Effects of Hydraulic Retention Time on Nutrient Film Technique Lettuce Production in a Decoupled Aquaponics System

by

Nathan Wallace-Springer

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Approved by

Daniel E. Wells, Chair, Assistant Professor of Horticulture
Jeremy M. Pickens, Assistant Research and Extension Professor of Horticulture
Joseph M. Kemble, Extension Specialist Professor of Horticulture

Abstract

A series of 28-d hydroponic and aquaponic experiments were conducted at Auburn University from April 2019-March 2020 to determine the effects of limited nitrogen (N) and hydraulic retention time (HRT) on 'Rex' butterhead lettuce using nutrient film technique (NFT), respectively. PROC GLIMMIX was used to conduct an analysis of variance on all responses using SAS version 9.4. Hydroponic experiments analyzing the effects of limited N on lettuce growth observed N treatments to be statistically different in terms of size 7 days after planting (DAP). After 28 DAP, average size index was observed to decrease 12%, from 20.4in to 18.3in as N was decreased from 150 ppm N to 50 ppm N. Plant fresh mass was linear between treatments, ranging from 189.7 to 241.7g, with treatment 125 ppm N yielding the highest fresh mass on average at 241.7g. Dry mass of plants showed treatment 150 ppm N had a similar dry mass to treatment 125 ppm N, indicating the difference in treatment mass was due to higher water absorption by treatment 125 ppm N. Once N fell below 125 ppm, plant growth suffered in all treatments. These results suggest a target value between 125-150 ppm N is better suited for lettuce growth.

Aquaponic experiments analyzing the effects of HRT on lettuce growth observed shorter HRT intervals (4d) exhibited better growth characteristics, producing more biomass and longer roots, over plants grown in longer HRT intervals (16d). After the initial experiment, iron supplementation was determined necessary to further evaluate HRT. In trial one without iron supplementation, aquaponic fresh mass and SPAD exhibited negative linear and quadratic trends 28 DAP respectively, decreasing 41% and 143%, from 203.43g to 143.81g and 18.7 to 7.6, as HRT increased from 4d to 16d. Foliar analysis revealed all HRT treatments absorbed excessive amounts of micronutrients. Shorter HRT intervals absorbed more micronutrients when compared to longer HRT intervals, with treatment 4d accumulating double the amount of Mn and Zn as

treatment 16d. In trial two experiments with iron supplementation, analysis of lettuce SPAD and size index found plants became statistically different in terms of color and size 14 DAP, and by 28 DAP, treatment 4d was observed to have the lowest SPAD average and largest size index average. Plant fresh mass decreased by 10%, from 162.25g to 147.09g, as HRT was increased from 4d to 16d. Analysis of water variables showed average nitrate and pH values increased as HRT increased from 4d to 16d, from 373 mg L^{-1} to 404 mg L^{-1} nitrate and 6.94 to 7.25 pH. Although average nitrate concentrations were higher for the longer HRT intervals, foliar analysis showed plant N% decreased in longer HRT intervals. Iron supplementation eliminated iron deficiencies in plants up to 14 DAP, but by 28 DAP HRT treatments were observed to be iron deficient along with the elements magnesium, calcium, boron, and copper. However, in contrast with trial one experiments, iron supplementation was observed to considerably reduce the uptake of the divalent cations manganese and zinc in plant tissues. Our findings suggest that smaller quantities of nutrients may be able to grow plants in aquaponics provided that faster hydraulic retention times are used and all essential nutrients are of high enough concentration.

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Chapter One: Literature Review

1.1 Introduction to Aquaponics

Aquaculture is currently one of the fastest growing food-producing sectors worldwide, and now accounts for 50% of the world's total fish and fish related products according to the United Nations Food and Agriculture Organization (FAO) [1,2]. Aquaponics is a system that seeks to integrate fish production with a hydroponic plant component to maximize resource use efficiencies and minimize negative environmental impacts [1,3–5]. In this developing system, aquaculture fish excrete waste in the form of ammonia (NH_3), which nitrifying bacteria convert into nitrate (NO_3^-), the predominant form of nitrogen demanded by plants. In most systems, this nutrient-rich water is then used to fertilize plants for production, where it is filtered, before being recirculated back to fish production or discharged to the environment [2–4,6–8].

The role of aquaponics and aquaculture systems are increasingly important, because in the 21st century the world will face many ecological challenges resulting directly from human population increase and climate change [1,9]. Currently, the global human population exceeds more than 7.6 billion, but this number is expected to rise to 8.5 billion by 2030, and 9.8 billion by 2050 with more than 75% of people expected to be living in urban areas [4,9]. Accompanied with this population increase will be an increase in demand placed on already stressed food, water, and energy resources needed to sustain its growth [1]. However, conventional agricultural systems are already constrained by diminishing arable land, declining freshwater resources, and increasing soil degradation [9,10]. Synthetic fertilizers, are non-renewable and can be environmentally damaging when improperly managed. Conventional fertilizer practices are not projected to meet the future demands of agriculture [9]. New agricultural systems that offer sustainable food production are required to meet these demands. Aquaponics offers a valid solution to this problem as it can provide food security, minimize the use of non-renewable resources, and produce multiple products at one time. Moreover, it has the potential to generate a high crop yield on a variety of plants, such as lettuce, spinach, basil, tomatoes, cucumbers, and bell peppers using limited land and water, and can be adapted to arid regions or metropolitan cities that need it the most [3–5,9,10].

1.2 Aquaponic Description/System Type

Aquaponic systems consist of five key elements: (1) a container tank to grow aquatic organisms, (2) a clarifier to remove system waste in the form of particulate matter, sludge, or feces, (3) a biofilter to convert toxic ammonia released by fish into a less toxic form, nitrate, that plants can use, (4) a hydroponic aspect that plants are grown in, and (5) a sump pump where water is collected and can be returned to the container tank [3]. A primary concern of these systems is water quality, which can be broken down into five major components: pH, nutrients, alkalinity, gas, and organic matter. Because plants, fish, and bacteria each operate more effectively at different pH levels and nutrient concentrations, which are directly affected by water alkalinity and organic matter particles in the water, compromises in the system must be made to optimize growth for all. This has led to the creation of coupled and decoupled aquaponic systems.

Coupled

At their core, coupled aquaponic systems or recirculating aquaponic systems (RAS), pass nutrient-rich effluent water from fish to plants, before recycling back to fish. However, due to the separate nature of aquaponic elements, distinct variations of RAS systems exist. For instance, some systems may convert NH_3 to NO_3^- in separate biofilters away from fish, while other systems choose to co-culture bacteria alongside of fish, such as in bio floc RAS systems. Apart from these variations, benefits associated with coupled aquaponic systems include increased recycling of water and nutrients to plants, increased water-use efficiencies, and the potential to reduce environmental impact [11–13]. Nonetheless, these systems do have several disadvantages associated with them as well. First, coupled systems may be more difficult to manage than decoupled systems. In coupled systems, all sub-systems must be properly managed simultaneously as problems in one stage could inadvertently put the whole system in jeopardy. Second, optimal growing conditions must be compromised between system components as plants require a pH of 5.5–6.5 to grow most efficiently, whereas nitrifying bacteria that are used to covert toxic NH_3 to NO_3^- are most effective at a pH $\geq$ 7.0. Subsequently, this can lead to a decrease in total yield. As a result, current literature suggests that RAS be maintained at a pH of 6.5-7.5 to achieve higher productivity [14,15].

Decoupled

Decoupled systems are split up into separate functional units where individual water cycles may be controlled independently [12]. Water is still recirculated between fish and bacteria; however, a portion of that water is used to irrigate crops and is not returned to the aquaculture unit. This can provide different benefits to aquaponic growers, as it allows for the adjustment of water pH to match plant requirements prior to crop irrigation, has higher nutrient concentrations than found in recirculating systems, and offers growers the potential to use pesticides or other applications on grown plants [16]. Additionally, due to the separateness of the system, one sub-system failing may not result in total system failure. Disadvantages associated with decoupled systems include a lower water-use efficiency and the potentially greater environmental impact compared to RAS as they increase nutrient effluent discharge. Increased water usage may not be applicable in all locations, such as arid regions. These factors should be considered before selection or construction of operating systems.

1.3 Hydroponic Systems

Current hydroponic systems used in aquaponics can be broken down into two distinct groups: systems that operate with substrate media and those that operate without. In substrate-based systems, a substrate is selected that anchors the plant's roots. Conversely, systems without substrate supply plants with nutrients via water-based techniques. Examples of these systems include nutrient film technique (NFT) and deep-water culture (DWC). Choice of hydroponic systems for aquaponics can vary based on life cycle assessment or perceived advantages over other systems [17].

Nutrient Film Technique (NFT)

Nutrient film technique (NFT) is a soilless hydroponic technique in which a continuous shallow stream of water carrying all essential plant nutrients flows down a channel or gully, where it comes in to contact with plant roots before making its way to a nutrient reservoir and is recirculated. Channels are set to a slight slope, usually 1-4%, to promote drainage by gravity and minimize pump usage [18]. Root aeration is ensured by providing a relatively large volume of air inside the channel chamber. In terms of aquaponics systems, water effluent serves as the main source of nutrients for crops. NFT systems are more suitable for smaller vegetation, such as lettuce, spinach or other leafy greens and herbs because channels can become clogged by plants that have larger root structures or by unfiltered aquaculture wastes [19]. However, due to their capital cost, NFT systems are less prevalent in commercial aquaponics [17].

Deep Water Culture (DWC)

Deep water culture (DWC) is a method of growing plants over a water solution, usually 8 to 24 inches deep. In this system, plants are placed onto a floating sheet, commonly constructed out of high density foam insulation boards and moved over a nutrient solution where plant roots are submerged in the nutrient solution [12,19]. Water is then slowly recirculated through this system and aeration is provided to ensure that the plant roots get adequate oxygen. In DWC, system root aeration can take place by two forms: active or passive. Active aeration is achieved by bubbling air into the nutrient solution through an air stone or by circulating the solution. Conversely, passive aeration is achieved by leaving air space between the sheet that supports plants being grown for plant roots to take up oxygen [19]. In aquaponic systems, the nutrient solution consists of the nutrient effluent from fish. DWC is frequently used in aquaponics because it allows for plants to absorb nutrients directly from the water and is not as prone to clogging as larger particles can fall to the bottom of the system to be removed [20].

Substrate-Based Culture

Substrate based aquaponic systems are used for the purpose of cultivating plants, in which nutrient-rich aquaponic effluent water is administered using drip irrigation to a growing media. Once transplanted in a substrate, the substrate in turn provides support for plants, encourages microbial community growth, and allows for increased filtration [12,17,21]. Moreover, it has the ability to filter out particle waste that may not have been removed during earlier aquaponic processes. Common substrate-based systems used in aquaponic include coco coir, perlite, rockwool, pine bark, or various expanded clay mixtures [12].

1.4 Significant Aquaponic Factors

Nutrients

Fish, plants, and bacteria require essential nutrients needed for growth and development. In aquaponic systems, this is supplied in the form of fish feed, which is composed of proteins, fats, fibers, ash minerals, and micronutrients. As feed is digested by fish, it is broken down into various components before being excreted in the form of waste and available for plants to use. Plants require 16 Macro and Micronutrients needed to facilitate healthy plant growth: Carbon (C), Oxygen (O), Hydrogen (H), Nitrogen (N), Phosphorus (P), Potassium (K), Sulfur (S), Calcium (Ca), Magnesium (Mg), Molybdenum (Mo), Iron (Fe), Copper (Cu), Manganese (Mn), Chlorine (Cl), Boron (B), and Zinc (Zn), the availability of which can be affected by water temperature, nutrient concentration, plant evapotranspiration, and pH [17,20–22]. Of these elements, N, Fe, K, and Ca can be variable or insufficient in quantity for aquaponic systems. These nutrients can be supplemented in different ways to meet the needs of the plants. For example, calcium can be added in the form calcium hydroxide ($Ca(OH)_2$) to counter the acidifying effects of fish waste, uneaten feed, and nitrification. Potassium or Fe can be added to water in the form KCl or chelated Fe to prevent nutrient deficiencies in plants [11]. Irrigation frequencies that are sufficient to prevent water stress may not be adequate to prevent nutrient deficiencies [4]. Periodic water quality analysis is necessary for aquaponic systems to verify water element compositions to maximize growth conditions of plants and fish.

TAN/Nitrogen

Nitrogen is an important element that must be monitored in aquaponic systems. It is vital for both plant and fish production and can be toxic to fish depending on its concentration or form. Plants and fish in turn use nitrogen to make deoxyribonucleic acid (DNA), ribonucleic acid (RNA), amino acids, protein, and other cell components [20]. The main source of nitrogen in aquaponic systems is fish feed, where more than 90% is excreted by fish in the form of ammonia [20]. In water, ammonia can exist in two forms: unionized ammonia (NH3) or ionized ammonium (NH4+). Together these two forms are referred to as Total Ammonium Nitrogen (TAN), written with the equilibrium reaction equation $NH_4 \leftrightarrow NH_3 + H^+$. The production rate of TAN can be approximated using the equation: pTAN = (F x PC x 0.092)/T; where pTAN represents the production rate of TAN (kg day^{-1}), F represents the feeding rate per day (kg day^{-1}), PC represents the protein content of fish feed, 0.092 represents the fraction of excreted TAN per protein input, and T represents 1 day [20]. It is important to monitor TAN, because unionized ammonia is toxic to fish at very low concentrations (0.05 mg L^{-1}), which can lead to convulsions, loss of equilibrium, lethargy, coma or death in fish [6,15]. To avoid accumulation of unionized ammonia, nitrification bacteria are used in aquaponic systems to convert ammonia into nitrate (NO_3^-), a form that is not toxic to fish except in high concentration [6,15,20,21]. In this process, ammonia-oxidizing bacteria (AOB) (e.g. *Nitrosomonas, Nitrosococcus, Nitrobacter, Nitrosolobus* sp., etc.) in the water are first used to oxidize ammonia into nitrite. The resulting nitrite is then converted to nitrate by nitrite oxidizing bacteria (NOB) (e.g., *Nitrobacter, Nitrococcus, Nitrospira, Nitrospina* sp., etc.) and then supplied to plants via water effluent [6,20].

Water Quality, pH, & Temperature

Water quality, pH, and temperature affect many parameters of aquaponic systems, including alkalinity, buffer capacity, electrical conductivity, nutrient availability, element toxicity, dissolved oxygen (DO), dissolved carbon dioxide (DOC), TAN, and the optimal growing conditions in which fish, plants, and nitrifying bacteria operate [11,13]. A primary challenge for aquaponics growers is balancing the needs of various organisms because each operate more efficiently under different conditions. For example, nitrifying bacteria have an optimal pH around 8.5, production crops have an optimal pH range of 5.5-6.5, and fish have an optimal pH range from 7.0-8.0 [23–25]. It is therefore likely that maximizing one-unit process requires compromising another unit process. Moreover, since pH is dynamic and constantly changing, its management requires constant attention. Additionally, water quality attributes such as alkalinity and TAN can influence other chemical characteristics such as nutrient availability and toxicity [26].

1.5 Environmental Sustainability

The concept of sustainability in aquaponics is linked to its use efficiencies of water, land, energy, and nutrients. Okemwa [4] defines this sustainability as systems that do not compromise the long-term viability of agricultural resources to meet present demands [4]. Aquaponics closely fits this definition, as it seeks to integrate aquaculture fish production with hydroponic plant production in order to maximize the efficiency of resources and minimize the environmental impact in a closed or semi-closed loop [15,27]. Nonetheless, aquaponics still has environmental concerns associated with it. Three current environmental concerns of aquaponics are: (1) the production of greenhouse gases, (2) the rapid discharging of water, and (3) the high use of electricity.

Due to high stocking densities of fish, aquaponic systems require air or oxygen to be pumped into water to support fish. However, dissolved oxygen in fish tanks is not always distributed uniformly, which can lead to the creation of anoxic zones. As oxygen becomes limited, denitrification begins to occur, in which nitrate is converted into nitrogen gas (N2). Two important byproducts of this process are nitrous oxide (N_2O) and methane (CH_4), both of which are potent greenhouse gasses that have warming potentials of 296 and 84 times that of carbon dioxide respectively [20,21,28]. According to Hu et al. [28], roughly 2% of current global N_2O emissions are generated from aquaculture systems and they estimate that this number could rise to 5.72% of global anthropogenic N_2O by 2030.

Another concern for aquaponics is the discharge of nutrient-rich effluent wastewater [29,30]. Aquaponic systems generate high amounts of system wastes in the form of suspended solids, particulate matter, sludge, and feces along with dissolved nitrates [19]. In order to maintain water quality, these systems discharge effluent and replace it with fresh water at varying rates ranging from 5-10% of the total recirculating water volume per day [17,31]. The reduction of water input and the treatment of this effluent water are equally significant, because water containing ammonia discharged into the ecosystem has the potential to cause eutrophication and other environmental hazards [11,17,32].

Finally, large-scale aquaponic systems consume large amounts of electricity as most always require pumps and motors to be run to prevent anoxia and fish fatality. Consequently, this requires constant power, which indirectly contributes to increased greenhouse gas emissions and increases the overall carbon footprint associated with each facility [30]. Properly designing and managing aquaponic systems to minimize these concerns has the potential to maintain the long-term sustainability of these enterprises [15,31].

1.6 Hydroponic Challenges in Aquaponics

To date, most aquaponic studies have focused exclusively on the commercialization of aquaculture systems, while few have focused on maximizing hydroponic systems. As a result, literature on the plant production side of aquaponics is limited [9,10].

According to Wahyuningsih, Effendi, and Wardiatno [6], one challenge for hydroponic systems is addressing the accumulation of system waste, such as particulate matter, sludge, or feces throughout a system. If not properly maintained, the authors state system waste can create blocks in the system that can lead to reduced water flow, unequal watering of plant crops, and water quality. Similarly, Endut et al. [2] found these system wastes can also hinder plant growth if not adequately addressed.

Comparatively, studies by Rakocy et al. [33], Saha, Monroe, and Day [9], and Pinho et al. [34] showed that management of available nutrient loads, specifically nitrogen and iron, are another challenge for hydroponic systems [33]. The total nitrogen available for plant production in a fish culture system is directly related to the number of stocked fish in the system, the protein content of the selected fish feed, and the feeding rate [2]. However, of initial nitrogen inputs, less than 1/3 of nitrogen inputs may ultimately become available for plant production as roughly ~35% of nitrogen is absorbed by fish tissues, ~18% is lost as uneaten feed, and ~13% becomes trapped as solid waste [35]. Moreover, Wongkiew et al. [20] observed that these percentages can fluctuate based on types of fish, seasonal weather, or water pH. As a result, the relative amount of nutrients made available in aquaponics tends to differ from the amount of nutrients supplied in normal hydroponic systems [2,12]. These results were also in agreeance with the prior studies, Silberbush and Ben-Asher [36] and Endut et al. [2], who reported that disproportionate accumulation or reduction rates can lead to inadequate concentrations of nutrients being supplied to plants that may cause nutrient deficiencies, toxic salt accumulation, or plant death if not checked routinely [2,13].

Lastly, Okemwa [4] noted that another challenge for these hydroponic systems is that irrigation frequencies that are sufficient to prevent water stress in plants may not be adequate to prevent nutrient deficiencies. Periodic water quality analysis is necessary to verify that nutrients are in sufficient ranges to maximize plant growth. The author proposed that more frequent flushing of media at lower nutrients concentrations may be able to counteract nutrient depletion between waterings, increasing nutrient use efficiency. Thus far, this hydraulic retention time strategy has not yet been evaluated.

1.7 NFT Challenges in Aquaponics

In a review of aquaponic studies that focused on hydroponic components, Maucieri et al. [17] found that from 1997-2017 research has favored deep water culture (43% of publications) and substrate-based (33% of publications) systems over NFT (17% of publications) system designs, with lettuce (*Lactuca sativa*), tomato (*Solanum lycopersicum*), and water spinach (*Ipomea aquatica*) selected as the most frequently used research crops. Moreover, this review found that comparative studies between different system types are also scarce (9% of publications), which the authors maintain pose a dilemma for commercial operations as commercial operators more often use NFT while researches more often use growth beds. Nevertheless, they concluded that NFT appears be an appropriate system design for aquaponics due to its low capital cost and ease of use, but future studies should be conducted on NFT in commercial settings under realistic conditions.

In a comparative study utilizing DWC, substate-based, and NFT systems, Lennard and Leonard [37] found NFT systems to be less efficient in terms of overall nutrient removal and nitrogen removal efficiency and to have smaller yield comparatively [37]. Similarly, Schmautz et al. [38] found this to also be the case in their study comparing aquaponic tomato yield in NFT, DWC, and substate-based systems. However, the authors presented one possible explanation for this may be due to the fact DWC and substate-media based systems have their entire roots in contact with either water or soil which allows them to assimilate more nutrients, whereas NFT systems are more restricted by narrow channels of water flow that limit a plant’s ability to uptake nutrients. Yet, due to the limited literature conducted on NFT systems, additional research is needed to expand on these results.

1.8 Conclusion

At present, aquaponics has the potential to offer a practical solution to real world issues. Available literature conducted thus far has shown it can increase food security and minimize use of non-renewable resources while able to grow a variety of products. Nonetheless, the majority of prior aquaponic studies have focused on the commercialization of aquaculture systems rather than the maximization of hydroponic systems. For this reason, there is a need for additional research into the plant production side of aquaponics. Equally important, future research should begin to align research objectives to match how commercial aquaponic enterprises are run to establish this industry. To date, however, this has been shown not to be the case as commercial operators continue to opt for NFT system designs while researchers have been shown to prefer DWC or substate-based systems. Thus, in order to make NFT systems a more viable aquaponic option, a comprehensive study is needed to address the lower yields of NFT systems observed in prior literature studies. Attempting to fill in the absence of knowledge from literature, this research intends to analyze the effects of hydraulic retention time on NFT lettuce production in a decoupled aquaponic system to determine: 1) can plant production be improved in NFT aquaponics by exchanging water more frequently and 2) if so, what is the optimal water exchange frequency for NFT aquaponics.

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Chapter Two: Evaluating the Effects of Limited Nitrogen on Hydroponic NFT Lettuce Production (*Lactuca sativa)* for Aquaponic Assessment

2.1 Abstract

A series of 28-day hydroponic experiments were conducted at Auburn University during the spring and summer months of 2019 to evaluate the effects of limited nitrogen (N) on nutrient film technique (NFT) 'Rex' butterhead lettuce (*Lactuca sativa*) production. PROC GLIMMIX was used to conduct an analysis of variance on all responses using SAS version 9.4. Average lettuce size was observed to be statistically different between N treatments by 7 days after planting (DAP). After 28 DAP, average size index was observed to decrease 12%, from 20.4 in to 18.3 to in as N was decreased from 150 ppm N to 50 ppm N. Plant fresh mass was linear between treatments, ranging from 189.7 to 241.7g, with treatment 125 ppm N yielding the highest fresh mass on average at 241.7g. Dry mass of plants showed treatment 150 ppm N had a similar dry mass to treatment 125 ppm N, indicating the difference in treatment mass was due to higher water absorption by treatment 125 ppm N. Once N fell below 125 ppm, plant growth suffered in all treatments. These results suggest a target value between 125-150 ppm N is better suited for lettuce growth.

2.2 Introduction

Hydroponics is the cultivation of plants using a complete nutrient solution to promote optimal plant growth in a soilless environment or substrate medium [1,2]. In general, there are three main types of hydroponic systems: deep water culture (DWC), substrate-media culture (SMC), and nutrient film technique (NFT). These systems offer a variety of benefits over other plant production practices, such as higher rates of production, improved crop quality, lower water requirements, reduced area required for production, and the potential for year round production

[2–4]. Conversely, aquaponics is a system of aquaculture production that seeks to integrate a hydroponic plant component in order to maximize resource use efficiencies and minimize negative environmental impacts associated with conventional fish production [5–8]. In this system, ammonia (NH_3) excreted by fish is captured and converted into nitrate (NO_3^-), the predominant form of nitrogen demanded by plants. This nitrate-rich water is then used to fertilize plants for production, where it is filtered and recirculated back to fish production or discharged to the environment [6,7,9–12].

However, to date, most aquaponic studies have focused exclusively on the commercialization of aquaculture systems, while few have focused on maximizing hydroponic components. In a review of those aquaponic studies that focused on hydroponic components, Maucieri et al. [13] found that from 1997-2017 research has disproportionately favored deep water culture designs (43% of publications) and substrate-based (33% of publications) systems over NFT (17% of publications) system designs [13]. This presents a problem, because of hydroponic system types, NFT systems are the most widely used to grow leafy green vegetables, most notably lettuce [2,14].

Lettuce is a cool-season, leafy-green vegetable that belongs to the plant family Asteraceae and annually accounts for more than 26 million tons of vegetable production [4,15,16]. Lettuce is only second behind the Irish potato in terms of land allotted to production and crop value in the United States, [4]. Quality and yield of lettuce production are both dependent upon the supply of essential nutrients, which has been shown to be a challenge in aquaponic systems [17–19]. For example, the element nitrogen can fluctuate in aquaponic systems due to a variety of factors, such as the type or number of stocked fish in the system, the protein content of the selected fish feed, and the feeding rate [9,18,19]. Nitrogen is also of particular importance to plants as it is required in the largest quantities and is an essential component of amino acids, proteins, and enzymes that make up DNA and RNA [17]. However, Neto and Ostrenksky [20] estimate that less than 1/3 of nitrogen inputs may ultimately become available for plant production in aquaponic systems as roughly ~35% of nitrogen is absorbed by fish tissues, ~18% is lost as uneaten feed, and ~13% becomes trapped as solid waste [20]. Nitrogen, more specially nitrate, has been shown to affect plant taste and quality [21,22], Therefore, it is necessary to continue to evaluate lettuce from the practical standpoint of enhancing production. The objective of this research was to study how lettuce responds to reduced levels of N under normal hydroponic conditions to serve as a baseline assessment for aquaponic research where literature has reported lower quantities of nitrogen.

2.3 Materials and Methods

Germination

‘Rex’ butterhead lettuce seeds (*Lactuca sativa* ‘Rex’; Johnny’s Selected Seeds) were sown in 5 flats of OASIS® horticubes (OASIS® Grower Solutions, Kent, Ohio) (2.54 cm ×3.18 cm × 3.81 cm). Flats were covered with clear plastic humidity domes until seedling emergence in a greenhouse at Auburn University (32° N, 85° W). After emergence, humidity seedlings began a fertilizer regiment containing 150, 80, 200, 150, and 35 mg• L-1 N, P, K, Ca, and Mg, respectively, from water-soluble 8N-6.5P-30K (Gramp’s Original Hydroponic Lettuce Fertilizer, Ballinger, TX), calcium nitrate (15.5N-0P-0K), and magnesium sulfate (10% Mg) for two weeks before 375 plants were transplanted into the NFT system.

Experimental Design

The experiment utilized a completely randomized block design, containing five blocks that were each comprised of five 4-m NFT channels (FarmTek, Connecticut, USA) Each channel held 15 plants spaced 20 cm apart and were supplied with a nutrient solution from one of five 113-liter treatments (Figures 2.1-2.3). Measurements were conducted on the middle 8 plants of each channel (n=40 per treatment). Treatments consisted of the five diminishing nitrogen rates 150 ppm N, 125 ppm N, 100 ppm, 75 ppm N and 50 ppm N with all other nutrients kept constant. pH was maintained between 5.8-6.2 using citric acid. The nitrogen rate 150 ppm N was selected as the starting point based on industry hydroponic lettuce usage as well as Cornell University literature guide [23]. Other rates were chosen to simulate reduced nitrogen levels that can be found in aquaponic systems.

Plant Measurements

Size index (SI) ([height + widest width + perpendicular width]/3) and SPAD chlorophyll were taken for the duration of the experiment once every seven and fourteen days, respectively, using standard ruler and a SPAD-502 meter (Spectrum Technologies Aurora, IL). Measurements were conducted on the middle eight plants from each channel (n=40 per treatment). Twenty-eight days after planting fresh mass, and root length were recorded. Dry mass was recorded after seven days in a forced air-drying oven at 75.5 C and root-shoot ratio and water composition were calculated.

Water Measurements

Nitrate concentration, electrical conductivity (EC), pH, and temperature were monitored and recorded four times a week from nutrient reservoirs for the duration of the experiment using a LAQUA twin NO3-N meter (Horiba, Kyoto, Japan), and a HANNA Instrument meter (Model HI 9813).

Figure 2.1: Hydroponic NFT Experimental Design

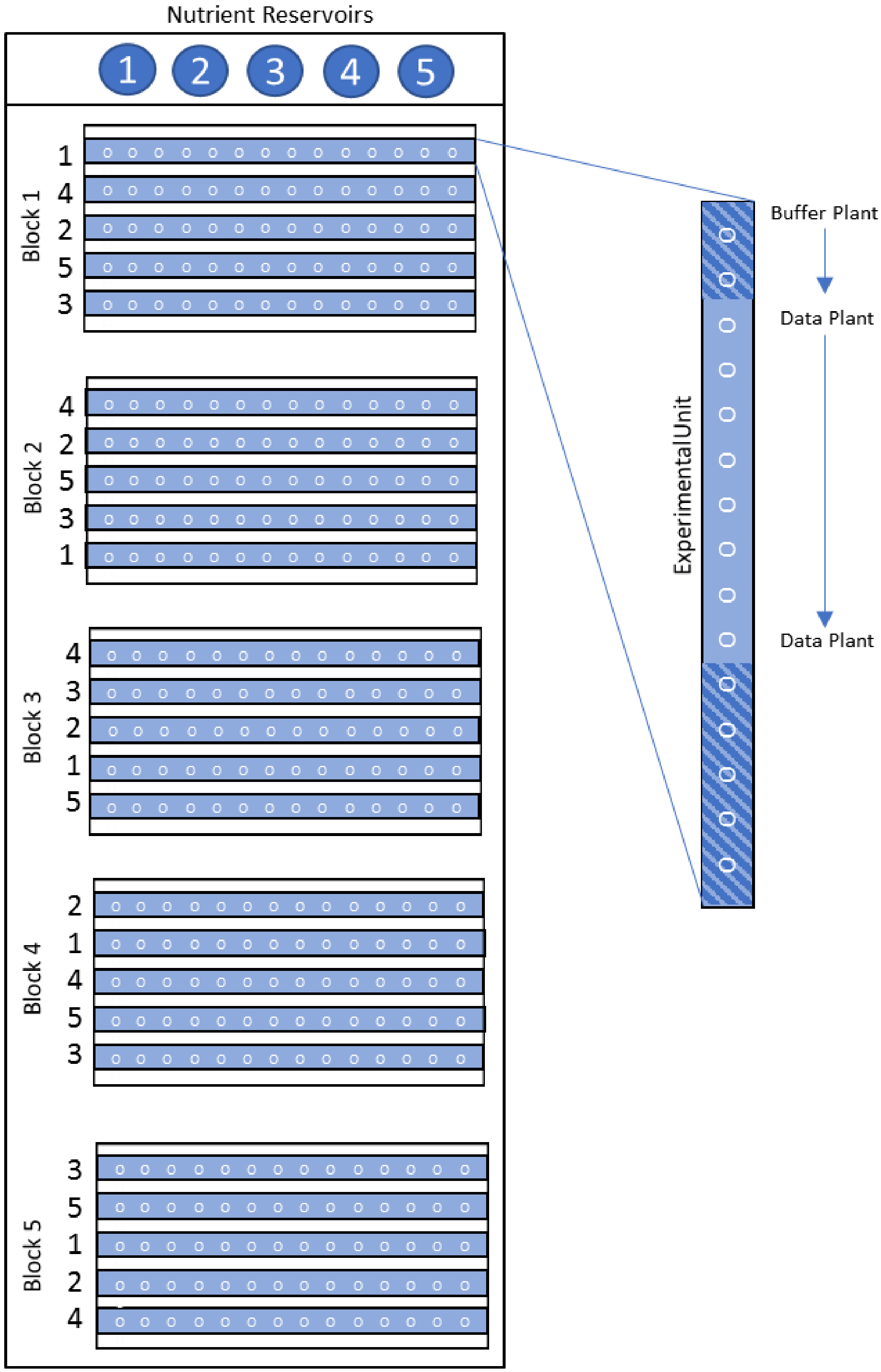


Figure 2.2: Hydroponic NFT System Flow

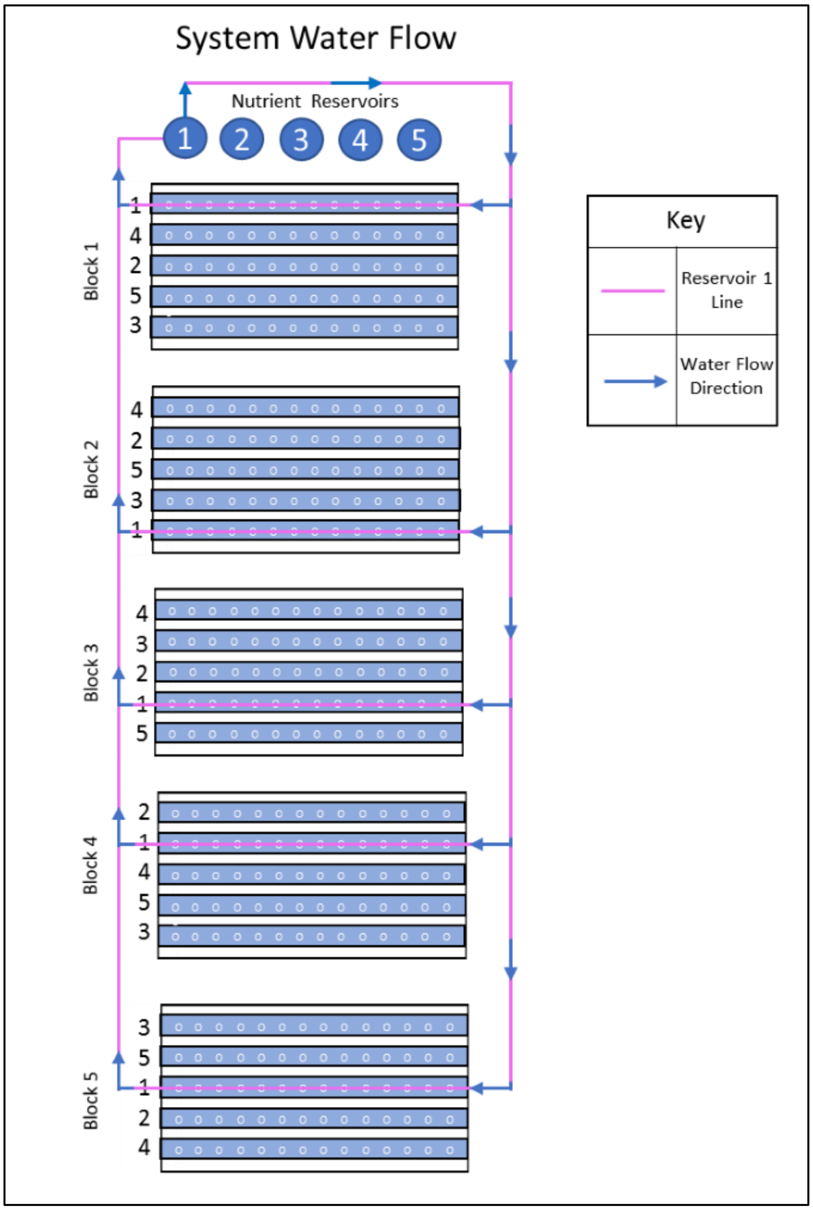
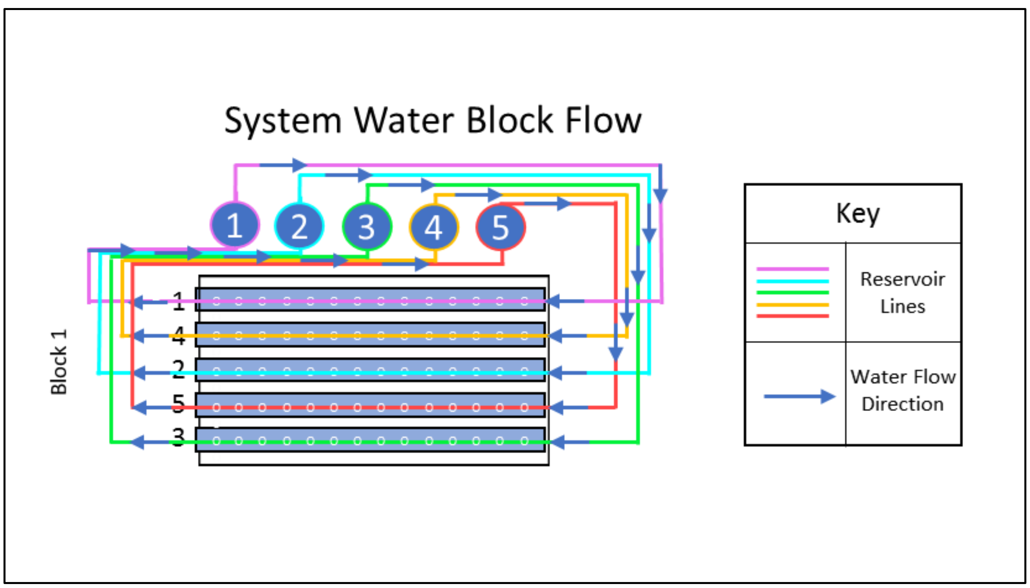


Figure 2.3: Hydroponic NFT Example Block Water Flow



Statistical Analysis

An analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). Size index was analyzed as a 2-way treatment design of N rate and time. The experimental design was a randomized complete block with time as repeated measures. Qualitative-quantitative model regressions were used to test linear and quadratic trends over N rate and time. Water analysis data was a 1-way treatment design of N rate, and the experimental design was completely randomized using sample times for replication. Linear and quadratic trends over N concentrations were tested using simple model regressions. Final data plant mass, shoot and root dry weight, and SPAD were analyzed as 1-way treatment designs of N concentration. Where residual plots and a significant COVTEST statement using the HOMOGENEITY option indicated heterogeneous variance among treatments, a RANDOM statement with the GROUP option was used to correct heterogeneity. All significances were at $\alpha = 0.05$ unless otherwise reported.

2.4 Results

Analysis of size index observed lettuce plant growth was statistically different between nitrogen treatments after 7 days after planting (DAP), showing a quadratic trend between treatments with growth lowest in treatment 50 ppm N solution and highest in treatment 150 ppm N (Table 2.1). This quadratic trend shifted into a linear trend by 21 DAP which remained after 28 DAP. At 28 DAP, size index increased by 12%, from 18.3 in to 20.4 in as N was increased from 50 ppm N to 150 ppm N. Analysis of plant final measurements found SPAD increased quadratically for hydroponic N treatments, from 23.9 to 28.9 as N was increased from 50 ppm N to 150 ppm (Table 2.2). Conversely, it was observed that root length had a negative linear trend, decreasing as N was increased from 50 ppm N to 150 ppm N. Plant fresh mass was linear between treatments, ranging from 189.7 to 241.7g, with treatment 125 ppm N yielding the highest fresh mass on average at 241.7g. Plants grown with 150 ppm N had a similar dry mass to those grown with 125 ppm N. This suggests differences in fresh mass between treatments was due to greater water absorption by treatment 125 ppm N. Plants grown with 125 ppm N had the highest water composition average. Dry Root dry mass was similar between treatments, ranging from 3.3-3.6; however shoot dry mass decreased more in proportion as N rate was decreased from 150 ppm N to 50 ppm N. As a result, root shoot ratio averages decreased as N rate increased from 50 ppm N to 150 ppm N. Analysis of water variables found recorded N for each treatment was less than each treatment's target value and that EC increased linearly from 50 ppm N to 150 ppm N, No differences were found regarding pH or temperature between treatments (Table 2.3).

2.5 Discussion

The purpose of this study was to evaluate how reduced amounts of N affect normal hydroponic NFT lettuce production prior to aquaponic research, which literature has shown to have reduced and fluctuating amounts of nitrogen. Our results demonstrated that as the concentration of N was decreased from 150 ppm N to 50 ppm N, plant size, mass, and SPAD chlorophyll content correspondingly decreased as well (Tables 2.1 and 2.2). These results were in agreement with Konstantopoulou et al. [24], who observed that lettuce increased in fresh mass, leaf number, leaf size, and chlorophyll concentration as N was increased from 80-260 ppm N. Similarly, Urlić et al. [25] observed that the fresh mass of two different lettuce cultivars, 'Lugano' and 'Satine', increased in size as solution N was increased and nitrate in plant tissues rose as nitrate was increased in nutrient solution for their NFT experiments. Our findings were also consistent with Stefanelli et al. [26], who observed that root fresh mass increased as the concentration N was increased up to 150 ppm N in their experiment. Equally, our results indicated that root length and root mass were inversely related in terms of N concentration, such that as N concentration decreased, root length increased and root mass decreased.

Altogether, this study provides additional evidence in support of the known roles of N in plants. For example, current literature has well documented N as an essential element that is vital for the creation of amino acids, proteins, and enzymes that make up plants [17]. Therefore, if the availability of N becomes limited, a plant will not be capable of carrying out these processes. As such, optimal growth will be compromised. Moreover, studies, such as Konstantopoulou et al. [24] and Broadley et al. [27], have shown that rates of plant transpiration, stomatal conductivity, and photosynthesis are also directly linked to N availability and that decreased N resulted in lower rates of each. While this study did not specifically evaluate these factors, those rates are known to influence a plant's fresh mass, leaf size, root length, and root mass, and likely had a role in determining the observed results in our experiments.

In conclusion, understanding and optimizing N for hydroponic NFT lettuce production is important from several different perspectives in regard to this research. First, identifying the optimal amount of N for NFT lettuce production will help avoid N overuse, making lettuce production more cost-effective and environmentally friendly, which are two issues associated with general aquaponics. Our experimental data showed that a target value between 125-150 ppm N is better suited for lettuce growth. Once N fell below 125 ppm, plant growth suffered in all treatments. Second, understanding N also helps to optimize lettuce quality and taste. For example, while too little N has been shown to be detrimental to plant growth, Liu et al. [21] and Alvarado-Camarillo et al. [22] reported that too high N, specifically in form nitrate, can influence lettuce taste through absorption and pose health risks to humans if found in high enough concentrations in lettuce leaves. While the later has not been reported in aquaponic literature, challenges regarding too low of N concentration in aquaponics have been reported. Therefore, it is still necessary to continue to evaluate lettuce from the standpoint of enhancing production in aquaponic systems.

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Chapter 2: Tables

Table 2.1 Size index of 'Rex' lettuce at various nitrogen rates, simple effects of nitrogen concentration at measurement week

Nitrogen Rate (ppm)[z]	Size Index[y] (cm)	Plant Length[x] (cm)	Plant Width[w] (cm)	Plant Height (cm)
		7 DAP		
50	8.9	11.2	10.4	5.1
75	8.8	10.6	11.0	4.8
100	8.0	10.2	9.7	4.0
125	9.0	11.1	11.0	5.0
150	9.0	11.1	11.1	4.8
Sign.	Q**	NS	NS	NS
		14 DAP		
50	14.6	18.1	18.7	6.8
75	14.3	17.9	18.2	6.7
100	13.7	17.1	17.6	6.4
125	15.3	18.8	19.7	7.4
150	15.3	19.0	20.0	6.9
Sign.	Q***	Q*	Q*	NS
		21 DAP		
50	16.5	20.0	19.7	9.6
75	17.1	20.3	20.8	10.0
100	17.0	20.5	21.2	9.2
125	18.1	21.5	22.2	10.6
150	18.1	21.4	22.3	10.7
Sign.	L***	L***	L***	L**
		28 DAP		
50	18.3	21.6	21.4	11.8
75	19.5	22.9	23.3	12.3
100	19.2	22.5	23.4	11.7
125	20.3	23.0	24.6	13.4
150	20.4	23.5	24.9	12.8
Sign.[v]	<0.0001	<0.0001	<0.0001	<0.0001
Sign.[u]	L***	L**	L***	L**

[z] Nitrogen rate refers to concentration of nitrogen found in each hydroponic solution in ppm
[y]Size index refers to (plant height + widest width + perpendicular width/3) as an average direction to plant growth
[x]Plant length refers to widest width of size index
[w]Plant width refers to perpendicular width of size index
[v]Significance established using PROC GLIMMIX. alpha=0.05
[u]Nonsignificant (NS) or significant (Sign.) linear (L) or quadratic (Q) trends using orthogonal contrasts at $P < 0.05$ (*), 0.01 (**) or 0.001 (***).

Table 2.2 Final plant measurement analysis of 'Rex' butterhead lettuce (*Lactuca sativa*) at various nitrogen concentrations

Nitrogen Rate (ppm)[z]	Root Length (cm)	Plant Mass (g)[y]	SPAD[x]	Dry Shoot Mass (g)	Dry Root Mass (g)	R/S Ratio[w]
50	44.2	189.7	23.9	9.1	3.3	0.37
75	41.6	184.3	26.1	9.5	3.2	0.34
100	43.0	184.8	26.9	9.2	3.2	0.35
125	39.0	241.7	28.7	10.9	3.6	0.33
150	40.6	220.2	28.9	10.8	3.6	0.34
P-value[v]	0.0278	<0.0001	<0.0001	0.0004	0.0010	0.0038
Sign.[u]	L**	L***	Q**	L***	Q*	L***

[z] Nitrogen rate refers to concentration of nitrogen found in each hydroponic solution in parts per million (ppm)
[y] Plant mass refers to lettuce head and root mass together
[x]SPAD values refer to the relative greenness of a plant. Measurements were taken with a SPAD-502 meter
[w]R/S refers to root shoot ratio. It is a measurement of a plant's root mass divided by its shoot mass and is used to evaluate the growth pattern of a plant
[v]Significance established using PROC GLIMMIX. alpha=0.05
[u]Nonsignificant (NS) or significant (Sign.) linear (L) or quadratic (Q) trends using orthogonal contrasts at P < 0.05 (*), 0.01 (**) or 0.001 (***).

Table 2.3 Water analysis of various nitrogen concentration treatments

Nitrogen Rate (ppm)[z]	**Nitrate (mg L^{-1})[y]**	**pH**	**EC[x]**	**Temp. (°C)**
50	198	6.05	1.79	21.24
75	298	6.09	1.87	21.14
100	394	6.13	1.90	21.59
125	505	6.04	1.98	21.48
150	585	6.01	1.90	21.85
P-Value[w]	<0.001	0.76	0.0016	0.96
Sign.[u]	L***	NS	L*	NS

[z]Nitrogen rate refers to concentration of nitrogen found in each hydroponic solution in parts per million (ppm)
[y]Nitrate-Nitrogen reading by LAQUA twin NO3-N meter in mg L^{-1}
[x]EC=Electrical conductivity of aquaponic effluent in mmho cm^{-1} measured by HANNAÒ meter (Model HI 9813)
[w]Significance established using PROC GLIMMIX. alpha=0.05
[u]Nonsignificant (NS) or significant (Sign.) linear (L) or quadratic (Q) trends using orthogonal contrasts at P<0.05(*).

Chapter 3: Effects of Hydraulic Retention Time on NFT Lettuce Production in a Decoupled Aquaponic System

3.1 Abstract

A series of 28-d aquaponic (AP) experiments were conducted at Auburn University from April 2019-March 2020 to determine the effects of hydraulic retention time (HRT) on 'Rex' butterhead lettuce using nutrient film technique (NFT) in a decoupled AP system. PROC GLIMMIX was used to conduct an analysis of variance on all responses using SAS version 9.4. Iron was later supplemented to evaluate HRT. Analysis revealed plants grown in shorter HRT intervals (4d) exhibited better growth characteristics over plants grown in longer HRT intervals (16d), producing more biomass and longer roots. In trial one experiments without iron supplementation, plant fresh mass and SPAD exhibited negative linear and quadratic trends 28 DAP respectively, decreasing 41% and 143%, from 203.43g to 143.81g and 18.7 to 7.6, as HRT increased from 4d to 16d. Foliar analysis revealed all HRT treatments absorbed excessive amounts of micronutrients, with treatment 4d accumulating double the amount of Mn and Zn as treatment 16d. In trial two experiments with iron supplementation, SPAD and size index were observed to be statistically different by 14 DAP, and by 28 DAP, treatment 4d was observed to have the lowest SPAD average and largest size index average. Plant fresh mass decreased by 10%, from 162.25g to 147.09g, as HRT was increased from 4d to 16d. Iron supplementation eliminated iron deficiencies in plants up to 14 DAP, but by 28 DAP HRT treatments were observed to be iron deficient along with the elements magnesium, calcium, boron, and copper. However, in contrast with trial one experiments, iron supplementation was observed to considerably reduce the uptake of the divalent cations manganese and zinc in plant tissues. Our findings suggest that smaller quantities of nutrients may be able to grow plants in aquaponics provided that faster hydraulic retention times are used and all essential nutrients are of high enough concentration.

3.2 Introduction

Currently, the global human population exceeds 7.6 billion, but this number is expected to rise to 8.5 billion by 2030, and 9.8 billion by 2050 with more than 75% of people expected to be living in urban areas [1,2]. Accompanied with this population increase will be an increase in demand placed on already stressed food, water, and energy resources needed to sustain its growth [3]. These summary statistics highlight the need for improved food systems that deliver high-quality calories in a sustainable way.

Aquaculture is currently one of the fastest growing food-producing sectors worldwide, and now accounts for 50% of the world's total fish and fish related products according to the United Nations Food and Agriculture Organization (FAO) [3,4]. Aquaponics is a system that seeks to integrate fish production with a hydroponic plant component to maximize resource use efficiencies and minimize negative environmental impacts [1,3,5,6]. In this developing system, aquaculture fish excrete waste in the form of ammonia (NH_3), which nitrifying bacteria convert into nitrate (NO_3^-), the predominant form of nitrogen demanded by plants. In most systems, this nutrient-rich water is then used to fertilize plants for production, where it is filtered, before being recirculated back to fish production or discharged to the environment [1,4,5,7–9].

Although aquaponics technology has obvious potential as a healthy food source for a growing population, there are challenges associated with adopting the technology [10]. Some of these challenges have been linked to the difficulties of integrating aquaculture and hydroponic components [10]. For example, nutrient film technique (NFT) is a popular, ergonomic, hydroponic system, but is not often used in commercial aquaponic systems. Moreover, in a review of aquaponic research that focused on hydroponic components, Maucieri et al. [11] found that from 1997-2017 NFT components accounted for only 17% of research studies. Of studies available evaluating different hydroponic component types together, NFT systems were reported to be less efficient in terms of overall nutrient removal, nitrogen removal efficiency, and yields comparatively [11–13].

Nonetheless, recent aquaponic developments have provided an opportunity for aquaponic system re-design with the use of decoupled systems, in which aquaculture effluent is utilized as a nutrient and water source by plants but is not returned to the aquaculture component [14]. This is mostly due to the fact that in a decoupled system aquaculture effluent can be diverted to multiple hydroponic components without as much regard for system sizing based on water volumes, as is the case in coupled systems. Hydroponic components can be linked in a series or can be independent of one another allowing for multiple system types, production strategies, and possibly higher plant yields, which may improve NFT aquaponics.

Another challenge for these aquaponic systems is that irrigation frequencies that are sufficient to prevent water stress in plants may not be adequate to prevent nutrient deficiencies due to lower or limited quantities of nutrients in aquaculture effluent [15]. This may be particularly true for NFT components in decoupled systems because NFT systems are typically recirculating within themselves. This is not a problem in a hydroponic system because nutrient concentrations are sufficiently high to allow for optimal yields.

Designing better decoupled systems may require either higher nutrient levels or optimal water exchange frequencies [15].Yet, literature evaluating the frequency of water exchanges, or hydraulic retention time (HRT), of NFT systems is limited [13,15]. Research is needed to evaluate if plant production can be improved in NFT aquaponics by exchanging water more frequently. Therefore, objective of this research was to determine the effects of HRT on lettuce growth and nutrient uptake in an NFT component of a decoupled aquaponics system and to predict optimal water exchange frequencies in such systems.

3.3 Materials and Methods

System Overview

Fish production was cultured in a double layer polyethylene greenhouse (9.1m x 29.3 m), containing two 102,000-L rectangular tanks that held approximately 20,000 Nile tilapia (*Oreochromis niloticus*) (Figure 3.1). This system utilized a modified biofloc-type biofiltration system where no additional nutrients were added to promote biofloc formation. Fish were fed twice daily until satiated using a commercial aquaculture feed containing 36% crude protein (Cargill, Franklinton, LA) and water quality of each tank was monitored for pH, ammonia, dissolved oxygen, and temperature. Calcium hydroxide was added as needed to maintain water pH ~6.5. All variables measured remained within acceptable levels for tilapia production for the experiment's duration.

Aquaculture Effluent

Prior to utilization for NFT treatments, aquaponic effluent (AE) underwent two filtration screenings to remove suspended solids (uneaten fish feed, fish feces, biofloc, large particles) that could obstruct NFT systems (Figure 3.1). In the first filtration, AE was passively screened through two 1500-L cone shaped clarifiers connected in series using an air lift that forced water to pass underneath a solid baffle before it flowed from one clarifier to the other. In the second filtration, AE was actively screened using a micron mesh material as it was pumped from the second clarifier to NFT treatment reservoirs. No problems regarding suspended solids were observed during the experiment. After trial one, iron supplementation was determined necessary to further evaluate HRT in NFT aquaponic lettuce production (Tables 3.1-3.4). Iron was supplemented at 2.5 ppm using Spring 330 Iron Chelate based off industry hydroponic lettuce formulas as well as from Cornell University's lettuce literature guide [16]. The purpose of supplementing iron was to further evaluate the effect of HRT on lettuce growth, not to analyze the effects of iron supplementation on lettuce growth.

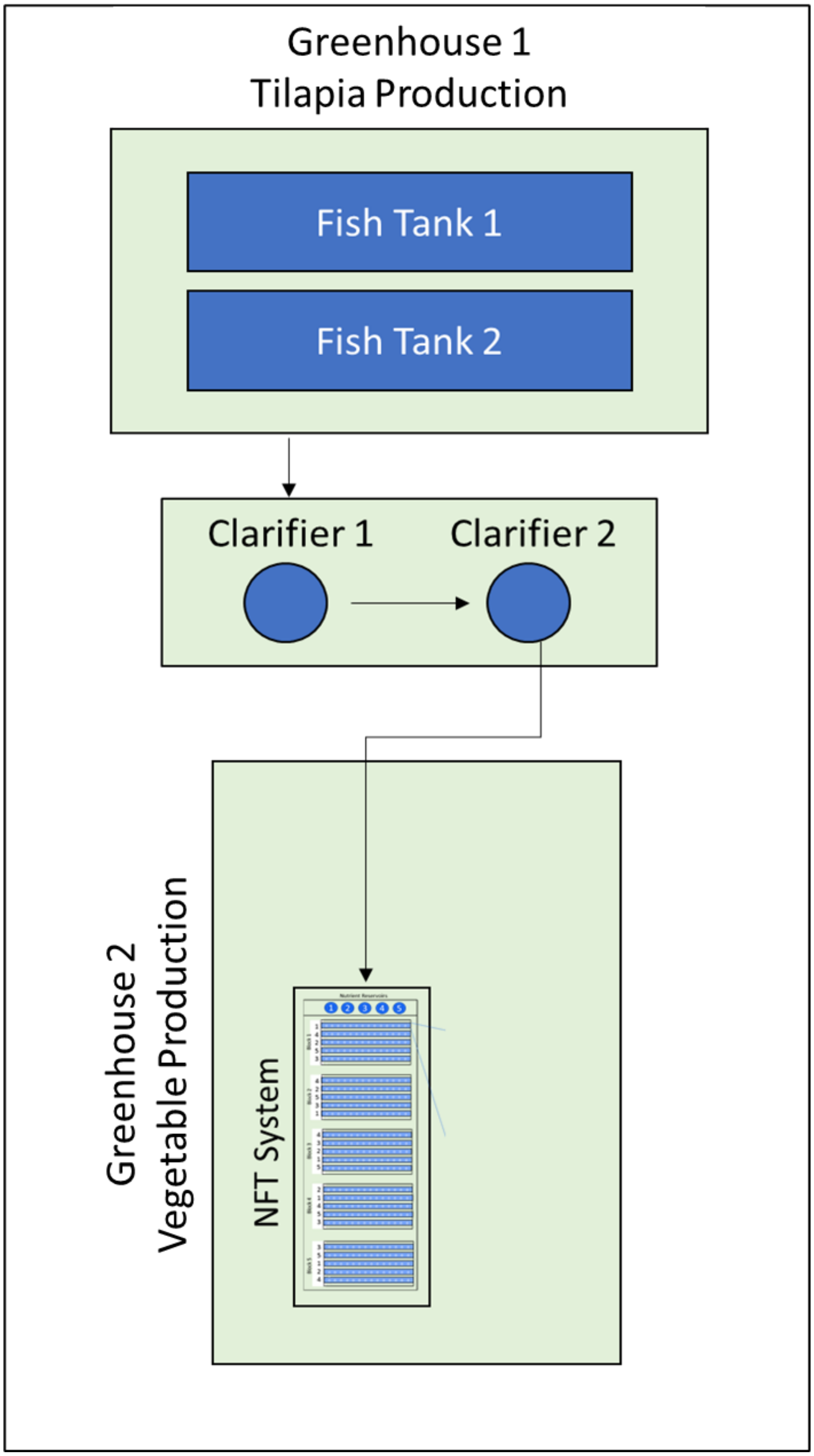
Germination

'Rex' butterhead lettuce seeds (*Lactuca sativa* 'Rex'; Johnny's Selected Seeds) were sown in 5 flats of OASIS® horticubes (OASIS® Grower Solutions, Kent, Ohio) (2.54 cm ×3.18 cm × 3.81 cm).Flats were covered with clear plastic humidity domes until seedling emergence in a greenhouse at Auburn University (32° N, 85° W). After emergence, humidity seedlings began a fertilizer regiment containing 150, 80, 200, 150, and 35 mg• L-1 N, P, K, Ca, and Mg, respectively, from water-soluble 8N-6.5P-30K (Gramp's Original Hydroponic Lettuce Fertilizer, Ballinger, TX), calcium nitrate (15.5N-0P-0K), and magnesium sulfate (10% Mg) for two weeks before plants were transplanted into the NFT system.

Experimental Design

The experiment was organized as a completely randomized block design. Five blocks were comprised of 4 four-meter NFT channels (FarmTek, Connecticut, USA) which held 15 plants spaced 20cm apart that were supplied with a solution from one of four 227-liter treatments (Figures 3.2-3.4). Measurements were conducted on the middle 8 plants of each channel (n=160). Four treatments consisted of aquaculture effluent exchanged at one of the pre-determined HRT intervals of four, eight, twelve, and sixteen days. Iron was later supplemented at the rate of 2.5 ppm into aquaculture effluent after antedating experiments revealed it was necessary. HRT Intervals were selected based off prior hydroponic experiments conducted on the system that showed solutions should be recycled after fourteen days to avoid nutrients depletion.

Figure 3.1: Aquaponic System Overview



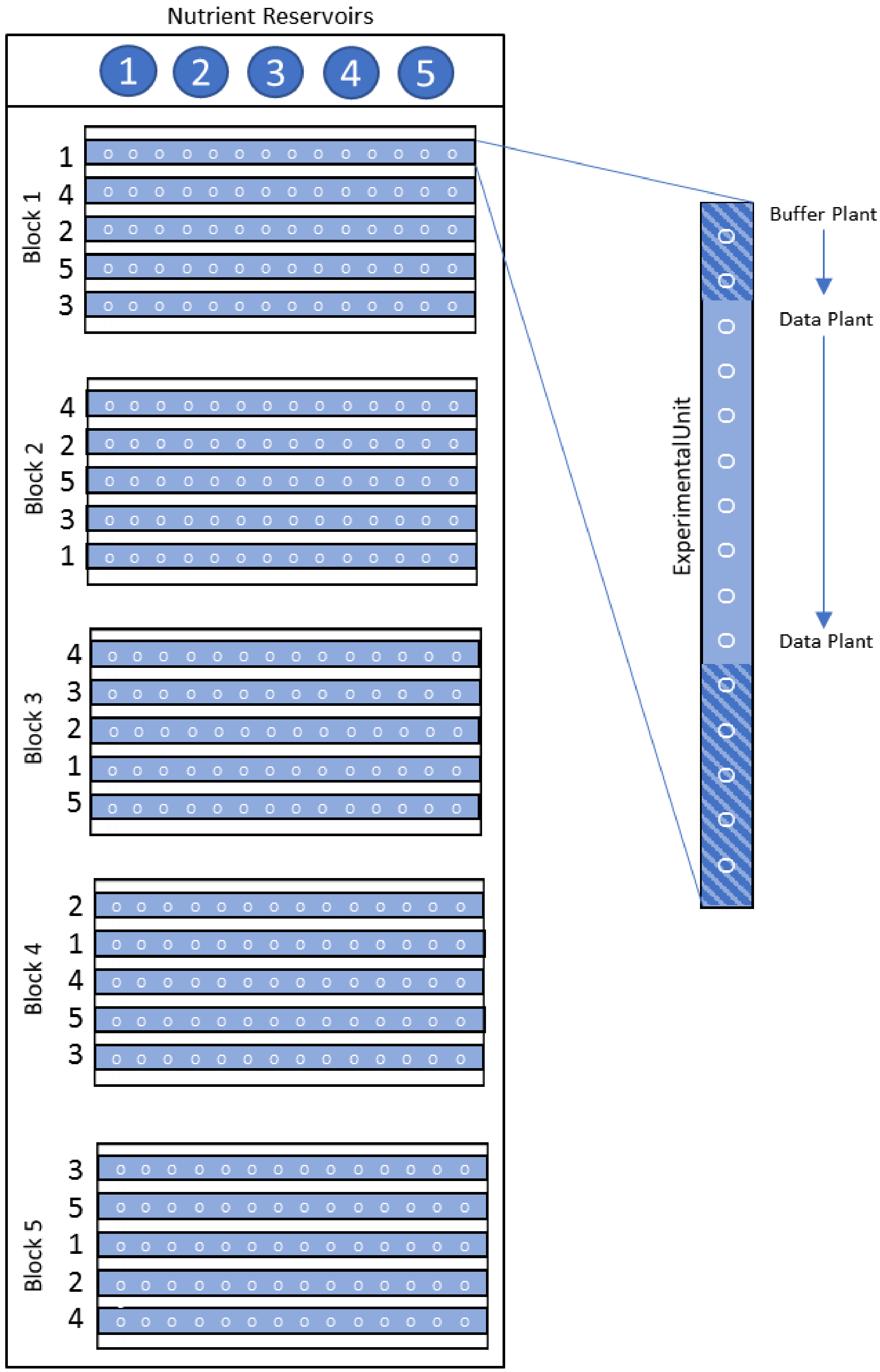


Figure 3.2: Aquaponic NFT Experimental Design

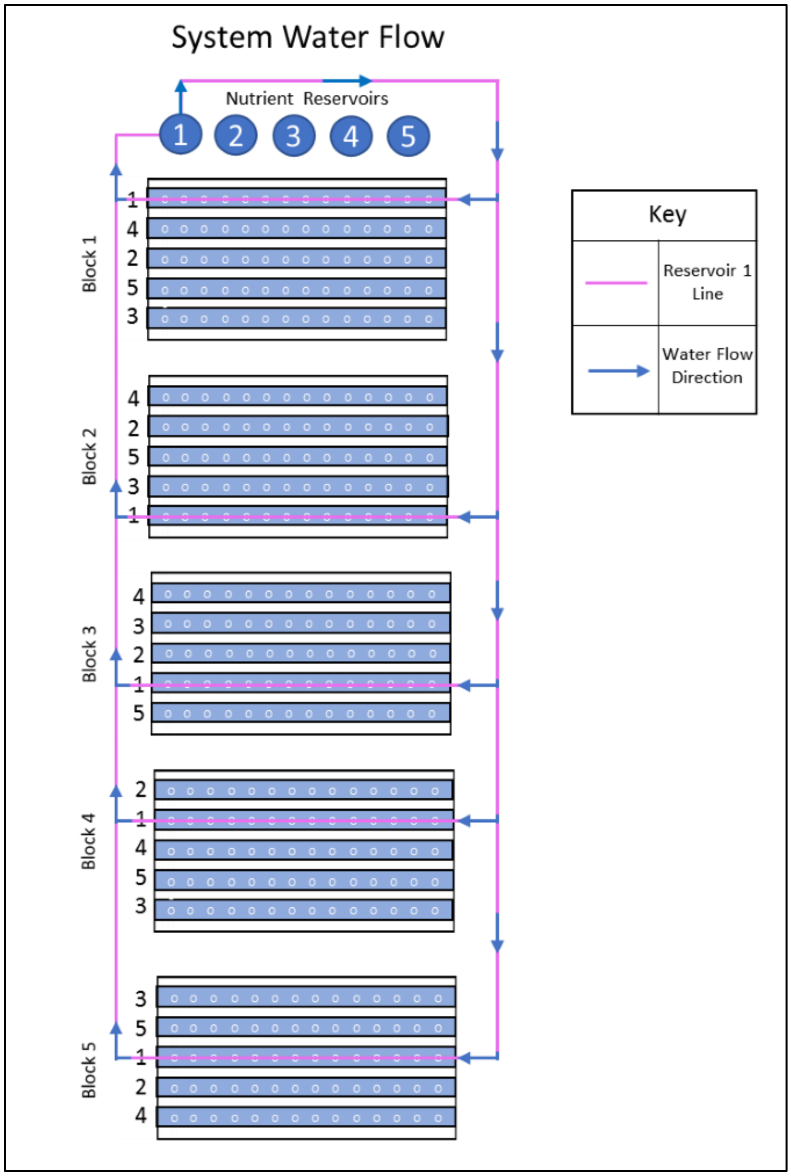


Figure 3.3: Aquaponic NFT System Water Flow

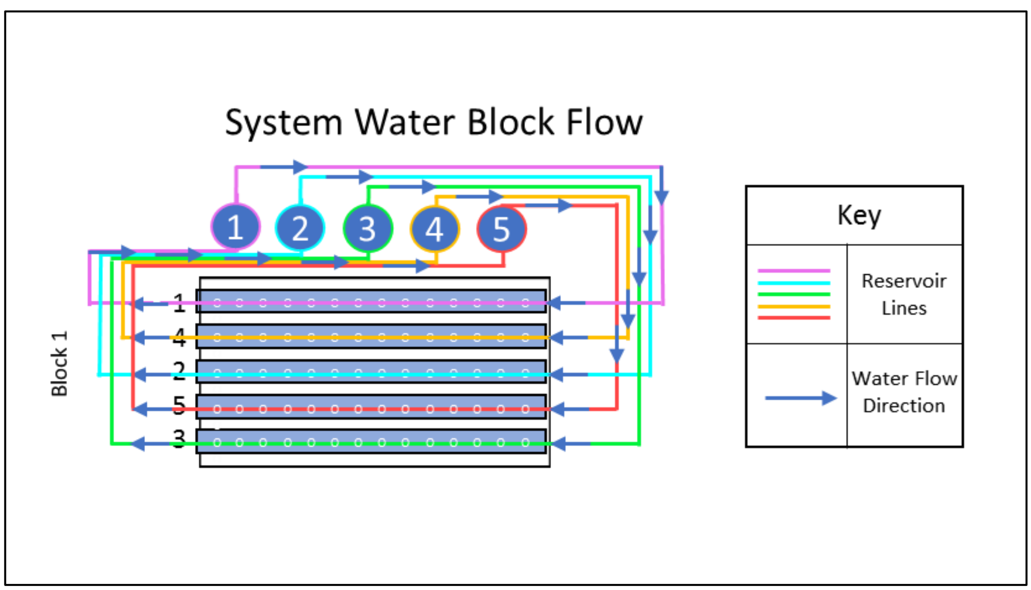


Figure 3.4: Aquaponic Example Block Water Flow

Plant Measurements

Size index (SI) ([height + widest width + perpendicular width]/3) and SPAD chlorophyll were taken for the duration of the experiment once every seven and fourteen days respectively using standard ruler and a SPAD-502 meter (Spectrum Technologies Aurora, IL). Measurements were conducted on the middle eight plants from each channel (n=40 per treatment). Twenty-eight days after planting fresh mass, and root length were recorded. Dry mass was recorded after seven days in a forced air-drying oven at 75.5 C and root-shoot ratio and water composition were calculated. Additionally, two lettuce plants were selected from each block's treatment channels from remaining plants and sent off for elemental analysis by Inductively Coupled Plasma-Emission Spectroscopy (ICP_ES) using AOAC official method 985.01(OMA, 2012) and Kjeldahl digestion at Waters Agricultural Laboratory in Camilla, Georgia. Observed ranges were then compared to sufficiency ranges found in Plant Analysis Handbook III, Micro-Macro Publishing, Inc on butterhead lettuce [17].

Water Measurements

Nitrate concentration, electrical conductivity (EC), pH, temperature, and oxygen concentration (ppm) were monitored and recorded four times a week from nutrient reservoirs using a HANNAÒ Instrument meter (Model HI 9813), LAQUA twin NO3-N meter (Horiba, Kyoto, Japan), and dissolved oxygen meter (OxyGuard Handy Polaris 2; Farum, Denmark).

Statistical Analysis

Trial 1: Without Iron Supplementation: This experiment was run one time and analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). Size index and SPAD were analyzed as 2-way treatment designs of hydraulic retention time (treatment) and measurement date (time). The experimental design was a generalized randomized complete block with time as repeated measures. For size index, orthogonal polynomials were used to determine linear and quadratic trends over time. For SPAD, F-tests were used to compare the 2 times. Water analysis data was a 1-way treatment design of treatment, and the experimental design was completely randomized using sample times for replication. Orthogonal polynomials were used to determine linear and quadratic trends over time. Final plant mass was analyzed as 1-way treatment design, and the experimental design was a generalized randomized complete block. Orthogonal polynomials were used to determine linear and quadratic trends over treatment. All significances were at $\alpha = 0.05$ unless otherwise reported.

Trial Two: With Iron Supplementation: This experiment was run four times and an analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). Size index, SPAD, and water analysis data were analyzed as 2-way treatment designs of hydraulic retention time (treatment) and measurement date (time). The experimental design was a randomized complete block with time as repeated measures and experimental run serving as a block (replication). Experimental run was considered a random effect in the analysis. Orthogonal polynomials were used to determine linear or quadratic trends over time or treatment. Where treatment by time interaction was significant ($P<0.05$), simple effects were presented, otherwise, the main effects of only treatment were presented. Final plant measurements were analyzed as 1-way treatment design, and the experimental design was a randomized complete block with experimental run serving as block (replicate). Orthogonal polynomials were used to determine linear or quadratic trends over treatment. All significances were at $\alpha = 0.05$ unless otherwise stated.

3.4 Results

Trial 1: Without Iron Supplementation

At 14 days after planting (DAP) differences were observed in size index and SPAD. (Table 3.1). By 28 DAP, size index and SPAD decreased by 8 and 143%, from 20.9 to 19.3 and 18.7 to 7.7 respectively, as HRT increased from 4d to 16d. Fresh mass was observed to decease 41%, from 203.4 g to 143.8 g as HRT increased from 4d to 16d, with 4d gaining the most mass at 203.4g (Table 3.2). Plants grown with HRT 8d had the largest dry mass of shoots and roots of all treatments, indicating mass differences between 4d and 8d were due to higher water absorption in treatment 4d. Analysis of HRT water variables found nitrate, electrical conductivity (EC), dissolved oxygen (DO), and pH to be statically different amongst treatments (Table 3.3). Average nitrate concentrations in aquaponics treatments ranged from 410 mg L^{-1} to 433.3 mg L^{-1} but were highest in treatment 4 day and 8d.

However, although average nitrate concentrations were higher for shorter HRT intervals, foliar analysis showed plant N% increased linearly from 5.4% to 6.0% as HRT interval was increased from 4d to 16d. (Table 3.4). Differences in leaf color and lettuce growth were likely the result of sub-optimal concentrations of micronutrients. Foliar analysis revealed that treatments were borderline insufficient-sufficient for Boron and Manganese and all treatments were iron deficient, which were shown to decrease as HRT increased from 4d to 16d, ranging from 87.7 ppm to 60.0 ppm. All treatments absorbed excessive amounts of micronutrients. Shorter HR intervals disproportionally absorbed more micronutrients when compared to longer HRT intervals. Plants grown with 4d accumulated double the amount of Mn and Zn in HRT 16d. Conversely, foliar boron concentrations showed the opposite trend, increasing from 23.2 to 33.6, as HRT increased from 4d to 16.

Trial 2: With Iron Supplementation

SPAD and size index became statistically different in terms of color and size 14 DAP, respectively (Table 3.5). By 28 DAP, SPAD and size index exhibited quadratic tends, ranging 22.3 to 24.4 and 21.7 to 22.5 respectively, with treatment 4d having the lowest SPAD readings and greatest size index. Plant fresh mass decreased by 10%, from 162.25g to 147.09g, as HRT was increased from 4d to 16d, with treatment 4d having the largest plant mass at 162.25 (Table 3.6). Plants grown with HRT of 8,12,16 days had comparable dry mass averages, ranging from 6.5-6.6g, while treatment 4d had the largest dry average at 7.00g. Analysis of water variables showed nitrate and pH values increased as HRT increased from 4d to 16d, from 373 mg L^{-1} to 404 mg L^{-1} nitrate and 6.94 to 7.25 pH, with the exception of nitrate in treatment 12d and pH in treatment 8d (Table 3.7).

Although nitrate concentrations were greater for the longer HRT intervals, foliar analysis showed plant N% decreased with longer HRT intervals, with the exception of 12d (Table 3.8). Iron supplementation eliminated Fe deficiency in plants up to 14 DAP, but by 28 DAP HRT treatments were still observed to be iron deficient. Though in contrast to trial one, iron supplementation was observed to considerably reduce the uptake of the divalent cations manganese and zinc, decreasing from 399-692 and 172-273, respectively in trial one, to 90-98 and 34-42 in trial two, and each were found to not be statistically significant between aquaponic HRT intervals. Nonetheless, the elements magnesium, calcium, boron, and copper were each found to be below optimal sufficiency ranges for all HRT intervals in trial two. Of all elements analyzed, only the elements nitrogen, copper, and magnesium were observed to experience any significant trends. Nitrogen and copper were observed to have quadratic trends among HRT intervals 14 DAP, but by 28 DAP these trends were not significant. Conversely, magnesium did not exhibit a trend 14 DAP, but by 28 DAP exhibited a quadratic trend, where the concentration of magnesium in plant tissues ranged from 0.32-0.37.

3.5 Discussion and Conclusion

Although aquaponic technology has the potential to be scalable to commercial levels, this has yet to happen on a large scale. Currently, there are two main system design approaches for aquaponics: recirculating aquaponic systems and decoupled aquaponic systems, the former of which has been more often applied than the latter. Nonetheless, research on nutrient film technique (NFT) components in each of these systems designs is limited, and prior literature has shown NFT components to be less efficient in terms of overall nutrient removal, nitrogen removal efficiency, and yield [11–13]. Therefore, this study sought to assess if NFT lettuce production could be improved by exchanging aquaponic effluent more frequently in decoupled aquaponics.

Our results found that plants grown in shorter hydraulic retention times (HRT) (4d) exhibited better growth characteristics, producing more biomass and growing longer roots, than plants grown in longer HRT intervals (16d) under normal aquaponic conditions . In terms of lettuce head marketability, all HRT intervals except treatment 16d would have met the recommended marketability fresh weight of 150g by Cornell University's CEA Hydroponic Lettuce Handbook [18]. Still, actual marketability may have suffered due to visible lettuce characteristics and observed nutrient deficiencies.

One potential explanation for our results is that plants in shorter HRT were exposed to a larger total supply of essential nutrients. Mahlangu et. [19] noted that quality and yield of lettuce production are both dependent upon the supply of essential nutrients during certain stages in a plant's growth cycle. A larger supply of essential nutrients at these points in the lettuce growth cycle could explain this result. However, while irrigation rates in our system were sufficient to prevent water stress, this did not prevent nutrient deficiencies from occurring in experiments. These results support Okemwa [1] who argued that irrigation frequencies sufficient to prevent water stress in hydroponic systems may not be adequate to prevent nutrient deficiencies because irrigation and fertilization are occurring simultaneously in soilless systems. Even in our shortest HRT interval, plants became nutrient deficient by 28 DAP if effluent was not supplemented with additional nutrients.

Additionally, contrasting Wahyuningsih et al. [7] who observed that the accumulation of system waste can be a problem in hydroponic components of aquaponic systems, we did not experience such problems in this study. This by design was likely due to our filtration system. However, this may have negatively affected our first series of HRT experiments. For example, in a review of iron in aquaponic system by Kasozi et. al [20], authors found aquaponic systems typically have between 0.35-1.7 mg L^{-1} iron whereas plants require 2.0-2.5 mg L^{-1} iron for optimal growth. Moreover, Blanchard et. al [21] found many of these essential nutrients, such as iron, are bound in system wastes and that their contact with the rootzone can be beneficial. Therefore, eliminating these solids should further restrict already limited essential nutrient concentrations. Nonetheless, this poses a dilemma for NFT systems, as NFT systems are more prone to clogging comparatively more than other system like DWC or substrate-media culture. If the removal of the system waste is necessary to prevent NFT system failures, but also directly reduces the availability of essential nutrients, supplementation of nutrients will be necessary.

In conclusion, supplementing iron to aquaponic effluent was necessary to assess how HRT affects lettuce growth in an NFT system. Under these conditions, shorter HRT intervals (4d) improved lettuce growth in our aquaponic NFT system, but our results found that the supplementation of additional microelements is necessary to prevent other nutrient deficiencies from forming. Our findings suggest that smaller quantities of nutrients may be able to grow plants in aquaponics provided that faster hydraulic retention times are used and all essential nutrients are of high enough concentration.

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Chapter 3: Tables

Table 3.1 Size index and SPAD of 'Rex' butterhead lettuce (*Lactuca sativa*) HRT without iron supplementation, simple effects of HRT at measurement week

Hydraulic Retention Time (d)[z]	Size Index[y] (cm)	Plant Length[x] (cm)	Plant Width[w] (cm)	Plant Height (cm)	SPAD[v] Index
			7 DAP		
4	6.6	8.5	8.7	2.6	
8	6.8	8.8	9.0	2.4	
12	6.4	8.2	8.6	2.4	
16	6.8	9.0	8.8	2.5	
Sign.	NS	NS	NS	NS	
			14 DAP		
4	12.8	16.6	17.0	4.9	14.9[u]
8	13.6	17.6	18.0	5.1	17.9
12	11.8	15.6	15.8	4.0	8.9
16	12.4	16.5	16.4	4.3	10.2
Sign.	L***	L***	L***	L***	L**
			21 DAP		
4	16.8	20.7	21.2	8.5	
8	17.2	21.4	21.7	8.6	
12	15.9	20.2	21.0	6.7	
16	15.6	19.8	20.6	6.3	
Sign.	L***	L***	L*	L***	
			28 DAP		
4	20.9	24.5	25.7	12.5	18.7
8	20.4	24.4	25.4	11.6	17.5
12	19.3	24.0	24.3	9.6	17.8
16	19.3	24.1	24.8	8.9	7.7
P-Value[t]	<0.0001	<0.0001	<0.0001	<0.001	0.0069
Sign.[t]	L***	NS	L**	L***	Q*

[z]Hydraulic retention time refers to how long aquaponic effluent is held in nutrient reservoirs before it is exchanged
[y]Size index refers to (plant height + widest width + perpendicular width/3) as an average direction to plant growth
[x]Plant length refers to widest width of size index
[w]Plant width refers to perpendicular width of size index
[v]SPAD values refer to the relative greenness of a plant. Measurements were taken with a SPAD-502 meter
[u]SPAD readings were only taken at the halfway and final points
[t]Significance established using PROC GLIMMIX. alpha=0.05
[s]Nonsignificant (NS) or significant (Sign.) linear (L) or quadratic (Q) trends using orthogonal contrasts at P < 0.05 (*), 0.01 (**) or 0.001 (***).

Table 3.2 Final plant measurement analysis of 'Rex' butterhead lettuce (*Lactuca sativa*) HRT without iron supplementation

Hydraulic Retention Time (d)[z]	**Root Length (cm)**	**Plant Mass (g)[y]**	**Dry Mass (g)**	**Dry Shoot Mass (g)**	**Dry Root Mass (g)**	**R/S Ratio[x]**
4	62.9	203.4	9.8	7.7	1.9	0.25
8	52.4	193.8	10.4	8.1	2.2	0.27
12	56.4	166.2	8.1	6.0	1.9	0.31
16	54.7	143.8	7.6	5.9	1.6	0.28
P-value[w]	*0.0312*	*<0.0001*	*<0.0001*	*<0.0001*	*0.0002*	*0.0024*
Sign.[v]	NS	L***	L***	L***	L*	Q*

[z] Hydraulic retention time refers to how long aquaponic effluent is held in nutrient reservoirs before it is exchanged

[y] Plant mass refers to lettuce head and root mass together

[x] R/S refers to root shoot ratio. It is a measurement of a plant's root mass divided by its shoot mass and is used to evaluate the growth pattern of a plant

[w] Significance established using PROC GLIMMIX. alpha=0.05

[v] Nonsignificant (NS) or significant (Sign.) linear (L) or quadratic (Q) trends using orthogonal contrasts at $P < 0.05$ (*), 0.01 (**) or 0.001 (***).

Table 3.3 Water analysis of HRT interval treatments without iron supplementation

Hydraulic Retention Time (d)[z]	Nitrate (mg L^{-1})[y]	EC[x]	pH	DO (%)[w]
4	427	1.31	6.84	6.25
8	433	1.33	6.67	6.72
12	410	1.36	7.00	6.53
16	424	1.28	6.98	6.68
P-Value[v]	<0.0001	<0.0001	<0.0001	0.0076
Sign.[u]	NS	NS	NS	NS

[z] Hydraulic retention time refers to how long aquaponic effluent is held in nutrient reservoirs before it is exchanged
[y] Nitrate-Nitrogen reading by LAQUA twin NO3-N meter in mg L^{-1}
[x]EC=Electrical conductivity of aquaponic effluent in mmho cm^{-1} measured by HANNAÒ meter (Model HI 9813)
[w] DO=Dissolved oxygen in water mg L^{-1} measured by OxyGuard Handy Polaris 2
[v]Significance established using PROC GLIMMIX. alpha=0.05
[u]Nonsignificant (NS) or significant (Sign.) linear (L) or quadratic (Q) trends using orthogonal contrasts at P<0.05(*).

Table 3.4 Selected foliar nutrient concentrations of 'Rex' butterhead lettuce (*Lactuca sativa*) HRT without iron supplementation, simple effects of HRT for final measurement

Hydraulic Retention Time (d)[z]	**Nitrogen**[y] **(% N)**	**Phosphorus (% P)**	**Potassium (% K)**	**Magnesium (% Mg)**	**Calcium (% Ca)**	**Manganese**[x] **(ppm Mn)**	**Boron (ppm B)**	**Copper (ppm Cu)**	**Zinc (ppm Zn)**	**Iron (ppm Fe)**
					28 DAP					
4	5.4	0.58	7.8	0.48	3.7	692.2	23.2	7.2	273.8	87.4
8	5.6	0.63	8.5	0.47	3.3	781.2	25.8	7.2	243.6	77.8
12	5.9	0.63	8.6	0.50	3.4	590.6	29.0	7.4	227.0	83.4
16	6.0	0.60	8.6	0.52	3.4	399.0	33.6	6.8	172.2	60
Sufficiency[w]	4.2 – 5.0	0.4 – 0.6	6.0 – 7.0	0.5 – 3.5	2.3 – 3.5	55 – 110	32 – 43	6 – 16	33 – 196	168 – 223
P-Value[v]	<0.0001	0.0148	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0302	<0.0001	0.0008
Sign.[u]	L***	Q**	L**	L***	Q*	Q***	L*	NS	L***	NS

[z]Hydraulic retention time refers to how long aquaponic effluent is held in nutrient reservoirs before it is exchanged

[y] Average percent composition of element found in foliar analysis

[x]Average parts per million of element found in foliar analysis

[w]Sufficiency ranges were identified from Plant Analysis Handbook III, Micro-Macro Publishing, Inc on butterhead lettuce

[v]Significance established using PROC GLIMMIX. alpha=0.05

[u]Nonsignificant (NS) or significant (Sign.) linear (L) or quadratic (Q) trends using orthogonal contrasts at $P < 0.05$ (*), or 0.01 (**).

Table 3.5 Size index and SPAD of 'Rex' butterhead lettuce (*Lactuca sativa*) HRT with iron supplementation, simple effects of HRT at measurement week

Hydraulic Retention Time (d)[z]	**Size Index[y] (cm)**	**Plant Length[x] (cm)**	**Plant Width[w] (cm)**	**SPAD[v] Value**	**Plant Height (cm)[u]**
			7 DAP		
4	8.4	10.9	10.9		8.6
8	8.5	11.2	10.7		8.0
12	8.3	10.8	10.7		8.3
16	8.1	10.4	10.7		8.3
P-Value	0.348	0.0719	0.9		0.0004
Sign.	NS	NS	NS		NS
			14 DAP		
4	16.0	19.8	20.2	22.4	
8	15.4	19.3	19.8	23.9	
12	16.0	19.8	20.3	22.4	
16	15.7	19.3	20.1	21.8	
P-Value	0.0127	0.0885	0.3098	<.0001	
Sign.	NS	NS	NS	Q***	
			21 DAP		
4	20.4	24.7	26.0	23.9	
8	19.1	23.2	24.3	24.9	
12	19.6	23.8	25.0	24.9	
16	19.8	24.1	25.3	23.5	
P-Value	<.0001	<.0001	<.0001	<.0001	
Sign.	Q***	Q***	Q***	Q***	
			28 DAP		
4	22.5	27.1	28.3	22.3	
8	21.7	26.1	27.1	24.4	
12	22.0	26.5	27.5	23.3	
16	22.1	26.6	27.5	23.8	
P-Value[t]	0.004	0.0147	0.0168	<.0001	
Sign.[s]	Q***	Q*	Q*	Q**	

[z] Hydraulic retention time refers to how long aquaponic effluent is held in nutrient reservoirs before it is exchanged
[y]Size index refers to (plant height + widest width + perpendicular width/3) as an average direction to plant growth
[x]Plant length refers to widest width of size index
[w]Plant width refers to perpendicular width of size index
[v]SPAD values refer to the relative greenness of a plant. Measurements were taken with a SPAD-502 meter, which began at week two when plants were large enough
[u]Significance established using PROC GLIMMIX. alpha=0.05
[t]Nonsignificant (NS) or significant (Sign.) linear (L) or quadratic (Q) trends using orthogonal contrasts at P < 0.05 (*), 0.01 (**) or 0.001 (***).
[s]Plant height is main effects, interaction of HRT by Time was not significant (P>0.05)

Table 3.6 Final plant measurement analysis of 'Rex' butterhead lettuce (*Lactuca sativa*) HRT with iron supplementation

Hydraulic Retention Time (d)[z]	**Root Length (cm)**	**Plant Mass (g)[y]**	**Dry Mass (g)**	**Dry Shoot Mass (g)**	**Dry Root Mass (g)**	**R/S Ratio[x]**	**Water %**	**Mass %**
4	47.1	162.2	7.0	5.0	1.9	0.41	0.95	0.05
8	45.4	157.7	6.5	4.5	1.9	0.44	0.96	0.04
12	41.5	155.5	6.5	4.6	1.8	0.42	0.95	0.05
16	43.8	147.1	6.6	4.7	1.8	0.40	0.95	0.05
P-value[w]	0.0004	0.011	0.0008	0.0005	0.0019	<.0001	0.0121	0.0121
Sign.[v]	NS	L*	Q*	Q*	L***	Q**	Q*	Q*

[z] Hydraulic retention time refers to how long aquaponic effluent is held in nutrient reservoirs before it is exchanged
[y] Plant mass refers to lettuce head and root mass together
[x] R/S refers to root shoot ratio. It is a measurement of a plant's root mass divided by its shoot mass and is used to evaluate the growth pattern of a plant
[w] Significance established using PROC GLIMMIX. alpha=0.05
[v] Nonsignificant (NS) or significant (Sign.) linear (L) or quadratic (Q) trends using orthogonal contrasts at P < 0.05 (*), 0.01 (**) or 0.001 (***).

Table 3.7 Water analysis of HRT interval treatments with iron supplementation

Hydraulic Retention Time (d)[z]	**Nitrate ($mg\ L^{-1}$)[y]**	**pH**	**EC[x]**
4	376	6.99	1.07
8	395	6.94	1.13
12	364	7.07	1.02
16	408	7.30	1.06
***P-Value*[w]**	0.0025	0.0028	0.0002
Sign.[v]	NS	L*	NS

[z] Hydraulic retention time refers to how long aquaponic effluent is held in nutrient reservoirs before it is exchanged
[y] Nitrate-Nitrogen reading by LAQUA twin NO3-N meter in $mg\ L^{-1}$
[x]EC=Electrical conductivity of aquaponic effluent in mmho cm^{-1} measured by HANNAÒ meter (Model HI 9813)
[w]Significance established using PROC GLIMMIX. alpha=0.05
[v]Nonsignificant (NS) or significant (Sign.) linear (L) or quadratic (Q) trends using orthogonal contrasts at P<0.05(*).

Table 3.8 Foliar analysis of 'Rex' butterhead lettuce (*Lactuca sativa*) HRT with iron supplementation, simple effects of HRT for each week measurement

Hydraulic Retention Time (d)[z]	**Nitrogen**[y] **(% N)**	**Phosphorus (% P)**	**Potassium (% K)**	**Magnesium (% Mg)**	**Calcium (% Ca)**	**Manganese**[x] **(ppm Mn)**	**Boron (ppm B)**	**Copper (ppm Cu)**	**Zinc (ppm Zn)**	**Iron (ppm Fe)**
					14 DAP					
4	6.5	0.9	9.0	0.3	1.1	83.87	22.20	7.1	53.80	165.60
8	5.9	0.8	7.7	0.3	1.0	84.67	22.80	4.9	42.00	135.73
12	6.4	0.9	9.4	0.3	1.2	78.27	24.33	6.7	48.47	141.07
16	6.4	0.9	9.2	0.3	1.2	90.33	24.33	6.7	43.93	122.00
Sufficiency[w]	4.2 – 5.0	0.4 – 0.6	6.0 – 7.0	0.5 – 3.5	2.3 – 3.5	55 – 110	32 – 43	6 – 16	33 – 196	168 – 223
P-Value	0.0001	0.0003	0.0001	0.065	0.0096	0.4776	0.0663	<.0001	0.0048	0.2348
Sign.	Q*	NS	NS	NS	NS	NS	NS	Q**	NS	NS
					28 DAP					
4	6.06	0.89	9.60	0.34	1.22	90.87	26.47	5.2	42.13	104.53
8	5.87	0.92	9.60	0.37	1.36	99.27	29.07	4.8	33.73	134.00
12	5.83	0.84	9.18	0.34	1.24	98.20	27.40	4.8667	37.33	110.07
16	5.57	0.82	9.27	0.32	1.26	98.60	27.53	4.7333	34.80	132.20
Sufficiency[w]	4.2 – 5.0	0.4 – 0.6	6.0 – 7.0	0.5 – 3.5	2.3 – 3.5	55 – 110	32 – 43	6 – 16	33 – 196	168 – 223
P-Value[v]	0.0215	0.0698	0.6104	0.0069	0.0268	0.6621	0.0699	0.7829	0.0798	0.4002
Sign.[u]	NS	NS	NS	Q*	NS	NS	NS	NS	NS	NS

[z] Hydraulic retention time refers to how long aquaponic effluent is held in nutrient reservoirs before it is exchanged
[y] Average percent composition of element found in foliar analysis
[x] Average parts per million of element found in foliar analysis
[w] Sufficiency ranges were identified from Plant Analysis Handbook III, Micro-Macro Publishing, Inc on butterhead lettuce
[v] Significance established using PROC GLIMMIX. alpha=0.05
[u] Nonsignificant (NS) or significant (Sign.) linear (L) or quadratic (Q) trends using orthogonal contrasts at P < 0.05 (*), or 0.01 (**).