy *et al.*, 1992). They found tomatoes were most productive in outdoor gravel-bed systems (Rakocy *et al.*, 1992). Production yields for tomatoes grown in this system were on average 21.0 lbs/plant (9.5 kg/plant) (Rakocy *et al.*, 1992). They found lettuce was most suitable for integrated aquaculture recirculating systems, since it grows abundantly in water with rich nitrogen concentrations (Rakocy *et al.*, 1992). They found that the methods best suited for lettuce cultivation included both the Nutrient Film Technique and deep flowing channels with polystyrene sheets for plant support (Rakocy *et al.*, 1992). Production yields for lettuce in the integrated recirculating aquaculture systems were on average 0.4 lbs/plant (0.2

kg/plant) (Rakocy *et al.*, 1992). They concluded that plant yields in integrated recirculating systems exceed that of field grown crops due to the higher plant densities that can be achieved, the control of the nutrient solutions, the constant availability of water, and the absence of competition from weeds (Rakocy *et al.*, 1992).

The Freshwater Institute has performed multiple studies to develop guidelines for an integrated recycle aquaculture and hydroponic system. The purpose of these guidelines is to provide operators interested in building such a system an introduction to some of the critical management issues (CFFI, 1998). The first issue discussed was that plants with a short life versus long life cycles tend to grow better in gravel bed aquaponic systems (CFFI, 1998). Such plants included lettuce, basil, watercress, parsley, spinach, oregano, mints, chives, mustard and cucumbers (CFFI, 1998). These vegetative plants were suggested since they favor the high nitrate levels found in recirculating aquaculture systems (CFFI, 1998). Other issues were the alkalinity, temperature and pH of the water. A pH maintained at approximately 7.0 was found to be the most optimal level for nutrient availability (CFFI, 1998). Alkalinity adjustments were found to work best through the use of potassium carbonate, since baking soda elevates sodium levels to a degree that is harmful to plants (CFFI, 1998). The optimum water temperature found for an aquaponic system was 24 °C for vegetable cultivation (CFFI, 1998).

Vincent and Downes evaluated the variation in nutrient removal from a stream via watercress (*Nasturtium officinale*) (Vincent & Downes, 1980). They theorized that a large watercress population in a stream leading into Lake Taupo in New Zealand was responsible for abundant seasonal variations in the removal of nitrate, phosphate and ammonia compounds (Vincent & Downes, 1980). The differences between upstream and

downstream nutrient concentrations reached a maximum level in January and February, which are in the summer season for New Zealand (Vincent & Downes, 1980). The average maximum temperature for the summer season in New Zealand ranges from 20-30 °C (68-86 °F) (Vincent & Downes, 1980). These nutrient concentrations were measured monthly from May 1973 to April 1974 (Vincent & Downes, 1980). This was also during the period of maximum watercress biomass in the stream (Vincent & Downes, 1980). In March 1975, late summer for New Zealand, twenty-four hour patterns of nutrient concentrations were investigated (Vincent & Downes, 1980). Nitrate concentrations were found to be lowest at midday and maximum at midnight (Vincent & Downes, 1980). Phosphate and ammonium loss were found to be relatively constant throughout the day (Vincent & Downes, 1980). In July 1979, midwinter for New Zealand, nitrate concentration differences upstream and downstream were almost insignificant, while phosphate and ammonium concentrations increased downstream (Vincent & Downes, 1980). Additionally, uptake of nitrate by watercress correlated with nitrate removal from the stream over a twenty-four hour period (Vincent & Downes, 1980). Phosphate concentrations were also reduced, but did not demonstrate a twenty-four hour pattern (Vincent & Downes, 1980).

Howard-Williams *et al.* examined the changes in plant biomass and growth, measured by shoot extensions, on a population of watercress (*Nasturtium officinale*) in a stream near Taupo, New Zealand (Howard-Williams *et al.*, 1982). Concentrations of nitrogen accumulated by the watercress were also examined. They found that a maximum relative growth rate of $0.0569 \text{ g g}^{-1} \text{ day}^{-1}$ occurred in the midsummer season (Howard-Williams *et al.*, 1982). Additionally, in the midsummer season nitrogen was found to

accumulate approximately $1.140 \text{ g m}^{-2} \text{ day}^{-1}$ (Howard-Williams *et al.*, 1982). Assuming this accumulation occurred within an area of 1 m^2 , the uptake rate of nitrogen via the watercress within this stream is equal to 1.140 g/day . A pattern of nitrate loss from the stream was also observed and correlated to watercress uptake of the nitrogen constituents (Howard-Williams *et al.*, 1982).

Cunningham examined the water characteristics, specifically the ammonia and nitrate levels of the effluent, of multiple aquaculture facilities located throughout West Virginia which reared rainbow trout (*Oncorhynchus mykiss*) exclusively (Cunningham, 2003). Of these aquaculture facilities, four used raceways for the cultivation of the fish (Cunningham, 2003). Three of the four raceway facilities had flow-through systems, while the third facility had a recirculating system (Cunningham, 2003). The first raceway facility reared 90,718 lbs (41,236 kg) of fish and had ammonia and nitrate concentrations of 0.31 mg/L and 1.28 mg/L in the effluent, respectively (Cunningham, 2003). The second raceway facility reared 13,608 lbs (6,186 kg) of fish and had ammonia and nitrate concentrations of 0.10 mg/L and 0.65 mg/L in the effluent, respectively (Cunningham, 2003). The third raceway facility reared 13,608 lbs (6,186 kg) of fish and had ammonia and nitrate concentrations of 0.21 mg/L and 0.07 mg/L in the effluent, respectively (Cunningham, 2003). The fourth raceway facility reared 9,000 lbs (4,091 kg) of fish and had ammonia and nitrate concentrations of 0.23 mg/L and 1.00 mg/L in the effluent, respectively (Cunningham, 2003). Although this case study does not deal with phytoremediation, it does present interrelated aquaculture effluent characteristics.

2.5.9 Plant Cultivation

There are a multitude of available plant species suitable for the use in different phytoremediation treatment techniques. For this aquaponics research project four plant species were cultivated. These species were watercress (*Nasturtium officinale* R. BR.), basil (*Ocimum basilicum*), dill (*Anethum graveolens*) and lettuce (*Lactuca sativa*).

2.5.9.1 Watercress

Watercress (*Nasturtium officinale* R. BR.) is a semi-aquatic perennial plant found naturally in still or slowly flowing shallow water systems such as springs, streams or ponds. It is a member of the family Brassicaceae, or cabbage family, and is originally native to Europe and central Asia. It can also grow to a height range of 50-120 cm (20-47 in) (Morgan, 2002).

The tissue of a watercress plant is exceptionally rich in many vitamins and minerals. It contains significant amounts of iron, calcium, potassium, folic acids, Vitamin A, Vitamin B-1, Vitamin B-3, Vitamin C and Vitamin K (USDA, 2004). Due to the abundance of these vitamins and minerals, watercress has long been valued as a dietary and medicinal plant (USDA, 2004). Benefits from eating watercress are that they act as a mild stimulant, are a source of antioxidants and act as a digestive aid (USDA, 2004).

Watercress is well suited to hydroponic cultivation, thriving most favorably in cool water that is slightly alkaline. Ideal water temperatures favorable for watercress cultivation range from approximately 12-18 °C (54-64 °F) (Morgan, 2002). Demand for watercress often exceeds supplies of agricultural markets, which adds to its value as a hydroponically grown crop.

2.5.9.2 Basil

Basil (*Ocimum basilicum*) is a tender aromatic annual herb that usually grows low to the ground. It is a member of the family Lamiaceae and is also commonly known as St. Joseph's Wort. Basil is originally native to tropical areas of Asia and can grow to a range of 20-60 cm (8-24 in) tall and 1-3 cm (0.4-1.2 in) broad (Mahr, 2003). Basil is very sensitive to cold climates and thus is cultivated best in hot climates with an ideal environmental temperature range of approximately 27-32 °C (80-90 °F) (Mahr, 2003). Although it is usually grown as a soil-based crop, basil can be cultivated hydroponically in warm water at the ideal temperature range.

2.5.9.3 Dill

Dill (*Anethum graveolens*) is a biennial herb known to have a short life cycle. It is part of the family Apiaceae and is originally native to southwest and central Asia and the Mediterranean. Dill can grow to a range of 40-60 cm (16-24 in) tall with leaves ranging 10-20 cm (4-8 in) long. Cultivation of dill is most favorable in warm climates with abundant light intensity. Ideal environmental temperatures for dill cultivation range from 6-26 °C (43-79 °F), though dill ideally prefer cooler temperatures around 7 °C (50 °F) (Simon *et al.*, 1984). Dill usually prefers rich soil, though it can be grown hydroponically in warm water at the ideal temperature range.

2.5.9.4 Lettuce

Lettuce (*Lactuca sativa*) is a temperate annual or biennial plant consisting of specifically bred cultivar groups. It is part of the family Asteraceae and is native to all parts of the world. Generally, lettuce prefers ideal environmental temperatures ranging from 16-18 °C (60-65 °F) (Sanders, 2001). It can tolerate a few days with a temperature

range of 27-29 °C (80-85 °F), provided that the nights are at the ideally cool temperatures (Sanders, 2001). Cultivation of lettuce is principally achieved as a soil-based crop, though hydroponic growth can be achieved in water at the ideal temperature range.

2.5.9.5 Plant Water Quality Criteria

The water quality criteria relevant to the commercial cultivation of hydroponically grown plants are typically based upon a nutrient solution recipe known as the Hoagland solution (Schwarz, 1968). The compounds comprised in the Hoagland solution are the “ideal” concentrations needed by most plants for development. The recipe for the Hoagland solution is as follows in *Table 2.5.9.5.1*:

Table 2.5.9.5.1 – Hoagland Solution for Ideal Plant Cultivation (Schwarz, 1968).

Chemical Compound	Concentration
-----	(mg/L)
Calcium Nitrate	540
Copper Sulfate	140
Ferrous Sulfate	130
Magnesium Sulfate	14
Manganese Sulfate	1.7
Monoammonia Phosphate	0.8
Monocalcium Phosphate	0.6
Potassium Nitrate	2
Zinc Sulfate	120

CHAPTER 3. MATERIALS & METHODS

3.1 Research Site

3.1.1 Site Location

The aquaponics research facility is located at West Virginia University's (WVU) Reymann Memorial Farm in Wardensville, West Virginia. This location was selected to study the nutrient removal via aquatic phytoremediation for three reasons: (1) the water at the site has nutrient constituents suitable for hydroponic growth; (2) there is available, though limited, electric power; (3) there is already a functioning aquaculture operation at the site with wastewater effluent containing bio-solids and soluble nutrients for a remediation study. A schematic illustration of the research site is presented in *Figure 3.1.1.1*:

Figure 3.1.1.1 – Research Site at Reymann Memorial Farm (Note: Not To Scale)

A photograph of the Reymann Memorial Farm is additionally presented in the *Appendix E-1*.

3.1.2 Environment

The research site is located geographically in a rural valley of West Virginia. The site has a typical climate of four seasons in a year, with average highest and lowest temperatures of 85 °F (29 °C) and 18 °F (-7 °C), respectively (NWS, 2006). On average, July is the warmest month of the year with a highest record temperature of 105 °F (40 °C) in 1988 (NWS, 2006). January is the coolest month of the year with a lowest record temperature of -24 °F (-31 °C) in 1930 (NWS, 2006). The average precipitation ranges from approximately 24-48 inches of rain per year, with the most precipitation occurring in the month of July (NWS, 2006).

3.1.3 Water Resource

The water resource used for the aquaculture operation and consequently, the aquatic phytoremediation system, is a natural spring. The spring is located about 182 m (600 ft) northwest of the aquaculture shelter. Spring water flows via gravity through a 12 in (0.3 m) intake pipe into the aquaculture system. Water flows via gravity through the rest of the raceway system, the polishing pond, and into the stream. The spring water has a consistent temperature and pH of 13-15 °C (55-59 °F) and 7.2, respectively.

3.2 Aquaculture Operation

3.2.1 Raceway Design

The aquaculture operation, which is located in a large shelter built on the farm, is comprised fundamentally of a head-box, raceway and tail-box components encompassing an area around 720 ft³ (19.44 m³). A photograph of the aquaculture shelter is presented in

Appendix E-2 and *E-3*. Structural components of the raceways are constructed of a honeycomb fiber reinforced polymer. The head-box accumulates influent water from the local spring that flows into the raceways at about 400 gal/min (1509 L/min). The head-box is a cube with 8 ft (2.4 m) sides and an approximate 8 ft (2.4 m) depth. Additionally, the head-box contains air diffusers to oxygenate the water. The diffusers are air supplied via a regenerative blower. A photograph of the head-box is presented in *Appendix E-9*.

The raceways consist of four tiered units in series, each containing two parallel and identical segments. A photograph of the raceways is presented in *Appendix E-7*. Each raceway segment is 3 ft (0.9 m) deep, 3 ft (0.9 m) wide and 30 ft (9 m) long and can hold a volume of 270 ft³ (7646 L) of water. A screen is located near the end of each raceway creating a quiescent zone where fish are not present. The quiescent zone allows for the settling of suspended particles from water. A stand pipe is situated vertically in the center of the quiescent zone, which can be detached for removal and cleaning of the settled particles. A photograph of the quiescent zone is presented in *Appendix E-10*. Water flowing out of each raceway is screened and aerated at the head of the subsequent raceway unit. A photograph of the spillways is presented in *Appendix E-12*. A simple schematic of a raceway unit is also presented in *Figure 3.2.1.1*:

Figure 3.2.1.1 – Aquaculture Raceway Unit (Note: Not To Scale)

The tail-box collects the effluent wastewater from the raceways, which is subsequently forced through a 12 in (0.3 m) discharge pipe to a polishing pond. The tail-box is a rectangle with 6 ft (1.8 m) and 3 ft (0.9 m) sides and a 3 ft (0.9 m) depth. The head-box, raceways and tail-box all have a frame thickness of 2-4 in (4.6-9.2 cm). From the polishing pond, the effluent wastewater eventually is discharged into a stream known as Moore’s Run and eventually the Cacapon River. Photographs of the polishing pond, Moore’s Run and the tail-box are presented in *Appendix E-5, E-6 and E-11*, respectively. Another schematic of the entire aquaculture operation system is presented in *Figure 3.2.1.2*:

Figure 3.2.1.2 – Aquaculture System (Note: Not To Scale)

3.2.2 Fish

The fish specie cultivated in the flow-through raceway system was brook trout (*Salvelinus fontinalis*). The brook trout (*Salvelinus fontinalis*) grown throughout the aquaponics study period were stocked on June 24, 2004. The density of the fish in the aquaculture raceways varied with the average fish size. Typically, no more than 1,100 lbs (499 kg) of fish were in a raceway unit at one time, giving an approximate weight-per-volume density equal to 4.1 lbs/ft³ (70 kg/m³). The entire aquaculture system typically is stocked with 6,000-8,000 lbs (2722-3629 kg) of fish.

3.2.3 Feed

The fish feed used in the aquaculture operation was *Zeigler Finfish Gold*. This feed includes both floating and slow-sinking pellets ranging in size from 3-5 mm (0.125-0.188 in) in diameter (ZBI, 2005). The feed is minimally guaranteed to contain 42 % protein, 16 % fat, 12 % moisture, 8 % ash and 4 % fiber (ZBI, 2005). *Finfish Gold* is typically best suited for the fish species of trout, flounder, striped bass, haddock, artic

charr, sea bass and sea bream. These effects enhanced the feed conversions for enhanced growth of the fish and improved flesh quality and texture of the fish with a lower average fat content. The manufacturer states that the feed was designed to generate lower soluble nitrogen and phosphorus effluent in the water (ZBI, 2005).

The feeding schedule for the brook trout at the aquaculture operation site was adjusted daily to meet the dietary needs of the fish. Adjustments were based and dependent upon the size and growth of the fish from day-to-day. Fundamentally, the fish were hand fed 50-100 lbs (23-45 kg) of daily rations that permitted maximum growth.

Assuming no feed waste, the total amount of byproduct solids generated by 1,000 lbs (454.5 kg) of feed is approximately equal to 240-330 lbs (109.1-150.0 kg) of solids by dry feed weight (IDEQ, 1998). This is based upon four factors: (1) fish feed is typically 92% dry, (2) fish are typically 26.15% dry weight, (3) 70-80% of feed is digested, and (4) approximately 90 lbs (40.9 kg) of dry feed is excreted as soluble solids (IDEQ, 1998). These solids and the chemical compound wastes excreted by fish account for the nutrient constituents found in aquaculture effluent. Waste generation at these levels would occur in the aquaculture operation at Reymann Memorial Farm every ten to twenty days.

3.3 Divergent Growth Conditions Study (# 1)

3.3.1 Purpose

The divergent growth conditions study was the initial experiment developed and conducted for the phytoremediation research, running from June 22, 2005 to October 8, 2005. The purpose of this study was to determine if watercress (*Nasturtium officinale* R. BR.) could be utilized, via aquatic phytoremediation, to reduce nutrients from aquaculture effluent in sufficient quantities to be useful as a nutrient remediation

technique and to determine operational and environmental conditions which affect nutrient removal and plant growth. Operational conditions varied between experimental treatment combinations of three flow velocities, three types of plant support media and three plant densities. A list of the experimental treatments randomly assigned to each aquatic phytoremediation channel is presented in *Table 3.3.1.1*:

Table 3.3.1.1 – Experimental Treatment Combinations (Study # 1)

Divergent Growth Conditions Study			
Sample Site	Plant Species	Flow Velocity	Plant Density
-----	-----	(ft/s)	(#)
Control Channel 1	Watercress	H	M
Control Channel 2	Watercress	L	M
Control Channel 3	Watercress	M	M
Experimental Channel 1	N/A	M	N/A
Experimental Channel 2	Watercress	M	M
Experimental Channel 3	Watercress	L	H
Experimental Channel 4	Watercress	H	L
Experimental Channel 5	Watercress	M	H
Experimental Channel 6	Watercress	L	M
Experimental Channel 7	Watercress	M	L
Experimental Channel 8	Watercress	H	H
Experimental Channel 9	Watercress	L	L
Experimental Channel 10	Watercress	H	M
Experimental Channel 11	Watercress	H	L
Experimental Channel 12	Watercress	M	M
Experimental Channel 13	Watercress	H	H
Experimental Channel 14	Watercress	M	L
Experimental Channel 15	N/A	L	N/A
Experimental Channel 16	Watercress	L	H
Experimental Channel 17	Watercress	L	L
Experimental Channel 18	Watercress	M	H
Experimental Channel 19	Watercress	H	M
Experimental Channel 20	Watercress	L	M
Experimental Channel 21	Watercress	H	H
Experimental Channel 22	Watercress	M	L
Experimental Channel 23	Watercress	M	H
Experimental Channel 24	Watercress	M	M
Experimental Channel 25	Watercress	L	H
Experimental Channel 26	Watercress	H	L
Experimental Channel 27	Watercress	L	L
Experimental Channel 28	Watercress	H	M
Experimental Channel 29	N/A	H	N/A
Experimental Channel 30	Watercress	L	M

The high, medium and low velocities or densities are designated in *Table 3.3.1.1* by the letters *H*, *M* and *L*, respectively. The actual magnitudes of these different velocity and density treatments can be found in subsequent sections of this chapter. The term *N/A* is

used to denote that no plants were grown at any density in the channel. These empty channels were used as a type of added control to the study.

The experimental treatments were selected for the initial study to determine if any differences in nutrient remediation were dependent upon the velocity and/or density of the water and plants in the channel. The empty experimental treatments were added to the study to act as another control to determine how nutrient concentrations would be effected in the absence of plants within the channels. The control channels were added to determine the differences in growth between plants cultivated in the nutrient enriched aquaculture effluent versus the natural spring influent.

Continual analysis of the nutrient concentrations of the effluent leaving the aquaponics system was required to resolve the research objectives. To do this, a large pilot and field-scale aquatic phytoremediation system was constructed inside the shelter in conjunction with the existing aquaculture operation.

3.3.2 Location

The divergent growth study was conducted in the shelter built upon WVU's Reymann Memorial Farm in Wardensville, West Virginia. A schematic of the entire aquaponics system, specifically the aquatic phytoremediation system setup, is presented in *Figure 3.3.2.1*:

Figure 3.3.2.1 – Aquaponics System Inside the Shelter (Note: Not To Scale)

3.3.3 Phytoremediation System

The aquatic phytoremediation system was constructed adjacent to the aquaculture operation. Effluent, which had passed through the suspended-solid-collecting quiescent zone, was pumped from the aquaculture tail-box into a large reservoir tank. The pump used was single phase and submersible Goulds 3887 Series Sewage Ejector Pump, which was capable of handling a maximum of two inch spherical solids. This was favorable since escaped fish could enter the pump and clog the system. Another characteristic of the pump was that it had only 1.5 horsepower with a maximum voltage and current rating of 230 V and 12.3 A, respectively. Additionally, the pump had a maximum flow rate of 200 gal/min (0.756 m³/min) a minimum pressure-head of 16 ft (4.878 m).

The tank used in the system was an ACE 1,000 gallon cone-bottom tank. The tank was constructed of heavy duty polyethylene that was translucent, had a specific gravity of

1.75 and was stabilized for ultraviolet light. Water from the tail-box was pumped into the top of the tank, which functioned as a “head tank”, and then released to the manifold conduit from a four inch outlet drilled at the point where the top of the cone met the rest of the tank. This allowed some particulate matter to settle onto the cone bottom of the tank, which could be cleaned via discharge through the bottom of the cone. The “head tank” provided the necessary pressure-head need for the water to be distributed throughout the aquatic phytoremediation channel system. A photograph of the tank is presented in *Appendix E-13*. A schematic of the tank is also presented in *Figure 3.3.3.3*:

Figure 3.3.3.3 – ACE 1,000 Gallon Cone Bottom Tank (Note: Not To Scale)

From the six inch manifold conduit, the water was distributed to each aquatic phytoremediation channel via one inch valves and piping drilled into the side of the manifold. After the water flowed through the channels, it was collected via two inch stand pipes which discharged the effluent into one inch piping drilled into a six inch

drainage pipe. The drainage pipe carried the effluent back to the tail-box and discharged the water directly into the twelve inch discharge pipe. The discharge pipe in the tail-box directed the effluent flow into the outside polishing pond.

A box containing channels used for the control side of the initial experiment was placed next to the head-box of the aquaculture system. Water was pumped from a similar pump through two and one inch piping to the control channels. Water then flowed through the control channels, drained into the stand pipes and was discharged back into the head-box. The purpose of these control channels was horticultural in nature, as they were used to determine differences in the development of plants cultivated in and out of the nutrient enriched aquaculture effluent.

3.3.3.1 Channel Design

The conduits used in the aquatic phytoremediation system were boxes containing three channels constructed of 0.75 in (1.9 cm) plywood. The boxes were supported by cinder blocks and arranged parallel to one another roughly 1.5 ft (0.5 m) apart. Each of the three channels within a box were 15 in (38 cm) in width and 8 ft (2.4 m) in length. The bottom of each channel was lined with a Styrofoam sheet to help prevent the channel from freezing. Each channel was then completely lined with a black rubber material, ethylene propylene diene monomer, to protect the plywood.

The inlet water was controlled by a one inch ball valve drilled into the manifold pipe and connected to the channels via one inch pipes and gaskets. The inlets of the channels were arranged roughly six to seven inches from the bottom of the channel, just above the water surface. This enabled easier calibration of the flow velocities and maintenance of the piping.

Three different velocities, based upon the flow velocity of the raceways, were permanently set and maintained throughout the study via these valves. These high, medium, and low velocities were 0.020 ft/s (0.006 m/s), 0.010 ft/s (0.003 m/s), and 0.002 ft/s (0.0006 m/s), respectively. The highest velocity was chosen to closely represent the velocity of the water in the aquaculture raceways. The velocity between the high velocity channels and the aquaculture raceways were not identical due to the hydraulic restriction of the aquatic phytoremediation system design. The medium and low velocities were simply division of the high velocity chosen to study the potential effects of water velocity on nutrient extraction by plants. Each individual channel's velocity setting for this initial experiment is presented previously in *Table 3.3.1.1*. The water level was regulated by six inch long stand pipes placed vertically at the end of each channel. Thus, the water level in the channels was maintained at a depth of six inches. The flow rates at the velocities set for the high, medium, and low channels are 14.0 gal/min (0.053 m³/min), 7.0 gal/min (0.026 m³/min), and 1.4 gal/min (0.005 m³/min), respectively. One exchange is the time that it would take the whole volume of water set at the high, medium and low velocities 2.6, 5.3, and 26.5 minutes, respectively, to flow entirely through an channel. Exchange rates for the channels were 23, 11, and 2.26 exchanges per hour for the high, medium, and low velocities, respectively.

The effluent from the channels drained through the stand pipes into a six inch drainage pipe and was ultimately sent to the outside polishing pond. Photographs of the aquatic phytoremediation channels are presented in *Appendix E-14* and *E-15*. A dimensional plan and profile schematic of the aquatic phytoremediation channels, including the inlet and outlets, is also presented in *Figure 3.3.4.1.1*:

Figure 3.3.4.1.1 – Aquatic Phytoremediation Box & Channels (Note: Not To Scale)

3.3.3.2 Plant Cultivation Design

Aquatic plants were grown hydroponically in channels to uptake and use the nutrients from the aquaculture effluent. Only watercress (*Nasturtium officinale* R. BR.) was cultivated for this initial study. The watercress seed, obtained from Johnny's

Selected Seed, were germinated and grown on rectangular rafts crafted from small polyvinylchloride (PVC) pipes and joints encompassing a thin and sturdy “Vexar” plastic meshing. The PVC pipes and joints had an interior diameter of one inch. Each raft was roughly 2.5 ft (76 cm) long and 14 in (36 cm) wide. Thus, each channel contained a series of three rafts. Individual rafts contained one of three different types of support media for the watercress. These support media included layers of paper towels, rock wool or oasis cubes. Oasis cubes are preformed, open-celled, water-absorbing foam, which uses capillary action to supply neutral water to plants.

Each channel contained a raft with each of the support media used for the plants. Therefore, the support media of the rafts in each channel was identical throughout the aquatic phytoremediation system. In addition, the watercress were cultivated at three different plant densities. The density of watercress on each individual raft were thus set at either a low (0.04 plants/cm^2), a medium (0.09 plants/cm^2) or a high (0.16 plants/cm^2) density. Three channels were setup as a blank for the study and incorporated only empty rafts. The arrangements of the plants densities for each channel in this initial experiment are presented previously in *Table 3.3.1.1*.

3.3.4 Sampling Regime

The divergent growth conditions study was conducted in the aquaculture shelter over a period of five months, from the end of June 2005 to the beginning of October 2005. Six sets of water samples were taken nearly every three weeks to be analyzed, with base initial samples taken on June 22, 2005. Each set of samples consisted of wastewater taken from multiple sites distributed among the aquaponics system. These sites consisted of all the thirty-three aquatic phytoremediation experimental and control channels, the

head-box, the ends of both raceways, the tail-box, the beginning of the manifold pipe, the polishing pond and the Moore's Run. At each collection site, four 250 mL bottles were rinsed and filled with water. In the case of the channels, each of the four bottles for a site would be filled with effluent immediately before the wastewater drained in the stand pipe. The samples were then preserved and transported in coolers back to the laboratory at West Virginia University in Morgantown, West Virginia for analysis. The water samples were analyzed for ammonia, nitrate, nitrite, and phosphate.

Settled waste and other materials were cleaned from the channels each sample date. The waste was simply swept down the discharge pipe. The plant rafts were also rotated upon each sample date to ensure an equal distribution of nutrients throughout the study.

3.4 Multiple Plant Species Winter Study (# 2)

3.4.1 Purpose

The multiple plant species winter study was the second experiment developed and conducted for the phytoremediation research, running from December 11, 2005 to March 3, 2006. The purpose of this study was again to determine if watercress (*Nasturtium officinale* R. BR.) could be utilized, via aquatic phytoremediation, to reduce nutrients from aquaculture effluent in sufficient quantities to be useful as a nutrient constituent remediation technique. In addition to the use of watercress, basil (*Ocimum basilicum*), dill (*Anethum graveolens*) and lettuce (*Lactuca sativa*) were also studied. The determination of operational and environmental conditions which affect nutrient removal and plant growth were again also scrutinized. In this study, operational conditions varied between experimental treatment combinations of two different flow velocities, two plant

densities and four plant species. A list of the experimental treatments randomly assigned to each aquatic phytoremediation channel is presented in *Table 3.4.1.1*:

Table 3.4.1.1 – Experimental Treatment Combination (Study # 2 & 3)

Multiple Plant Species Winter/Spring Study			
Sample Site	Plant Species	Flow Velocity	Plant Density
-----	-----	(ft/s)	(#)
Experimental Channel 1	Basil	L	H
Experimental Channel 2	Dill	L	H
Experimental Channel 3	Lettuce	L	H
Experimental Channel 4	Lettuce	L	H
Experimental Channel 5	Basil	L	H
Experimental Channel 6	Dill	L	H
Experimental Channel 7	Dill	L	H
Experimental Channel 8	Lettuce	L	H
Experimental Channel 9	Basil	L	H
Experimental Channel 10	Watercress	L	H
Experimental Channel 11	Watercress	H	H
Experimental Channel 12	Watercress	L	L
Experimental Channel 13	Watercress	L	L
Experimental Channel 14	Watercress	H	H
Experimental Channel 15	Watercress	H	L
Experimental Channel 16	Watercress	H	H
Experimental Channel 17	Watercress	H	L
Experimental Channel 18	Watercress	L	H
Experimental Channel 19	Watercress	L	L
Experimental Channel 20	Watercress	L	H
Experimental Channel 21	Watercress	H	L
Long Channel 22	Watercress	L	H
Long Channel 23	Watercress	L	H
Long Channel 24	Watercress	L	H
Old Shelter Channel 25	Watercress	L	H
Old Shelter Channel 26	Watercress	L	H
Old Shelter Channel 27	Watercress	L	H

The high and low velocities or densities are designated in *Table 3.4.1.1* by the letters *H* and *L*, respectively. The actual magnitudes of these different velocity and density treatments can be found in subsequent sections of this chapter.

These experimental treatments were selected for the second and third study for several reasons. The addition of basil, dill and lettuce cultivated channels was to analyze how effective different plant species, which prefer both warm and cool environmental temperatures, are at the remediation of nutrient compounds. The medium velocity and medium density experimental treatments from the first study were removed for the second study. By only using low and high extremes for the velocity and density of the experimental treatments, differences in nutrient removal would be more notable. The long channels were added to the experimental treatments to determine if more contact time between the nutrients and plant roots would demonstrate greater reductions in the concentrations within the water samples. The old shelter channels were kept within the study to determine any differences between the initial summer study and the second and third winter and spring study. The majority of the new experimental treatments were set to low velocities and high densities due to the lack of channels within the greenhouse to create the necessary replications required for additional velocity-density combinations. The low velocity and high density treatment was selected since it allowed the greatest amount of contact time between nutrient constituents and plant roots.

As in the previous study continuous analysis of the nutrient concentrations throughout the colder winter months was required to meet these objectives. To conduct this winter experiment, a greenhouse was constructed near the aquaculture operation shelter, in which the aquatic phytoremediation system was reconstructed using many of the original channel boxes used in the initial study.

3.4.2 Location

The multiple plant species winter study was conducted within the greenhouse built upon WVU's Reymann Memorial Farm in Wardensville, West Virginia. The greenhouse, constructed during fall 2005, was built next to the aquaculture shelter and the polishing pond, as shown in *Figure 3.1.1.1*. A photograph of the greenhouse is presented in *Appendix E-2* and *E-4*. A schematic of the aquatic phytoremediation system within the greenhouse is presented in *Figure 3.4.2.1*:

Figure 3.4.2.1 – Aquatic Phytoremediation System Inside the Greenhouse (Note: Not To Scale)

3.4.3 Phytoremediation System

Wastewater was pumped to the greenhouse, via a 1 horsepower pump similar used in the first experiment, from the tail-box, through six inch piping and into a six inch manifold conduit. From the manifold conduit the wastewater was discharged into eight boxes or twenty-four individual experimental channels, constructed for the original study. From there, the effluent drained directly through two inch stand pipes into a six inch

outlet pipe. The outlet pipe ultimately connected with the twelve inch discharge pipe from the tail-box, which in turn discharged into the polishing pond.

3.4.3.1 Channel Design

The channels used for this study consisted of the same channels used in the initial experiment. The boxes were, however, painted to prevent molding and were placed upon small bricks for support. Each channel was set at either a high or low velocity of 0.020 ft/s (0.094 gal/s) and 0.002 ft/s (0.009 gal/s), respectively. The velocity settings for each channel in the second experiment are presented previously in *Table 3.4.1.1*. A new box was constructed for this study that was identical to the original boxes except that it was roughly twice as long, or 18 ft (5.5 m) in length. In this box, the velocities were all adjusted to the low setting, and seven rafts per channel were used to support plants of the same density. This was done in an effort to allow more contact time between the plants and the nutrient constituents in the wastewater. A single box of three old experimental channels was left next to the aquaculture operation to compare the differences between the use of the greenhouse and the shelter. A photograph of the aquatic phytoremediation channels within the greenhouse is presented in *Appendix E-8*.

3.4.3.2 Plant Cultivation Design

Multiple species of plants were grown hydroponically in the channels to uptake and use the nutrients from the aquaculture effluent. The species of the plants cultivated were primarily watercress, though limited numbers of channels were used to grow basil, dill and lettuce. In this study, seed for all plant species were obtained from Park Seed. All the plants were germinated and grown upon only paper towels on the rafts. The density of plants grown on each individual raft were set at either a low (0.04 plants/cm²) or a high

(0.16 plants/cm²) density. The locations of these density settings throughout the aquatic phytoremediation system for the second experiment are presented previously in *Table 3.4.1.1*. Watercress was also grown in the three channels of a box left inside the aquaculture shelter from the initial experiment.

3.4.4 Sampling Regime

The multiple plant species winter study was conducted in the aquaponics greenhouse over a period of four months from the middle of December 2005 to the beginning of March 2006. Within that period, five sets of water samples were taken approximately every three weeks with initial samples taken on January 5, 2006. Each set of samples consisted of wastewater taken from multiple sites distributed among the aquaponics system. These sites consisted of all the twenty-seven aquatic phytoremediation experimental channels, the head-box, the ends of both raceways, the tail-box, the polishing pond and the Moore's Run. The sampling procedure was the same as described for the previous study.

For this second study, each sampling trip was conducted as scheduled and planned except for the sampling conducted on February 20, 2006. This sampling trip should have been made on February 11, 2006. It had to be postponed due to poor weather and travel conditions.

3.5 Multiple Plant Species Spring Study (# 3)

3.5.1 Purpose

The multiple plant species spring study was the third experiment developed and conducted for the phytoremediation research, running from March 11, 2006 to June 2, 2006. The purpose of this study was to determine if watercress (*Nasturtium officinale* R.

BR.), basil (*Ocimum basilicum*), dill (*Anethum graveolens*) or lettuce (*Lactuca sativa*) can be utilized, via aquatic phytoremediation, to reduce nutrients from aquaculture effluent in sufficient quantities to be useful as a nutrient constituent remediation technique. The determination of operational and environmental conditions which affected nutrient removal and plant growth were also scrutinized. In this study, operational conditions varied between treatment combinations of two different flow velocities, two plant densities and four plant species. The experimental treatments used in this study are identical to the combinations used throughout the second experiment. The main difference between these two experiments was thus the winter and spring climate conditions. A list of the treatment combinations assigned to each aquatic phytoremediation channel was presented previously in *Table 3.4.1.1*.

The environmental conditions would only include any changes in the water characteristics and climate over the span of the study. Continual analysis and monitoring of the nutrient concentrations throughout the warmer spring to summer months was required to resolve these objectives. To conduct this spring experiment, the greenhouse and the aquatic phytoremediation system was again utilized.

3.5.2 Location

The multiple plant species spring study was conducted within the greenhouse built upon WVU's Reymann Memorial Farm in Wardensville, West Virginia. The greenhouse was improved from the second experiment via adding an interior vent shutter and fan to help regulate the inside temperature. A schematic of the aquatic phytoremediation system in the greenhouse was presented previously in *Figure 3.4.2.1*.

3.5.3 Phytoremediation System

The aquatic phytoremediation system for this study was utilized in the same manner as the second experiment. Wastewater was again pumped from the tail-box, through four inch piping and into a six inch manifold conduit. From the manifold conduit the wastewater was discharged into eight boxes or twenty-four individual experimental channels. From there, the effluent drained directly through two inch stand pipes into a six inch outlet pipe. The outlet pipe ultimately connected with the twelve inch discharge pipe from the tail-box, which in turn sent the effluent into the polishing pond.

3.4.3.1 Channel Design

The channels used for this study consisted of the same channels used in the second experiment. Each channel was set at either a high or low velocity of 0.020 ft/s (0.094 gal/s) and 0.002 ft/s (0.009 gal/s), respectively. These velocity settings of each individual channel for this third experiment are presented previously in *Table 3.4.1.1*. A single box of three channels was left next to the aquaculture operation to continue to compare the differences between the use of the greenhouse and the shelter.

3.4.3.2 Plant Cultivation Design

Multiple species of plants were grown hydroponically in the channels to uptake and use the nutrients from the aquaculture effluent. The species of the plants cultivated were still primarily watercress with limited growth of basil, dill and lettuce. In this study, seed for all plant species were obtained from Park Seed. All the plants were germinated and grown upon only paper towels on the rafts. The density of plants grown on each individual raft were set at either a low (0.04 plants/cm²) or a high (0.16 plants/cm²) density. The density configuration throughout the aquatic phytoremediation system for

this third experiment is presented previously in *Table 3.4.1.1*. The three channels inside the aquaculture shelter were only used for the cultivation of watercress.

3.4.4 Sampling Regime

The multiple plant species spring study was conducted in the aquaponics greenhouse over a period of three months from the middle of March 2006 to the beginning of June 2006. Four sets of water samples were again taken nearly every three weeks to be analyzed, with a base initial sample taken on April 1, 2006. Each set of samples consisted of wastewater taken from the same sites distributed among the aquaponics system as in the second experiment. These sites consisted of all the twenty-seven aquatic phytoremediation experimental channels, the head-box, the ends of raceways, the tail-box, the polishing pond and the Moore's Run. The sampling procedure was the same as described for the previous two studies.

3.6 Experimental Methodology

3.6.3 Water Sample Analysis

The water samples taken from the aquaponics research site were analyzed for nitrogen and phosphorus concentrations in the forms of ammonia, nitrate, nitrite and phosphate. Two of the sample bottles from each sampling location were refrigerated and analyzed for ammonia and nitrite within a twenty-four hour span from the time of sampling. The other two sample bottles were frozen for analysis of nitrate and phosphate, at a later time. Ammonia, nitrate, nitrite and phosphate concentration were determined by the selective electrode method, the cadmium reduction method, the colorimetric method and the ascorbic acid method, respectively.

3.6.1.1 Ammonia-Selective Electrode Method (4500-NH₃ D.)

The selective electrode method was used to determine the ammonia (NH₃-N) concentration in the water samples. In this method, ammonia levels are found by chemically transforming all forms of ammonia in solution into ammonia gas and detecting the concentration via a Hach Company Sensor ammonia probe connected to a Hach Company EC40 pH/ISE meter. The sample measurements are thus evaluated against the standard measurements and the calibration curve to determine the actual concentrations. To transform the ammonia in solution into ammonia gas, 1 mL of a 10 N sodium hydroxide-EDTA (NaOH-EDTA) solution was added to the sample solution to raise the pH above 11 (APHA, 1998). If more than 1 mL of the 10 N sodium hydroxide-EDTA solution was necessary to shift the pH, notation of the actual volume used was required for subsequent calculations to determine the actual ammonia concentration. The pH was monitored via an Accumet pH probe. The formula used for the calculation is as follows in *Equation 3.6.1.1.1*:

$$mg / L NH_3^- N = A \times B \times \frac{[100 + D]}{[100 + C]}$$

Equation 3.6.1.1.1 – Adjusted Ammonia Concentration (APHA, 1998).

In *Equation 3.6.1.1.1*, A is equal to the dilution factor, B is equal to the concentration of ammonia from the calibration curve (mg/L), C is equal to the volume of 10 N sodium hydroxide-EDTA solution added to calibration standards (mL) and D is equal to the volume of 10 N sodium hydroxide-EDTA solution added to the sample (mL) (APHA, 1998).

The ammonia-selective electrode used to detect the ammonia gas had a hydrophobic gas-permeable membrane to separate the sample solution from an internal

electrode solution of ammonium chloride (NH_4Cl) (APHA, 1998). Potentiometric measurements are made with a pH meter having an expanded millivolt scale of 0.1 mV resolution between -700 mV and +700 mV (APHA, 1998). This method is applicable to the measurement of ammonia gas at a range of 0.03-1400 mg/L (APHA, 1998). Water samples tested by this method had to be refrigerated at 4 °C and analyzed within a 24 hour period (APHA, 1998).

3.6.1.2 Nitrate Cadmium Reduction Method (4500- NO_3^- E.)

The cadmium reduction method was used to determine the nitrate (NO_3^-) concentration in the water samples. In this method, nitrate levels are found first by reducing nitrate to nitrite (NO_2^-) through a cadmium packed glass column in which the granules were washed with 6 N hydrochloric acid (HCl) and treated with a copper sulfate (CuSO_4) solution (APHA, 1998). The sample solution passed through the reduction column was initially mixed with an ammonium chloride-EDTA (NH_4Cl -EDTA) solution to prevent metals and turbid restrictions from lowering the reduction efficiency (APHA, 1998). Second, nitrite produced and recovered by the reduction column was diazotized with a highly colored azo dye and measured colorimetrically, via a Thermo-Electron Corporation Genesys scanning spectrophotometer set at 543 nm, to determine the concentration (APHA, 1998). The azo dye consists of sulfanilamide ($\text{C}_6\text{H}_8\text{N}_2\text{SO}_2$) and an ammonium hydroxide (NH_4OH) solution mixed with an 85 % phosphoric acid (H_3PO_4) solution (APHA, 1998). The azo dye gave the sample solution a reddish-purple coloration. The nitrite concentration found through this method consisted of a total of both nitrate converted to nitrite and nitrite initially present in the sample solution. Nitrate concentrations are thereby determined by measuring the difference between the total

nitrite levels and the nitrite levels found in a corresponding water solution tested for nitrite but not passed through a nitrate reduction column. The sample measurements are evaluated against the standard measurements and the calibration curve to determine the actual concentrations. The applicable range for the cadmium reduction method for nitrate is 0.01-1.00 mg/L (APHA, 1998). Water samples tested by this method did not have to be immediately analyzed but must be frozen for proper preservation (APHA, 1998).

3.6.1.3 Nitrite Colorimetric Method (4500-NO₂⁻ B.)

The colorimetric method was used to determine the initial nitrite (NO₂⁻N) concentration in the water samples. In this method, nitrite levels are found by mixing the sample solution with an azo dye, consisting of sulfanilamide (C₆H₈N₂SO₂) and an ammonium hydroxide (NH₄OH) solution mixed with an 85 % phosphoric acid (H₃PO₄) solution (APHA, 1998). The azo dye gave the sample solution a reddish-purple coloration. The sample solutions treated with the azo dye were then measured colorimetrically via a Thermo-Electron Corporation Genesys scanning spectrophotometer set at 543 nm to determine the nitrite concentration (APHA, 1998). The sample measurements were then evaluated against the standard measurements and the calibration curve to determine the actual concentrations. The applicable range of the colorimetric method for nitrite is 10-1000 µg/L (APHA, 1998). Water samples tested by this method can only be preserved short-term for one to two days by freezing at -20 °C or storing at 4 °C (APHA, 1998).

3.6.1.4 Phosphate Ascorbic Acid Method (4500-P E.)

The ascorbic acid method was used to determine the unhydrolyzed orthophosphate (PO₄⁻P) concentration in the water samples. In this method, phosphate

levels were found by mixing the water sample solutions with a lime-green color reagent and measuring them colorimetrically by a Thermo-Electron Corporation Genesys scanning spectrophotometer set at 880 nm (APHA, 1998). The color reagent consisted of a combination of 5 N sulfuric acid (H_2SO_4), potassium antimonyl tartrate ($\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot \text{H}_2\text{O}$) solution, ammonium molybdate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$) and 0.1 M ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$) (APHA, 1998). The ammonium molybdate and potassium antimonyl tartrate reacted in the acid medium of sulfuric acid with phosphate to form a heteropoly acid, or phosphomolybdic acid, which was reduced to intensely colored molybdenum blue by the ascorbic acid (APHA, 1998). The molybdenum blue of the sample solution was comparable to a cobalt-blue coloration. The sample measurements were lastly evaluated against the standard measurements and the calibration curve to determine the actual concentrations. Adjustments were made to sample and standard measurements so compensate for the addition 8.0 mL of the color reagent. The formula used for the calculation is as follows in *Equation 3.6.1.4.1*:

$$\frac{\text{mg}}{\text{L}} \text{ P} = \frac{\text{mg P (in approximately 58 mL final volume)} \times 1000}{\text{mL sample}}$$

Equation 3.6.1.4.1 – Adjusted Phosphate Concentration (APHA, 1998).

The approximate applicable range for the ascorbic acid method for phosphate is 0.15-1.30 mg/L at a light path of 1.0 cm (0.4 in) (APHA, 1998).

3.6.2 Analytic Quality Assurance & Control

For each test of each sampling set analyzed, at least one statistical triplicate was examined to insure and control the quality and validity of the experiment results. This was done according to the assessment guidelines established by the USEPA for correct data analysis (USEPA, 1998). Additionally, a set of standards incorporating at least five

measurements was prepared for each sampling set and individual nutrient test. Tables containing all the triplicate and standards data for each sample date and nutrient test for all three experiments are presented in *Appendix C-1* through *C-4* and *D-1* through *D-3*, respectively. The average concentrations and standard deviations of the triplicates will also be included in the correlating appendices.

3.6.3 Statistical Data Analysis

The statistical analysis conducted for all the raw data computed for each of the three different studies was done using Sigma Stat 2.03. The primary test from this program used to analyze the data was the one-way ANOVA test. When running these tests, Sigma Stat 2.03 would initially and automatically conduct a test for normality and equal variances upon the data sets. If the test for normality would fail, Sigma Stat 2.03 would instead execute the Kruskal-Wallis test, to analyze the Non-Gaussian data sets. If significant statistical differences existed among the data sets, Sigma Stat 2.03 would then execute the Tukey test to compare and determine which data sets had significant differences among one another.

3.6.3.1 Outliers Test

A test of outliers within the data sets was not conducted in the data analysis. There were multiple reasons for this decision. First, none of the outlier tests utilized for data analysis by the United States Environmental Protection Agency fit within the aspects of the data sets for each study. Second, many of the potential outliers were much lower than the values expected to be seen. These potential outliers were thus overlooked since the point of this research was to obtain lower nutrient concentrations. Third, it was impossible to compare potential outliers to other data, since the treatments for the

majority of the channels differed from one another. Any outliers, above or below the acceptable detection limit range of the four nutrient tests used in this research, were set to their closest corresponding detection limit concentration. This was done since most outliers were below the lower detection limit of each test. Thus, the detection limit concentration was the only acceptable known value for the low outlier.

3.6.3.2 Normality Test

A normality test assesses whether a set of data approximates a normal probability distribution (Mendenhall *et al.*, 1999). A data set with a normal probability distribution, or a Gaussian population, will have a frequency distribution histogram with an approximate shape of a mound (Mendenhall *et al.*, 1999). A normal or Gaussian data set is also commonly termed to be parametric, while a non-Gaussian distribution of data is also termed nonparametric. A large number of random variables observed in natural processes have been found to have a normal probability distribution (Mendenhall *et al.*, 1999).

3.6.3.3 One-Way ANOVA Test

A one-way ANOVA test, analysis of variances, examines whether variances within groups of data sets have any significant differences when compared to one another (Mendenhall *et al.*, 1999). This test is run based on the assumptions or knowledge that the data sets are normally distributed with equal variances (Mendenhall *et al.*, 1999). This test is designed for the comparison between three or more independent, or unmatched, groups of normal data (Mendenhall *et al.*, 1999). Typically, the one-way ANOVA test looks for significant differences in variation at a probability less than or equal to 5.0 % ($P \leq 0.050$).

3.6.2.4 Kruskal-Wallis Test

The Kruskal-Wallis test, also known as the H or ANOVA on ranks test, is the nonparametric alternative to a one-way ANOVA test. The Kruskal-Wallis test examines whether a significant difference exists between the comparisons of the means of data sets (Mendenhall *et al.*, 1999). This test is also designed for the comparison between three or more unmatched groups of nonparametric data (Mendenhall *et al.*, 1999). A value known as the H statistic is calculated for this test to determine if there is a significant difference among the data sets (Mendenhall *et al.*, 1999). The larger the value of the H statistic, the greater the difference in the positions among the data set distributions will be (Mendenhall *et al.*, 1999). This indicates that the data set distributions are not identical for large values of the H statistic (Mendenhall *et al.*, 1999).

3.6.3.5 Tukey Test

The Tukey test, or studentized range test, is a statistical method used to find paired comparisons of significant differences found in the evaluations between multiple groups of data sets (Mendenhall *et al.*, 1999). This test is based upon the usual analysis of variance assumptions (Mendenhall *et al.*, 1999). In addition, the Tukey test assumes that the sample means are independent and established on data sets of equal population size (Mendenhall *et al.*, 1999). The Tukey test evaluated which pairs within a data set have significant differences (Mendenhall *et al.*, 1999). The probability used to determine these significant pairwise differences is typically less than 5.0 % ($P < 0.050$).

CHAPTER 4. RESULTS

4.1 Study Results Foreword

4.1.1 Experimental Treatment Nomenclature

Each sampling site within the aquaculture shelter, greenhouse, and the rest of the aquaponics system was cataloged based upon the sampling site, flow velocity, plant species, and plant density, where such experimental treatment conditions applied. A complete list of all these treatment conditions and nutrient concentrations for each group of samples, according to date, for this first study is presented in *Table 3.3.1.1*, as well as in *Appendix A*. *Appendix A* includes raw nutrient concentration data, as well as computed averages and standard deviations based upon combined identical treatments.

The previously mentioned experimental treatments used throughout each study consisted of a combination of the various operational conditions set for each individual aquatic phytoremediation channel. Within the channels of the aquatic phytoremediation system, groups of three channels will possessed the same operational conditions or experimental treatments. For example, three channels, located randomly in the aquatic phytoremediation system, will have treatment conditions consisting of a low flow velocity and a high plant density (LVHD). These channels might also only be used for the cultivation of watercress. Thus, the three channels have identical treatment conditions and can be grouped together for analysis of the entire data set.

Groups of channels with identical treatment conditions for the first experiment are previously presented in *Table 3.3.1.1* and *3.4.1.1*. Furthermore, the nutrient concentrations of the raceways, tail-box and manifold were grouped together due to their nutrient concentration similarities. A table listing the different acronyms for experimental

treatment conditions and aquaponic system locations used in all the nutrient analysis result charts and tables for each study is presented in *Table 4.1.1*:

Table 4.1.1.1 – Study Treatment / Location Acronyms

Treatment / Location Acronym	Definition
HB	Head-box
RWM	Raceways & Manifold Pipe
LVLD	Low Velocity Low Density Channels
LVMD	Low Velocity Medium Density Channels
LVHD	Low Velocity High Density Channels
MVLD	Medium Velocity Low Density Channels
MVMD	Medium Velocity Medium Density Channels
MVHD	Medium Velocity High Density Channels
HVLD	High Velocity Low Density Channels
HVMD	High Velocity Medium Density Channels
HVHD	High Velocity High Density Channels
CC	Control Channels
EC	Empty Channels
RWTB	Raceways & Tail-box
LC	Longer Channels
OC	Old Shelter Channels
Basil	Basil Cultivation Channels
Dill	Dill Cultivation Channels
Lettuce	Lettuce Cultivation Channels

In *Table 4.1.1.1*, all channels which are not explicitly labeled for a plant species were used for the cultivation of watercress. The flow velocity and density parameters used for the basil, dill, lettuce, control, long, empty, and old shelter channels can be found within *Appendix A-4* through *A-9* or previously in *Table 3.3.1.1* and *3.4.1.1*.

4.1.2 Nutrient Data Transformations

To present the data most efficiently, raw nutrient concentration data was transformed into percent nutrient removal or recovery. By transforming the data in this manner, the actual remediation performance of the plants is made more apparent throughout each of the studies.

Two different types of percent nutrient removals were determined: (1) the average percent removal for each nutrient, experimental treatment, and sample date within a study, and (2) the average total percent removal for each nutrient and experimental treatment throughout an entire study. To compute the average percent removal, the nutrient concentration of each identical group of experimental treatments per sample date were first averaged. The formula then used to calculate the average percent removal is as follows in *Equation 4.1.2.1*:

$$Avg. \% Removal = \frac{(C_{in} - C_{out})}{C_{in}} \times 100$$

Equation 4.1.2.1 – Average Percent Nutrient Removal

In *Equation 4.1.2.1*, C_{in} is equal to the average nutrient concentration of the aquaculture raceway effluent, the tail-box, and the aquatic phytoremediation manifold influent for in individual sample date, as the effluent from these locations are fundamentally the same. C_{out} is equal to the average concentration of the effluent from identical groups of experimental treatments within the same individual sample date.

To determine the average total percent removal, the total average percent removal was first computed. To calculate the total percent removal, the nutrient concentration for every sample date within a study were first summed together for each individual experimental treatment. The formula then used to calculate the total percent removal is as follows in *Equation 4.1.2.2*:

$$Total \% Removal = \frac{(Sum C_{in} - Sum C_{out})}{Sum C_{in}}$$

Equation 4.1.2.2 – Total Percent Nutrient Removal

In Equation 4.1.2.2, $\text{Sum } C_{\text{in}}$ is equal to the average total summation of the nutrient concentrations of the aquaculture raceway effluent, the tail-box, and the aquatic phytoremediation manifold influent for all the sample dates within a study, as the effluent from these locations are fundamentally the same. $\text{Sum } C_{\text{out}}$ is equal to the total summation of the nutrient concentrations of the effluent from each individual experimental treatment for all the sample dates within a study. The average total percent removal was then determined by averaging the total percent removal of identical experimental treatments within a study.

4.2 Divergent Growth Conditions Study (# 1)

4.2.1 Overview

For divergent growth conditions study, each sampling trip was conducted as scheduled and planned. In total, six sets of water samples were taken from the aquaponics research site. The water samples were analyzed for ammonia, nitrate, nitrite and phosphate. The four nutrient tests for each sampling set were performed without any identified laboratory equipment failures and in accordance to the quality assurance and control standards.

The ammonia analysis for the sampling date of September 17, 2005 was removed from further analysis. On this sample date, all the ammonia concentrations found for each sampling site within the aquaponics system was elevated an average ten times larger than the values found on every other sampling date. This elevation in ammonia in the aquaculture and aquatic phytoremediation system was likely due to an external source further enriching the spring influent that entered the head-box.

Additionally, the analysis of nitrite for all sampling dates for all three studies was removed from any further analysis, though the raw concentration results were tabulated and are presented in the *Appendix A-7, A-8 and A-9*. Every single nitrite concentration, experimentally analyzed for each sampling site of the aquaponics system of six sampling dates, was below the applicable detection limit (10 µg/L) of the colorimetric nitrite test method. Likely, all nitrite was converted to other nitrogenous forms through oxidation, nitrification or denitrification to nitrate, ammonia or nitrogen gas, respectively. When considering the pH of the water system is consistently equal to 7.2, the nitrite is most probably being oxidized to nitrate and/or denitrified to nitrogen gas.

4.2.2 Percent Removal Results

A compilation of the average percent removal results for ammonia in the first study is presented in *Figure 4.2.2.1*:

Figure 4.2.2.1 – Average Percent Ammonia Removal (Study # 1)

A compilation of the average percent removal results for nitrate in the first study is presented in *Figure 4.2.2.2*:

Figure 4.2.2.2 – Average Percent Nitrate Removal (Study # 1)

A compilation of the average percent removal results for phosphate in the first study is presented in *Figure 4.2.2.3*:

Figure 4.2.2.3 – Average Percent Phosphate Removal (Study # 1)

A compilation of the average total percent removal results for all the nutrients for the first study is presented in *Figure 4.2.2.4*:

Figure 4.2.2.4 – Average Total Percent Nutrient Removal (Study # 1)

Tables with complete raw concentration data for ammonia, nitrate, and phosphate are presented in the *Appendix A-1, A-4, and A-10*, respectively for the first study.

4.3 Multiple Plant Species Winter Study (# 2)

4.3.1 Overview

For multiple plant species winter study, each sampling trip was conducted as scheduled and planned. In total, five sets of water samples were taken from the aquaponics research site. The water samples were analyzed for ammonia, nitrate, nitrite and phosphate. The four nutrient tests for each sampling set were performed in accordance to the quality assurance and control standards. There was an identified laboratory equipment failure for the ammonia analysis that should have been conducted for the samples taken on January 22, 2006. The probe used in the selective ammonia test

was damaged and unusable for the examination of ammonia. Since the ammonia had a default hold-time of twenty-four hours, the samples were unfortunately discarded from subsequent nutrient analysis.

The analysis of nitrite for all six sampling dates was removed from any further data analysis, as all nitrite concentrations were below the detection limits of the colorimetric test. Nonetheless, the nitrite results were tabulated and presented in the *Appendix A-7, A-8 and A-9*.

4.3.2 Percent Removal Results

A compilation of the average percent removal results for ammonia in the second study is presented in *Figure 4.3.2.1*:

Figure 4.3.2.1 – Average Percent Ammonia Removal (Study # 2)

A compilation of the average percent removal results for nitrate in the second study is presented in *Figure 4.3.2.2*:

Figure 4.3.2.2 – Average Percent Nitrate Removal (Study # 2)

A compilation of the average percent removal results for phosphate in the second study is presented in *Figure 4.3.2.3*:

Figure 4.3.2.3 – Average Percent Phosphate Removal (Study # 2)

A compilation of the average total percent removal results for all the nutrients for the second study is presented in *Figure 4.3.2.4*:

Figure 4.3.2.4 – Average Total Percent Nutrient Removal (Study # 2)

Tables with complete raw concentration data for ammonia, nitrate, and phosphate are presented in the *Appendix A-2, A-5, and A-11*, respectively for the second study.

4.4 Multiple Plant Species Spring Study (# 3)

4.4.1 Overview

For the multiple plant species spring study, each sampling trip was conducted as scheduled and planned. In total, four sets of water samples were taken from the aquaponics research site. Each of the four nutrient tests for each sampling set were performed without any identified lab equipment failures and in accordance to the quality assurance and control standards.

The examination and analysis of the channels, in which basil, dill and lettuce were cultivated, was eventually disregarded, due to the fact the plant species did not

successfully germinate and grow. No nutrient concentration data transformations or analysis was conducted for the basil, dill and lettuce experimental treatments.

The analysis of nitrite for all six sampling dates was removed from any further data analysis, as all nitrite concentrations were below the detection limits of the colorimetric test. Nonetheless, the nitrite results were tabulated and presented in the *Appendix A-7, A-8 and A-9*.

4.4.2 Percent Removal Results

A compilation of the average percent removal results for ammonia in the third study is presented in *Figure 4.4.2.1*:

Figure 4.4.2.1 – Average Percent Ammonia Removal (Study # 3)

A compilation of the average percent removal results for nitrate in the third study is presented in *Figure 4.4.2.2*:

Figure 4.4.2.2 – Average Percent Nitrate Removal (Study # 3)

A compilation of the average percent removal results for phosphate in the third study is presented in *Figure 4.4.2.3*:

Figure 4.4.2.3 – Average Percent Phosphate Removal (Study # 3)

A compilation of the average total percent removal results for all the nutrients for the second study is presented in *Figure 4.4.2.4*:

Figure 4.4.2.4 – Average Total Percent Nutrient Removal (Study # 3)

Tables with complete raw concentration data for ammonia, nitrate, and phosphate are presented in the *Appendix A-3, A-6, and A-12*, respectively for the second study.

4.5 Environmental Data-logger Results

4.5.1 Overview

The data-loggers, setup in both the aquaculture shelter and the aquaponics greenhouse, were used to measure both the atmospheric temperature in degrees Celsius and the photosynthetically active radiation (PAR) in $\mu\text{mol}/\text{m}^2/\text{s}$. The PAR measures the radiant power or the spectral range of solar light from 400 to 700 nanometers that is useful to plants in the process of growth and photosynthesis (Gates, 1980). The location of the data-loggers is presented in *Figures 3.3.2.1* and *3.4.2.1*. The data-loggers were setup to take measurements of the temperature and PAR every hour. For the purpose of

this research, data-logger measurements were taken within the shelter, greenhouse, or both between the dates of November 19, 2005 and June 2, 2006.

4.5.2 Data-logger Results

A compilation of the average monthly temperature results, transformed to degrees Fahrenheit, is presented as follows in *Figure 4.5.2.1*:

Figure 4.5.2.1 – Average Monthly Temperatures

A compilation of the average monthly PAR results is presented as follows in *Figure 4.5.2.2*:

Figure 4.5.2.2 – Average Monthly Photosynthetically Active Radiation

CHAPTER 5. ANALYSIS & DISCUSSION

5.1 Statistical Analysis

Statistical analyses were performed to test two statistical questions: (1) do significant differences in the average total percent nutrient removal exist between the different experimental treatments within an individual study, and (2) do significant differences in average total percent nutrient removals exist between identical experimental treatments between the studies. These questions were used to analyze for any significant statistical differences among the experiment treatments and studies for each of the nutrients. The first question would help determine any operational conditions or experimental treatments which are more effective in the remediation of nutrients. The second question would help determine if the different environmental conditions existing between the studies had any effect on remediation of nutrients via identical experimental treatments.

An example of the first question would be to ask if any significant statistical difference existed between the high velocity-high density (HVHD) and low velocity-low density (LVLD) experimental treatments for the remediation of ammonia in the first study. In this case there was not a significant statistical difference between these treatments for ammonia in the first study. These particular data presented in *Table 5.1.1.1*.

An example of the second question was to ask if any significant statistical difference existed between the high velocity-high density (HVHD) experimental treatment between the first and second study. In this case there was not a significant

statistical difference apparent between these two studies for that particular experimental treatment. These particular data are presented in *Table 5.1.1.2*.

Each nutrient, except for nitrite which was not considered in further analysis, was investigated separately, though the premises of the statistical analysis were identical for ammonia, nitrate and phosphate. The statistical analysis results are also presented in *Appendix B*.

5.1.1 Statistic Results

A summary of statistical results based on statistical question, nutrient constituent, study and experimental treatment, are presented in *Tables 5.1.1.1* and *5.1.1.2*:

Table 5.1.1.1 – Statistical Analysis Results (Study # 1, 2, & 3)

Differences Between the Average Total Percent Removal of the Different Experimental Treatments within a Study				
Research Study	Nutrient Constituent	Passed Normality Test? (Y/N)	Analytical Statistical Test(s) Used	Statistically Significant Difference (Y/N ; P<0.05)
Study # 1	Ammonia	Y	One-Way ANOVA	N
	Nitrate	Y	One-Way ANOVA	N
	Phosphate	N	Kruskal-Wallis	N
Study # 2	Ammonia	N	Kruskal-Wallis	N
	Nitrate	N	Kruskal-Wallis	N
	Phosphate	N	Kruskal-Wallis	N
Study # 3	Ammonia	Y	One-Way ANOVA	N
	Nitrate	N	Kruskal-Wallis	N
	Phosphate	Y	One-Way ANOVA	N

Table 5.1.1.2 – Statistical Analysis Results (Study # 1, 2, & 3)

Differences Between the Average Total Percent Removal of the Identical Experimental Treatments Between the Studies				
Nutrient Constituent	Experimental Treatment	Passed Normality Test? (Y/N)	Analytical Statistical Test(s) Used	Statistically Significant Difference (Y/N ; P<0.05)
Ammonia	LVLD	Y	One-Way ANOVA	N
	LVHD	Y	One-Way ANOVA	N
	HVLD	Y	One-Way ANOVA	N
	HVHD	Y	One-Way ANOVA	N
	LC	Y	One-Way ANOVA	N
Nitrate	LVLD	Y	One-Way ANOVA	N
	LVHD	Y	One-Way ANOVA	N
	HVLD	Y	One-Way ANOVA	N
	HVHD	Y	One-Way ANOVA	N
	LC	Y	One-Way ANOVA	N
Phosphate	LVLD	Y	One-Way ANOVA	N
	LVHD	Y	One-Way ANOVA	N
	HVLD	Y	One-Way ANOVA	N
	HVHD	Y	One-Way ANOVA	N
	LC	Y	One-Way ANOVA	N

5.2 Horticulture Analysis

Horticulture analysis of the plants used throughout the three studies of the aquaponics research was performed by colleagues in the Davis College of Agriculture, Forestry and Consumer Sciences, Division of Plant and Soil Sciences at West Virginia University. This analysis was supervised and performed by Dr. Todd West and Nichole Smith, a horticulture professor and graduate student, respectively. Tests were conducted on plant biomass samples taken throughout these experiments to determine fresh weights, dry weights and heights.

From the analysis of horticultural data and results from all three experiments and based upon plant biomasses related to the experimental treatments, the high velocity-high density (HVHD) treatments were observed to be best suited for the cultivation of

watercress. According to the results concluded from the divergent growth conditions study, it was also observed that the growth medium most effective at supporting plant cultivation was the paper media.

5.3 Discussion

The statistical analysis of the percent nutrient removal data for the experimental treatments within and between each study resulted in no significant differences found in reference to the two statistical questions investigated. The lack of significant differences signifies that no particular experimental treatment within each of the studies was more effective, statistically, at the remediation of ammonia, nitrate, or phosphate. Additionally, the lack of significant differences between identical experimental treatments between the studies signifies that plant cultivation and nutrient remediation were not more effective, statistically, within either the aquaculture shelter or the greenhouse. Since no significant differences were found within the nutrient data through statistical analysis, notable differences and trends within the nutrient data must be found through the examination of the percent removal results and charts. Additionally, trends within the temperature and the photosynthetically active radiation data must also be found through the examination of the data-logger results and charts.

When examining the data-logger results, the first observation that can be made is that the PAR within the aquaculture shelter is only on average 10% of the values recorded in the greenhouse used in the second and third study. PAR values are presented in *Figure 4.5.2.2*. These results undeniably confirm that there is much more natural light available to plants cultivated within the greenhouse.

From the temperature data-logger results, the second observation that can be made is that temperature ranges appropriate for each of the plant species cultivated existed at some period throughout each study. Based upon the ideal temperature ranges for each of the plant species, basil, which prefers warm climates, would be cultivated best throughout the month of June. The average temperature measured for the month of June was 78 °F. Dill, which prefers cool climates, would be cultivated best throughout the months of November, December, January, and February when the average temperature was 48 °F. Lettuce and watercress, which prefer cool to warm climates, would be cultivated best throughout the months of March, April, and May when the average temperature was 61 °F.

For the first study, the first notable trend is that the majority of the experimental treatments, all of which cultivated watercress, were most effective at the remediation of nitrate as is presented in *Figure 4.2.2.4*. The remediation of ammonia and phosphate was not highly effective. Of these experimental treatments, the low velocity-low density (LVLD) treatment was the most effective at the removal of nitrate. The low velocity-low density (LVLD) treatment had an average total percent removal of 8.8% of the entire nitrate concentration passing through the aquatic phytoremediation channel during the first study. Also, the low velocity-medium density (LVMD), low velocity-high density (LVHD), and medium velocity-medium density (MVMD) treatments had a total percent nitrate removal of 8.0, 7.5, and 7.5%, respectively.

Most of the experimental treatments removed the highest average percentage of nitrate towards the end of the first study. Specifically, the low velocity-low density (LVLD) treatment had an average percent nitrate removal of 41% on the final sample

date of October 8, 2005, as is presented in *Figure 4.2.2.2*. Additionally, the medium velocity-low density (MVLD) and high velocity-high density (HVHD) treatments had an average percent nitrate removal of 40 and 34%, respectively, on the final sample date.

One other notable detail is that the empty channel (EC) experimental treatment had an average total percent nitrate removal of 4%. This detail suggests that not all the nitrate removal was necessarily due to the uptake of plants. The reduction of the total nitrate concentration passing through the empty channels could be due to the denitrification of nitrate to free nitrogen gas within the aquatic phytoremediation system.

For the second study, the first notable trend is that the majority of the experimental treatments, which cultivated basil, dill, lettuce, and watercress, were most effective at the removal of ammonia as is presented in *Figure 4.3.2.4*. Of these experimental treatments, the lettuce treatment was the most effective at the removal of ammonia. The lettuce treatment had an average total percent removal of 11.2% of the entire ammonia concentration passing through the aquatic phytoremediation channel during the second study. Also, the basil, low velocity-low density (LVLD), high velocity-low density (HVLD), and long channel (LC) treatments had a total percent ammonia removal of 9.6, 7.0, 7.0, and 7.0%, respectively. The remediation of nitrate and phosphate was not effective. The basil treatment had an average total percent nitrate removal of 6%.

The basil, dill, and lettuce treatments removed the most ammonia at the beginning of the second study. Lettuce, specifically, had an average percent ammonia removal of 53% on the sample date of January 8, 2006 as is presented in *Figure 4.3.2.1*. These plants also removed the largest amounts of nitrate and phosphate upon the first two sample dates for the second study, although these percent removals were not as large as they were for

ammonia. Additionally, the average total percent ammonia removal of the low-velocity-high density (LVHD) experimental treatments within the greenhouse was 1.5% higher than the old channel (OC) treatment in the aquaculture shelter, which was set at a low velocity and a high density. This observation demonstrates that ammonia remediation within the greenhouse is slightly more effective than within the aquaculture shelter, due to the PAR differences which affect plant growth and nutrient requirements.

For the third study, the first notable trend is that the majority of the experimental treatments, which eventually only cultivated watercress, were most effective at the removal of ammonia and phosphate as is presented in *Figure 4.4.2.4*. Of these experimental treatments, the low velocity-high density (LVHD) treatment was the most effective at the removal of phosphate. The low velocity-high density (LVHD) treatment had an average total percent removal of 4.9% of the entire phosphate concentration passing through the aquatic phytoremediation channel during the third study. The long channel (LC) treatment was the most effective at the remediation of ammonia, having an average total percent ammonia removal of 4.1% throughout the third study.

Most of the phosphate removed occurred at the end of the third study, as is presented in *Figure 4.4.2.3*. Most of the ammonia removal occurred at the beginning of the third study, as is presented in *Figure 4.4.2.1*. Overall, the removal of nutrients within this study was much lower than the previous two. This is likely due to the plant cultivation period being reduced by three to six weeks when compared to the other studies. If the plants had been given more time to grow, higher average total percent removals may have been achieved.

The phosphate removal via lettuce cultivation in the second study can be compared with the case study of Adler *et al.* Adler *et al.* found that 57% of the phosphorus passing through their aquatic phytoremediation system was removed via the lettuce. This value is much greater than the highest average phosphate removal of 4% achieved via the lettuce on the sample date of December 11, 2005, which is presented in *Figure 4.3.2.3*.

There are several reasons why they obtained a much higher percent removal of phosphorus. First, they measured total phosphorus removal compared to only the analysis of phosphate. Second, they used a recirculating aquaponics system with higher concentrations of nutrients. For example, their total phosphorus and nitrate concentrations were equal to 25 and 0.7 mg/L, respectively. In contrast, the average phosphate and nitrate concentrations in the aquaculture system for the second study were equal to 0.3 and 0.55 mg/L, respectively. Third, they loaded their system with other essential nutrients in order to make phosphorus the most limiting nutrient. By doing this, they would enhance the requirement and removal of phosphorus via the lettuce. The addition of nutrients to enhance specific constituent removal in a flow-through aquaponics system is impractical. Fourth, the densities of lettuce plants within their aquatic phytoremediation system exceeded the densities within the lettuce treatment channels of the second study. More plants would promote greater removals of phosphorus.

The ammonia and phosphate removal of all the studies can be compared to the case study of Johnson. In this case study, watercress was used to remediate the effluent of an aquaculture operation rearing 25,000 lbs of fish, which far exceeds the 6,000-8,000 lbs of fish reared within the aquaculture operation at the Reymann Memorial Farm. The

flowrate of the aquaculture operation in the case study was only 55 gal/min, which is much lower than the 400 gal/min flowrate at the Reymann Memorial Farm. Johnson observed that approximately 74 and 58% of ammonia and phosphorus, respectively, was removed from the system via the watercress. These values exceed the highest average percent removal of 53 and 16.6% measured within the three studies for ammonia and phosphate, respectively. The ammonia and phosphate values are presented in *Figures 4.2.2.3 and 4.3.2.1*, respectively.

There are a couple reasons why Johnson obtained a much higher percent removal of ammonia and phosphorus. First, Johnson's aquaponics system had a higher quantity of fish and a much slower flowrate. These characteristics would provide the system with a much more abundant concentration of ammonia and phosphorus, as well as increase the contact time between the nutrient constituents and the plant roots. Second, Johnson measured total phosphorus rather than only phosphate, which would increase the amount of phosphorus constituents measured to be removed from the effluent samples.

The percent nutrient removal results from all the studies can be compared with the case study by Vincent and Downes. Vincent and Downes studied the nutrient remediation effects, specifically the removal of ammonia and nitrate, of watercress naturally growing in a stream in New Zealand. They found that nutrient removal was the greatest during the summer months of January and February in the country. This observation does not correlate with the ammonia and nitrate results from the three aquaponics studies. The highest average total percent removals of ammonia and nitrate occurred during the fall and winter months of October, December, and January. These values can be observed in *Figures 4.2.2.2 and 4.3.2.1*. If the watercress cultivated in the third study, which was

conducted in the spring and summer season, had been allowed to grow for a longer period of time, the same nutrient removal trend observed by Vincent and Downes may have occurred.

One final notable trend from the examination of the three studies is that the watercress removed a greater average total percentage of nitrates during the cooler fall months when temperatures ranged from 40-50 °F and PAR levels were approximately 100 $\mu\text{mol}/\text{m}^2/\text{s}$ within the aquaculture shelter. Subsequently, the watercress removed a greater average total percentage of ammonia and phosphate during the warmer winter, spring, and summer months when temperatures ranged from 40-80 °F and PAR levels were on average 275 $\mu\text{mol}/\text{m}^2/\text{s}$ within the greenhouse. The differences in the constituents removed throughout each of the three studies could be caused by changes in the watercress's nutrient requirements or metabolism due to differences in temperature, light intensity, or combinations of both parameters.

Theoretically, the environmental conditions within the aquaculture shelter were most advantageous to the remediation of nitrate via watercress, while the environmental conditions within the greenhouse were most beneficial to the remediation of ammonia and phosphate via watercress. However, the fact that the initial study was longer in duration than the second and third study may be a cause to the increased nitrate removal. The requirement for nitrate may intensify as the biomass in a plant, specifically watercress, increases. This fact would negate the environmental conditions being the cause of increased nitrate removal. This fact can also be fundamentally disputed since the average percent nitrate removal for each sample date during the first study, is larger than the percent removals observed for the final two studies.

CHAPTER 6. CONCLUSIONS

6.1 Research Objectives

The objectives of the aquaponics research project were (1) to determine if watercress (*Nasturtium officinale* R. BR.), dill (*Anethum graveolens*), lettuce (*Lactuca sativa*) or basil (*Ocimum basilicum*) can be cultivated and utilized, via aquatic phytoremediation, to reduce nutrients from aquaculture effluent in sufficient quantities to be useful as an effective treatment or remediation technique, and (2) to determine operational and environmental conditions which affect such nutrient removal and plant growth.

From the examination of the percent nutrient removal results, several operational and environmental conditions were determined. The low velocity-low density (LVLD) treatment was most effective at the remediation of nitrate, capable of removing 8.8% of the total nitrate concentration which passed through the aquaponics research system. The low velocity-high density (LVHD) treatment was most effective at the remediation of phosphate, capable of removing 4.9% of the total phosphate concentration which passed through the aquaponics research system. The lettuce treatment was most effective at the remediation of ammonia, capable of removing 11.2 % of the total ammonia concentration which passed through the aquaponics research system. The low velocity-low density (LVLD) treatment was the most effective at the remediation of ammonia via watercress, capable of removing 7.1% of the total ammonia concentration which passed through the aquaponics research system. The environmental conditions within the aquaculture shelter were most beneficial to the remediation of nitrate via watercress. The environmental conditions within the greenhouse were most beneficial to the remediation of ammonia

and phosphate via watercress. The high velocity-high density (HVHD) treatment was the most effective combination for the cultivation of watercress, capable of producing more abundant plant biomass and height.

From these results, the conclusion can be made that watercress is effective at aquatic phytoremediation of ammonia, nitrate, and phosphate only at the previously mentioned percent removal levels. Additionally, the conclusion that lettuce is effective at the aquatic phytoremediation of ammonia at the previously mentioned percent removal levels can also be made. These conclusions are only valid when dealing with aquaculture effluent and environmental characteristics analogous to the conditions present at the research site at the Reymann Memorial Farm.

The characteristics of the aquaculture effluent which could have affected the aquaponics system are the initial concentrations of the nutrients in the wastewater. Within a non-recycling water system, any significant changes in nutrient levels, due to extraction of constituents via plants, could be impossible to detect at average concentrations of 0.20, 0.25 and 0.55 mg/L for ammonia, nitrate and phosphate, respectively. Additionally, system design parameters, such as the short contact-times between the plants and constituents, insufficient densities of cultivated plant biomass, inadequate levels of light intensity, and the species of plants hydroponically cultivated within the aquatic phytoremediation system, could have also affected to the remediation of nutrients at the existing concentrations.

6.2 Research Recommendations

From the results and conclusions established during the three preliminary studies, many operation adjustments and modifications can be made to the aquaponics system

design throughout further studies. These changes would be specific to the improvement of nutrient remediation within a system comparable to the operation at Reymann Memorial Farm. Ultimately, the aquaponic system would be developed into a highly effective low concentration nutrient removal treatment with supplementary profitable cultivation of crops.

Of the alterations that could be made to the aquaponics system design, the factor which cannot be changed beyond the limits of the aquaculture raceway design and maximum fish rearing density is the low concentration of nutrients within the aquaculture effluent. This factor would act as an autonomous variable of the aquaponic system design. Thus, all of the alterations made would be to compensate for this low nutrient concentration variable. However, if significant increases in nutrient concentration could be achieved via the addition of more fish to the aquaculture system, the percent removal of the constituents and plant growth would most likely be enhanced.

One potential alteration that could be made to the aquatic phytoremediation system is the plant species which are hydroponically cultivated and used for the uptake of nutrients at the low existing concentrations. The determination of these plant species at a field-scale level would overall squander a lot of time, money and effort. Lab-scale uptake-rate experiments would need to be developed to more efficiently verify plant species most effective at low-level nutrient remediation. These experiments would need to be highly representative of the environmental characteristics of the aquaponics system at Reymann Memorial Farm. Although the findings from a lab-scale experiment are not completely representative of the conditions present at the field-scale level, such experiments would help to narrow the scope of potentially useful plant species. The

results from these lab-scale experiments could also be used further to create a biochemical and hydrological modeling program of the field-scale aquaponics system. Such a model would additionally aid in the successful selection of useful plant species.

Other potential modifications to the aquatic phytoremediation system include an increase in the contact-time between plant roots and nutrient constituents, as well as an enlargement in the plant biomass cultivated throughout the channels. These changes could be achieved through alterations made to the existing field-scale aquatic phytoremediation channel design. Once more, these adjustments would be specifically correlated to the parameters which are similar to the setup at Reymann Memorial Farm.

To maximize the contact-time between plants and constituents either the water velocities within the channels would need to be decreased, or the dimensions of the channels, specifically the lengths, would need to be increased. Decreasing the water velocities within the channels would be unfavorable for two reasons. First, it was already concluded from the three preliminary studies that the high velocity channels were most effective at the removal of the most prominent nutrient, nitrate. The high velocity channels also promoted greater plant growth. Second, the high velocity of the aquatic phytoremediation channels is the most representative of the velocities present in the aquaculture raceways. This is an important aspect since a distant goal of the aquaponic research is to eventually design as a free-flowing aquatic phytoremediation system adjacent to the aquaculture operation.

Increasing the length or other dimensions of the aquatic phytoremediation channels would be the most beneficial alteration that could be made to the aquaponics system design. Longer and wider channels would increase the contact-time between the

plant roots and nutrient constituents. This would grant the plants more chances overall to extract increased quantities of nutrients from the aquaculture effluent. Additionally, longer and wider channels would allow a larger amount of plant biomass to be cultivated throughout the aquatic phytoremediation system. An example of a hypothetical aquatic phytoremediation system design for the already existing greenhouse located at the aquaponics research site is presented in *Figure 6.2.1*:

Figure 6.2.1 – Hypothetical Aquatic Phytoremediation System Design

In this schematic, the lengths and width of each channel would be dependent upon the space allotted by the greenhouse. Connecting each corresponding groups of channels together would moreover increase the amount of contact-time between the plants and nutrients. Segmenting sections of each group of channels would also allow for the appropriate amount of experimental replication required any further studies.

Increasing the levels of light intensity could also affect the growth of plants and consequently the remediation of nutrients. Structural changes to the greenhouse or additions of artificial light sources would be impractical both economically and

topographically at a rural site, such as the Reymann Memorial Farm. However, the addition of reflective liner within the aquatic phytoremediation channels would increase the intensity of the radiating natural light in the greenhouse.

Other factors which could eventually be studied include the combined effects of foreign organisms in the aquaponics system such as algae, bacteria or duckweed, as well as the complexities of plant biochemistry which induce or retard the uptake of nutrients throughout the span of a plants development and growth. The study of such dynamics would aid in making an existing successful aquaponics system even more efficient.

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APPENDIX A. NUTRIENT ANALYSIS RESULTS

A-1. Ammonia Results (Study # 1)

Ammonia (NH ₃ N) Concentration									
Divergent Growth Conditions Study (Experiment # 1)									
Sample Site	Plant Species	Flow Velocity	Plant Density	Sample Date					
				6/22/2005	7/16/2005	8/7/2005	8/27/2005	9/17/2005	10/8/2005
-----	-----	(ft/s)	(#)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Head Box	N/A	N/A	N/A	0.030	0.060	0.030	0.030	2.052	0.075
Raceway A	N/A	N/A	N/A	0.163	0.189	0.083	0.127	2.251	0.178
Raceway B	N/A	N/A	N/A	0.219	0.205	0.127	0.152	2.166	0.217
Manifold	N/A	N/A	N/A	0.214	0.206	0.120	0.174	1.928	0.218
Experimental Channel 1	N/A	M	N/A	0.210	0.186	0.114	0.320	1.921	0.276
Experimental Channel 2	Watercress	M	M	0.200	0.205	0.109	0.240	1.951	0.241
Experimental Channel 3	Watercress	L	H	0.192	0.171	0.107	0.192	1.614	0.242
Experimental Channel 4	Watercress	H	L	0.200	0.179	0.106	0.229	1.523	0.248
Experimental Channel 5	Watercress	M	H	0.201	0.174	0.107	0.274	1.717	0.231
Experimental Channel 6	Watercress	L	M	0.202	0.180	0.110	0.245	1.460	0.234
Experimental Channel 7	Watercress	M	L	0.208	0.209	0.111	0.209	1.454	0.240
Experimental Channel 8	Watercress	H	H	0.202	0.221	0.112	0.212	1.943	0.217
Experimental Channel 9	Watercress	L	L	0.185	0.244	0.094	0.179	1.652	0.228
Experimental Channel 10	Watercress	H	M	0.193	0.216	0.110	0.204	1.454	0.236
Experimental Channel 11	Watercress	H	L	0.192	0.227	0.106	0.201	1.906	0.237
Experimental Channel 12	Watercress	M	M	0.191	0.221	0.107	0.212	1.834	0.230
Experimental Channel 13	Watercress	H	H	0.190	0.249	0.118	0.186	1.415	0.213
Experimental Channel 14	Watercress	M	L	0.184	0.236	0.109	0.212	1.535	0.227
Experimental Channel 15	N/A	L	N/A	0.195	0.197	0.116	0.174	2.108	0.231
Experimental Channel 16	Watercress	L	H	0.197	0.238	0.036	0.184	1.981	0.224
Experimental Channel 17	Watercress	L	L	0.191	0.203	0.100	0.159	2.004	0.178
Experimental Channel 18	Watercress	M	H	0.174	0.191	0.112	0.210	2.217	0.217
Experimental Channel 19	Watercress	H	M	0.186	0.244	0.116	0.183	2.432	0.225
Experimental Channel 20	Watercress	L	M	0.204	0.262	0.110	0.154	1.966	0.187
Experimental Channel 21	Watercress	H	H	0.213	0.206	0.109	0.154	2.349	0.213
Experimental Channel 22	Watercress	M	L	0.189	0.205	0.120	0.186	2.199	0.225
Experimental Channel 23	Watercress	M	H	0.179	0.236	0.090	0.204	2.174	0.239
Experimental Channel 24	Watercress	M	M	0.178	0.207	0.119	0.169	2.004	0.206

Experimental Channel 25	Watercress	L	H	0.174	0.223	0.107	0.177	2.092	0.209
Experimental Channel 26	Watercress	H	L	0.183	0.254	0.133	0.163	2.242	0.200
Experimental Channel 27	Watercress	L	L	0.176	0.246	0.109	0.144	2.149	0.211
Experimental Channel 28	Watercress	H	M	0.180	0.207	0.116	0.164	2.286	0.212
Experimental Channel 29	N/A	H	N/A	0.181	0.221	0.133	0.147	1.848	0.172
Experimental Channel 30	Watercress	L	M	0.179	0.197	0.113	0.142	2.509	0.180
Tail Box	N/A	N/A	N/A	0.203	0.199	0.096	0.139	1.171	0.198
Polishing Pond	N/A	N/A	N/A	0.102	0.171	0.053	0.240	2.067	0.231
Control Channel 1	Watercress	H	M	0.030	0.054	0.030	0.030	1.806	0.089
Control Channel 2	Watercress	L	M	0.030	0.064	0.030	0.030	2.116	0.086
Control Channel 3	Watercress	M	M	0.030	0.060	0.030	0.030	2.277	0.064

	Treatment Conditions	Sample Date					
		6/22/2005	7/16/2005	8/7/2005	8/27/2005	9/17/2005	10/8/2005
		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Average Concentration	RWM	0.199	0.200	0.110	0.151	2.115	0.204
	LVLD	0.184	0.231	0.101	0.160	1.935	0.206
	LVMD	0.195	0.213	0.111	0.180	1.978	0.200
	LVHD	0.187	0.210	0.084	0.184	1.896	0.225
	MVLD	0.194	0.217	0.113	0.202	1.729	0.231
	MVMD	0.190	0.211	0.112	0.207	1.930	0.226
	MVHD	0.184	0.200	0.103	0.229	2.036	0.229
	HVLD	0.192	0.220	0.115	0.197	1.891	0.229
	HVMD	0.186	0.222	0.114	0.184	2.057	0.224
	HVHD	0.202	0.225	0.113	0.184	1.903	0.214
	CC	0.030	0.059	0.030	0.030	2.066	0.080
	EC	0.195	0.201	0.121	0.214	1.959	0.226
Standard Deviation	RWM	0.031	0.009	0.023	0.024	0.167	0.023
	LVLD	0.007	0.024	0.008	0.017	0.256	0.025
	LVMD	0.014	0.043	0.002	0.057	0.525	0.029
	LVHD	0.012	0.035	0.041	0.007	0.250	0.017
	MVLD	0.012	0.017	0.006	0.014	0.409	0.008
	MVMD	0.011	0.009	0.006	0.036	0.087	0.018
	MVHD	0.014	0.032	0.011	0.039	0.277	0.011
	HVLD	0.008	0.038	0.016	0.033	0.360	0.025
	HVMD	0.007	0.019	0.003	0.020	0.528	0.012
	HVHD	0.012	0.022	0.005	0.029	0.468	0.003
	CC	0.000	0.005	0.000	0.000	0.240	0.013
	EC	0.015	0.018	0.010	0.093	0.134	0.052

A-2. Ammonia Results (Study # 2)

Ammonia (NH ₃ -N) Concentration								
Multiple Plant Species Winter Study (Experiment # 2)								
Sample Site	Plant Species	Flow Velocity	Plant Density	Sample Date				
				12/11/2005	1/8/2006	1/22/2006	2/20/2006	3/3/2006
-----	-----	(ft/s)	(#)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Head Box	N/A	N/A	N/A	0.031	0.104	N/A	0.098	0.037
Raceway A	N/A	N/A	N/A	0.110	0.145	N/A	0.115	0.096
Raceway B	N/A	N/A	N/A	0.114	0.144	N/A	0.113	0.097
Tail Box	N/A	N/A	N/A	0.127	0.190	N/A	0.146	0.115
Experimental Channel 1	Basil	L	H	0.127	0.103	N/A	0.110	0.080
Experimental Channel 2	Dill	L	H	0.145	0.082	N/A	0.112	0.126
Experimental Channel 3	Lettuce	L	H	0.141	0.091	N/A	0.124	0.133
Experimental Channel 4	Lettuce	L	H	0.143	0.077	N/A	0.111	0.131
Experimental Channel 5	Basil	L	H	0.139	0.080	N/A	0.106	0.129
Experimental Channel 6	Dill	L	H	0.155	0.085	N/A	0.116	0.124
Experimental Channel 7	Dill	L	H	0.149	0.061	N/A	0.130	0.146
Experimental Channel 8	Lettuce	L	H	0.136	0.057	N/A	0.112	0.086
Experimental Channel 9	Basil	L	H	0.151	0.117	N/A	0.103	0.117
Experimental Channel 10	Watercress	L	H	0.133	0.140	N/A	0.112	0.101
Experimental Channel 11	Watercress	H	H	0.139	0.156	N/A	0.118	0.122
Experimental Channel 12	Watercress	L	L	0.148	0.096	N/A	0.093	0.155
Experimental Channel 13	Watercress	L	L	0.162	0.123	N/A	0.097	0.131
Experimental Channel 14	Watercress	H	H	0.135	0.118	N/A	0.106	0.097
Experimental Channel 15	Watercress	H	L	0.125	0.111	N/A	0.098	0.087
Experimental Channel 16	Watercress	H	H	0.138	0.121	N/A	0.110	0.126
Experimental Channel 17	Watercress	H	L	0.132	0.131	N/A	0.132	0.107
Experimental Channel 18	Watercress	L	H	0.133	0.127	N/A	0.107	0.111
Experimental Channel 19	Watercress	L	L	0.137	0.126	N/A	0.107	0.083
Experimental Channel 20	Watercress	L	H	0.133	0.140	N/A	0.112	0.133
Experimental Channel 21	Watercress	H	L	0.131	0.160	N/A	0.112	0.107
Long Channel 22	Watercress	L	H	0.126	0.169	N/A	0.123	0.080
Long Channel 23	Watercress	L	H	0.126	0.145	N/A	0.114	0.107
Long Channel 24	Watercress	L	H	0.125	0.127	N/A	0.102	0.099
Old Shelter Channel 25	Watercress	L	H	0.128	0.128	N/A	0.107	0.096
Old Shelter Channel 26	Watercress	L	H	0.121	0.157	N/A	0.119	0.115
Old Shelter Channel	Watercress	L	H	0.123	0.153	N/A	0.117	0.099

Polishing Pond	N/A	N/A	N/A	0.125	0.223	N/A	0.141	0.094
River	N/A	N/A	N/A	0.097	0.120	N/A	0.104	0.032

	Treatment Conditions	Sample Date				
		12/11/20	1/8/20	1/22/20	2/20/20	3/3/20
		05	06	06	06	06
Average Concentration		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
	RWTB	0.117	0.159	N/A	0.125	0.103
	LVL	0.149	0.115	N/A	0.099	0.123
	LVD	0.133	0.136	N/A	0.111	0.115
	HVL	0.129	0.134	N/A	0.114	0.100
	HVD	0.137	0.132	N/A	0.111	0.115
	LC	0.126	0.147	N/A	0.113	0.095
	OC	0.124	0.146	N/A	0.115	0.103
	Basil	0.139	0.100	N/A	0.106	0.108
	Dill	0.150	0.076	N/A	0.119	0.132
Standard Deviation	Lettuce	0.140	0.075	N/A	0.116	0.117
	RWTB	0.009	0.026	N/A	0.018	0.011
	LVL	0.013	0.016	N/A	0.007	0.037
	LVD	0.000	0.008	N/A	0.003	0.017
	HVL	0.004	0.025	N/A	0.017	0.011
	HVD	0.002	0.021	N/A	0.007	0.015
	LC	0.000	0.021	N/A	0.010	0.014
	OC	0.003	0.016	N/A	0.006	0.010
	Basil	0.012	0.019	N/A	0.004	0.026
	Dill	0.005	0.013	N/A	0.009	0.012
	Lettuce	0.004	0.017	N/A	0.007	0.027

A-3. Ammonia Results (Study # 3)

Ammonia (NH ₃ -N) Concentration							
Multiple Plant Species Spring Study (Experiment # 3)							
Sample Site	Plant Species	Flow Velocity (ft/s)	Plant Density (#)	Sample Date			
				4/2/2006 (mg/L)	4/23/2006 (mg/L)	5/11/2006 (mg/L)	6/2/2006 (mg/L)
Head Box	N/A	N/A	N/A	0.040	0.042	0.040	0.023
Raceway A	N/A	N/A	N/A	0.064	0.064	0.069	0.048
Raceway B	N/A	N/A	N/A	0.065	0.074	0.067	0.055
Tail Box	N/A	N/A	N/A	0.071	0.068	0.067	0.030
Experimental Channel 1	Basil	L	H	0.062	0.060	N/A	N/A
Experimental Channel 2	Dill	L	H	0.064	0.073	N/A	N/A
Experimental Channel 3	Lettuce	L	H	0.066	0.077	N/A	N/A
Experimental Channel 4	Lettuce	L	H	0.076	0.063	N/A	N/A
Experimental Channel 5	Basil	L	H	0.057	0.073	N/A	N/A
Experimental Channel 6	Dill	L	H	0.062	0.055	N/A	N/A
Experimental Channel 7	Dill	L	H	0.066	0.065	N/A	N/A
Experimental Channel 8	Lettuce	L	H	0.060	0.061	N/A	N/A
Experimental Channel 9	Basil	L	H	0.061	0.053	N/A	N/A
Experimental Channel 10	Watercress	L	H	0.064	0.071	0.069	0.061
Experimental Channel 11	Watercress	H	H	0.065	0.082	0.071	0.048
Experimental Channel 12	Watercress	L	L	0.062	0.062	0.081	0.052
Experimental Channel 13	Watercress	L	L	0.061	0.057	0.077	0.048
Experimental Channel 14	Watercress	H	H	0.060	0.064	0.079	0.050
Experimental Channel 15	Watercress	H	L	0.060	0.053	0.068	0.041
Experimental Channel 16	Watercress	H	H	0.063	0.065	0.071	0.044
Experimental Channel 17	Watercress	H	L	0.064	0.076	0.080	0.051
Experimental Channel 18	Watercress	L	H	0.062	0.066	0.075	0.047
Experimental Channel 19	Watercress	L	L	0.059	0.060	0.077	0.051
Experimental Channel 20	Watercress	L	H	0.071	0.068	0.071	0.048
Experimental Channel 21	Watercress	H	L	0.066	0.066	0.065	0.057
Long Channel 22	Watercress	L	H	0.073	0.066	0.065	0.037
Long Channel 23	Watercress	L	H	0.065	0.062	0.066	0.040
Long Channel 24	Watercress	L	H	0.069	0.068	0.078	0.049
Old Shelter Channel 25	Watercress	L	H	0.060	0.071	0.079	0.087
Old Shelter Channel 26	Watercress	L	H	0.064	0.068	0.071	0.063
Old Shelter Channel 27	Watercress	L	H	0.069	0.064	0.078	0.056
Polishing Pond	N/A	N/A	N/A	0.086	0.095	0.122	0.154
River	N/A	N/A	N/A	0.046	0.043	0.040	0.025

	Treatment Conditions	Sample Date			
		4/2/2006	4/23/2006	5/11/2006	6/2/2006
		(mg/L)	(mg/L)	(mg/L)	(mg/L)
Average Concentration	RWTB	0.067	0.069	0.068	0.044
	LVLD	0.061	0.060	0.078	0.050
	LVHD	0.066	0.069	0.072	0.052
	HVLD	0.063	0.065	0.071	0.050
	HVHD	0.063	0.070	0.074	0.048
	LC	0.069	0.065	0.070	0.042
	OC	0.064	0.068	0.076	0.069
	Basil	0.060	0.062	N/A	N/A
	Dill	0.064	0.064	N/A	N/A
	Lettuce	0.068	0.067	N/A	N/A
Standard Deviation	RWTB	0.003	0.005	0.001	0.013
	LVLD	0.001	0.002	0.003	0.002
	LVHD	0.005	0.003	0.003	0.008
	HVLD	0.003	0.012	0.008	0.008
	HVHD	0.003	0.010	0.005	0.003
	LC	0.004	0.003	0.007	0.006
	OC	0.005	0.004	0.004	0.017
	Basil	0.002	0.010	N/A	N/A
	Dill	0.002	0.009	N/A	N/A
	Lettuce	0.008	0.009	N/A	N/A

A-4. Nitrate Results (Study # 1)

Nitrate (NO ₃ -N) Concentration									
Divergent Growth Conditions Study (Experiment # 1)									
Sample Site	Plant Species	Flow Velocity	Plant Density	Sample Date					
				6/22/2005	7/16/2005	8/7/2005	8/27/2005	9/17/2005	10/8/2005
-----	-----	(ft/s)	(#)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Head Box	N/A	N/A	N/A	0.287	0.268	0.288	0.248	0.186	0.200
Raceway A	N/A	N/A	N/A	0.348	0.296	0.266	0.279	0.282	0.225
Raceway B	N/A	N/A	N/A	0.313	0.288	0.262	0.289	0.292	0.233
Manifold	N/A	N/A	N/A	0.354	0.281	0.272	0.296	0.345	0.195
Experimental Channel 1	N/A	M	N/A	0.362	0.276	0.206	0.223	0.277	0.214
Experimental Channel 2	Watercress	M	M	0.306	0.269	0.176	0.205	0.303	0.190
Experimental Channel 3	Watercress	L	H	0.287	0.264	0.201	0.208	0.307	0.147
Experimental Channel 4	Watercress	H	L	0.313	0.261	0.290	0.274	0.276	0.187
Experimental Channel 5	Watercress	M	H	0.355	0.273	0.262	0.278	0.294	0.240
Experimental Channel 6	Watercress	L	M	0.345	0.238	0.276	0.286	0.316	0.077
Experimental Channel 7	Watercress	M	L	0.353	0.268	0.278	0.277	0.290	0.010
Experimental Channel 8	Watercress	H	H	0.346	0.257	0.212	0.305	0.331	0.039
Experimental Channel 9	Watercress	L	L	0.340	0.219	0.235	0.296	0.269	0.010
Experimental Channel 10	Watercress	H	M	0.360	0.259	0.188	0.331	0.310	0.176
Experimental Channel 11	Watercress	H	L	0.357	0.226	0.222	0.288	0.333	0.246
Experimental Channel 12	Watercress	M	M	0.411	0.263	0.229	0.304	0.305	0.185
Experimental Channel 13	Watercress	H	H	0.344	0.260	0.184	0.301	0.308	0.193
Experimental Channel 14	Watercress	M	L	0.422	0.234	0.221	0.292	0.312	0.204
Experimental Channel 15	N/A	L	N/A	0.362	0.287	0.174	0.295	0.286	0.217
Experimental Channel 16	Watercress	L	H	0.377	0.285	0.269	0.155	0.328	0.209
Experimental Channel 17	Watercress	L	L	0.405	0.266	0.282	0.190	0.297	0.197
Experimental Channel 18	Watercress	M	H	0.343	0.271	0.297	0.177	0.341	0.227
Experimental Channel 19	Watercress	H	M	0.352	0.289	0.286	0.237	0.310	0.202
Experimental Channel 20	Watercress	L	M	0.356	0.257	0.269	0.286	0.304	0.206
Experimental Channel 21	Watercress	H	H	0.333	0.282	0.297	0.319	0.369	0.199
Experimental Channel 22	Watercress	M	L	0.335	0.264	0.309	0.308	0.301	0.180
Experimental Channel 23	Watercress	M	H	0.393	0.286	0.300	0.313	0.290	0.193
Experimental Channel 24	Watercress	M	M	0.391	0.184	0.296	0.232	0.308	0.188
Experimental Channel 25	Watercress	L	H	0.344	0.273	0.289	0.276	0.313	0.195
Experimental	Watercress	H	L	0.337	0.286	0.298	0.317	0.305	0.177

Channel 26									
Experimental Channel 27	Watercress	L	L	0.342	0.265	0.272	0.308	0.293	0.181
Experimental Channel 28	Watercress	H	M	0.336	0.275	0.277	0.212	0.306	0.186
Experimental Channel 29	N/A	H	N/A	0.451	0.251	0.266	0.302	0.305	0.206
Experimental Channel 30	Watercress	L	M	0.366	0.047	0.264	0.294	0.325	0.190
Tail Box	N/A	N/A	N/A	0.294	0.284	0.293	0.277	0.284	0.170
Polishing Pond	N/A	N/A	N/A	0.280	0.216	0.222	0.266	0.269	0.194
Control Channel 1	Watercress	H	M	0.194	0.278	0.265	0.183	0.286	0.087
Control Channel 2	Watercress	L	M	0.445	0.223	0.262	0.275	0.267	0.202
Control Channel 3	Watercress	M	M	0.281	0.263	0.205	0.211	0.288	0.201

	Treatment Conditions	Sample Date					
		6/22/2005	7/16/2005	8/7/2005	8/27/2005	9/17/2005	10/8/2005
		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Average Concentration	RWM	0.338	0.288	0.267	0.288	0.306	0.218
	LVLD	0.363	0.250	0.263	0.265	0.286	0.129
	LVMD	0.356	0.180	0.270	0.289	0.315	0.158
	LVHD	0.336	0.274	0.253	0.213	0.316	0.183
	MVLD	0.370	0.255	0.269	0.292	0.301	0.131
	MVMD	0.369	0.238	0.234	0.247	0.305	0.188
	MVHD	0.364	0.277	0.286	0.256	0.308	0.220
	HVLD	0.335	0.258	0.270	0.293	0.304	0.203
	HVMD	0.350	0.274	0.251	0.260	0.309	0.188
	HVHD	0.341	0.266	0.231	0.308	0.336	0.144
	CC	0.306	0.255	0.244	0.223	0.281	0.163
	EC	0.391	0.271	0.216	0.273	0.289	0.212
Standard Deviation	RWM	0.022	0.008	0.005	0.009	0.034	0.020
	LVLD	0.037	0.027	0.025	0.065	0.015	0.103
	LVMD	0.010	0.116	0.006	0.005	0.010	0.070
	LVHD	0.046	0.011	0.046	0.061	0.011	0.033
	MVLD	0.046	0.019	0.045	0.015	0.011	0.106
	MVMD	0.056	0.047	0.060	0.051	0.003	0.002
	MVHD	0.026	0.008	0.021	0.070	0.029	0.024
	HVLD	0.022	0.030	0.042	0.022	0.029	0.037
	HVMD	0.012	0.015	0.054	0.063	0.003	0.013
	HVHD	0.007	0.014	0.059	0.010	0.030	0.091
	CC	0.127	0.029	0.034	0.047	0.012	0.066
	EC	0.052	0.018	0.047	0.044	0.014	0.005

A-5. Nitrate Results (Study # 2)

Nitrate (NO ₃ -N) Concentration								
Multiple Plant Species Winter Study (Experiment # 2)								
Sample Site	Plant Species	Flow Velocity	Plant Density	Sample Date				
				12/11/2005	1/8/2006	1/22/2006	2/20/2006	3/3/2006
-----	-----	(ft/s)	(#)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Head Box	N/A	N/A	N/A	0.310	0.346	0.348	0.332	0.346
Raceway A	N/A	N/A	N/A	0.365	0.375	0.380	0.364	0.346
Raceway B	N/A	N/A	N/A	0.343	0.361	0.363	0.356	0.334
Tail Box	N/A	N/A	N/A	0.351	0.362	0.370	0.360	0.321
Experimental Channel 1	Basil	L	H	0.332	0.277	0.334	0.328	0.313
Experimental Channel 2	Dill	L	H	0.327	0.319	0.323	0.319	0.346
Experimental Channel 3	Lettuce	L	H	0.320	0.338	0.339	0.326	0.320
Experimental Channel 4	Lettuce	L	H	0.362	0.387	0.359	0.358	0.332
Experimental Channel 5	Basil	L	H	0.338	0.352	0.376	0.373	0.363
Experimental Channel 6	Dill	L	H	0.337	0.339	0.347	0.330	0.375
Experimental Channel 7	Dill	L	H	0.376	0.329	0.348	0.325	0.374
Experimental Channel 8	Lettuce	L	H	0.364	0.346	0.348	0.357	0.358
Experimental Channel 9	Basil	L	H	0.365	0.284	0.319	0.362	0.355
Experimental Channel 10	Watercress	L	H	0.387	0.337	0.344	0.335	0.367
Experimental Channel 11	Watercress	H	H	0.352	0.342	0.351	0.339	0.363
Experimental Channel 12	Watercress	L	L	0.370	0.369	0.368	0.363	0.338
Experimental Channel 13	Watercress	L	L	0.372	0.362	0.384	0.367	0.385
Experimental Channel 14	Watercress	H	H	0.393	0.363	0.390	0.358	0.341
Experimental Channel 15	Watercress	H	L	0.358	0.369	0.375	0.358	0.370
Experimental Channel 16	Watercress	H	H	0.351	0.362	0.352	0.357	0.340
Experimental Channel 17	Watercress	H	L	0.358	0.369	0.372	0.359	0.352
Experimental Channel 18	Watercress	L	H	0.365	0.365	0.381	0.352	0.355
Experimental Channel 19	Watercress	L	L	0.379	0.308	0.373	0.299	0.336
Experimental Channel 20	Watercress	L	H	0.371	0.379	0.385	0.368	0.351
Experimental Channel 21	Watercress	H	L	0.358	0.363	0.374	0.362	0.370
Long Channel 22	Watercress	L	H	0.363	0.350	0.369	0.356	0.331
Long Channel 23	Watercress	L	H	0.376	0.377	0.385	0.372	0.350
Long Channel 24	Watercress	L	H	0.383	0.381	0.394	0.381	0.344
Old Shelter Channel 25	Watercress	L	H	0.361	0.376	0.389	0.358	0.339
Old Shelter Channel 26	Watercress	L	H	0.359	0.390	0.375	0.372	0.355
Old Shelter Channel	Watercress	L	H	0.365	0.374	0.386	0.383	0.349

Polishing Pond	N/A	N/A	N/A	0.320	0.355	0.396	0.360	0.290
River	N/A	N/A	N/A	0.360	0.384	0.410	0.380	0.285

	Treatment Conditions	Sample Date				
		12/11/20 05	1/8/20 06	1/22/20 06	2/20/20 06	3/3/20 06
		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Average Concentration	RWTB	0.353	0.366	0.371	0.360	0.334
	LVL	0.374	0.347	0.375	0.343	0.353
	LVHD	0.374	0.361	0.370	0.352	0.358
	HVL	0.358	0.367	0.374	0.359	0.364
	HVHD	0.365	0.355	0.364	0.351	0.348
	LC	0.374	0.369	0.383	0.370	0.342
	OC	0.362	0.380	0.383	0.371	0.348
	Basil	0.345	0.304	0.343	0.354	0.344
	Dill	0.347	0.329	0.339	0.325	0.365
	Lettuce	0.349	0.357	0.349	0.347	0.337
Standard Deviation	RWTB	0.011	0.008	0.008	0.004	0.012
	LVL	0.005	0.034	0.008	0.038	0.028
	LVHD	0.011	0.022	0.023	0.017	0.008
	HVL	0.000	0.003	0.001	0.002	0.010
	HVHD	0.024	0.012	0.022	0.011	0.013
	LC	0.010	0.017	0.013	0.013	0.010
	OC	0.003	0.009	0.008	0.013	0.008
	Basil	0.017	0.042	0.030	0.023	0.027
	Dill	0.026	0.010	0.014	0.005	0.017
	Lettuce	0.025	0.026	0.010	0.018	0.019

A-6. Nitrate Results (Study # 3)

Nitrate (NO ₃ -N) Concentration							
Multiple Plant Species Spring Study (Experiment # 3)							
Sample Site	Plant Species	Flow Velocity (ft/s)	Plant Density (#)	Sample Date			
				4/2/2006 (mg/L)	4/23/2006 (mg/L)	5/11/2006 (mg/L)	6/2/2006 (mg/L)
Head Box	N/A	N/A	N/A	0.342	0.351	0.347	0.324
Raceway A	N/A	N/A	N/A	0.349	0.354	0.353	0.331
Raceway B	N/A	N/A	N/A	0.348	0.359	0.348	0.328
Tail Box	N/A	N/A	N/A	0.342	0.351	0.339	0.319
Experimental Channel 1	Basil	L	H	0.348	0.354	N/A	N/A
Experimental Channel 2	Dill	L	H	0.353	0.359	N/A	N/A
Experimental Channel 3	Lettuce	L	H	0.342	0.347	N/A	N/A
Experimental Channel 4	Lettuce	L	H	0.335	0.341	N/A	N/A
Experimental Channel 5	Basil	L	H	0.360	0.347	N/A	N/A
Experimental Channel 6	Dill	L	H	0.351	0.352	N/A	N/A
Experimental Channel 7	Dill	L	H	0.364	0.353	N/A	N/A
Experimental Channel 8	Lettuce	L	H	0.362	0.360	N/A	N/A
Experimental Channel 9	Basil	L	H	0.371	0.373	N/A	N/A
Experimental Channel 10	Watercress	L	H	0.367	0.378	0.369	0.324
Experimental Channel 11	Watercress	H	H	0.354	0.358	0.347	0.318
Experimental Channel 12	Watercress	L	L	0.347	0.366	0.360	0.329
Experimental Channel 13	Watercress	L	L	0.375	0.348	0.350	0.318
Experimental Channel 14	Watercress	H	H	0.366	0.346	0.349	0.310
Experimental Channel 15	Watercress	H	L	0.362	0.345	0.367	0.306
Experimental Channel 16	Watercress	H	H	0.351	0.339	0.369	0.300
Experimental Channel 17	Watercress	H	L	0.348	0.349	0.374	0.318
Experimental Channel 18	Watercress	L	H	0.348	0.357	0.380	0.330
Experimental Channel 19	Watercress	L	L	0.349	0.357	0.357	0.331
Experimental Channel 20	Watercress	L	H	0.349	0.358	0.350	0.331
Experimental Channel 21	Watercress	H	L	0.356	0.364	0.336	0.322
Long Channel 22	Watercress	L	H	0.355	0.345	0.350	0.321
Long Channel 23	Watercress	L	H	0.340	0.350	0.361	0.319
Long Channel 24	Watercress	L	H	0.347	0.348	0.364	0.320
Old Shelter Channel 25	Watercress	L	H	0.348	0.347	0.373	0.325
Old Shelter Channel 26	Watercress	L	H	0.354	0.353	0.371	0.325
Old Shelter Channel 27	Watercress	L	H	0.353	0.370	0.358	0.318
Polishing Pond	N/A	N/A	N/A	0.296	0.305	0.334	0.325
River	N/A	N/A	N/A	0.292	0.359	0.364	0.336

	Treatment Conditions	Sample Date			
		4/2/2006	4/23/2006	5/11/2006	6/2/2006
		(mg/L)	(mg/L)	(mg/L)	(mg/L)
Average Concentration	RWTB	0.346	0.354	0.346	0.326
	LVLD	0.357	0.357	0.356	0.326
	LVHD	0.355	0.364	0.366	0.328
	HVLD	0.355	0.353	0.359	0.315
	HVHD	0.357	0.348	0.355	0.309
	LC	0.348	0.348	0.358	0.320
	OC	0.352	0.357	0.367	0.323
	Basil	0.360	0.358	N/A	N/A
	Dill	0.356	0.355	N/A	N/A
	Lettuce	0.346	0.350	N/A	N/A
Standard Deviation	RWTB	0.004	0.004	0.007	0.006
	LVLD	0.015	0.009	0.005	0.007
	LVHD	0.011	0.012	0.015	0.004
	HVLD	0.007	0.010	0.020	0.009
	HVHD	0.008	0.010	0.012	0.009
	LC	0.008	0.002	0.008	0.001
	OC	0.003	0.012	0.008	0.004
	Basil	0.011	0.013	N/A	N/A
	Dill	0.007	0.004	N/A	N/A
	Lettuce	0.014	0.010	N/A	N/A

A-7. Nitrite Results (Study # 1)

Nitrite (NO ₂ -N) Concentration									
Divergent Growth Conditions Study (Experiment # 1)									
Sample Site	Plant Species	Flow Velocity	Plant Density	Sample Date					
				6/22/2005	7/16/2005	8/7/2005	8/27/2005	9/17/2005	10/8/2005
----	-----	(ft/s)	(#)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Head Box	N/A	N/A	N/A	0.000	0.000	0.001	-0.001	0.000	0.000
Raceway A	N/A	N/A	N/A	0.001	0.001	0.000	0.000	0.000	0.001
Raceway B	N/A	N/A	N/A	0.001	0.001	0.001	0.001	0.001	0.001
Manifold	N/A	N/A	N/A	0.002	0.002	0.001	0.001	0.002	0.002
Experimental Channel 1	N/A	M	N/A	0.001	0.005	0.001	0.002	0.002	0.003
Experimental Channel 2	Watercress	M	M	0.001	0.004	0.002	0.004	0.004	0.003
Experimental Channel 3	Watercress	L	H	0.001	0.003	0.001	0.003	0.002	0.004
Experimental Channel 4	Watercress	H	L	0.001	0.003	0.002	0.004	0.003	0.004
Experimental Channel 5	Watercress	M	H	0.001	0.003	0.001	0.006	0.004	0.004
Experimental Channel 6	Watercress	L	M	0.002	0.002	0.001	0.003	0.002	0.004
Experimental Channel 7	Watercress	M	L	0.001	0.004	0.001	0.004	0.003	0.006
Experimental Channel 8	Watercress	H	H	0.001	0.002	0.001	0.004	0.003	0.006
Experimental Channel 9	Watercress	L	L	0.001	0.002	0.001	0.002	0.001	0.004
Experimental Channel 10	Watercress	H	M	0.001	0.004	0.001	0.004	0.002	0.007
Experimental Channel 11	Watercress	H	L	0.001	0.004	0.002	0.003	0.003	0.003
Experimental Channel 12	Watercress	M	M	0.001	0.005	0.002	0.004	0.003	0.004
Experimental Channel 13	Watercress	H	H	0.001	0.003	0.002	0.002	0.002	0.004
Experimental Channel 14	Watercress	M	L	0.001	0.004	0.001	0.003	0.002	0.005
Experimental Channel 15	N/A	L	N/A	0.002	0.002	0.001	0.002	0.001	0.003
Experimental Channel 16	Watercress	L	H	0.002	0.002	0.001	0.002	0.002	0.006
Experimental Channel 17	Watercress	L	L	0.001	0.002	0.001	0.002	0.003	0.005
Experimental Channel 18	Watercress	M	H	0.001	0.003	0.002	0.003	0.003	0.003
Experimental Channel 19	Watercress	H	M	0.001	0.003	0.003	0.002	0.003	0.005
Experimental Channel 20	Watercress	L	M	0.001	0.005	0.001	0.002	0.002	0.004
Experimental Channel 21	Watercress	H	H	0.001	0.002	0.003	0.003	0.002	0.004
Experimental Channel 22	Watercress	M	L	0.001	0.006	0.003	0.005	0.002	0.006
Experimental Channel 23	Watercress	M	H	0.001	0.004	0.003	0.007	0.003	0.004
Experimental Channel 24	Watercress	M	M	0.001	0.004	0.002	0.004	0.003	0.006
Experimental Channel 25	Watercress	L	H	0.001	0.003	0.002	0.002	0.002	0.007
Experimental	Watercress	H	L	0.001	0.004	0.002	0.004	0.002	0.005

Channel 26									
Experimental Channel 27	Watercress	L	L	0.001	0.003	0.001	0.003	0.002	0.004
Experimental Channel 28	Watercress	H	M	0.001	0.004	0.003	0.004	0.002	0.006
Experimental Channel 29	N/A	H	N/A	0.001	0.018	0.002	0.003	0.003	0.003
Experimental Channel 30	Watercress	L	M	0.001	0.003	0.001	0.002	0.001	0.008
Tail Box	N/A	N/A	N/A	0.001	0.002	0.001	0.002	0.002	0.002
Polishing Pond	N/A	N/A	N/A	0.002	0.004	0.002	0.005	0.005	0.006
Control Channel 1	Watercress	H	M	0.000	0.001	0.001	-0.001	0.000	0.001
Control Channel 2	Watercress	L	M	0.000	0.002	0.000	-0.001	0.000	0.001
Control Channel 3	Watercress	M	M	0.000	0.001	0.000	-0.001	0.000	0.001

	Treatment Conditions	Sample Date					
		6/22/2005	7/16/2005	8/7/2005	8/27/2005	9/17/2005	10/8/2005
		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Average Concentration	RWM	0.001	0.001	0.001	0.001	0.001	0.001
	LVLD	0.001	0.003	0.001	0.002	0.002	0.005
	LVMD	0.001	0.003	0.001	0.002	0.002	0.005
	LVHD	0.001	0.003	0.001	0.002	0.002	0.006
	MVLD	0.001	0.005	0.002	0.004	0.002	0.006
	MVMD	0.001	0.004	0.002	0.004	0.003	0.004
	MVHD	0.001	0.003	0.002	0.005	0.003	0.004
	HVLD	0.001	0.003	0.002	0.003	0.003	0.004
	HVMD	0.001	0.004	0.002	0.003	0.002	0.006
	HVHD	0.001	0.002	0.002	0.003	0.002	0.005
	CC	0.000	0.001	0.000	-0.001	0.000	0.001
	EC	0.001	0.008	0.001	0.002	0.002	0.003
Standard Deviation	RWM	0.000	0.001	0.000	0.001	0.001	0.000
	LVLD	0.000	0.001	0.000	0.001	0.001	0.001
	LVMD	0.000	0.002	0.000	0.000	0.001	0.002
	LVHD	0.000	0.001	0.000	0.000	0.000	0.002
	MVLD	0.000	0.001	0.001	0.001	0.000	0.001
	MVMD	0.000	0.001	0.000	0.000	0.001	0.001
	MVHD	0.000	0.000	0.001	0.002	0.001	0.001
	HVLD	0.000	0.001	0.000	0.000	0.001	0.001
	HVMD	0.000	0.000	0.001	0.001	0.000	0.001
	HVHD	0.000	0.000	0.001	0.001	0.000	0.001
	CC	0.000	0.000	0.000	0.000	0.000	0.000
	EC	0.000	0.008	0.001	0.000	0.001	0.000

A-8. Nitrite Results (Study # 2)

Nitrite (NO ₂ -N) Concentration								
Multiple Plant Species Winter Study (Experiment # 2)								
Sample Site	Plant Species	Flow Velocity	Plant Density	Sample Date				
				12/11/2005	1/8/2006	1/22/2006	2/20/2006	3/3/2006
-----	-----	(ft/s)	(#)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Head Box	N/A	N/A	N/A	0.000	0.000	0.001	0.000	0.001
Raceway A	N/A	N/A	N/A	0.001	0.001	0.001	0.001	0.001
Raceway B	N/A	N/A	N/A	0.001	0.001	0.001	0.001	0.002
Tail Box	N/A	N/A	N/A	0.001	0.001	0.001	0.001	0.002
Experimental Channel 1	Basil	L	H	0.002	0.003	0.002	0.002	0.004
Experimental Channel 2	Dill	L	H	0.001	0.002	0.002	0.002	0.004
Experimental Channel 3	Lettuce	L	H	0.001	0.002	0.001	0.001	0.004
Experimental Channel 4	Lettuce	L	H	0.001	0.002	0.002	0.003	0.004
Experimental Channel 5	Basil	L	H	0.001	0.002	0.002	0.001	0.004
Experimental Channel 6	Dill	L	H	0.001	0.002	0.001	0.001	0.004
Experimental Channel 7	Dill	L	H	0.001	0.001	0.002	0.002	0.004
Experimental Channel 8	Lettuce	L	H	0.001	0.002	0.001	0.002	0.004
Experimental Channel 9	Basil	L	H	0.001	0.002	0.003	0.003	0.004
Experimental Channel 10	Watercress	L	H	0.002	0.002	0.002	0.002	0.003
Experimental Channel 11	Watercress	H	H	0.001	0.002	0.002	0.002	0.004
Experimental Channel 12	Watercress	L	L	0.002	0.002	0.002	0.002	0.004
Experimental Channel 13	Watercress	L	L	0.001	0.002	0.002	0.002	0.003
Experimental Channel 14	Watercress	H	H	0.001	0.002	0.002	0.001	0.004
Experimental Channel 15	Watercress	H	L	0.001	0.002	0.002	0.002	0.004
Experimental Channel 16	Watercress	H	H	0.001	0.002	0.002	0.002	0.005
Experimental Channel 17	Watercress	H	L	0.001	0.003	0.002	0.003	0.005
Experimental Channel 18	Watercress	L	H	0.002	0.002	0.002	0.002	0.004
Experimental Channel 19	Watercress	L	L	0.001	0.002	0.001	0.001	0.003
Experimental Channel 20	Watercress	L	H	0.001	0.002	0.002	0.002	0.003
Experimental Channel 21	Watercress	H	L	0.002	0.002	0.001	0.002	0.004
Long Channel 22	Watercress	L	H	0.002	0.002	0.001	0.002	0.004
Long Channel 23	Watercress	L	H	0.002	0.002	0.001	0.002	0.003
Long Channel 24	Watercress	L	H	0.002	0.002	0.001	0.002	0.004
Old Shelter Channel 25	Watercress	L	H	0.002	0.003	0.001	0.002	0.004
Old Shelter Channel 26	Watercress	L	H	0.002	0.004	0.002	0.002	0.004
Old Shelter Channel	Watercress	L	H	0.002	0.003	0.002	0.002	0.005

Polishing Pond	N/A	N/A	N/A	0.001	0.004	0.002	0.002	0.006
River	N/A	N/A	N/A	0.005	0.003	0.003	0.003	0.006

	Treatment Conditions	Sample Date				
		12/11/2005	1/8/2006	1/22/2006	2/20/2006	3/3/2006
		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Average Concentration	RWTB	0.001	0.001	0.001	0.001	0.002
	LVL	0.001	0.002	0.002	0.002	0.003
	LVD	0.002	0.002	0.002	0.002	0.004
	HVL	0.002	0.002	0.002	0.002	0.004
	HVD	0.001	0.002	0.002	0.002	0.004
	LC	0.002	0.002	0.001	0.002	0.004
	OC	0.002	0.003	0.002	0.002	0.004
	Basil	0.001	0.002	0.002	0.002	0.004
	Dill	0.001	0.002	0.002	0.002	0.004
	Lettuce	0.001	0.002	0.001	0.002	0.004
Standard Deviation	RWTB	0.000	0.000	0.000	0.000	0.000
	LVL	0.000	0.000	0.000	0.000	0.000
	LVD	0.000	0.000	0.000	0.000	0.001
	HVL	0.000	0.001	0.000	0.001	0.001
	HVD	0.000	0.000	0.000	0.000	0.000
	LC	0.000	0.000	0.000	0.000	0.000
	OC	0.000	0.001	0.000	0.000	0.000
	Basil	0.000	0.001	0.001	0.001	0.000
	Dill	0.000	0.001	0.000	0.001	0.000
	Lettuce	0.000	0.000	0.000	0.001	0.000

A-9. Nitrite Results (Study # 3)

Nitrite (NO ₂ -N) Concentration							
Multiple Plant Species Spring Study (Experiment # 3)							
Sample Site	Plant Species	Flow Velocity (ft/s)	Plant Density (#)	Sample Date			
				4/2/2006 (mg/L)	4/23/2006 (mg/L)	5/11/2006 (mg/L)	6/2/2006 (mg/L)
Head Box	N/A	N/A	N/A	0.001	0.002	0.002	0.002
Raceway A	N/A	N/A	N/A	0.002	0.002	0.003	0.002
Raceway B	N/A	N/A	N/A	0.001	0.002	0.002	0.002
Tail Box	N/A	N/A	N/A	0.001	0.002	0.003	0.002
Experimental Channel 1	Basil	L	H	0.002	0.002	N/A	N/A
Experimental Channel 2	Dill	L	H	0.002	0.002	N/A	N/A
Experimental Channel 3	Lettuce	L	H	0.002	0.002	N/A	N/A
Experimental Channel 4	Lettuce	L	H	0.002	0.002	N/A	N/A
Experimental Channel 5	Basil	L	H	0.002	0.003	N/A	N/A
Experimental Channel 6	Dill	L	H	0.002	0.002	N/A	N/A
Experimental Channel 7	Dill	L	H	0.003	0.002	N/A	N/A
Experimental Channel 8	Lettuce	L	H	0.002	0.002	N/A	N/A
Experimental Channel 9	Basil	L	H	0.002	0.002	N/A	N/A
Experimental Channel 10	Watercress	L	H	0.002	0.002	0.003	0.002
Experimental Channel 11	Watercress	H	H	0.002	0.003	0.002	0.002
Experimental Channel 12	Watercress	L	L	0.002	0.002	0.002	0.002
Experimental Channel 13	Watercress	L	L	0.002	0.002	0.002	0.002
Experimental Channel 14	Watercress	H	H	0.002	0.002	0.002	0.002
Experimental Channel 15	Watercress	H	L	0.002	0.002	0.002	0.002
Experimental Channel 16	Watercress	H	H	0.002	0.002	0.002	0.002
Experimental Channel 17	Watercress	H	L	0.002	0.002	0.003	0.002
Experimental Channel 18	Watercress	L	H	0.002	0.003	0.002	0.002
Experimental Channel 19	Watercress	L	L	0.002	0.002	0.002	0.002
Experimental Channel 20	Watercress	L	H	0.002	0.003	0.003	0.002
Experimental Channel 21	Watercress	H	L	0.002	0.003	0.003	0.002
Long Channel 22	Watercress	L	H	0.002	0.002	0.002	0.001
Long Channel 23	Watercress	L	H	0.002	0.002	0.002	0.002
Long Channel 24	Watercress	L	H	0.002	0.002	0.002	0.002
Old Shelter Channel 25	Watercress	L	H	0.002	0.002	0.002	0.002
Old Shelter Channel 26	Watercress	L	H	0.002	0.003	0.003	0.002
Old Shelter Channel 27	Watercress	L	H	0.003	0.003	0.003	0.003
Polishing Pond	N/A	N/A	N/A	0.001	0.001	0.002	0.001
River	N/A	N/A	N/A	0.003	0.004	0.005	0.003

	Treatment Conditions	Sample Date			
		4/2/2006	4/23/2006	5/11/2006	6/2/2006
		(mg/L)	(mg/L)	(mg/L)	(mg/L)
Average Concentration	RWTB	0.001	0.002	0.003	0.002
	LVLD	0.002	0.002	0.002	0.002
	LVHD	0.002	0.002	0.003	0.002
	HVLD	0.002	0.002	0.003	0.002
	HVHD	0.002	0.002	0.002	0.002
	LC	0.002	0.002	0.002	0.002
	OC	0.002	0.003	0.003	0.002
	Basil	0.002	0.002	N/A	N/A
	Dill	0.002	0.002	N/A	N/A
	Lettuce	0.002	0.002	N/A	N/A
Standard Deviation	RWTB	0.000	0.000	0.000	0.000
	LVLD	0.000	0.000	0.000	0.000
	LVHD	0.000	0.000	0.000	0.000
	HVLD	0.000	0.001	0.001	0.000
	HVHD	0.000	0.000	0.000	0.000
	LC	0.000	0.000	0.000	0.000
	OC	0.000	0.000	0.001	0.000
	Basil	0.000	0.001	N/A	N/A
	Dill	0.000	0.000	N/A	N/A
	Lettuce	0.000	0.000	N/A	N/A

A-10. Phosphate Results (Study # 1)

Phosphate (PO ₄ ³⁻ P) Concentration									
Divergent Growth Conditions Study (Experiment # 1)									
Sample Site	Plant Species	Flow Velocity	Plant Density	Sample Date					
				6/22/2005	7/16/2005	8/7/2005	8/27/2005	9/17/2005	10/8/2005
----	-----	(ft/s)	(#)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Head Box	N/A	N/A	N/A	0.446	0.458	0.460	0.314	0.449	0.497
Raceway A	N/A	N/A	N/A	0.548	0.642	0.528	0.464	0.613	0.729
Raceway B	N/A	N/A	N/A	0.534	0.539	0.579	0.559	0.610	0.616
Manifold	N/A	N/A	N/A	0.574	0.586	0.497	0.455	0.576	0.653
Experimental Channel 1	N/A	M	N/A	0.594	0.698	0.557	0.657	0.613	0.613
Experimental Channel 2	Watercress	M	M	0.622	0.645	0.559	0.519	0.579	0.647
Experimental Channel 3	Watercress	L	H	0.523	0.739	0.503	0.380	0.602	0.638
Experimental Channel 4	Watercress	H	L	0.562	0.667	0.596	0.510	0.607	0.605
Experimental Channel 5	Watercress	M	H	0.531	0.645	0.571	0.461	0.593	0.596
Experimental Channel 6	Watercress	L	M	0.599	0.617	0.562	0.475	0.590	0.655
Experimental Channel 7	Watercress	M	L	0.611	0.712	0.477	0.403	0.650	0.678
Experimental Channel 8	Watercress	H	H	0.571	0.661	0.554	0.438	0.565	0.636
Experimental Channel 9	Watercress	L	L	0.588	0.675	0.511	0.470	0.568	0.689
Experimental Channel 10	Watercress	H	M	0.588	0.667	0.559	0.432	0.596	0.777
Experimental Channel 11	Watercress	H	L	0.548	0.672	0.588	0.449	0.576	0.650
Experimental Channel 12	Watercress	M	M	0.560	0.756	0.508	0.472	0.602	0.695
Experimental Channel 13	Watercress	H	H	0.557	0.695	0.593	0.481	0.630	0.610
Experimental Channel 14	Watercress	M	L	0.560	0.659	0.523	0.539	0.610	0.681
Experimental Channel 15	N/A	L	N/A	0.588	0.745	0.573	0.619	0.613	0.630
Experimental Channel 16	Watercress	L	H	0.551	0.661	0.585	0.582	0.650	0.644
Experimental Channel 17	Watercress	L	L	0.511	1.300	0.528	0.441	0.633	0.678
Experimental Channel 18	Watercress	M	H	0.554	1.300	0.576	0.628	0.602	0.630
Experimental Channel 19	Watercress	H	M	0.500	0.698	0.534	0.657	0.630	0.672
Experimental Channel 20	Watercress	L	M	0.591	0.620	0.531	0.602	0.576	0.692
Experimental Channel 21	Watercress	H	H	0.503	0.731	0.514	0.602	0.647	0.678
Experimental Channel 22	Watercress	M	L	0.551	0.759	0.551	0.645	0.616	0.734
Experimental Channel 23	Watercress	M	H	0.571	0.742	0.568	0.521	0.667	0.641
Experimental Channel 24	Watercress	M	M	0.528	0.636	0.571	0.556	0.627	0.720
Experimental Channel 25	Watercress	L	H	0.526	0.681	0.599	0.622	0.562	0.723
Experimental	Watercress	H	L	0.568	0.686	0.551	0.591	0.602	0.743

Channel 26									
Experimental Channel 27	Watercress	L	L	0.582	0.661	0.494	0.625	0.585	0.726
Experimental Channel 28	Watercress	H	M	0.497	0.661	0.627	0.642	0.588	0.732
Experimental Channel 29	N/A	H	N/A	0.571	0.703	0.562	0.570	0.610	0.734
Experimental Channel 30	Watercress	L	M	0.588	0.238	0.520	0.605	0.548	0.622
Tail Box	N/A	N/A	N/A	0.571	0.650	0.528	0.556	0.613	0.545
Polishing Pond	N/A	N/A	N/A	0.523	0.541	0.486	0.602	0.497	0.655
Control Channel 1	Watercress	H	M	0.452	0.500	0.525	0.400	0.446	0.449
Control Channel 2	Watercress	L	M	0.494	0.371	0.452	0.369	0.461	0.537
Control Channel 3	Watercress	M	M	0.412	0.480	0.472	0.429	0.452	0.526

	Treatment Conditions	Sample Date					
		6/22/2005	7/16/2005	8/7/2005	8/27/2005	9/17/2005	10/8/2005
		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Average Concentration	RWM	0.552	0.589	0.535	0.493	0.600	0.666
	LVLD	0.561	0.879	0.511	0.512	0.595	0.698
	LVMD	0.593	0.491	0.538	0.561	0.572	0.656
	LVHD	0.533	0.694	0.562	0.528	0.605	0.669
	MVLD	0.574	0.710	0.517	0.529	0.625	0.698
	MVMD	0.570	0.679	0.546	0.516	0.603	0.687
	MVHD	0.552	0.896	0.572	0.537	0.621	0.622
	HVLD	0.560	0.675	0.578	0.517	0.595	0.666
	HVMD	0.528	0.675	0.573	0.577	0.605	0.727
	HVHD	0.544	0.696	0.554	0.507	0.614	0.641
	CC	0.453	0.450	0.483	0.399	0.453	0.504
	EC	0.584	0.715	0.564	0.616	0.612	0.659
Standard Deviation	RWM	0.020	0.052	0.041	0.058	0.020	0.058
	LVLD	0.043	0.365	0.017	0.099	0.034	0.025
	LVMD	0.006	0.220	0.022	0.074	0.022	0.035
	LVHD	0.016	0.041	0.052	0.130	0.044	0.047
	MVLD	0.032	0.050	0.037	0.121	0.021	0.032
	MVMD	0.048	0.067	0.033	0.042	0.024	0.037
	MVHD	0.020	0.354	0.004	0.085	0.040	0.024
	HVLD	0.010	0.010	0.024	0.071	0.017	0.071
	HVMD	0.052	0.020	0.048	0.126	0.022	0.052
	HVHD	0.036	0.035	0.040	0.085	0.043	0.034
	CC	0.041	0.069	0.038	0.030	0.007	0.048
	EC	0.012	0.026	0.009	0.043	0.002	0.066

A-11. Phosphate Results (Study # 2)

Phosphate (PO ₄ ³⁻ P) Concentration								
Multiple Plant Species Winter Study (Experiment # 2)								
Sample Site	Plant Species	Flow Velocity	Plant Density	Sample Date				
				12/11/2005	1/8/2006	1/22/2006	2/20/2006	3/3/2006
-----	-----	(ft/s)	(#)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Head Box	N/A	N/A	N/A	0.482	0.466	0.471	0.504	0.416
Raceway A	N/A	N/A	N/A	0.557	0.510	0.525	0.534	0.528
Raceway B	N/A	N/A	N/A	0.551	0.554	0.492	0.525	0.555
Tail Box	N/A	N/A	N/A	0.589	0.554	0.549	0.552	0.525
Experimental Channel 1	Basil	L	H	0.551	0.480	0.528	0.534	0.588
Experimental Channel 2	Dill	L	H	0.545	0.551	0.519	0.549	0.576
Experimental Channel 3	Lettuce	L	H	0.504	0.531	0.597	0.528	0.626
Experimental Channel 4	Lettuce	L	H	0.542	0.536	0.555	0.525	0.626
Experimental Channel 5	Basil	L	H	0.560	0.528	0.594	0.603	0.573
Experimental Channel 6	Dill	L	H	0.573	0.531	0.537	0.537	0.520
Experimental Channel 7	Dill	L	H	0.551	0.466	0.540	0.543	0.638
Experimental Channel 8	Lettuce	L	H	0.576	0.457	0.561	0.576	0.534
Experimental Channel 9	Basil	L	H	0.507	0.516	0.525	0.531	0.617
Experimental Channel 10	Watercress	L	H	0.560	0.516	0.486	0.477	0.573
Experimental Channel 11	Watercress	H	H	0.557	0.531	0.787	0.552	0.579
Experimental Channel 12	Watercress	L	L	0.548	0.581	0.549	0.552	0.579
Experimental Channel 13	Watercress	L	L	0.604	0.510	0.591	0.561	0.502
Experimental Channel 14	Watercress	H	H	0.560	0.525	0.558	0.549	0.608
Experimental Channel 15	Watercress	H	L	0.576	0.563	0.591	0.618	0.588
Experimental Channel 16	Watercress	H	H	0.523	0.536	0.582	0.585	0.597
Experimental Channel 17	Watercress	H	L	0.564	0.539	0.543	0.546	0.605
Experimental Channel 18	Watercress	L	H	0.579	0.516	0.531	0.516	0.614
Experimental Channel 19	Watercress	L	L	0.614	0.531	0.570	0.606	0.585
Experimental Channel 20	Watercress	L	H	0.576	0.507	0.531	0.486	0.600
Experimental Channel 21	Watercress	H	L	0.526	0.534	0.564	0.585	0.591
Long Channel 22	Watercress	L	H	0.564	0.498	0.549	0.543	0.511
Long Channel 23	Watercress	L	H	0.529	0.513	0.531	0.534	0.614
Long Channel 24	Watercress	L	H	0.548	0.661	0.549	0.558	0.520
Old Shelter Channel 25	Watercress	L	H	0.585	0.377	0.570	0.567	0.617
Old Shelter Channel 26	Watercress	L	H	0.573	0.504	0.534	0.531	0.523
Old Shelter Channel	Watercress	L	H	0.535	0.761	0.570	0.624	0.511

Polishing Pond	N/A	N/A	N/A	0.573	0.548	0.555	0.585	0.502
River	N/A	N/A	N/A	0.507	0.061	0.067	0.094	0.070

	Treatment Conditions	Sample Date				
		12/11/2005	1/8/2006	1/22/2006	2/20/2006	3/3/2006
		(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
Average Concentration	RWTB	0.566	0.539	0.522	0.537	0.536
	LVL	0.589	0.540	0.570	0.573	0.555
	LVHD	0.572	0.513	0.516	0.493	0.596
	HVL	0.555	0.545	0.566	0.583	0.595
	HVHD	0.547	0.531	0.643	0.562	0.595
	LC	0.547	0.557	0.543	0.545	0.548
	OC	0.565	0.547	0.558	0.574	0.550
	Basil	0.540	0.508	0.549	0.556	0.593
	Dill	0.556	0.516	0.532	0.543	0.578
	Lettuce	0.541	0.508	0.571	0.543	0.596
Standard Deviation	RWTB	0.020	0.026	0.029	0.014	0.016
	LVL	0.036	0.036	0.021	0.029	0.046
	LVHD	0.010	0.005	0.026	0.021	0.021
	HVL	0.026	0.016	0.024	0.036	0.010
	HVHD	0.021	0.006	0.126	0.020	0.015
	LC	0.017	0.090	0.010	0.012	0.057
	OC	0.026	0.196	0.021	0.047	0.058
	Basil	0.028	0.025	0.039	0.041	0.023
	Dill	0.015	0.045	0.011	0.006	0.059
	Lettuce	0.036	0.044	0.023	0.029	0.053

A-12. Phosphate Results (Study # 3)

Phosphate (PO ₄ ³⁻ P) Concentration							
Multiple Plant Species Spring Study (Experiment # 3)							
Sample Site	Plant Species	Flow Velocity (ft/s)	Plant Density (#)	Sample Date			
				4/2/2006 (mg/L)	4/23/2006 (mg/L)	5/11/2006 (mg/L)	6/2/2006 (mg/L)
Head Box	N/A	N/A	N/A	0.373	0.416	0.385	0.361
Raceway A	N/A	N/A	N/A	0.488	0.488	0.500	0.485
Raceway B	N/A	N/A	N/A	0.470	0.458	0.464	0.460
Tail Box	N/A	N/A	N/A	0.488	0.503	0.497	0.479
Experimental Channel 1	Basil	L	H	0.485	0.494	N/A	N/A
Experimental Channel 2	Dill	L	H	0.482	0.497	N/A	N/A
Experimental Channel 3	Lettuce	L	H	0.479	0.488	N/A	N/A
Experimental Channel 4	Lettuce	L	H	0.428	0.479	N/A	N/A
Experimental Channel 5	Basil	L	H	0.461	0.509	N/A	N/A
Experimental Channel 6	Dill	L	H	0.446	0.473	N/A	N/A
Experimental Channel 7	Dill	L	H	0.470	0.461	N/A	N/A
Experimental Channel 8	Lettuce	L	H	0.473	0.482	N/A	N/A
Experimental Channel 9	Basil	L	H	0.458	0.488	N/A	N/A
Experimental Channel 10	Watercress	L	H	0.455	0.509	0.416	0.394
Experimental Channel 11	Watercress	H	H	0.452	0.512	0.470	0.442
Experimental Channel 12	Watercress	L	L	0.479	0.488	0.485	0.470
Experimental Channel 13	Watercress	L	L	0.473	0.482	0.461	0.473
Experimental Channel 14	Watercress	H	H	0.494	0.521	0.473	0.433
Experimental Channel 15	Watercress	H	L	0.479	0.497	0.431	0.427
Experimental Channel 16	Watercress	H	H	0.428	0.500	0.491	0.421
Experimental Channel 17	Watercress	H	L	0.449	0.488	0.476	0.442
Experimental Channel 18	Watercress	L	H	0.455	0.527	0.449	0.470
Experimental Channel 19	Watercress	L	L	0.473	0.542	0.461	0.409
Experimental Channel 20	Watercress	L	H	0.458	0.521	0.467	0.421
Experimental Channel 21	Watercress	H	L	0.473	0.618	0.464	0.442
Long Channel 22	Watercress	L	H	0.476	0.488	0.431	0.430
Long Channel 23	Watercress	L	H	0.485	0.518	0.434	0.409
Long Channel 24	Watercress	L	H	0.458	0.536	0.473	0.470
Old Shelter Channel 25	Watercress	L	H	0.473	0.545	0.455	0.473
Old Shelter Channel 26	Watercress	L	H	0.470	0.518	0.482	0.418
Old Shelter Channel 27	Watercress	L	H	0.491	0.515	0.515	0.482
Polishing Pond	N/A	N/A	N/A	0.277	0.252	0.274	0.235
River	N/A	N/A	N/A	0.382	0.168	0.428	0.427

	Treatment Conditions	Sample Date			
		4/2/2006	4/23/2006	5/11/2006	6/2/2006
		(mg/L)	(mg/L)	(mg/L)	(mg/L)
Average Concentration	RWTB	0.482	0.483	0.487	0.475
	LVLD	0.475	0.504	0.469	0.450
	LVHD	0.456	0.519	0.444	0.428
	HVLD	0.467	0.534	0.457	0.437
	HVHD	0.458	0.511	0.478	0.432
	LC	0.473	0.514	0.446	0.436
	OC	0.478	0.526	0.484	0.457
	Basil	0.468	0.497	N/A	N/A
	Dill	0.466	0.477	N/A	N/A
	Lettuce	0.460	0.483	N/A	N/A
Standard Deviation	RWTB	0.010	0.023	0.020	0.013
	LVLD	0.003	0.033	0.014	0.036
	LVHD	0.002	0.009	0.026	0.038
	HVLD	0.016	0.073	0.023	0.009
	HVHD	0.034	0.011	0.011	0.011
	LC	0.014	0.024	0.024	0.031
	OC	0.011	0.017	0.030	0.034
	Basil	0.015	0.011	N/A	N/A
	Dill	0.018	0.018	N/A	N/A
	Lettuce	0.028	0.005	N/A	N/A

APPENDIX B. STATISTICAL ANALYSIS RESULTS

B-1. Ammonia Statistical Results (Question # 1)

Differences Between the Average Total Percent Removal of the Different Experimental Treatments within a Study

Research Study	Nutrient Constituent	Passed Normality Test? (Y/N)	Analytical Statistical Test(s) Used	Statistically Significant Difference (Y/N ; P<0.05)
Study # 1	Ammonia	Y	One-Way ANOVA	N
	Nitrate	Y	One-Way ANOVA	N
	Phosphate	N	Kruskal-Wallis	N
Study # 2	Ammonia	N	Kruskal-Wallis	N
	Nitrate	N	Kruskal-Wallis	N
	Phosphate	N	Kruskal-Wallis	N
Study # 3	Ammonia	Y	One-Way ANOVA	N
	Nitrate	N	Kruskal-Wallis	N
	Phosphate	Y	One-Way ANOVA	N

B-2. Ammonia Statistical Results (Question # 2)

Differences Between the Average Total Percent Removal of the Identical Experimental Treatments Between the Studies

Nutrient Constituent	Experimental Treatment	Passed Normality Test? (Y/N)	Analytical Statistical Test(s) Used	Statistically Significant Difference (Y/N ; P<0.05)
Ammonia	LVLD	Y	One-Way ANOVA	N
	LVHD	Y	One-Way ANOVA	N
	HVLD	Y	One-Way ANOVA	N
	HVHD	Y	One-Way ANOVA	N
	LC	Y	One-Way ANOVA	N
Nitrate	LVLD	Y	One-Way ANOVA	N
	LVHD	Y	One-Way ANOVA	N
	HVLD	Y	One-Way ANOVA	N
	HVHD	Y	One-Way ANOVA	N
	LC	Y	One-Way ANOVA	N
Phosphate	LVLD	Y	One-Way ANOVA	N
	LVHD	Y	One-Way ANOVA	N
	HVLD	Y	One-Way ANOVA	N
	HVHD	Y	One-Way ANOVA	N
	LC	Y	One-Way ANOVA	N

APPENDIX C. TRIPLICATE DATA RESULTS

C-1. Ammonia Triplicate Results (Study # 1, 2 & 3)

Ammonia (NH ₃ -N) Test Result Triplicates (Study # 1, 2 & 3)									
Sample Date	Sample Site	Trial 1		Trial 2		Trial 3		Concentration Mean	Concentration Standard Deviation
		Concentration (mg/L)	Potential (mV)	Concentration (mg/L)	Potential (mV)	Concentration (mg/L)	Potential (mV)		
6/22/2005	Raceway A	0.163	74.6	0.166	74.3	0.163	74.6	0.164	0.001
	Experimental Channel 1	0.210	69.6	0.197	70.9	0.187	71.9	0.198	0.011
	Control Channel 1	0.016	120.8	0.017	119.4	0.033	106.4	0.022	0.010
7/16/2005	Head Box	0.060	73.0	0.586	38.0	0.085	67.8	0.244	0.297
	Experimental Channel 13	0.249	51.2	0.542	39.2	0.291	48.8	0.361	0.159
	Experimental Channel 30	0.197	54.8	0.116	62.9	0.062	72.5	0.125	0.068
8/7/2005	Raceway A	0.083	96.0	0.075	98.5	0.073	98.9	0.077	0.005
	Raceway B	0.127	86.4	0.116	88.5	0.119	87.8	0.121	0.006
	Experimental Channel 16	0.036	114.9	0.028	120.6	0.029	119.9	0.031	0.004
8/27/2005	Raceway A	0.127	58.3	0.106	60.6	0.108	60.4	0.114	0.012
	Experimental Channel 21	0.154	55.8	0.148	56.3	0.138	57.2	0.147	0.008
	Experimental Channel 30	0.142	56.9	0.135	57.5	0.119	59.1	0.132	0.011
9/17/2005	Manifold	1.928	-17.0	2.191	-20.3	2.269	-21.2	2.129	0.178
	Experimental Channel 13	1.415	-9.0	1.477	-10.1	1.421	-9.1	1.437	0.034
	Experimental Channel 30	2.509	-23.8	2.538	-24.1	2.480	-23.5	2.509	0.029
10/8/2005	Experimental Channel 1	0.276	62.5	0.278	62.3	0.286	61.5	0.280	0.005
	Experimental Channel 29	0.172	75.4	0.177	74.6	0.164	76.6	0.171	0.006
	Control Channel 3	0.064	102.1	0.072	99.1	0.082	95.6	0.072	0.009
12/11/2005	Experimental Channel 13	0.162	79.7	0.159	80.0	0.159	80.1	0.160	0.002
	Experimental Channel 21	0.131	83.5	0.133	83.2	0.132	83.4	0.132	0.001
	Polishing Pond	0.125	84.4	0.126	84.2	0.124	84.5	0.125	0.001
1/8/2006	Experimental Channel 1	0.103	36.5	0.185	30.0	0.190	29.7	0.159	0.048
	Experimental Channel 7	0.061	42.4	0.063	42.0	0.061	42.4	0.062	0.001
	Experimental Channel 8	0.057	43.2	0.068	41.2	0.058	43.0	0.061	0.006
1/22/2006	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Tail Box	0.146	92.2	0.140	93.1	0.117	97.1	0.134	0.015
2/20/2006	Experimental Channel 12	0.093	102.3	0.094	102.1	0.097	101.5	0.095	0.002
	Experimental Channel 15	0.098	101.2	0.099	101.0	0.094	102.1	0.097	0.002
3/3/2006	Experimental Channel 1	0.080	30.1	0.080	30.1	0.08	30.1	0.08	0.000
4/2/2006	Experimental Channel 4	0.076	93.5	0.073	94.6	0.061	99.6	0.070	0.008
4/23/2006	Experimental Channel 9	0.053	104.6	0.055	103.5	0.054	104.1	0.054	0.001
5/11/2006	Experimental Channel 12	0.081	93.9	0.083	93.5	0.086	92.4	0.083	0.002
6/2/2006	Experimental Channel 10	0.061	105.3	0.061	105.3	0.061	105.3	0.061	0.000

C-2. Nitrate Triplicate Results (Study # 1, 2 & 3)

Nitrate (NO₃-N) Test Result Triplicates (Study # 1, 2 & 3)

Sample Date	Sample Site	Trial 1		Trial 2		Trial 3		Concentration Mean	Concentration Standard Deviation
		Concentration	Absorbance	Concentration	Absorbance	Concentration	Absorbance		
----	-----	(mg/L)	-----	(mg/L)	-----	(mg/L)	-----	(mg/L)	(mg/L)
	Raceway A	0.349	0.561	0.352	0.565	0.353	0.566	0.351	0.002
6/22/2005	Control Channel 1	0.194	0.357	0.208	0.376	0.199	0.363	0.200	0.007
	Control Channel 2	0.445	0.688	0.447	0.691	0.474	0.726	0.455	0.016
	Experimental Channel 9	0.220	0.380	0.222	0.382	0.220	0.379	0.221	0.001
7/16/2005	Experimental Channel 24	0.187	0.329	0.190	0.333	0.189	0.331	0.189	0.001
	Experimental Channel 30	0.050	0.118	0.049	0.117	0.056	0.127	0.051	0.004
8/7/2005	Experimental Channel 15	0.175	0.291	0.176	0.293	0.181	0.301	0.177	0.004
8/27/2005	Experimental Channel 10	0.335	0.527	0.328	0.517	0.324	0.510	0.329	0.006
9/17/2005	Experimental Channel 9	0.270	0.446	0.266	0.440	0.270	0.446	0.269	0.002
10/8/2005	Experimental Channel 7	-0.061	0.058	-0.059	0.060	-0.030	0.101	-0.050	0.017
12/11/2005	Experimental Channel 14	0.395	0.726	0.391	0.721	0.372	0.694	0.386	0.012
1/8/2006	Experimental Channel 3	0.340	0.592	0.147	0.232	0.339	0.590	0.275	0.111
1/22/2006	Experimental Channel 9	0.322	0.555	0.322	0.556	0.321	0.553	0.322	0.001
2/20/2006	Old Shelter Channel 27	0.385	0.703	0.383	0.699	0.377	0.688	0.381	0.004
3/3/2006	Experimental Channel 1	0.317	0.548	0.324	0.561	0.321	0.555	0.321	0.004
4/2/2006	Experimental Channel 13	0.377	0.650	0.376	0.648	0.374	0.644	0.376	0.002
4/23/2006	Experimental Channel 16	0.341	0.584	0.343	0.588	0.350	0.601	0.345	0.005
5/11/2006	Experimental Channel 18	0.382	0.671	0.382	0.671	0.380	0.668	0.381	0.001
6/2/2006	Experimental Channel 16	0.302	0.543	0.308	0.555	0.306	0.552	0.305	0.003

C-3. Nitrite Triplicate Results (Study # 1, 2 & 3)

Nitrite (NO₂-N) Test Result Triplicates (Study # 1, 2 & 3)

Sample Date	Sample Site	Trial 1		Trial 2		Trial 3		Concentration Mean	Concentration Standard Deviation
		Concentration	Absorbance	Concentration	Absorbance	Concentration	Absorbance		
		(mg/L)	-----	(mg/L)	-----	(mg/L)	-----		
6/22/2005	Raceway A	0.001	0.018	0.001	0.018	0.001	0.019	0.001	0.000
	Experimental Channel 1	0.001	0.023	0.001	0.022	0.001	0.024	0.001	0.000
	Control Channel 1	0.000	0.011	0.000	0.011	0.000	0.011	0.000	0.000
7/16/2005	Experimental Channel 12	0.005	0.039	0.005	0.037	0.005	0.037	0.005	0.000
	Experimental Channel 22	0.006	0.041	0.006	0.047	0.006	0.047	0.006	0.001
	Experimental Channel 29	0.018	0.125	0.018	0.127	0.018	0.127	0.018	0.000
8/7/2005	Experimental Channel 21	0.003	0.033	0.003	0.029	0.003	0.030	0.003	0.000
	Experimental Channel 23	0.003	0.030	0.003	0.028	0.003	0.028	0.003	0.000
	Experimental Channel 28	0.003	0.035	0.003	0.034	0.003	0.036	0.003	0.000
8/27/2005	Manifold	0.001	0.020	0.002	0.021	0.002	0.021	0.001	0.000
	Experimental Channel 5	0.006	0.053	0.007	0.060	0.006	0.056	0.006	0.000
	Experimental Channel 23	0.007	0.059	0.007	0.060	0.007	0.064	0.007	0.000
9/17/2005	Experimental Channel 2	0.004	0.034	0.003	0.029	0.003	0.029	0.003	0.000
	Experimental Channel 4	0.003	0.030	0.003	0.030	0.003	0.028	0.003	0.000
	Experimental Channel 23	0.003	0.032	0.004	0.034	0.003	0.033	0.003	0.000
10/8/2005	Experimental Channel 15	0.003	0.027	0.003	0.027	0.003	0.027	0.003	0.000
	Experimental Channel 25	0.007	0.055	0.006	0.051	0.007	0.055	0.007	0.000
	Experimental Channel 30	0.008	0.059	0.008	0.059	0.008	0.060	0.008	0.000
12/11/2005	Experimental Channel 1	0.002	0.014	0.001	0.013	0.001	0.013	0.002	0.000
	Experimental Channel 22	0.002	0.016	0.002	0.015	0.002	0.016	0.002	0.000
	Experimental Channel 24	0.002	0.016	0.002	0.015	0.002	0.015	0.002	0.000
1/8/2006	Experimental Channel 12	0.002	0.015	0.002	0.016	0.002	0.016	0.002	0.000
	Experimental Channel 16	0.002	0.014	0.002	0.015	0.002	0.014	0.002	0.000
	Experimental Channel 17	0.003	0.021	0.003	0.020	0.003	0.021	0.003	0.000
1/22/2006	Experimental Channel 3	0.001	0.008	0.001	0.009	0.001	0.009	0.001	0.000
	Experimental Channel 7	0.002	0.014	0.002	0.012	0.002	0.015	0.002	0.000
	River	0.003	0.019	0.003	0.019	0.003	0.020	0.003	0.000
2/20/2006	Experimental Channel 2	0.002	0.018	0.002	0.017	0.002	0.018	0.002	0.000
	Experimental Channel 19	0.001	0.008	0.001	0.008	0.001	0.008	0.001	0.000
	River	0.003	0.021	0.003	0.020	0.003	0.019	0.003	0.000
3/3/2006	Raceway B	0.002	0.013	0.002	0.016	0.002	0.015	0.002	0.000
	Experimental Channel 10	0.003	0.023	0.003	0.023	0.003	0.023	0.003	0.000
	Experimental Channel 16	0.005	0.033	0.005	0.034	0.005	0.033	0.005	0.000
4/2/2006	Head Box	0.001	0.006	0.001	0.006	0.001	0.006	0.001	0.000
	Polishing Pond	0.001	0.006	0.002	0.010	0.002	0.008	0.002	0.000
	River	0.003	0.021	0.003	0.020	0.003	0.019	0.003	0.000
4/23/2006	Experimental Channel 23	0.002	0.005	0.002	0.006	0.002	0.005	0.002	0.000
5/11/2006	Experimental Channel 22	0.002	0.004	0.002	0.005	0.002	0.004	0.002	0.000
6/2/2006	Experimental Channel 22	0.001	0.003	0.001	0.003	0.002	0.004	0.001	0.000

C-4. Phosphate Triplicate Results (Study # 1, 2 & 3)

Phosphate (PO_4^{3-}P) Test Result Triplicates (Study # 1, 2 & 3)

Sample Date	Sample Site	Trial 1		Trial 2		Trial 3		Concentration Mean	Concentration Standard Deviation
		Concentration	Absorbance	Concentration	Absorbance	Concentration	Absorbance		
		(mg/L)	-----	(mg/L)	-----	(mg/L)	-----	(mg/L)	(mg/L)
6/22/2005	Raceway A	0.548	0.163	0.543	0.161	0.534	0.158	0.542	0.007
	Experimental Channel 1	0.594	0.179	0.645	0.197	0.545	0.162	0.595	0.050
	Control Channel 1	0.452	0.129	0.443	0.126	0.438	0.124	0.444	0.007
7/16/2005	Experimental Channel 3	0.739	0.230	0.561	0.166	0.550	0.162	0.617	0.106
	Experimental Channel 6	0.617	0.186	0.617	0.186	0.606	0.182	0.613	0.006
	Experimental Channel 17	1.612	0.543	0.594	0.178	0.583	0.174	0.930	0.591
8/7/2005	Manifold	0.497	0.135	0.517	0.142	0.534	0.148	0.516	0.018
	Experimental Channel 3	0.503	0.137	0.508	0.139	3.748	1.285	1.587	1.872
	Experimental Channel 17	0.528	0.146	0.559	0.157	1.306	0.421	0.798	0.440
8/27/2005	Manifold	0.455	0.100	0.467	0.104	0.455	0.100	0.459	0.007
	Experimental Channel 3	0.380	0.074	0.374	0.072	0.397	0.080	0.384	0.012
	Experimental Channel 22	0.645	0.166	0.680	0.178	0.709	0.188	0.678	0.032
9/17/2005	Manifold	0.576	0.134	0.593	0.140	0.582	0.136	0.584	0.009
	Experimental Channel 7	0.650	0.160	0.650	0.160	0.647	0.159	0.649	0.002
	Experimental Channel 23	0.667	0.166	0.650	0.160	0.653	0.161	0.656	0.009
10/8/2005	Experimental Channel 10	0.777	0.205	0.749	0.195	0.771	0.203	0.766	0.015
	Experimental Channel 22	0.734	0.190	0.734	0.190	0.740	0.192	0.736	0.003
	Experimental Channel 26	0.743	0.193	0.746	0.194	0.749	0.195	0.746	0.003
12/11/2005	Experimental Channel 3	0.504	0.173	0.501	0.172	0.504	0.173	0.503	0.002
	Experimental Channel 6	0.573	0.195	0.576	0.196	0.573	0.195	0.574	0.002
	Experimental Channel 10	0.560	0.191	0.564	0.192	0.564	0.192	0.562	0.002
1/8/2006	Experimental Channel 12	0.581	0.193	0.572	0.190	0.581	0.193	0.578	0.005
	Experimental Channel 24	0.661	0.220	0.631	0.210	0.640	0.213	0.644	0.015
	Polishing Pond	0.548	0.182	0.572	0.190	0.575	0.191	0.565	0.015
1/22/2006	Raceway B	0.492	0.157	0.501	0.160	0.495	0.158	0.496	0.005
	Experimental Channel 4	0.555	0.178	0.540	0.173	0.552	0.177	0.549	0.008
	Experimental Channel 11	0.787	0.255	0.784	0.254	0.727	0.235	0.766	0.034
2/20/2006	Head Box	0.504	0.161	0.513	0.164	0.498	0.159	0.505	0.008
	Experimental Channel 10	0.477	0.152	0.474	0.151	0.477	0.152	0.476	0.002
	Experimental Channel 27	0.624	0.201	0.615	0.198	0.627	0.202	0.622	0.006
3/3/2006	Tail Box	0.525	0.176	0.537	0.180	0.555	0.186	0.539	0.015
	Experimental Channel 13	0.502	0.168	0.502	0.168	0.511	0.171	0.505	0.005
	Polishing Pond	0.502	0.168	0.505	0.169	0.508	0.170	0.505	0.003
4/2/2006	Experimental Channel 16	0.428	0.138	0.434	0.140	0.437	0.141	0.433	0.005
4/23/2006	Experimental Channel 21	0.618	0.201	0.633	0.206	0.642	0.209	0.631	0.012
5/11/2006	Experimental Channel 27	0.515	0.167	0.491	0.159	0.500	0.162	0.502	0.012
6/2/2006	Head Box	0.361	0.119	0.367	0.121	0.367	0.121	0.365	0.003

APPENDIX D. STANDARDS DATA RESULTS

D-1. Nutrient Test Standards (Study # 1)

Nutrient Test Standards							
Divergent Growth Conditions Study (Study # 1)							
		Sample Date					
		6/22/2005	7/16/2005	8/7/2005	8/27/2005	9/17/2005	10/8/2005
R ² =		0.9885	0.9513	0.9939	0.9309	0.9879	0.9978
Ammonia (NH ₃ -N)	Concentration (mg/L)	Potential (mV)	Potential (mV)	Potential (mV)	Potential (mV)	Potential (mV)	Potential (mV)
	0.01	125.4	92.2	140.5	82.4	116.3	156.3
	0.05	101.2	80.7	110.9	73.8	78.9	119.8
	0.10	88.4	71.5	95.5	68.5	55.4	90.1
	0.50	53.5	42.5	54.0	42.5	26.9	43.3
	1.00	35.1	25.6	37.7	28.0	-6.3	30.9
R ² =		0.9980	0.9993	0.9988	0.9988	0.9997	0.9937
Nitrate (NO ₃ -N)	Concentration (mg/L)	Absorbance -----	Absorbance -----	Absorbance -----	Absorbance -----	Absorbance -----	Absorbance -----
	0.00	0.120	0.029	0.024	0.024	0.034	0.160
	0.05	0.140	0.117	0.106	0.106	0.119	0.240
	0.10	0.218	0.197	0.179	0.179	0.197	0.325
	0.20	0.375	0.347	0.324	0.324	0.351	0.351
	0.50	0.790	0.838	0.807	0.807	0.797	0.817
	1.00	1.404	1.561	1.486	1.486	1.517	1.563
R ² =		1.0000	0.9999	0.9999	0.9997	0.9999	0.9999
Nitrite (NO ₂ -N)	Concentration (mg/L)	Absorbance -----	Absorbance -----	Absorbance -----	Absorbance -----	Absorbance -----	Absorbance -----
	0.000	0.004	0.000	0.002	0.003	0.007	0.003
	0.005	0.063	0.008	0.014	0.017	0.015	0.011
	0.010	0.127	0.038	0.049	0.048	0.048	0.040
	0.020	0.242	0.075	0.094	0.087	0.079	0.079
	0.050	0.585	0.345	0.446	0.388	0.363	0.368
	0.100	1.168	0.685	0.871	0.749	0.729	0.721
R ² =		1.0000	0.9997	0.9974	1.0000	1.0000	1.0000
Phosphate (PO ₄ ³⁻ -P)	Concentration (mg/L)	Absorbance -----	Absorbance -----	Absorbance -----	Absorbance -----	Absorbance -----	Absorbance -----
	0.00	-0.033	-0.039	-0.050	-0.057	-0.071	-0.071
	0.05	-0.012	-0.016	-0.024	-0.040	-0.053	-0.053
	0.10	0.006	0.001	-0.012	-0.025	-0.035	-0.035
	0.50	0.146	0.141	0.164	0.114	0.108	0.108
	1.00	0.324	0.333	0.306	0.292	0.286	0.286
	2.00	0.674	0.678	0.662	0.635	0.637	0.637

D-2. Nutrient Test Standards (Study # 2)

Nutrient Test Standards						
Multiple Plant Species Winter Study (Study # 2)						
Sample Date						
		12/11/2005	1/8/2006	1/22/2006	2/20/2006	3/3/2006
R ² =		0.9744	0.9528	N/A	0.9958	0.9284
Ammonia (NH ₃ -N)	Concentration	Potential	Potential	Potential	Potential	Potential
	(mg/L)	(mV)	(mV)	(mV)	(mV)	(mV)
	0.01	122.5	56.3	N/A	150.4	97.7
	0.05	105.4	49.8	N/A	121.2	64.7
	0.10	93.4	39.5	N/A	100.2	30.7
	0.50	60.2	19.7	N/A	64.2	21.9
	1.00	43.5	8.8	N/A	48.1	14.4
R ² =		0.9971	0.9889	0.9982	0.9958	0.9985
Nitrate (NO ₃ -N)	Concentration	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance
	(mg/L)	-----	-----	-----	-----	-----
	0.00	0.161	0.000	0.000	0.002	0.001
	0.05	0.244	0.014	0.045	0.068	0.054
	0.10	0.333	0.052	0.098	0.103	0.102
	0.20	0.435	0.456	0.345	0.432	0.350
	0.50	0.825	0.880	0.898	0.905	0.880
	1.00	1.615	1.796	1.796	1.850	1.806
R ² =		0.9999	0.9999	0.9998	0.9996	0.9997
Nitrite (NO ₂ -N)	Concentration	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance
	(mg/L)	-----	-----	-----	-----	-----
	0.000	0.001	0.002	0.002	0.001	0.002
	0.005	0.010	0.008	0.008	0.008	0.006
	0.010	0.040	0.036	0.037	0.035	0.036
	0.020	0.072	0.070	0.073	0.081	0.073
	0.050	0.344	0.340	0.354	0.364	0.345
	0.100	0.693	0.668	0.730	0.755	0.715
R ² =		0.9999	0.9999	0.9998	0.9998	0.9999
Phosphate (PO ₄ ³⁻ -P)	Concentration	Absorbance	Absorbance	Absorbance	Absorbance	Absorbance
	(mg/L)	-----	-----	-----	-----	-----
	0.00	0.011	-0.005	-0.005	-0.005	-0.003
	0.05	0.027	0.013	0.012	0.012	0.014
	0.10	0.042	0.035	0.030	0.030	0.035
	0.50	0.175	0.163	0.155	0.155	0.166
	1.00	0.334	0.333	0.322	0.322	0.339
	2.00	0.649	0.675	0.660	0.660	0.673

D-3. Nutrient Test Standards (Study # 3)

Nutrient Test Standards					
Multiple Plant Species Spring Study (Study # 3)					
		Sample Date			
		4/2/2006	4/23/2006	5/11/2006	6/2/2006
	R ² =	0.9711	0.9799	0.9685	0.9973
Ammonia (NH ₃ N)	Concentration (mg/L)	Potential (mV)	Potential (mV)	Potential (mV)	Potential (mV)
	0.01	140.1	141.3	140.2	143.1
	0.05	114.2	115.3	120.3	112.6
	0.10	88.6	88.6	90.5	94.5
	0.50	46.7	46.0	44.1	61.2
	1.00	12.4	18.6	20.4	42.5
Nitrate (NO ₃ N)	Concentration (mg/L)	Absorbance -----	Absorbance -----	Absorbance -----	Absorbance -----
	0.00	0.000	0.000	0.003	0.001
	0.05	0.048	0.048	0.056	0.066
	0.10	0.099	0.099	0.108	0.125
	0.20	0.342	0.342	0.360	0.375
	0.50	0.875	0.875	0.888	0.912
Nitrite (NO ₂ N)	Concentration (mg/L)	Absorbance -----	Absorbance -----	Absorbance -----	Absorbance -----
	0.000	0.000	0.000	0.000	0.000
	0.005	0.003	0.002	0.001	0.002
	0.010	0.030	0.027	0.020	0.022
	0.020	0.068	0.066	0.059	0.060
	0.050	0.342	0.339	0.345	0.352
Phosphate (PO ₄ ³⁻ P)	Concentration (mg/L)	Absorbance -----	Absorbance -----	Absorbance -----	Absorbance -----
	0.00	-0.001	-0.001	-0.001	-0.002
	0.05	0.010	0.010	0.010	0.012
	0.10	0.035	0.035	0.035	0.041
	0.50	0.148	0.148	0.148	0.153
	1.00	0.340	0.340	0.340	0.342
Phosphate (PO ₄ ³⁻ P)	Concentration (mg/L)	Absorbance -----	Absorbance -----	Absorbance -----	Absorbance -----
	2.00	0.655	0.655	0.655	0.661

APPENDIX E. AQUAPONICS RESEARCH PHOTOGRAPHS

E-1. West Virginia University's Reymann Memorial

E-2. Aquaculture Shelter, Greenhouse & Polishing Pond

E-3. Aquaculture / Aquaponics Shelter

E-4. Aquatic Phytoremediation Greenhouse

E-5. Polishing Pond / Aquaponic Effluent Discharge

E-6. Moore's Run / Polishing Pond Discharge

E-7. Aquaculture Raceways

E-8. Greenhouse Aquatic Phytoremediation Channels

E-9. Aquaculture Operation Head-box

E-10. Aquaculture Operation Quiescent Zone

E-11. Aquaculture Operation Tail-box

E-12. Aquaculture Operation Raceway Segment Spillway

E-13. Aquaponic System Tank

E-14. Aquatic Phytoremediation Channel Group

E-15. Aquatic Phytoremediation Channel Group