horticulturae

MDPI

Article

Integrating Greenhouse Cherry Tomato Production with Biofloc Tilapia Production

Jeremy M. Pickens [1,*], Jason J. Danaher [2], Jeff L. Sibley [1], Jesse A. Chappell [3] and Terry R. Hanson [3]

1 Department of Horticulture, Auburn University, 101 Funchess Hall, Auburn, AL 36849, USA; sibleje@auburn.edu
2 Lake County Water Authority, 27351 SR 19, Tavares, FL 32778, USA; jdanaher@lcwa.org
3 School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University, 203 Swingle Hall, Auburn, AL 36849, USA; chappj1@auburn.edu (J.A.C.); hansontr@auburn.edu (T.R.H.)
* Correspondence: jeremy.pickens@auburn.edu; Tel.:+1-(251)-342-2366

Received: 30 November 2019; Accepted: 30 July 2020; Published: 4 August 2020

Abstract: Integration of intensive aquaculture systems with greenhouse plant production has been shown to improve aquaculture water quality conditions and improve plant nutrient use efficiency. The majority of the focus of integrated systems has involved raft culture or true hydroponics. Little work has been done on soilless culture utilizing drip irrigation. This study investigates the feasibility of integrating biofloc Nile tilapia (*Oreochromis niloticus*) production with greenhouse cherry tomato production (*Solanum lycopersicum* var. *cerasiforme*). Nile tilapia (157 g/fish) were stocked at 40 fish/m^3 and grown for 149 days. The cherry tomato cvs. "Favorita" and "Goldita" were grown with aquaculture effluent (AE) waste and compared to plants grown with conventional fertilizer (CF) in soilless culture. Plants were grown for 157 days. Few differences in yield occurred between treatments until fish harvest (117 DAT). Post fish harvest, there was an 18.4% difference in total yield between CF and AE at crop termination for "Favorita". Differences in yield between AE and CF were observed for "Goldita" at fish harvest (117 DAT) and crop termination (157 DAT). Results from this study suggest the potential for successful integration of cherry tomato grown in a substrate-based system with AE from a tilapia biofloc production system.

Keywords: aquaponics; hydroponics; recirculating aquaculture system (RAS); decoupled aquaponics

1. Introduction

Feed can account for over 50% of production costs in an aquaculture system [1]. Consequently, it is important to effectively convert feed into sellable products. Fish are among the most efficiently cultured animals in regard to feed conversion, but there is still a considerable amount of wasted nutrients associated with fish [2,3]. Recirculating aquaculture systems (RASs) improve water and space utilization over traditional pond-based systems, but traditional RASs do little to improve the nutrient use efficiency (NUE) of a system.

Biofloc technology (BFT) is a form of RAS that does not use traditional biofilters. BFT relies on the constant mixing of suspended solids in the water column. Solids in suspension in BFT-cultured water provide a surface area for heterotrophic and autotrophic bacterial growth. Most BFTs are operated where nitrogenous waste is primarily handled through mineralization by utilizing heterotrophic bacteria. Nitrogenous waste (primarily ammonia) is assimilated into microbial protein, converting N into a nontoxic form [4,5]. This technique is enhanced by increasing the C:N ratio of food, adding highly available carbon sources, or by lowering the protein content in feed [6,7]. BFT improves the feed conversion ratio (FCR) over clearwater systems, which enhances NUE of the system [7]. The BFT

system contains high concentrations of settleable solids that include microbial flocs, uneaten feed, and fecal waste.

Hydroponic vegetable production lends itself to integration into RASs, improving NUE. The integration of RASs with hydroponic vegetable production is commonly referred to as aquaponics [8]. Aquaponic systems improve NUE, decrease water consumption, and improve water quality over conventional RASs [9–12]. Quillere et al. [13] reported that 60% of applied nitrogen was recovered, with 28% being assimilated into plant biomass and 31% being assimilated into fish biomass when fish production was integrated with hydroponic tomato (*Solanum lycopersicum* L) production.

Aquaponic research has primarily involved leafy greens [9–11,14,15] and tomato [16–20]. Savidov et al. [21] evaluated 24 different plant species grown in an aquaponic system, demonstrating the variety of crops that can be grown aquaponically.

Little research has addressed integrating drip-irrigated plant production systems used in the greenhouse vegetable industry, with RASs. Soilless systems utilize highly porous substrates, allowing growers to manipulate nutrients in the root zone with frequent short irrigation cycles. Clogging of the micro-orifices associated with microirrigation with fish waste is a concern with aquaponics. High concentrations of settleable solids associated with BFT have had limited research on its integration with substrate-based growing systems. The purpose of this research is to evaluate the integration of BFT aquaculture effluent (AE) as a nutrient solution for greenhouse cherry tomato using a substrate-based hydroponic system.

2. Materials and Methods

Facilities used in this research consisted of 2 commercial-size greenhouses located at the E.W. Shell Fisheries Center, North Auburn Unit, approximately 10 km north of Auburn, Alabama, USA (32.649171° N, −85.486725° E). The fish culture system was housed in a 267.6 m^2 double-polyethylene covered greenhouse (9.1 × 29.2 m) with an east to west orientation and consisted of 2 rectangular tanks (1.2 × 3 × 26.8 m), each with an average volume of 100 m^3, operated as a biofloc system [5]. A 1.9 m^3 cone-bottomed clarifier (30% slope), adjacent to the greenhouse, was used to reduce suspended solids concentration from the system (Figure 1). Water flowed through the clarifier at an approximate flow rate of 18.9 $L{\cdot}min^{-1}$ and entered a 1.1 m^3 cone-bottomed irrigation sump of a similar design before re-entering the fish production tank. Both vessels had an uninterrupted and constant flow of water, driven by airlift pumps. The clarifier and irrigation sump were flushed of collected solids twice daily.

The 267.5 m^2 (9.1 × 29.2 m) plant greenhouse was covered with double-layered polyethylene sheeting with a north to south orientation. The plant greenhouse was outfitted for soilless vine crop production, with a steel cable trellis system running the length of the greenhouse and cables suspended approximately 2.1 m above the greenhouse floor. Two cables were suspended above each row, approximately 0.1 m from the row center. Each row was 1.5 m apart, and the plant-growing containers were spaced 40.6 cm apart within rows. Both greenhouses were equipped with environmental controls for year-round production.

For the purposes of this study, the south-facing tank was stocked with 3000 Nile tilapia (*Oreochromis niloticus*; 157 g/fish), 40 fish/m^3, on 29 August 2012. Fish were hand-fed a 36% protein floating feed, with 6.0% crude fat and 3.5% crude fiber (Cargill®, Franklinton, LA, USA), at 13% body weight/day twice daily (8:30 a.m. and 4:00 p.m.) for approximately 20 min. Calcium-hydroxide ($Ca(OH)^2$) was applied after each feeding to maintain a targeted pH of 6.8 to 7.0 [8]. Oxygen was supplied by two 1118.5 W (1.5 horsepower) regenerative blowers feeding the air-diffusing tubing that was suspended around the walls of the fish tank. Dissolved oxygen and temperature of the fish culture water were recorded twice daily (YSI 550A, YSI Inc., Yellow Springs, OH, USA). Fish were harvested 150 days after stocking (24 January 2013). The water for fish production was sourced from a reservoir that is fed by the surrounding watershed.

To evaluate the yields of tomatoes grown with AE against conventionally grown plants, a commercially available hydroponic fertilizer, Bag Culture Tomato Special 3-13-29, (3.0% N; 13.0% P;

29.0% K; 0.1% B, Cu, and Mn; 0.34% Fe; 5.4% Mg; 0.01% Mo; 11.0% S; 0.045% Zn; Total Grow^{TM}, Winnsboro, LA, USA) and greenhouse-grade calcium nitrate (15.5N-0P-0K) were used for the control treatment. Plants were irrigated and fertilized at rates following recommendations by Hanna [22] (Table 1). The irrigation water source for CF-grown plants was supplied by the local municipal water source. The cherry tomato (*S. lycopersicum* var. *cerasiforme*) cvs. Favorita and Goldita (Paramount Seeds, Stuart, FL, USA) were used. On 1 October 2012, 8-week-old tomato transplants were transplanted into 11 L Bato pots (Bato Plastics B.V. Zevenbergon, The Netherlands) filled with commercial grade perlite. Two tomatoes were planted in each pot, resulting in a plant density of 3.2 plants/m^2, and placed in the high wire trellis system previously described. Each pot served as a single experimental unit.

Table 1. Fertilization schedule for greenhouse tomato production [Z].

Week Following Transplanting	Days Following Seeding	Fertilizer Components [Y]		Times of Irrigation per Day	N ppm	K ppm
		3-13-29	Calcium Nitrate			
1	35	45	30	3	56	100
2	42	52	37	4	77	110
3	49	60	45	5	90	130
4	56	67	52	6	99	150
5	63	75	60	7	113	170
6	70	82	67	8	129	190
7	77	90	67	9	129	200
8	84	97	67	10	129	220
9	91	105	67	11	131	240
10	98	105	67	12	135	260

[Z] From Hanna [22]. [Y] Grams (dry weight) per 100 L.

This study consisted of two treatments: aquaculture effluent (AE) and commercial fertilizer (CF). AE was pumped from the irrigation sump adjacent to the fish greenhouse. The tomato varieties were evaluated simultaneously but in separate experiments. Plants were arranged in a completely randomized design, with 10 replicates for both treatments of "Favorita". "Goldita" had 9 replicates of AE-grown plants and 11 replicates of CF-grown plants. Tomato harvest began 61 days after transplanting (DAT), and fish harvest occurred at 117 DAT of the tomato crop. Tomato harvest continued daily until the termination of the study (157 DAT). Tomato fruits were harvested based on ripeness, with fruit color used as an indicator. Tissue samples were taken at the final harvest.

The irrigation sump was used to access clarified water for the drip irrigation system for the soilless culture of cherry tomatoes in the adjacent greenhouse. A 1118.5 W (1.5 horsepower) irrigation pump was plumbed to the previously described irrigation sump, where it drew AE water from $\frac{1}{2}$ the depth of the tank and delivered pressurized settled water at 206.8 kPa (30 psi) to the tomato irrigation system (Figure 1). The pressure was regulated by bleeding excess pressure back into the irrigation sump. Both treatments were delivered to plants using a clog-resistant pressure-compensated emitter (Bowsmith Nonstop Emitter, Bowsmith Inc. Exeter, CA, USA) at a flow rate of 3.785 L·h^{-1}. Each container was outfitted with two emitters. Plants grown with AE received water directly from the irrigation sump. Plants grown with CF received water and fertilizer through 2 fertilizer injectors (Model DM11, Dosatron, Clearwater, FL, USA). This allowed separate, but simultaneous, injections of the hydroponic fertilizer blend and calcium nitrate. Solenoid valves responsible for delivering the respective treatments were wired in tangent so that both treatments were applied at the same time.

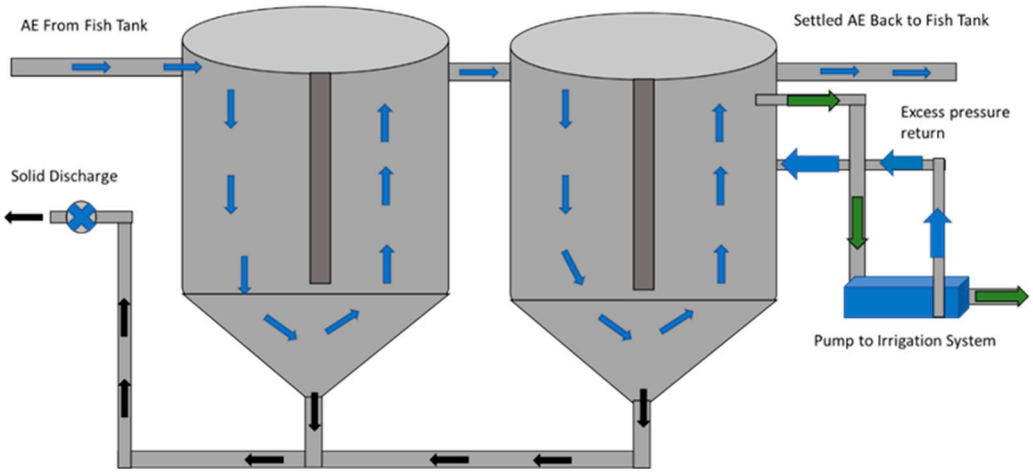

Figure 1. Schematic of water clarifier and irrigation system.

A three-liter sample of the fish culture water and AE from the irrigation sump was collected and analyzed once weekly to characterize total ammonia nitrogen (TAN), nitrate, nitrite, potassium, and orthophosphate of the water being used to irrigate the tomato plants receiving AE. Each sample was filtered using a 40-micron Whatman™ glass fiber filter (VWR International, Radnor, PA, USA). Standard curves were fit for TAN, nitrate–nitrogen, potassium, and orthophosphate on a GENESYS 20 visible spectrophotometer (Spectronic Unicam, Rochester, NY, USA). Nessler method 8038 (Hach Company, Loveland, CO, USA) was used to determine TAN; the ascorbic acid method 8048 (Hach Company, Loveland, CO, USA) was used to determine orthophosphate. Nitrate–nitrogen and potassium were analyzed using Cardy twin nitrate and potassium meters (Spectrum Technologies, Inc., Plainfield, IL, USA). Titration method 8329, using ethylenediamine–tetraacetic acid (Hach Company, Loveland, CO, USA), was used to determine calcium and magnesium. Total phosphorus and total nitrogen were determined through persulfate digestion [23]. Digestates of nitrate and orthophosphate were determined using spectrophotometric screening and an ascorbic acid method [23–25].

Plant tissue was collected at termination from the third leaf from the terminal shoot. Samples were analyzed at Brookside Laboratories (New Bremen, OH, USA) for N. Combustion analysis was used to determine total nitrogen [26] using a Carlo Erba 1500 series analyzer (CE Elantech, Inc., Lakewood, NJ, USA). Minerals (P, K, Ca, Mg, S, B, Fe, Mn, Cu, Zn, and Al) were extracted using methods described by [26] and analyzed with a Thermo 6500 duo ICP (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Settleable solids were determined for water contained in the fish tank and water returning to the tank from the irrigation sump using an adopted procedure of Standard Method 2540 F [23]. Avliminech [27] reported that floc particles became reanimated if left undisturbed for the 1-h recommended settling time in the procedure described in Standard Method 2450 due to gas bubbles forming. For purposes of this study, a 30-min period was used for settling. Suspended solids were measured according to Standard Method 2540 D [23] using glass fiber filtration, followed by gravimetric analysis. The pH of AE of the samples was taken twice daily at approximately 9:00 a.m. and 4:00 p.m.

An analysis of variance was performed on all responses using a generalized linear model (PROC GLIMMIX in SAS version 9.4 SAS Institute, Cary, NC, USA). The experimental design for yield was a randomized design, with each variety analyzed separately. Due to a mistake at planting, the varieties were not randomized together; thus, no comparisons are made between varieties. The treatment design for yield was a two-way factorial of nutrient source and harvest date. Data recorded over harvest dates were analyzed as repeated measures using a heterogeneous compound symmetry covariance structure. Linear and quadratic trends were tested using qualitative/quantitative model regressions, and differences between fertility types least squares means were tested using F-tests. The experimental design for two yield totals (pre and post fish harvest) was a completely randomized design. Differences in yield between fertility types and between yield totals at fish harvest and

termination were tested using F-tests. Where residual plots and a significant covariance test for homogeneity (H_0 = homogeneity) indicated heterogeneous variance among treatments, a RANDOM statement with the GROUP option was used to correct heterogeneity.

Means comparisons for plant tissue were analyzed using a *t*-test (Proc TTest SAS, ver. 9.2, SAS Institute, Cary, NC.). If variances were equal, the pooled method was used to determine significance. If variances were unequal, the Satterthwaite method was used to determine significance.

3. Results

3.1. Fish Production

Fish were grown in the biofloc system for 149 days. The final harvested biomass was 1502 kg (15.0 kg/m^3) live weight of tilapia (Table 2). The total harvested fish biomass produced (final–initial) was 1032 kg of fish (10.3 kg/m^3; Table 2). Survival was approximately 96%, with 3000 fish stocked and 2872 fish harvested. This yield represents a 220% increase in growth over 149 days of production, and fish grew at a rate of 2.3 g/day/fish.

Table 2. Inputs and outputs of a 149-day tilapia crop in a 100-m^3 production system.

	Total per 100 m^3	per m^3 of Fish Production [Z]	per kg of Fish
Final Biomass (kg) [Y]	1502	15.0	-
Beginning biomass (kg)	470	4.7	-
Feed (kg)	2010	20.1	1.9
Power use (kWh) [X]	5338	53.4	5.2
Water (m^3)	168	1.7	0.2
Base (kg) [W]	159	1.6	0.2

[Z] Calculated from 100 m^3 fish production unit. [Y] Final biomass of Nile tilapia (*Oreocrhromis niloticus*) and 96% survival rate. [X] Power included energy consumption from regenerative blowers and greenhouse fans. [W] Calcium hydroxide was used as base source.

The biofloc system used in this study was managed strictly as an autotrophic system with no supplemental carbon inputs. Water exchange was minimal and typically only involved make-up water due to loss from solid removal and plant water needs. Total water use was approximately 168 m^3 and translated to 6.14 kg/m^3 per kg of fish biomass produced (total water use/fish biomass produced; Table 2). The power required was 5.2 kW/kg of tilapia biomass produced and translated to 35.8 kW/day (Table 2). Base addition using calcium hydroxide would be considered a minor input of 158.9 kg or 0.2 kg per kg of fish biomass gained. Feed inputs totaled 2010 kg (20.1 kg/m^3) and represented an FCR of 1.9 (Table 2).

Total ammonia nitrogen was averaged to 2.3 ± 0.95 mg/L in the fish production tanks (Table 3). The mean nitrite within fish production tanks was 6.2 ± 1.5 mg/L., above recommended levels. Dissolved oxygen (DO) concentrations averaged 5.7 to 4.9 mg/L for morning and afternoon, respectively (Table 4). DO concentrations were approximately 16% higher in the morning than in the evening (Table 4). The difference observed in the mean temperatures, between morning (26.9 °C) and afternoon (27.8 °C), in combination with feed inputs, were likely the reason for DO temperature fluctuations. Water pH within the fish culture tank was maintained at approximately 6.7 (Table 4).

Table 3. Water quality parameters as related to fish health during the 149-day production cycle in the minimum water exchange biofloc production system.

Parameter	Location	Mean [Z]
Total Ammonia Nitrogen (mg/L)	Production Tank	2.3 ± 0.95
	Exiting Clarifier	2.2 ± 1.15
Nitrite (mg/L)	Production Tank	6.2 ± 1.50
	Exiting Clarifier	6.1 ± 1.30
Nitrate-N (mg/L)	Production Tank	330.6 ± 99.70
	Exiting Clarifier	331.0 ± 106.00
Total Hardness (mg/L)	Production Tank	1217 ± 368.00
	Exiting Clarifier	1232 ± 368.00
Total Suspended Solids (mg/L)	Production Tank	508.8 ± 210.00
	Exiting Clarifier	463.6 ± 170.60
Settleable Solids (ml/L)	Production Tank	21.1 ± 21.60
	Exiting Clarifier	11.6 ± 15.90

[Z] Standard deviations of the means were calculated from water samples taken weekly.

Table 4. Daily water quality parameters as related to fish health in the minimum water exchange biofloc production system.

Parameter	Time Measured	Mean [Z]
Dissolved Oxygen (mg/L)	AM	5.7 ± 0.8
	PM	4.9 ± 0.5
Temperature (°C)	AM	27 ± 3.0
	PM	28 ± 3.0
pH	AM	6.7 ± 0.2
	PM	6.7 ± 0.2

[Z] Standard deviations of the means were calculated from weekly water samples.

3.2. Cherry Tomato Production

For tomato production before fish harvest (117 DAT), few differences in yield were observed between plants grown with AE and CF for each harvest date for the cherry tomato 'Favorita' (Tables 3, 5 and 6). Some differences were seen between treatments before fish harvest in 'Goldita' (Table 6). At fish harvest, the total yields across all harvest dates for 'Favorita' were 5.9 kg/m^2 (CF) and 5.5 (AE) kg/m^2 and were not different (Table 7). However, for 'Goldita', yields were 5.5 kg/m^2 (CF) and 4.3 kg/m^2 (AE) kg/m^2 and were significant (Table 7). The fish production system went 22 days without feed input until a new crop of fish was stocked. At crop termination, differences in total yield were observed between AE and CF and were higher for CF-grown plants. Total yield at tomato crop termination (157 DAT) for 'Favorita' was 11.4 kg/m^2 for CF-grown plants and 9.3 kg/m^2 for AE-grown plants (Table 7). 'Goldita' plants produced 10.54 kg/m^2 of fruit for CF-grown plants and 7.4 kg/m^2 for AE-grown plants (Table 7).

Table 5. Probability values from analysis of variance for yield, shown by cultivar and harvest date.

Cv. Goldita			
Yield by Harvest Date		Total Yield	
ANOVA	Pr > F	ANOVA	Pr > F
Nutrient Source	<0.0001	Nutrient Source	<0.0001
Harvest Time	<0.0001	Harvest Time (117 and 157 days)	<0.0001
Nutrient Source × Harvest Time	<0.0001	Nutrient Source × Harvest Time	0.0006
Cv. Favorita			
Yield by Harvest Date		Total Yield	
ANOVA	Pr > F	ANOVA	Pr > F
Nutrient Source	<0.0001	Nutrient Source	0.0013
Harvest Time	<0.0001	Harvest Time (117 and 157 days)	<0.0001
Nutrient Source × Harvest Time	<0.0001	Nutrient Source × Harvest Time	0.0172

Table 6. Fertility type influence on yield by harvest date and variety.

			Cv. Goldita (kg·m^2) by Harvest Time [Z]										
DAT	59	67	73	80	88	98	105	116	130	141	150	157	Sign. [y]
CF [X]	-	0.1	0.6	0.3	0.7	1.1	1.1	1.5	1.7	1.4	0.9	1.0	Q ***
AE [W]	-	0.1	0.5	0.2	0.7	0.9	0.9	1.0	1.0	0.8	0.6	0.7	Q ***
Significance [V]		ns	*	*	ns	ns	ns	*	*	*	*	*	
			Cv. Favorita (kg·m^2) by Harvest Time										
DAT	59	67	73	80	88	98	105	116	130	141	150	157	Sign. [y]
CF [X]	0.1	0.5	0.7	0.4	0.6	1.4	0.9	1.2	1.8	1.4	1.2	1.2	Q ***
AE [W]	0.1	0.5	0.6	0.4	0.7	1.4	1.0	0.8	1.1	0.8	1.0	0.8	Q ***
Significance [V]	ns	ns	ns	ns	ns	ns	ns	*	*	*	ns	*	

[Z] Nutrient source by harvest date interaction was significant at $p < 0.05$ (*). [y] Significant quadratic (Q) trends using qualitative/quantitative model regressions at $p < 0.001$ (***). [X] CF = Conventional fertilizer treatment. [W] AE = Aquaculture effluent treatment. [V] Least squares means comparisons between fertility types using F-tests at $p < 0.05$. ns = not significant.

Table 7. Nutrient source influence on total yield at fish harvest (117 DAT) and crop termination (157 DAT). [z]

	Goldita				**Favorita**		
	Yield at Fish Harvest	Yield at Termination	Sign. [Y]		Yield at Fish Harvest	Yield at Termination	Sign. [Y]
CF [X]	5.5	10.5	*	CF	5.9	11.4	*
AE [W]	4.3	7.4	*	AE	5.5	9.3	*
Sign. [Y]	*	*		Sign.	ns	*	

[z] Nutrient source by harvest date interaction was significant at $p < 0.05$ (*). [Y] Least squares means comparisons between fertility types (columns) and harvest date totals (rows) using F-tests at $p < 0.05$ (*). ns = not significant. [X] CF = Conventional fertilizer treatment. [W] AE = Aquaulture effluent treatment.

ANOVA was performed on all responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). The experimental design for yield was a completely randomized design with each cultivar analyzed separately.

3.3. Aquaculture Effluent Nutrient Parameters

Macronutrients were only recorded up to fish harvest, with the exception of one sample analyzed at the termination of the tomato crop (Table 8). Nitrate–nitrogen was considerably higher for AE when compared to CF throughout the study and ranged from 150 to 540 mg/L. At crop termination, nitrate–nitrogen was 4 times the concentration in AE than CF. Available phosphorus was considerably lower for AE than CF and likely influenced yield. Concentrations of PO_4-P in AE ranged from 7 to 25 mg/L and averaged 14 mg/L for AE and 60 mg/L for CF in the last 9 weeks of production. The supply of K was adequate for plant growth throughout the study. As a result of daily additions of CaOH to maintain pH and the minimum exchange of water, calcium was in excess for AE throughout the entire study and ranged from 184 to 688 mg/L. At termination, AE had 3 times as much Ca when compared to the CF treatment. Magnesium ranged from 24 to 71 mg/L but was typically within ranges found in common nutrient solutions [28].

Table 8. Macronutrient concentrations for aquaculture effluent and conventional fertilizer treatments used to grow cherry tomato.

	NO_3-N		PO_4-P		Total P	K		Ca		Mg	
DAP [Z]	CF [YX]	AE	CF	AE	AE	CF	AE	CF	AE	CF	AE
5	60	150	26	17	60	108	170	88	184	24	29
12	73	170	29	17	39	125	170	109	212	28	42
19	88	210	34	17	65	144	220	133	280	32	49
23	101	250	38	16	-	161	250	153	268	36	46
33	116	250	43	13	82	181	220	177	328	41	24
41	128	300	46	10	81	197	250	197	412	44	-
47	131	320	51	7	60	217	240	197	428	49	24
54	133	340	55	11	77	233	245	197	444	52	29
61	135	340	60	8	70	253	250	197	468	57	42
68	135	360	60	11	68	253	240	197	488	57	39
80	135	400	60	9	52	253	240	197	536	57	29
83	135	430	60	10	-	253	240	197	568	57	39
89	135	370	60	21	74	253	240	197	504	57	54
86	135	440	60	13	-	253	290	197	568	57	54
103	135	460	60	14	-	253	240	197	616	57	32
110	135	520	60	25	90	253	278	197	592	57	71
152	135	540	60	12	-	253	240	197	688	57	49

[Z] DAP = Days after planting. [Y] CF = Conventional fertilizer treatment, AE = Aquaculture effluent treatment. [X] Conventional fertilizer nutrients were calculated based off fertilizer formulations and rates applied.

3.4. Nutrient Concentrations in Cherry Tomato Tissue

No visual symptoms of nutrient deficiencies were observed throughout the study. Leaf tissue concentration of nutrients at crop termination was generally lower for AE plants when compared to CF for both cultivars (Tables 9 and 10). For "Favorita", no difference was observed in N tissue concentration between nutrient sources. "Golidata" plants grown with CF were higher in N concentrations when compared to AE. Calcium was significantly higher in AE-grown plants, across varieties, by a factor of 1.5. Sulfur, zinc, and aluminum were lower in leaf nutrient concentrations for AE when compared to CF with Favorita. Nutrients were generally lower for all elements in AE Goldita plants when compared to CF, with the exception of zinc and calcium.

Table 9. Nutrient concentration of cherry tomato "Favorita" leaf tissue.

Treatment	Percent Macronutrient Found in Leaf Tissue [Z]					
	Nitrogen	**Phosphorous**	**Magnesium**	**Potassium**	**Calcium**	**Sulfur**
CF [Y]	2.77	0.44	0.51	4.48	3.89	1.92
AE [X]	2.62	0.27	0.33	3.20	6.01	1.97
Significance	NS	*	*	*	*	NS
Treatment	Concentration (mg/L of micronutrient found in leaf tissue [Z]					
	Boron	Iron	Manganese	Copper	Zinc	Aluminum
CF	113.30	115.00	711.30	11.90	33.50	16.10
AE	49.67	73.00	243.00	6.07	38.13	21.33
Significance	*	*	*	*	NS	NS

[Z] Means were analyzed using Proc T-test (SAS Version 9.2 SAS Institute, Cary, NC). If variances were found to be equal the Pooled method was used to determine significance. If variances were unequal Satterthwaite method was used to determine significance. $p \leq 0.05$ (*); NS = nonsignificant. [Y] CF = Conventional fertilizer treatment. [X] AE = Aquaculture Effluent treatment.

Table 10. Nutrient concentration of cherry tomato "Goldita" leaf tissue.

Treatment	Percent Macronutrient Found in Leaf Tissue [Z]					
	Nitrogen	**Phosphorous**	**Magnesium**	**Potassium**	**Calcium**	**Sulfur**
CF [Y]	2.93	0.31	0.78	4.87	4.35	1.99
AE [X]	2.63	0.23	0.47	3.33	6.41	1.61
Significance	*	*	*	*	*	*
Treatment	Concentration (mg/L) of micronutrient found in leaf tissue [Z]					
	Boron	Iron	Manganese	Copper	Zinc	Aluminum
CF	145.67	126.67	736.33	10.63	28.80	22.13
AE	38.43	56.87	179.33	5.20	65.23	14.33
Significance	*	*	*	*	*	*

[Z] Means were analyzed using Proc T-test (SAS Version 9.2 SAS Institute, Cary, NC). If variances were found to be equal the Pooled method was used to determine significance. If variances were unequal Satterthwaite method was used to determine significance. $p \leq 0.05$ (*); NS = nonsignificant. [Y] CF = Conventional fertilizer treatment. [X] AE = Aquaculture effluent treatment.

Nitrogen in AE and CF plants, for both varieties, were lower than levels recommended by Snyder [29] (Table 11), with 4.0% to 5.5% N. However, it was found to be within ranges proposed by Jones [28] (2.5% to 4.0%). Phosphorus was lower for AE-grown Favorita and Goldita plants and was also below concentrations proposed by Snyder [29]. Magnesium was lower for AE-grown plants, across both varieties, and was also lower than levels recommended by Snyder [29] but were near the lower end of recommendations suggested by Jones [28]. For both varieties, potassium was lower than the level recommended by Snyder [29] for AE-grown plants. Potassium leaf nutrient sufficiency ranges were considerably lower (1.25% to 2.5%) than recommended by Snyder [28], and K tissue concentrations for AE plants were above this level for varieties. Calcium was significantly higher for AE plants when compared to CF-grown plants. No differences were seen in S tissue concentrations between the two treatments for Favorita, but S was slightly lower in AE-grown Goldita plants when compared to CF. Across treatments and varieties, S was above the recommended levels proposed by Jones [28].

Table 11. Optimum levels of nutrient elements in greenhouse tomato leaf tissue.

	Percent Macronutrient Found in Leaf Tissue [Z]					
	Nitrogen	**Phoshorus**	**Potassium**	**Calcium**	**Magnesium**	
Snyder [Z]	4.0–5.5	0.3–1.0	4.0–7.0	1.0–5.0	0.4–1.5	
Jones	2.8–4.5	0.3–0.75	2.5–4.0	1.5–4.0	0.4–1.3	
	Concentration (mg/L) of micronutrient found in leaf tissue					
	Iron	Zinc	Manganese	Copper	Boron	Molybdenum
Snyder	100–250	30–150	40–300	5–25	35–100	0.15–5.0
Jones	40–300	20–100	40–400	20-May	25–100	0.1–10

[Z] From Snyder (1992) and Jones (2005).

Differences between treatments and micronutrients were substantially greater than what was observed in the macronutrients. Boron was within sufficiency ranges but were 56% and 73% lower in AE tissue when compared to CF for Favorita and Goldita, respectively [29]. AE-grown plants were 27% ("Favorita") and 55% ("Goldita") lower than CF-grown plants and were below the suggested levels of Snyder [29] but within the acceptable levels of Jones [28]. Sufficiency ranges for Mn have a wide range, and AE plants for both varieties fell within these ranges; however, they were 65% ("Favorita") and 75% ("Goldita") lower than CF tissue concentrations. Copper for AE-grown plants was within sufficiency ranges, but at the lower end, and was nearly half of the concentrations in CF-grown plants. No differences were seen in Zn tissue between treatments for Favorita, but AE-grown Goldita plants contained nearly twice the amount of Zn in AE plants when compared to CF.

4. Discussion

4.1. Fish Production Parameters

The biomass load in this study (15.0 kg/m^3) is comparable to that in the study of Rakocy et al. [30], with a similarly managed outdoor system (14.4 kg/m^3 and 13.7 kg/m^3) and similar tank volume and horsepower aeration (0.56 kW/100 m^3). Timmons and Ebeling [31] described 40 kg/m^3 as the maximum biomass that can be produced through aeration with no supplemental oxygen. Growth rates for biofloc tilapia production (2.3 g/day/fish) were lower than reported by Rakocy et al. [30] (3.0 to 4.0 g/day/fish). FCRs in this experiment were comparable to FCR reported by Rakocy et al. [30] of 2.2 to 1.8 g of feed to grams of net fish biomass. Reduced growth rates could be due to chronic high nitrite concentrations experienced throughout production. It was observed that the fish response to feed was less aggressive; however, mortality remained less than 4% throughout the production cycle. Total ammonia nitrogen averaged to 2.3 ± 0.95 mg/L in the fish production tanks (Table 3). The mean nitrite within fish production tanks was 6.2 ± 1.5 mg/L, above recommended levels. Dissolved oxygen (DO) concentrations averaged 5.7 to 4.9 mg/L for morning and afternoon, respectively (Table 4). DO concentrations were approximately 16% higher in the morning than in the evening (Table 4). The difference observed in mean temperatures between morning (26.9 °C) and afternoon (27.8 °C), in combination with feed inputs, was likely the reason for DO temperature fluctuations. Water pH within the fish culture tank was maintained at approximately 6.7 (Table 4.)

4.2. Tomato Production

This study shows the potential for the integration of BFT and hydroponic cherry tomato production, but improvements are necessary to increase yield. The control (conventional fertilizer) used in this study was comparable to yields reported in the literature [32,33]. Testa et al. [33] reported yields from 30 cherry tomato greenhouse operations in Sicily. The Sicilian growers used "Creative F1", had an average plant density of 3.2, and carried crops to the 20 and 22 fruit clusters. In our study, "Favorita"

plants grown with AE were near the lower end of the range reported by Testa [33] when comparing the calculated yield per fruit cluster. Nevertheless, AE-grown "Favorita" yields from our study were considerably lower than those reported by Halmann and Kobryn [32] when comparing the calculated yield per fruit cluster.

Reduced yield in AE-grown plants was likely a result of plant nutrition. Plants grown with AE were generally lower in concentrations of nutrients in leaf tissue; however, no symptoms of deficiency were visually observed throughout the study (Tables 9 and 10). Phosphorus concentrations may have been the most limiting nutrient as AE-grown plants were 26% ("Goldita") and 30% ("Favorita") lower in P than CF-grown plants. Phosphorus concentrations in tissue were also found to be below the recommendations reported by Snyder [29]. Available phosphorus was in undersupply throughout the study (Table 8); however, this did not explain the distinction in yield difference pre and post fish harvest. Supraoptimal Ca concentrations due to twice daily calcium hydroxide applications could have influenced the availability of P. Calcium phosphate formation would have been intensified if pH increased within the irrigation system or pore water within the substrate, but pH was not measured in these areas.

Excessive Ca concentrations may have resulted in lower available P. In a later study using the same system, Blanchard et al. [34] investigated the response of pH on nutrient assimilation of nutrients in aquaponically grown cucumbers; however, Ca concentrations were considerably lower than levels observed in this study. Da Cerozi and Fitzsimmons [34] demonstrated the effects of pH on P availability; however, a significant reduction was not realized until pH reached 10. In this same study, Ca-binding compounds (dissolved organic carbon and alkalinity) were determined to play a significant role in preventing mineralization of orthophosphate. These compounds bind to available Ca, thereby reducing the opportunity for calcium phosphate formation [35]. In our study, Ca was in plentiful supply while alkalinity was low (data not shown). Dissolved organic carbon was not measured. More work is needed to determine if increasing the availability of P through pH modification in a similar situation, with extreme Ca availability, would increase yield.

Many of the macronutrients (N, P, K, and Mg) were found to be lower in AE-grown plants despite concentrations being greater than the recommended levels. We speculate that denitrification may have occurred in the irrigation systems due to anaerobic conditions associated with the hydraulic retention time between irrigation cycles. Denitrification would reduce nitrate in the system and, subsequently, increase pH. The case for denitrification could be made as NO_3-N concentrations were 4 times greater in AE when compared to the CF solution, yet AE was found to have lower concentrations in tissue than CF. Water samples used in the analysis were taken at the irrigation sump instead of the drip emitter; consequently, it cannot be determined if nutrient concentration or availability changed between the irrigation sump and the drip emitter.

5. Conclusions

This study demonstrates that substrate-based hydroponic systems have the potential to be integrated with biofloc production systems. However, further refinements are needed to increase yields to commercial levels. Future work in decoupled systems should investigate the potential for denitrification occurring in the drip irrigation system. Evaluation of alternatives to CaOH for pH management in the system may also improve the yield of fruiting crops grown with BFT effluent by reducing free Ca in the system.

Author Contributions: Conceptualization, J.M.P., J.J.D., J.L.S., J.A.C., and T.R.H.; methodology, J.M.P.; formal analysis, J.M.P.; data curation, J.J.P. and J.J.D.; writing—original draft preparation, J.M.P.; writing—review and editing, J.M.P., J.L.S., J.A.C., and T.R.H.; visualization, J.M.P.; supervision, J.M.P.; project administration, J.A.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Soy Aquaculture Alliance and Alabama Agriculture Experiment Station.

Acknowledgments: The authors wish to thank Raymond Kessler for his assistance with data analysis and Luke Foshee and Micah Fern in their assistance with data collection and project maintenance.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Anonymous. *Impact of Rising Feed Ingredient Prices on Aquafeeds and Aquaculture Production*; Food and Agriculture Organization of the United Nations, FAO Fisheries Department: Rome, Italy, 2009.
2. Heinen, J.M.; Hankins, J.A.; Adler, P.R. Water quality and waste production in a recirculating trout-culture system with feeding of a higher-energy or a lower-energy diet. *Aquac. Res.* **1996**, *27*, 699–710. [CrossRef]
3. Rafiee, G.; Saad, C.R. Nutrient cycle and sludge production during different stages of red tilapia (*Oreochromis* sp.) growth in a recirculating aquaculture system. *Aquaculture* **2005**, *244*, 109–118. [CrossRef]
4. De Schryver, P.; Crab, R.; Defoirdt, T.; Boon, N.; Verstraete, W. The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture* **2008**, *277*, 125–137. [CrossRef]
5. Avnimelech, Y. Bio-filters: The need for a new comprehensive approach. *Aquac. Eng.* **2006**, *34*, 172–178. [CrossRef]
6. Azim, M.E.; Little, D.C. The Biofloc technology (BFT) in indoor tanks: Water quality, biolfoc composition and growth and welfare of Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **2008**, *283*, 29–35. [CrossRef]
7. Avnimelech, Y. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture* **1999**, *176*, 227–235. [CrossRef]
8. Rakocy, J.E.; Masser, M.P.; Lasordo, T.M. *Recirculating Aquaculture Tank Production Systems: Aquaponics-Integrating Fish and Plant Culture*; Publication No. 454; Southern Regional Aquaculture Center: Stoneville, MI, USA, 2006.
9. Rakocy, J.E. *Hydroponic Lettuce Production in a Recirculating Fish Culture System*; University of the Virgin Islands, AES: St. Croix, VI, USA, 1988; Volume 3, pp. 5–10. Available online: https://dloc.com/CA01300009/00001/3j (accessed on 30 July 2020).
10. Clarkson, R.; Lane, S.D. Use of a small-scale nutrient-film hydroponic technique to reduce mineral accumulation in aquarium water. *Aquac. Fish. Manag.* **1991**, *22*, 37–45. [CrossRef]
11. Al-Hafedh, Y.S.; Alam, A.; Beltagi, M.S. Food production and water conservation in a recirculating aquaponic system in Saudi Arabia at different ratios and fish feed and plants. *J. World Aquac. Soc.* **2008**, *39*, 510–520. [CrossRef]
12. Takeda, F.; Adler, P.R.; Glenn, D.M. Strawberry production linked to aquaculture wastewater treatment. *Acta Hortic.* **1997**, *439*, 637–677. [CrossRef]
13. Quillere, I.; Roux, L.; Marie, D.; Roux, Y.; Gosse, F.; Morot-Gaudry, J.F. An artificial productive ecosystem based on a fish/bacteria/plant association 2 Performance. *Agric. Ecosyst. Environ.* **1995**, *53*, 19–30. [CrossRef]
14. Chaves, P.A.; Beltrao, J.; Laird, L.M.; Sutherland, R. Lettuce (*Lactuca sativa* sp.) response to fish culture effluent. *Acta Hortic.* **2000**, *537*, 705–708. [CrossRef]
15. Rakocy, J.E.; Schultz, R.C.; Bailey, D.S.; Thoman, E.S. Aquaponic production of tilapia and basil: Comparing a batch and staggered cropping system. *Acta Hortic.* **2004**, *648*, 63–69. [CrossRef]
16. Lewis, W.M.; Yopp, J.H.; Schramm, H.L.; Brandenburg, A.M. Use of hydroponics to maintain quality recirculated water in a fish culture system. *Trans. Am. Fish. Soc.* **1978**, *107*, 92–99. [CrossRef]
17. Watten, B.J.; Busch, R.L. Tropical production of tilapia (*Sarotherodon aurea*) and tomatoes (*Lycopersicon esculentum*) in a small-scale recirculating water system. *Aquaculture* **1984**, *41*, 271–283. [CrossRef]
18. McMurty, M.R.; Sanders, D.C.; Nelson, P.V.; Nash, A. Mineral nutrient concentration and uptake by tomato irrigated with recirculating aquaculture water influenced by quantity of fish waste products supplied. *J. Plant Nutr.* **1993**, *16*, 407–419. [CrossRef]
19. Mariscal-Lagarda, M.M.; Paez-Osuna, F.J.L.; Esquer-Mendez, I.; Guerrero-Monroy, A.; del Vivar, A.R.; Felix-Gastelum, R. Integrated culture of white shrimp (*Litopenaeus vannamei*) and tomato (*Lycopersicon esculentum* Mill) with low salinity groundwater: Management and production. *Aquaculture* **2012**, *366*, 76–84. [CrossRef]
20. Castro, R.S.; Azevedo, C.M.S.B.; Bezerra-Neto, F. Increasing cherry tomato yield using fish effluent as irrigation water in Northeast Brazil. *Sci. Hortic.* **2006**, *110*, 44–50. [CrossRef]
21. Savidov, N.A.; Hutchings, E.; Rakocy, J.E. Fish and plant production in a recirculating aquaponic system: A new approach to sustainable agriculture in Canada. *Acta Hortic.* **2007**, *742*, 209–221. [CrossRef]
22. Hanna, H. Hanna Tomato Book, Baton Rouge, Lousiana. 2013. Available online: https://www.lsuagcenter.com/NR/rdonlyres/38D013C6-A7BF-4702-88DC-921E038778F8/10111/HannaTomatoBook.pdf (accessed on 30 July 2020).

23. Rice, E.W.; Baird, R.B.; Eaton, A.D.; Clesci, L.S. (Eds.) *Standard Methods for Examination of Water and Wastewater*, 22nd ed.; American Water Works Assn: Washington, DC, USA, 2012.
24. Prapaiwong, N.; Boyd, C.E. Effluent volume and pollutant loads at an inland, low-salinity, shrimp farm in Alabama. *Aquac. Eng.* **2012**, *48*, 1–5. [CrossRef]
25. Gross, A.; Boyd, C.E. A digestion procedure for simultaneous determination of total nitrogen and total phosphorus in pond water. *J. World Aquacult. Soc.* **1998**, *29*, 300–303. [CrossRef]
26. Gavlak, R.G.; Horneck, D.A.; Miller, R.O.; Kotuby-Amacher, J. *Soil, Plant, and Water Reference Methods for the Western Region*, 2nd ed.; WREP-125: Corvalis, OR, USA, 2003.
27. Avnimelech, Y. Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. *Aquaculture* **2007**, *264*, 140–147. [CrossRef]
28. Jones, J.B. *Hydroponics: A Practical Guide for the Soilless Grower*, 2nd ed.; CRC Press: Baco Raton, FL, USA, 2005; p. 423.
29. Snyder, R.G. *Greenhouse Tomato Handbook*; Extension ServiceBullington, P1828; Soneville: Washington, MS, USA, 2019; p. 199.
30. Rakocy, J.E.; Danaher, J.J.; Bailey, D.S.; Shultz, R.C. Development of a Biofloc System for the Production of Tilapia. Better Science, Better Fish, Better Life. In Proceedings of the Ninth International Symposium on Tilapia in Aquaculture, Shanghai, China, 22–24 April 2011; pp. 344–348.
31. Timmons, M.B.; Ebeling, J.M. *Recirculating Aquaculture*, 3rd ed.; Ithaca Publishing: Ithaca, NY, USA, 2013.
32. Halmann, E.; Kobryn, E. Yield and quality of cherry tomato (*Lycopersicon esclentum* var. *cerasiforme*) cultivated on rockwool and cocofiber. *Acta Hortic.* **2003**, *614*, 693–697. [CrossRef]
33. Testa, R.; Trapani, A.M.; Sgroi, F.; Tudisca, S. Economic sustainability of Italian greenhouse cherry tomato. *Sustainability* **2014**, *6*, 7967–7981. [CrossRef]
34. Blanchard, C.; Wells, D.E.; Pickens, J.M.; Blersch, D.M. Effect of pH on Cucumber Growth and Nurient Availability in a Decoupled Aquaponic System with Minimal Solids Removal. *Horticulturae* **2020**, *6*, 10. [CrossRef]
35. Da Cerozi, B.S.; Fitzsimmons, K. The effect of pH on phosphorus availability and speciation in an aquaponics nutrient solution. *Bioresour. Technol.* **2016**, *219*, 778–781. [CrossRef] [PubMed]