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Effects of Hydraulic Retention Time of Aquaculture Effluent on Nutrient Film Technique Lettuce Productivity

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Abstract: Nutrient film technique (NFT) is a popular, ergonomic, hydroponic system, but is not often used in commercial aquaponic systems due to lower efficiency in overall nutrient removal. Experiments were conducted to assess if NFT lettuce production could be improved by exchanging aquaculture effluent more frequently, and if so, determine the optimal water exchange rate. The AE was taken from a biofloc-based nile tiapia production system. Treatments consisted of increasing hydraulic retention time (HRT (d)) viz: four-, eight-, twelve-, or sixteen-day water exchanges arranged in a randomized complete block design with five blocks. In one trial (trial 1) where iron (Fe) was not supplemented, there was one replication. There were three replications for the second trial with iron supplementation. The analysis of lettuce plant size index and chlorophyll index (SPAD units) in trial 1 was statistically different among the HRTs beginning 14 days after planting, exhibiting negative linear trends with increasing HRT. However, most foliar micronutrients were borderline sufficient, and all treatments showed foliar Fe deficiency. After iron supplementation (trial 2), lettuce plant chlorophyll and size index exhibited quadratic trends with increasing HRT on 14 and 21 DAP, respectively. In trial 2, plant fresh mass decreased linearly from 162.1 g/head to 147.1 g/head, with increasing HRT. Furthermore, iron supplementation eliminated Fe deficiencies in the plants albeit only up to 14 DAP. Our findings suggest that shorter hydraulic retention times provide a solution to using the NFT system in aquaponics especially with iron supplementation.

Keywords: aquaponics; iron supplementation; size index; butterhead lettuce; chlorophyll index; biofloc tilapia

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1. Introduction

Aquaculture, or the farming of fish and other aquatic organisms, currently accounts for 50% of the world's total fish and fish-related products. Aquaculture is one of the fastest growing food-producing sectors worldwide [1,2]. Total aquaculture production comprising human food aquatic animals reached 87.5 million tonnes in 2022 even amid the COVID-19 pandemic [3]. Integrating aquaculture with hydroponic plant production, also known as aquaponics, seeks to maximize resource use efficiencies and minimize negative environmental impacts of aquaculture [2,4–6]. In these integrated systems, ammonia (NH_3) waste excreted by fish converted by nitrifying bacteria into nitrate (NO_3^-), forms the basis for pairing hydroponics with aquaculture. In the system, the nutrient-rich aquaculture effluent is used to fertilize plants for production, where the plants filter the water, before being recirculated back to the aquaculture unit or discharged to the environment or re-used in some other way [1,4,6–9].

Although integrating aquaculture with hydroponics has obvious potential as a source of healthy food for a growing population, there are challenges associated with adopting the technology [10]. Some of these challenges have been linked to the type of hydroponics system used [10]. For example, the nutrient film technique (NFT) is a popular, ergonomic, hydroponic system, but not often used commercially for integrated fish-plant cultures. Moreover, Maucieri et al. [11] found in a review that from 1997–2017, NFT use in integrated aquaculture-hydroponics systems accounted for only 17% of research studies. For studies 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].

Another challenge for integrating aquaculture with hydroponics 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 the aquaculture effluent [6]. Therefore, understanding the effects of hydraulic retention time on crop productivity might improve the use of the NFT in integrated aquaponics systems.

Furthermore, recent developments have provided an opportunity for system re-design with the use of non-circulating fish-plant cultures in which aquaculture effluent is utilized as a nutrient and water source by plants but is not returned to the aquaculture unit [14].

More frequent water exchanges, or shorter hydraulic retention time (HRT) has been suggested for NFT systems especially with aquaculture effluent [6,11].

Aquaponics has the potential to be scalable to commercial levels. However, this has yet to happen on a large scale worldwide. Currently, there are two main system design approaches for integration: recirculating or single loop systems and decoupled or multi-loop systems, the former has been more often applied than the latter [15]. Despite the interest in single loop systems, recent studies are shifting to multi-loop systems due to ease of manipulation [14]. Yet, research on the nutrient film technique (NFT) designs in both system types are under-evaluated, which presents a problem as NFT systems are the most widely used hydroponic systems to grow small, leafy-green vegetables [16]. Thus, this study sought to assess if NFT lettuce production could be improved by exchanging aquaculture effluent more frequently, and if so, determine the optimal water exchange rate.

Generally, in some decoupled systems, there is a pump sump [14] in which the water is recirculated within the hydroponics unit before it is discarded or continuously used. The goal of our study was to determine the optimal retention time which the aquaculture effluent recirculates between this pump sump and the hydroponics NFT unit before being discarded.

2. Materials and Methods

2.1. Aquaculture System Overview

The aquaculture effluent was drawn from a pilot commercial scale biofloc tilapia (*Oreochromis niloticus*) production system. The system was housed in a double layer polyethylene greenhouse (9.1 m × 29.3 m). Nile tilapia was cultured in two 102,000-L rectangular tanks (Figure 1). However, water for this trial was drawn from only one of the tanks (Fish Tank 2). In the biofloc system, bacteria floc was maintained by constant aeration to keep the microbial column in flocculation. Fish were fed twice daily until satiated using a commercial aquaculture feed containing either 36% or 40% crude protein (Cargill, Franklinton, LA, USA) depending on fish age.

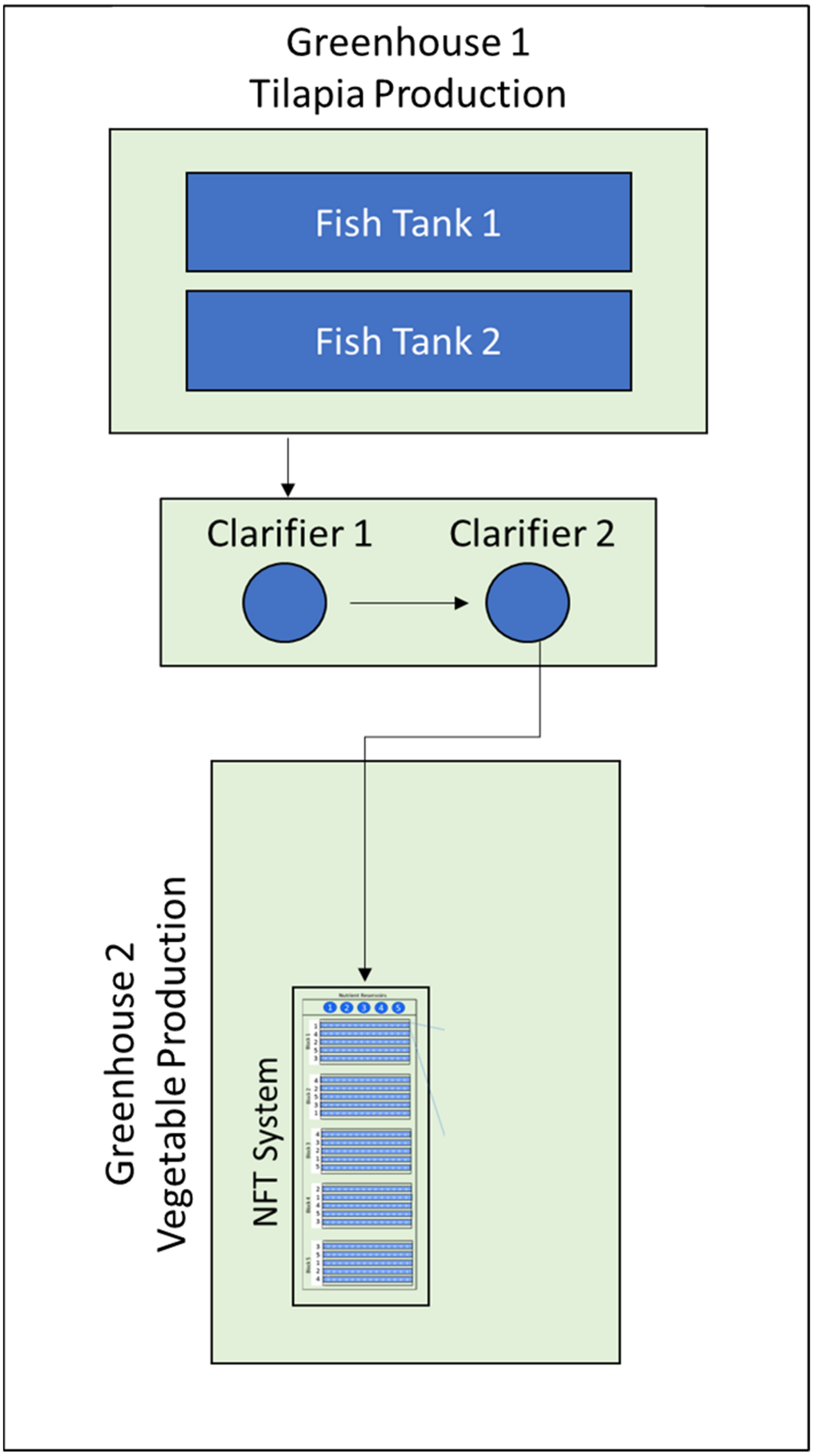

Figure 1. System. Overview. Water for hydraulic retention time trial was drawn from Fish tank 2 in this diagram.

Before the commencement of the trial without iron supplementation, a total of 5492 tilapia fishes were stocked between 17 April 2019 and 22 April 2019 with a total weight of 566.3 kg (1248.4 lbs) and an average fish live weight of ca. 181 g (6.4 oz). By the end of the trial, a total of 5234 tilapia fishes had been harvested from the fish tank with an average weight of 331.1 g (11.7 oz) per fish. The feed weights within the period were 888.5 kg (1958.8 lbs) of 3606 feed and 938.9 kg (2070 lbs) of 4010 feed. The 3606 feed contains 36% crude protein whereas the 4010 feed contains 40% crude protein. Restocking and feeding of fish is done in similar manner for system in year 2020. Water quality of each tank was monitored for pH, ammonia, dissolved oxygen, and temperature (data not shown). To maintain water pH at ca. 6.5, hydrated lime was added as needed. All variables measured remained within acceptable levels for tilapia production during the experiment.

2.2. Filtration of Aquaculture Effluent

Filtration the aquaculture effluent (AE) was done in two stages. The first stage involved a passive removal of suspended solids (uneaten fish feed, fish feces, biofloc, large particles) (Figure 1). Two 1500-L cone-shaped clarifiers connected in series using an air lift forced water to pass underneath a solid baffle before it flowed from one clarifier to the other. Prior to utilization in the NFT treatments, the passively clarified 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.

2.3. Germination

'Rex' butterhead lettuce seeds (*Lactuca sativa* 'Rex; Johnny's Selected Seeds) were sown in OASIS® Horticubes (OASIS® Grower Solutions, Kent, Ohio) (2.54 cm × 3.18 cm × 3.81 cm) and grown for two weeks in a greenhouse at Auburn University (32° N, 85 W) before transplanted into the NFT system. For the first four days at the nursery, a humidity dome was used to help seeds germinate. After germination, four days from seeding, seedlings were fertilized with a nutrient solution containing 150, 80, 200, 150, and 35 $mg \cdot L^{-1}$ N, P, K, Ca, and Mg, respectively from water-soluble 8N-6.5P-30K (Gramp's Original Hydroponic Lettuce Fertilizer, Ballinger, TX, USA), calcium nitrate (15.5N-19Ca), and magnesium sulfate (10Mg-13S) for two weeks before transplanted into a NFT system located at E.W. Shell Fisheries Center, Auburn, Alabama, USA. The experimental timelines from transplanting to harvest were as follows: trial 1 (no iron supplementation) was between 16 July 2019 and 14 August 2019; trial 2 (with iron supplementation) replication 1 was between 28 September 2019 and 26 October 2019; trial 2 replication 2 was between 15 November 2019 and 15 December 2019 and trial 2 replication 3 was between 4 February 2020 and 3 March 2020.

2.4. Experimental Design

The experiment was organized as a randomized complete block design. There were five four-meter NFT channels (FarmTek, South Windsor, CT, USA) which held 15 plants each that were supplied with a solution from one of four 60-gallon (ca. 227.1 L) treatments (Figures 2 and 3). There were three replications over time for trials with with iron supplementation and only one replication for trial without iron supplementation. Measurements were conducted on the middle eight plants in each channel (n = 160). Four treatments consisted of aquaculture effluent exchanged at one of the pre-determined HRT intervals of four, eight, twelve, or sixteen days. HRT Intervals were selected based on prior hydroponic experiments conducted on the system that showed solutions should be recycled after fourteen days to avoid nutrient depletion (data not shown). During the iron supplementation trial, a rate of 2.5 ppm chelated iron was added into aquaculture effluent. Rate of iron was supplemented was based on industry hydroponic lettuce formulas as well as from Cornell University's lettuce literature guide after previous experiments revealed it was necessary [17].

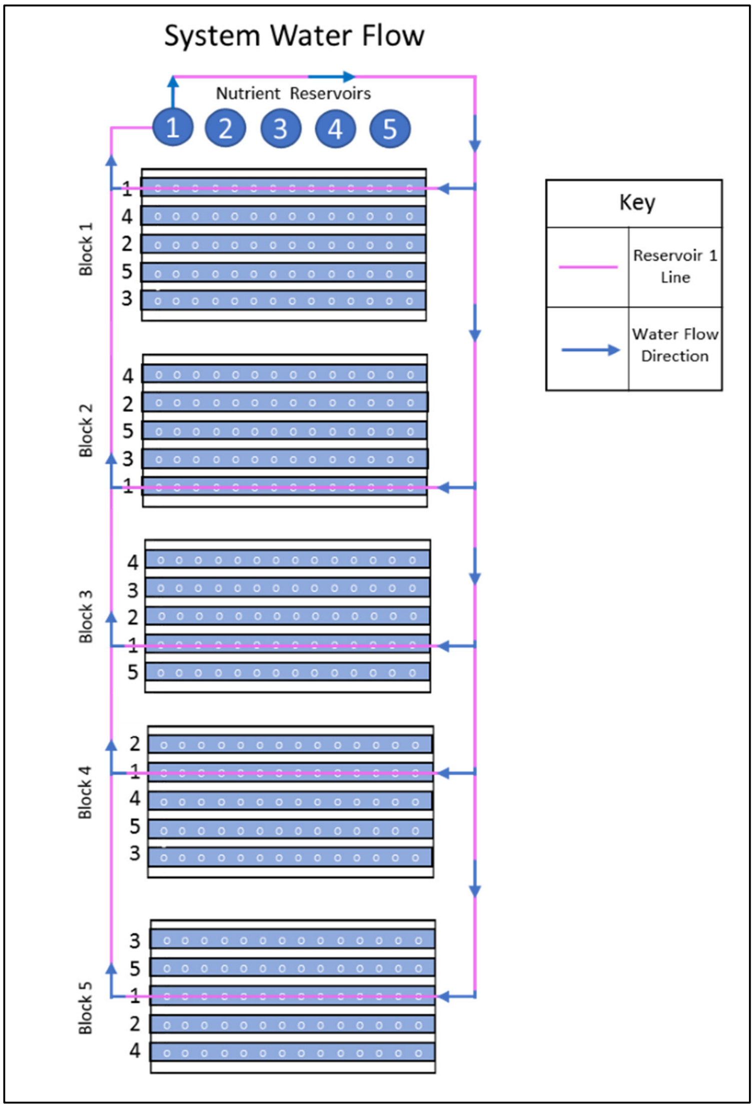

Figure 2. NFT System Water Flow.

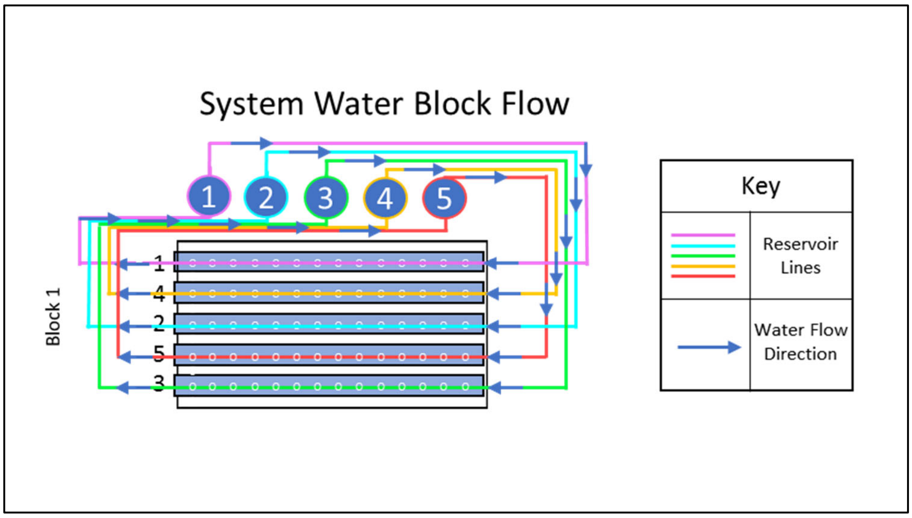

Figure 3. Aquaponic Example Block Water Flow.

2.5. Plant Measurements

Size index (SI) was measured as ([height + widest width + perpendicular width]/3) using a standard ruler and leaf greenness was measured using a SPAD-502 m (Konica-Minolta) for the duration of the experiment every seven and fourteen days, respectively. Measurements were conducted on the middle eight plants from each channel (n = 200). Upon completion of the experiment, final fresh mass (lettuce head + lettuce roots) and root length of experimental plants were recorded using a scale and standard ruler, before being dried in Grieveâ SC-350 heating oven at 75.5 °C for seven days. After drying, total dry mass, shoot dry mass, root dry mass, root-shoot ratio, and water composition were determined. Additionally, two plants from each HRT treatment in each block (50 plants total) were analyzed for nutrient content by Inductively Coupled Plasma-Emission Spectroscopy (ICP_ES) using AOAC official method 985.01 [18] at Waters Agricultural Laboratory in Camilla, Georgia. Nutrient concentrations were compared with ranges found in Plant Analysis Handbook III, Micro-Macro Publishing, Inc for butterhead lettuce [19].

2.6. Water Measurements

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

2.7. Statistical Analysis

An analysis of variance (ANOVA) was performed on all responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC, USA) after testing to ensure the data met the assumptions for ANOVA. The experimental design was a randomized complete block with repeated measures for SI and SPAD. Qualitative-quantitative model regressions were used to test linear and quadratic trends over the HRT. Water analysis data was a 1-way treatment design of HRT, and the experimental design was completely randomized using sample times for replication. Final total dry mass, shoot and root dry mass, and SPAD were analyzed as 1-way treatment designs of HRT. 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.

3. Results

3.1. Without Iron Supplementation

At 14 DAP, size index and leaf greenness decreased linearly, from 12.8 cm to 12.4 cm and 14.9 SPAD units to 10.2 SPAD units, respectively, as hydraulic retention time (HRT) increased from 4-d to 16-d. At 28 DAP, the leaf greenness had shifted from linear to quadratic decreasing from 18.7 SPAD units to 7.7 SPAD units with increasing HRT (Table 1). Furthermore, at 28 DAP, plant fresh mass was observed to decrease linearly by 34% from 203.4 g/head to 143.8 g/head, as HRT increased from 4-d to 16-d. (Table 2). Total plant dry mass, dry shoot mass, and dry root mass were all highest for the 8-d HRT treatment indicating differences in average mass between 4-d and 8-d were due to higher water uptake in 4-d treatment. Differences in leaf color and lettuce growth were likely the result of sub-optimal concentrations of micronutrients.

Table 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}	Plant Length ^{x}	Plant Width ^{w}	Plant Height	SPAD ^{v} Index
	cm				
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	
Polynomial trends	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
Polynomial trends	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	
Polynomial trends	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
ANOVA ^{t}	<0.0001	<0.0001	<0.0001	<0.001	0.0069
Polynomial trends ^{t}	L ***	NS	L **	L ***	Q *

^{z} Hydraulic retention time refers to how long aquaculture 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 m. ^{u} SPAD readings were only taken at the halfway and final points. ^{t} Analysis of variance (ANOVA) significance established using PROC GLIMMIX. alpha = 0.05. ^{s} Polynomial trends at $p > 0.05$ (NS), $p < 0.05$ (*), 0.01 (**) or 0.001 (***). Linear (L) or quadratic (Q) trends were tested using orthogonal contrasts.

Table 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 ^{y}	Dry Mass	Dry Shoot Mass	Dry Root Mass	R/S Ratio ^{x}
		Gram				
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	1.9	0.31
16	54.7	143.8	7.6	5.9	1.6	0.28
ANOVA ^{w}	0.0312	<0.0001	<0.0001	<0.0001	0.0002	0.0024
Polynomial trends ^{v}	NS	L ***	L ***	L ***	L *	Q *

^{z} Hydraulic retention time refers to how long aquaculture 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} Analysis of variance (ANOVA) significance established using PROC GLIMMIX. alpha = 0.05. ^{v} Polynomial trends at $p > 0.05$ (NS), $p < 0.05$ (*) or 0.001 (***). Linear (L) or quadratic (Q) trends were tested using orthogonal contrasts.

There were no significant trends for water nitrate, pH, electrical conductivity (EC), dissolved oxygen (DO), or temperature (Table 3). Average nitrate concentrations in the aquaculture effluent ranged from 410 mg·L^{-1} to 433.3 mg·L^{-1}, but were highest in treatment 4-d and 8-d. However, although average nitrate concentrations were higher for shorter HRT intervals, foliar analysis showed plant N (% dry matter) increased linearly from 5.4% to 6.0% with increasing HRT (Table 4). Higher plant N recorded by longer HRT treatments could be attributed to nutrient dilution. Larger plants recorded by shorter HRT (Table 2) diluted the N concentration resulting in lower N. Foliar analysis revealed that treatments were borderline sufficient for boron and copper whereas manganese and zinc were above the upper sufficiency limit. All treatments were deficient in iron. Foliar iron decreased from 87.7 mg·kg^{-1} to 60.0 mg·kg^{-1} dry mass with increasing HRT. Additionally, the foliar analysis revealed that shorter HRT intervals disproportionally absorbed more of some micronutrients compared to longer HRT intervals, such that 4-d accumulated close to double the amount of manganese (Mn) and zinc (Zn) as treatment 16-d. Conversely, foliar boron concentrations showed the opposite trend, increasing from 23.2 to 33.6 mg·kg^{-1} dry matter with increasing HRT. In conclusion, decreasing HRT of aquaculture effluent improved lettuce growth in the NFT system, but growth and quality of all treatments were ultimately limited. due to sub-optimal micronutrient concentrations in aquaculture effluent. Iron supplementation was deemed necessary because the foliar analysis showed iron was about twice lower than the lower sufficiency requirement for lettuce. The lowered iron concentration might have resulted in the increased uptake of manganese and zinc to maintain charge balance.

Table 3. Water measurements of HRT interval treatments without iron supplementation. Measurements were taken four times per week during the trial from nutrient reservoirs.

Hydraulic Retention Time (d) ^{z}	Nitrate (mg·L^{-1}) ^{y}	EC ^{x}	pH	DO (%) ^{w}
4	427	1.3	6.8	6.3
8	433	1.3	6.7	6.7
12	410	1.4	7.0	6.5
16	424	1.3	7.0	6.7
ANOVA ^{v}	<0.0001	<0.0001	<0.0001	0.0076
Polynomial trends ^{u}	NS	NS	NS	NS

^{z} Hydraulic retention time refers to how long aquaculture 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} Analysis of variance (ANOVA) significance established using PROC GLIMMIX. alpha = 0.05. ^{u} Polynomial trends at $p > 0.05$ (NS) Linear (L) or quadratic (Q) trends were tested using orthogonal contrasts.

Table 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}	Nitrogeny	Phosphorus	Potassium	Magnesium	Calcium	Manganese ^{x}	Boron	Copper	Zinc	Iron
		g 100 g−1 Dry Matter					mg·kg−1 Dry Matter			
					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	60.0
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
ANOVA ^{v}	<0.0001	0.0148	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0302	<0.0001	0.0008
Polynomial trends ^{u}	L ***	Q **	L **	L ***	Q *	Q ***	L *	NS	L ***	NS

^{z} Hydraulic retention time refers to how long aquaculture 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} Analysis of variance (ANOVA) significance established using PROC GLIMMIX. alpha = 0.05. ^{u} Polynomial trends at $p > 0.05$ (NS), $p < 0.05$ (*), 0.01 (**), or 0.001 (***). Linear (L) or quadratic (Q) trends were tested using orthogonal contrasts.

3.2. With Iron Supplementation

Analysis of lettuce leaf greenness and size indices found plants became statistically different in terms of color and size at 14 and 21 DAP, respectively, both exhibiting quadratic trends with increasing HRT (Table 5). These quadratic trends each remained at 28 DAP, with leaf chlorophyll and size indices ranging from 22.3 to 24.4 SPAD units, and 21.7 to 22.5 cm respectively. Treatment 4-d had the lowest average chlorophyll index and largest average size index. Additionally, at 28 DAP, plant fresh mass was observed to decrease linearly for the treatments, ranging from 162.25 g/head to 147.09 g/head with increasing HRT, with treatment 4-d having the largest plant mass at 162.25 g/head (Table 6). Examining the dry mass revealed treatments that treatments 8-, 12-, and 16-d had comparable dry mass averages, ranging from 6.48–6.57 g/head, while treatment 4-d had the largest dry average at 7.00 g/head. Analysis of water variables showed that average nitrate and pH values increased with increasing from 373 $mg \cdot L^{-1}$ to 404 $mg \cdot L^{-1}$ nitrate and 6.94 to 7.25 pH, except for nitrate in treatment 12-d and pH in treatment 8-d (Table 7). Although average nitrate concentrations were higher for the longer HRT intervals, foliar analysis showed plant N (% dry matter) decreased in longer HRT intervals, except for 12-d (Table 8). Additionally, foliar analysis showed iron supplementation eliminated iron deficiency in plants up to 14 DAP, but by 28 DAP, all 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 such as manganese and zinc, decreasing from 399–692 $mg \cdot kg^{-1}$ and 172–273 $mg \cdot kg^{-1}$, respectively, in trial one to 90–98 $mg \cdot kg^{-1}$ and 34–42 $mg \cdot kg^{-1}$ in trial two, each were found to not be statistically significant ($p > 0.05$) 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 have 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 g 100 g^{-1}.

Table 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}	**Plant Length** ^{x}	**Plant Width** ^{w}	**Plant Height** ^{u}	**SPAD** ^{v} **Value**
	cm				
			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	
ANOVA	0.348	0.0719	0.9	0.0004	
Polynomial trends	NS	NS	NS	NS	
			14 DAP		
4	16	19.8	20.2		22.4
8	15.4	19.3	19.8		23.9
12	16	19.8	20.3		22.4
16	15.7	19.3	20.1		21.8
ANOVA	0.0127	0.0885	0.3098		<0.0001
Polynomial trends	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
ANOVA	<0.0001	<0.0001	<0.0001		<0.0001
Polynomial trends	Q ***	Q ***	Q ***		Q ***

Table 5. *Cont.*

Hydraulic Retention Time (d)^{z}	Size Index ^{y}	Plant Length ^{x}	Plant Width ^{w}	Plant Height ^{u}	SPAD ^{v} Value
	cm				
			28 DAP		
4	22.5	27.1	28.3		22.3
8	21.7	26.1	27.1		24.4
12	22	26.5	27.5		23.3
16	22.1	26.6	27.5		23.8
ANOVA ^{t}	0.004	0.0147	0.0168		<0.0001
Polynomial trends	Q ***	Q *	Q *		Q **

^{z} Hydraulic retention time refers to how long aquaculture 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 m, which began at week two when plants were large enough. ^{u} Analysis of variance (ANOVA) significance established using PROC GLIMMIX. alpha = 0.05. ^{t} Polynomial trends at $p > 0.05$ (NS), $p < 0.05$ (*), 0.01 (**), or 0.001 (***). Linear (L) or quadratic (Q) trends were tested using orthogonal contrasts. Plant height is main effects, interaction of HRT by Time was not significant ($p > 0.05$).

Table 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 ^{y}	Dry Mass	Dry Shoot Mass	Dry Root Mass	R/S Ratio ^{x}	Water	Mass
		gram					%	
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
ANOVA ^{w}	0.0004	0.011	0.0008	0.0005	0.0019	<0.0001	0.0121	0.0121
Polynomial trends ^{v}	NS	L *	Q *	Q *	L ***	Q **	Q *	Q *

^{z} Hydraulic retention time refers to how long aquaculture 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} Analysis of variance (ANOVA) significance established using PROC GLIMMIX. alpha = 0.05. ^{v} Polynomial trends at $p > 0.05$ (NS), $p < 0.05$ (*), 0.01 (**), or 0.001 (***). Linear (L) or quadratic (Q) trends were tested using orthogonal contrasts.

Table 7. Water measurements of HRT interval treatments with iron supplementation. Measurements were taken four times per week during the trial from nutrient reservoirs.

Hydraulic Retention Time (d) ^{z}	Nitrate ($mg \cdot L^{-1}$) ^{y}	pH	EC ^{x}
4	376	7.0	1.1
8	395	6.9	1.1
12	364	7.1	1.0
16	408	7.3	1.1
ANOVA ^{w}	0.0025	0.0028	0.0002
Polynomial trends ^{v}	NS	L *	NS

^{z} Hydraulic retention time refers to how long aquaculture effluent is held in nutrient reservoirs before it is exchanged. ^{y} Nitrate-Nitrogen reading by LAQUA twin NO3-N meter in $mg \cdot L^{-1}$. ^{x} EC = Electrical conductivity of aquaponic effluent in $mmho \cdot cm^{-1}$ measured by HANNAÒ meter (Model HI 9813). ^{w} Analysis of variance (ANOVA) significance established using PROC GLIMMIX. alpha = 0.05. ^{v} Polynomial trends at $p > 0.05$ (NS), $p < 0.05$ (*). Linear (L) or quadratic (Q) trends were tested using orthogonal contrasts.

Table 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]	Phosphorus	Potassium	Magnesium	Calcium	Manganese [x]	Boron	Copper	Zinc	Iron
	g 100 g^{-1} Dry Mass					mg·kg^{-1} Dry Mass				
					14 DAP					
4	6.5	0.9	9.0	0.3	1.1	83.9	22.2	7.1	53.8	165.6
8	5.9	0.8	7.7	0.3	1.0	84.7	22.8	4.9	42.0	135.7
12	6.4	0.9	9.4	0.3	1.2	78.3	24.3	6.7	48.5	141.1
16	6.4	0.9	9.2	0.3	1.2	90.3	24.3	6.7	43.9	122.0
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
ANOVA	0.0001	0.0003	0.0001	0.065	0.0096	0.4776	0.0663	<0.0001	0.0048	0.2348
Polynomial trend	Q *	NS	NS	NS	NS	NS	NS	Q **	NS	NS
					28 DAP					
4	6.1	0.9	9.6	0.3	1.2	90.9	26.5	5.2	42.1	104.5
8	5.9	0.9	9.6	0.4	1.4	99.3	29.1	4.8	33.7	134.0
12	5.8	0.8	9.2	0.3	1.2	98.2	27.4	4.9	37.3	110.1
16	5.6	0.8	9.3	0.3	1.3	98.6	27.5	4.7	34.8	132.2
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	616	33–196	168–223
ANOVA [v]	0.0215	0.0698	0.6104	0.0069	0.0268	0.6621	0.0699	0.7829	0.0798	0.4002
Polynomial trend. [u]	NS	NS	NS	Q *	NS	NS	NS	NS	NS	NS

[z] Hydraulic retention time refers to how long aquaculture 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] Analysis of variance (ANOVA) significance established using PROC GLIMMIX. alpha = 0.05. [u] Polynomial trends at $p > 0.05$ (NS), $p < 0.05$ (*), or 0.01 (**). Linear (L) or quadratic (Q) trends were tested using orthogonal contrasts.

4. Discussion

Our results found that under normal aquaculture production without iron supplementation, plants grown in shorter hydraulic retention times (HRT) exhibited better growth characteristics, producing more biomass, and growing longer roots, than plants grown in longer HRT intervals. This shows that refreshing the nutrient solution at a faster rate might produce better results. One potential explanation for this result is that plants in shorter HRT were exposed to a larger total supply of essential nutrients. Mahlangu et al. [20] 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, in our current study even replenishing the nutrient solution at every four days did not produce desirable results. Furthermore, while irrigation rates in our system were sufficient to prevent water stress, this did not prevent nutrient deficiencies from occurring in experiments. This might be due to the intrinsically low nutrient concentration of the aquaculture effluent used. These results support Tyson et al. [6] 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. Therefore, we observed that even in our shortest HRT interval, plants became nutrient deficient by twenty-eight days after planting, the normal production cycle for lettuce.

Removal of solids is necessary in NFT-systems so as not to clog pumps and pipes. However, this comes at a penalty of reduced nutrient concentrations which might results from mineralization over time. We observed that due to our filtration system, our first HRT experiment was affected when iron was not supplemented. For example, in a review of iron in similar systems by Kasozi et al. [21], the authors found aquaculture effluent 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. [22] found that many of these essential nutrients, such as iron, are bound in system solid waste and that their contact with the rootzone can be beneficial. Therefore, eliminating these solids further restricted the already limited essential nutrient concentrations.

Therefore, there exists a dilemma for adopting NFT systems which is an efficient and ergonomic system yet more prone to clogging comparative to other systems like floating raft or substrate-media culture. That is, If the removal of the system waste is necessary to prevent NFT system failures, but also directly reduces the availability of essential nutrients for them, supplementation of nutrients is necessary to make up the difference. Furthermore, as shown by our study, reducing the hydraulic retention time to every 4-days will improve nutrient the system as nutrients are replenished more frequently. Our study also provides further evidence that the supplementation of nutrients in aquaculture effluent is not only necessary, but also vital to optimize its coupling with NFT hydroponic system.

Supplementing iron at the rate of 2.5 ppm eliminated iron deficiencies albeit only up to 14 DAP. However, lettuce plants were observed to be iron deficient by 28 DAP. Therefore, higher rates or multiple application of similar rates of iron may be required to extend sufficient iron availability. Furthermore, the effect of pH on the nutrient availability should be investigated because the pH values were high and might have contributed to low Fe availability. Nonetheless, iron supplementation was shown to considerably reduce the divalent cation uptake of manganese and zinc found in trial one experiments back to sufficient levels. Thus, with iron supplementation, decreasing HRT from 16-d to 4-d improved lettuce growth and mass in the NFT system, but supplementation of additional micronutrients is required to improve growth and nutrient sufficiency.

5. Conclusions

In conclusion, shorter HRT intervals (4 d) improved lettuce growth in our NFT system, but our results found that the supplementation of additional microelements to the aquaculture effluent would be necessary to prevent other nutrient deficiencies from forming. Furthermore, iron supplementation improved lettuce growth, yield and foliar concentrations. Our findings suggest that smaller nutrient concentrations of aquaculture effluent successfully produced a lettuce crop provided shorter hydraulic retention times were used in combination with iron supplementation.

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