water

Article

Fish Welfare in Urban Aquaponics: Effects of Fertilizer for Lettuce (*Lactuca sativa* L.) on Some Physiological Stress Indicators in Nile Tilapia (*Oreochromis niloticus* L.)

Morris Villarroel [1,*], Genaro C. Miranda-de la Lama [2], Rafael Escobar-Álvarez [3] and Rubén Moratiel [1]

[1] CEIGRAM, Technical University of Madrid (UPM), Ciudad Universitaria s/n, 28040 Madrid, Spain; ruben.moratiel@upm.es
[2] Department of Animal Production & Food Science, Agri-Food Institute of Aragon (IA2), University of Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain; genaro@unizar.es
[3] Center for Practical Teaching and Research in Animal Production and Health, Faculty of Veterinary Medicine and Animal Husbandry, National Autonomous University of Mexico, San Miguel Topilejo, Mexico 04510, DF, Mexico; rafaelescobar51@gmail.com
* Correspondence: morris.villarroel@upm.es; Tel.: +34-91-067-10-95

Abstract: The combination of hydroponics and aquaculture, or aquaponics, normally requires adding fertilizer to recirculated water to ensure optimal plant growth, but the effect of that fertilizer on fish welfare has not been studied in detail, especially for small urban and coupled aquaponic systems. In this study, a commercial fertilizer was placed in two small aquaponic systems (less than 250 liters each) to test its effect on the stress levels of Nile tilapia (*Oreochromis niloticus*) compared to controls without any fertilizer. Fish production parameters were not significantly different between treatments, nor were physiological indicators of fish stress (plasma cortisol, glucose, and triglycerides). On the other hand, lettuce growth (leaf and root weight and length) was up to three times higher in the treatment that received fertilizer.

Keywords: aquaponics; Nile tilapia; lettuce; fish welfare

Citation: Villarroel, M.; Miranda-de la Lama, G.C.; Escobar-Álvarez, R.; Moratiel, R. Fish Welfare in Urban Aquaponics: Effects of Fertilizer for Lettuce (*Lactuca sativa* L.) on Some Physiological Stress Indicators in Nile Tilapia (*Oreochromis niloticus* L.). *Water* **2022**, *14*, 935. https://doi.org/10.3390/w14060935

Academic Editor: Wayne O'Connor

Received: 11 February 2022
Accepted: 14 March 2022
Published: 17 March 2022

1. Introduction

The global agri-food industry is developing new practices to produce local and sustainable food [1]. One possible solution is to save and recycle water and nutrients [2]. This is precisely the basis of aquaponics, which can be defined as a bio-integrated and multi-trophic system combining elements of recirculating aquaculture (RAS) and hydroponics, in which nutrient-enriched fish tank water is used for plant growth [3]. This mutual exchange depends on the action of two different groups of bacteria in RAS, *Nitrosomonas* spp. and *Nitrobacter* spp., which oxidize the ammonia excreted by fish into nitrates, which are more easily absorbed by plant roots [4]. When a proper balance between fish waste generation and plant nutrient uptake is achieved, daily water consumption can be reduced to just the amount of water needed to replace losses by evapotranspiration [5]. Therefore, aquaponic systems represent a form of food production model tied to the circular economy paradigm, especially relevant in urban contexts, because they have a small footprint, can mimic natural systems, and provide locally supplied products [6,7].

In Europe, tilapia (*Oreochromis niloticus*) is the main fish species used in aquaponics [8]. Tilapia are the second most important farmed food fish after carp and the preferred fish for both experimental and commercial aquaponics-based production systems [9]. Nile tilapia is the predominant species in the world's inland fish farms due to its rapid growth, disease resistance, ability to survive in poor water quality, low mortality rate, resistance to low oxygen concentrations, tolerance to high stocking densities, flexibility in feed acceptance, adaptability to tank-based culture systems, and ease of management [10,11]. In recirculation and aquaponic systems, fish welfare depends mainly on abiotic factors, such as water quality and temperature, and biotic factors, such as stocking density, parasites and pathogens, behavior, body condition, different physiological parameters, feed quality and distribution, and growth performance, as well as anthropogenic influences, such as sorting and handling [12]. However, physiological parameters such as stress hormones and metabolites, together with external body condition, are considered valid, viable, and reliable indicators of an organism's ability to cope with the challenges of the production environment [13].

Many crops can be grown in hydroponics, but species with a short cycle, small size, and fast growth, such as lettuce, are the best adapted to these cultivation systems [14]. Lettuce also performs well in aquaponic systems. Different authors have suggested different concentrations of nutrients for fertilization. For example, Schon [15] recommends a nutrient solution with 200 mg/L nitrogen, 50 mg/L phosphorus, 300 mg/L potassium, 200 mg/L calcium, and 65 mg/L magnesium. Other commercial formulations of commercial nutrient solutions, such as Hoagland®, used in lettuce growth suggest 210 mg/L nitrogen, 31 mg/L phosphorus, 235 mg/L potassium, 200 mg/L calcium, 48 mg/L magnesium, and 64 mg/L sulfur, plus micronutrients [16]. In aquaponics, nitrogenous waste produced by fish is used to grow plants, but several important minerals are needed to provide for optimal plant growth [4]. Although some minerals are indirectly provided by fish feed, most commercial companies supplement or "fertilize" the water used in the hydroponic unit with one or more ions [3]. The addition of fertilizer increases production costs and may affect fish welfare by changing the osmotic balance of their surrounding water [17]. This is especially the case in coupled aquaponics, as opposed to decoupled systems, in which growers can better separate the two water systems [18]. In coupled systems, it is necessary to analyze the possible direct detrimental effects of fertilizers on physiological indicators related to fish welfare, so that appropriate modifications of water quality practices can be recommended to minimize the biological cost of the animals during the growth process. Therefore, the objective of this study was to analyze the effects of lettuce (*Lactuca sativa* L.) fertilizer on certain indicators of physiological stress in Nile tilapia.

2. Materials and Methods

The present study was carried out in accordance with the EU Directive 2010/63/EU for animal experiments and approved by the Animal Ethics Committee of the Polytechnic University of Madrid (Spain), in compliance with the Spanish guidelines for the care and use of animals in research [19].

2.1. Study Description

The trials were carried out at the aquaculture facilities in the Department of Agricultural Production of the ETSIAAB-UPM (Madrid, Spain), using four separate aquaponic units installed in a glass greenhouse. Every unit consisted of a rectangular tank (250 L) where the tilapias were kept, each connected to a solids separator and biofilter, and an New Growing System (NGS®) hydroponic table with five rows (Minicamp, Ltd., Pulpí, Almeria, Spain), where the lettuce was grown. The solids separator consisted of an EHEIM Classic 250 filter filled with sponge, which was cleaned every two weeks. The biofilter was a trickling filter, 1.5 m tall, with five plastic containers set on top of one another filled with small plastic tubing pieces [20]. Each fish tank had a heater and an air blower to maintain temperature and oxygen at appropriate levels (approximately 22 °C and 6 ppm, respectively, but see full details in Results). Two months before the start of the experiment, a fishless nitrogen cycle was carried out as described in López-Luna et al. [21]. Briefly, 5 mL of ammonia (5%) was added to each tank twice per week until nitrites and, later, nitrates began to build up, which indicated that *Nitrobacter* and *Nitrosomonas* populations were healthy. Fish were added to the tanks when ammonia (TAN) and nitrite levels decreased to near zero.

2.2. Tilapia

Once nitrite and nitrate levels decreased to a non-toxic amount, a total of 32 tilapia, originally purchased from Valenciana de Acuicultura (Puçol, Spain) were anesthetized using eugenol, weighed, and measured. Each fish was also identified by inserting a microchip (PIT-TAG, Trovan, Ltd, Madrid, Spain) subcutaneously under the dorsal fin. Fish were distributed equally among the four experimental tanks (8 tilapia/tank) and given extruded feed (Biomar; 4.5 mm particle size), with 38% protein, 24% lipids, 26% carbohydrates, 3.5% fiber, 6% ashes, and 1.1% phosphorus. The food was supplied twice per day (at 10:00 h and 18:00 h) every day of the week except Sunday, at a rate of 1.2% live weight per tank.

At the end of the trial, fish were again weighed and measured to calculate gain in weight and length. Half of the fish per tank were anesthetized and blood sampled (0.5 mL) to measure basal levels of plasma indicators of stress, while the other half were stressed by crowding to test their reaction to acute stress, following Kittilsen et al. [22]. Briefly, for the crowding stress, 4 fish were placed in a small tank (10 L volume) to generate a high stocking density, where they were maintained for 20 min (with oxygenation) and then their blood sampled afterward. The plasma stress indicators measured at the end of the trial and after the crowding stress were cortisol, glucose, and triglycerides. After obtaining the blood samples, they were immediately centrifuged at 3500 rpm for 10 min to obtain blood plasma, then frozen at −20 °C until further analysis. Cortisol was measured by an immune enzymatic assay using a commercial Cortisol EIA kit (Radim Ibérica S.A., Barcelona, Spain). Glucose and triglycerides were determined using a spectrophotometer (Reflotron® System, Roche Diagnostics, Indianapolis, IN, USA) based on the methods described by Trinder [22] and Bucolo and David [23].

2.3. Lettuce

We planted 25 lettuce plants (*Lactuca sativa var. capitata* L. cv. '*Maravilla de Verano*') per aquaponic system supplied by Semillas Fito® (Barcelona, Spain), which were bought from a local producer in the seedling phase in jiffy pots (Jiffy Growing Solutions, Zwijndrecht, the Netherlands). The plants were transplanted to New Growing System (NGS®, Almería, Spain), a hydroponic recirculating system without soil, specially designed for horticultural crops, with a phenological stage of 13–14 BBCH [24]. These plants were introduced into the system one week after introducing the fish to allow the fish time to acclimatize to their new environment. On the same day that the plants were placed in the NGS®, we added fertilizer to the tank water in two randomly chosen tanks. The chemical composition of the fertilizer added to the treatment tanks included 580 ppm $CaNO_3$, 280 ppm KNO_3, 490 ppm $MgSO_4$, 270 ppm K_2PO_4, and 48 ppm Nutrel C YaraVita™ (with 0,4% B; 0.16% Cu; 4.84% Fe; 2% Mn; 0.12% Mo; 0.33% Zn). Plant cover was measured every week using the Canopeo app [25], which calculates the percentage of area covered on the hydroponics table using colorimetric contrast.

At the beginning and end of the trial, we measured root length, root weight, aerial leaf length, aerial weight, and total plant weight, using a ruler, digital caliper (150 mm RSPRO®, Kwai Chung, Hong Kong), and a precision scale ME1002T/00 (Metter Toledo®, Columbus, OH, USA). The aerial part of the plant was separated from the roots at the level of the soil in the jiffy pot. Once the fresh weight data were obtained, the leaves were placed in an oven at 65 °C for 4 days to calculate the dry weight of the leaves and calculate their dry matter. Dry matter, nitrogen, ashes, and crude protein were calculated following the method of the Official Association of Analytical Chemists [26]. To determine leaf color, a Minolta CM-2500c reflectance spectrophotometer was used as an indirect measure of chlorophyll content and N content, measuring between 360 to 740 nm with a wavelength pitch of 10 nm. There are many chlorophyll indices used to assess nutrition in plants but, in this study, we used the single wavelength at 700 nm as an index, according to Gitelson and Merzlyak [27], and 550 nm, according to Carter [28]. Four measurements were obtained from each leaf, on both sides of the follicle apex.

2.4. Water Quality Parameters

Water quality measurements were taken regularly during the trial, approximately once every 2 or 3 days. The controlled parameters were: pH, temperature, dissolved oxygen (OD), electrical conductivity (EC), nitrite (NO_2^-), nitrate (NO_3^-), and total ammonia nitrogen (NH_3^+ NH_4). The pH was measured using a HANNA® Instruments HI 98130 instrument. These values were maintained around 6.0 with the addition of sodium bicarbonate ($NaHCO_3$) when necessary, due to the decrease in pH produced by nitrification. Underwater sensors (Hobo-U11, ONSET, Bourne, MA, USA) were used to measure the water temperature, which recorded the values of this parameter every 15 min. To keep the water temperature at 22 °C, Atman AT-150 W heaters were used. Dissolved oxygen (DO) was measured using a Thermo Scientific™ Orion 3 Star DO, keeping the concentration above 4 mg/L. Electrical conductivity (EC) was measured once a week using a HANNA® HI 98130 instrument. To measure nitrogen compounds, we used a HANNA® Instruments HI 8320 Aquaculture Photometer, together with the corresponding reagents: HI 93728-0 Nitrate Reagent, HI (number) Nitrite High Range Reagent, HI 93715A-0 Ammonia MR Reagent A, and HI 93715B-0 Ammonia MR Reagent B. The measurements were taken following the manufacturer's instructions. At the end of the experiment, water samples were collected from all systems to analyze the contents of phosphate, potassium, and sulfate using a spectrophotometric analysis, and carbonates and bicarbonates by colorimetry.

2.5. Statistical Analysis

Data were analyzed using Statistix 9 (Analytical Software, Miller Landing Rd, Tallahassee, FL, USA). For all results, the analysis of variance (ANOVA) was performed where $p < 0.05$.

3. Results

3.1. Tilapia

Production parameters were not significantly different across treatments (Table 1), although the initial and final weights of fish with fertilized water (FW) were slightly lower, and weight gain was slightly higher for fish in water without fertilizer (WF). The basal levels of cortisol, glucose, and triglycerides concentrations at the end of the trial were not significantly different between treatments. After the crowding stress, however, the cortisol levels in FW fish were significantly lower than in WF fish (Table 2).

Table 1. Means (±SE) of initial weight, final weight, and weight gain, as well as the level of significance (*p*) for each measure.

Variable	Without Fertilizer (WF)	Fertilizer (FW)	*p*
Initial weight (g)	63.5 ± 9.9	52.6 ± 10.5	0.45
Final weight (g)	75.1 ± 12.1	69.9 ± 12.9	0.76
Weight gain (g/fish)	11.7 ± 1.2	16.7 ± 5.3	0.31

Table 2. Means (±SE) of cortisol, glucose, and triglyceride measurements, as well as the level of significance (*p*) for each measure for without fertilizer (WF), without fertilizer + stress (WF + S), fertilizer (FW) and fertilizer + stress (FW + S), and the effect of each treatment and their interaction (fertilizer and stress).

Variable	WF (n = 6)	WF + S (n = 9)	FW (n = 5)	FW + S (n = 6)	*p* Fertilizer	*p* Stress	*p* F × S
Cortisol (ng/mL)	3.9 ± 0.69	3.5 ± 0.55	5.17 ± 0.64	3.5 ± 0.60	0.20	0.89	0.17
Glucose (mg/dL)	80.3 ± 3.16	89.8 ± 4.17	79.8 ± 3.35	84.3 ± 4.8	0.91	0.41	0.30
Triglycerides (mg/dL)	76.6 ± 8.8	91.8 ± 12.7	80.7 ± 11.2	81.6 ± 17.1	0.78	0.64	0.33

3.2. Lettuce

The effects of fertilizer addition and treatments on lettuce can be seen in Table 3. Total plant weight was significantly higher in FW lettuce, which was approximately 276% higher than in WF lettuce. Leaf and root lengths were significantly different between treatments, being longer in FW lettuce. Leaf weight was significantly higher in FW lettuce, but root weight was not. Regarding the nutritional content of lettuce, the crude protein, dry matter, and ashes were significantly higher ($p < 0.05$) in FW lettuce. Total plant cover on the NFT tables was not significantly different between treatments at the beginning of the experiment but were significantly higher in FW by the end of the trial. Regarding leaf color, a* was significantly greener (shifted to negative values) in lettuce leaves given FW, while b* and L* were significantly lower than in WF. Color photographs of the plants grown in our study can be seen in Figure 1.

Table 3. Means (±SE) of root length, root weight, aerial length, leaf weight, total weight, ratio, spectral index, initial area covered, final area covered, crude protein, crude protein in relation to dry matter (CP/DM), dry matter, and ashes, as well as the level of significance (p) for each measure. Same letters within the same row do not present significant differences at the 5% level.

Variable	Without Fertilizer (WF)	Fertilizer (FW)	p
Root length (cm)	14.37 ± 1.22^{a}	23.31 ± 2.42^{b}	0.003
Root weight (g)	31.71 ± 0.87	35.14 ± 2.14	0.15
Aerial length (cm)	12.72 ± 0.97^{a}	26.36 ± 1.11^{b}	<0.001
Leaf weight (g)	20.02 ± 2.59^{a}	108.11 ± 16.96^{b}	<0.001
Total weight (g)	51.73 ± 3.27^{a}	143.25 ± 18.38^{b}	<0.001
Ratio (Shoot/Root, g)	0.62 ± 0.07^{a}	3.01 ± 0.39^{b}	<0.001
Spectral Index ($1/R_{700}$)	0.02227 ± 0.012^{a}	0.02846 ± 0.015^{b}	0.012
Spectral Index (R_{550})	44.02 ± 2.14^{a}	34.83 ± 1.55^{b}	0.006
	Area covered		
Initial area covered (%)	6.99 ± 1.29	6.69 ± 1.23	0.6
Final area covered (%)	68.47 ± 2.92^{a}	94.7 ± 4.27^{b}	0.03
	Composition		
Crude Protein (%)	16.44 ± 0.84^{a}	33.24 ± 0.90^{b}	<0.0001
CP/DM (%)	18.28 ± 0.94^{a}	35.91 ± 0.96^{b}	<0.0001
Dry Matter (%)	89.96 ± 0.42^{a}	92.56 ± 0.38^{b}	0.001
Ashes (%)	9.83 ± 0.47^{a}	22.29 ± 0.60^{b}	<0.0001
	Color characteristics		
L* (D65)	62.86 ± 0.81^{a}	55.88 ± 0.70^{b}	<0.0001
a* (D65)	-12.31 ± 0.20^{a}	-13.24 ± 0.13^{b}	0.0003
b* (D65)	43.36 ± 0.73^{a}	36.97 ± 0.71^{b}	<0.0001

3.3. Water Quality Parameters

Total ammonia nitrogen ($NH_3 + NH_4^+$), nitrite (NO_2^-), and nitrate (NO_3^-) were significantly higher in FW tanks ($p < 0.05$) (Table 4), while oxygen, pH, and temperature were not significantly different between treatments ($p > 0.05$). At the end of the experiment, phosphate, potassium, and sulfate were significantly higher in FW (Table 4).

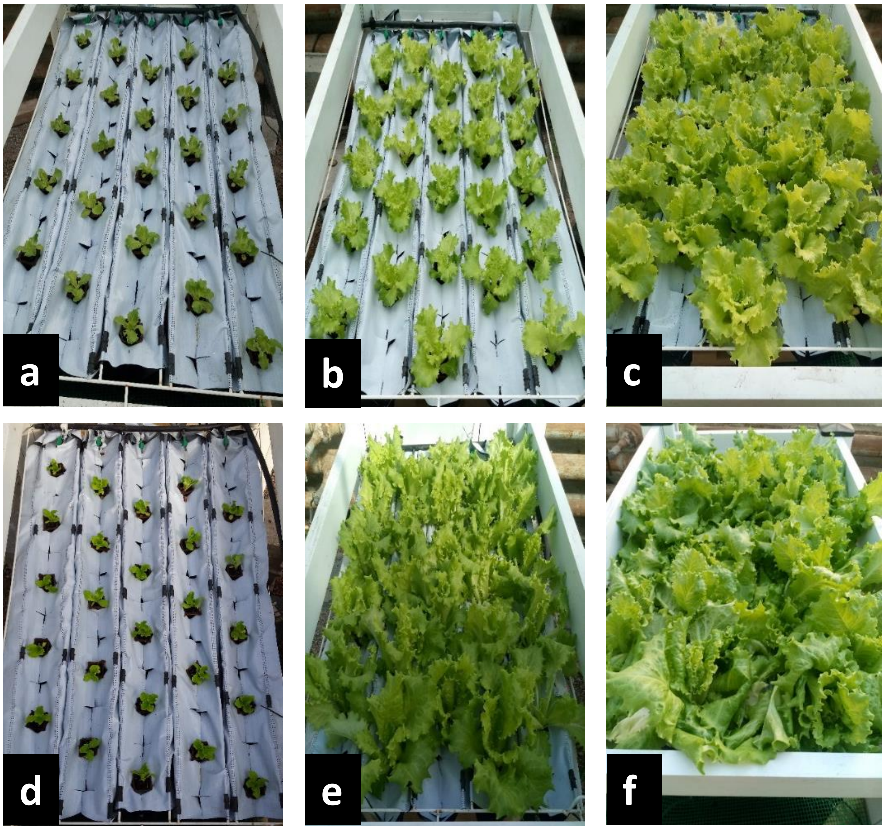

Figure 1. (**a**) Lettuce without fertilizer day 0; (**b**) lettuce without fertilizer day 10; (**c**) lettuce without fertilizer day 24; (**d**) lettuce cover with fertilizer day 0; (**e**) lettuce cover with fertilizer day 10; (**f**) lettuce cover with fertilizer day 24. Photographs show the evolution of growth but are not to scale.

Table 4. Means (±SE) of nitrite (NO_2^-), nitrate (NO_3^-), total ammonia nitrogen (NH_3^+ NH_4^+), dissolved oxygen (DO), temperature, pH, phosphate (μg/mL), potassium (μg/mL), sulfate (μg/mL), and bicarbonate (mg/L) of water samples during the experiment; level of significance ($p < 0.05$). Same letters within the same row do not present significant differences at the 5% level.

Variable	Without Fertilizer (WF)	Fertilizer (FW)	*p*
NO_2^- (mg/L)	2.40 ± 1.38 ^{a}	24.6 ± 1.69 ^{b}	<0.0001
NO_3^- (mg/L)	21.3 ± 1.21 ^{a}	25.5 ± 1.49 ^{b}	0.032
NH_3^+ NH_4^+ (mg/L)	0.62 ± 0.40 ^{a}	6.70 ± 0.49 ^{b}	<0.0001
DO (mg/L)	6.13 ± 0.23	5.87 ± 0.26	0.48
pH	6.62 ± 0.40	5.81 ± 0.49	0.21
Temperature (°C)	23.0 ± 0.22 ^{a}	23.7 ± 0.28 ^{b}	0.05
Phosphate (μg/mL)	25.24 ± 7.12 ^{a}	101.02 ± 17.7 ^{b}	0.0007
Potassium (μg/mL)	4.13 ± 1.01 ^{a}	175.85 ± 23.7 ^{b}	<0.0001
Sulfate (μg/mL)	0 ^{a}	1.14 ± 0.25 ^{b}	0.02
Bicarbonate (mg/L)	9.15 ± 0.06	8.72 ± 0.06	0.59

4. Discussion

Two of the main goals of modern aquaculture are sustainability and resilience. In this sense, urban aquaponics represents a strategic possibility to take advantage of resources and productive efforts to obtain quality plant and fish products [14]. The integration of food safety, animal welfare, and productivity is a technological challenge for aquaponics, guided by legal requirements and the market [29]. In this context, water quality is one of the main concerns in hazard identification for fish welfare risk assessment; therefore, aquaponic systems are not different from aquaculture. Fish reared in aquaponic systems require good water quality conditions, which means that parameters such as dissolved oxygen, carbon dioxide, ammonia, nitrate, nitrite, and pH must be within acceptable limits for each species [17]. In terms of production indices, there appears to be no significant effect of adding fertilizer on tilapia growth. However, we used relatively few fish and low densities, replicating typically small urban aquaponic systems. With a more even weight distribution, a higher number of fish, higher densities, and a higher feeding rate, those results could vary.

Regarding fish welfare, it is preferable to use a range of physiological, behavioral, and biochemical parameters to accurately estimate stress levels [30]. In fish, it has been shown that abnormally high levels of stress affect the metabolism of amino acids, causing a delay in growth [31,32], thereby affecting flesh quality [9,33,34]. In our study, the blood plasma indicators (cortisol, glucose, and triglycerides) were not significantly different between fish from either treatment, suggesting that the tilapia were able to adapt to the addition of fertilizer. The addition of a salt-rich fertilizer could have been a major stressor but our physiological results reinforce the idea that, at the exposure levels we used, tilapia do not appear to experience salinity stress in the water or, in any case, are able to cope. This result may owe to the fact that salinity produces a mitigating effect on bacterial contamination and allows tilapia to maintain a hemostatic balance even during periods of stress, as demonstrated by Juanito et al. [35] during fish road transport. Nile tilapia are effectively reared in freshwater; therefore, high levels of water salinity can increase oxygen demand and decrease fish growth and welfare [36]. Following transfer to progressively higher salinities, where fish are permitted to acclimate to each salinity at longer-term intervals from days to weeks, tilapia reach a threshold at near 60–65 parts per thousand (‰), after which their ability to maintain osmoregulatory balance is compromised [37]. In future studies, it may be a good idea to analyze fish blood electrolytes to better quantify that osmoregulatory balance.

Concerning the plants, the poor growth of the lettuce without fertilizer supports studies by other authors suggesting that additional minerals need to be added to the fish waste in the recirculated waste in order to promote optimal growth and be profitable [38]. Many of these nutrients are necessary for plants to carry out photosynthesis; their absence causes reductions in growth and chlorosis and a decrease in the intensity of the green color [39], as shown by our finding that FW plants had significantly higher absorbance values for green and WF plants for yellow. Nitrogen may be the only nutrient that is present in adequate concentration in fish waste to support plant growth, since 70–80% of feed is lost as nitrogen, which dissolves in the water [40]. Other studies have found that using fish waste water can save 63% of the mineral fertilizers used in commercial hydroponics [41]. However, the balance in the ion solutions of hydroponics and aquaponics differs quite a bit [42,43], with their concentrations being lower in aquaponics, with the exception of microelements such as iron, boron, copper, and manganese, among others. The variables measured in lettuce, analyzed in Table 3, show that the values are lower for the unfertilized solution. These same results have been reported previously in other crops such as tomato, for which it is necessary to add nutrients for optimal growth using aquaponics, rendering it essential to continuously measure and adjust nutrients from the fish waste water [44,45]. In our study, lettuce production data show the same trend as the trials reported by Delaide et al. [43], with productions greater than 70% in solutions with mineral fertilizers incorporated in the aquaponics solution (Aquaponic Complemented, CAP).

Finally, water quality, oxygen, pH, and temperature were kept constant, while the rest of the parameters varied between treatments. For good growth of plants in aquaponic systems, macronutrients such as nitrogen, phosphorous, calcium, magnesium, potassium, and sulfur are required [4], and all of them were supplied with fertilizer. All of these macronutrients were still present in FW lettuce but significantly lower in WF lettuce, coinciding with the lower level of growth of lettuce. As Tidwell [4] mentions, the iron derived from the fish feed is not sufficient for hydroponic production and must be supplemented.

5. Conclusions

Our results suggest that in rather small, low-density urban aquaponic systems, the addition of a commercial fertilizer greatly improved lettuce growth compared to controls with no fertilizer, and did not cause an increase in physiological stress levels in tilapia, while fish growth was similar between treatments. In addition, when a stress test was applied to the fish *a posteriori*, tilapia from both treatments responded similarly. It is clear, therefore, that in closed aquaponic systems, farmers must find a balance between adding enough fertilizer to promote plant growth, while avoiding any negative effects on the osmoregulatory balance or welfare of fish. In this sense, decoupled aquaponic systems may have an advantage but more studies are needed to compare the two, especially in terms of fish welfare measurements and with a reasonable number of replicates (e.g., 2–3 aquaponic systems per treatment). However, our results suggest that the joint growth of fish and lettuce is still quite feasible in coupled systems. Adding fertilizer increased lettuce growth by almost three times more than without fertilizer. This implies, based on the control treatment, that the fish used, at least in the number and densities described above, cannot contribute enough nutrients for the lettuce to reach its full growth potential. Finally, fish welfare, which has not always been considered in aquaponics, can be used as an additional attribute within a broad multidimensional concept of sustainability that may favor the future use of aquaponics in the urban environment.

Author Contributions: Conceptualization, M.V. and R.M.; methodology, M.V. and R.M.; formal analysis, G.C.M.-d.l.L., R.E.-Á. and M.V.; investigation, M.V. and R.M.; data curation, M.V.; resources, M.V.; writing—original draft preparation, M.V., R.M. and G.C.M.-d.l.L.; visualization, G.C.M.-d.l.L.; funding acquisition, M.V. and R.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: All animal handling processes performed in this project were part of routine animal husbandry practices, and according to the Legislation for the Protection of Animals used for Scientific Purposes (EU Directive 2010-63-EU and Spanish RD 53/2013), no procedure was performed. Therefore, this project was exempted from an ethical review by the Ethical Committee of the Technical University of Madrid.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author Morris Villarroel by contacting morris.villarroel@upm.es.

Conflicts of Interest: The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Dorick, J.; Hayden, M.; Smith, M.; Blanchard, C.; Monu, E.; Wells, D.; Huang, T.-S. Evaluation of *Escherichia coli* and Coliforms in Aquaponic Water for Produce Irrigation. *Food Microbiol.* **2021**, *99*, 103801. [CrossRef] [PubMed]
2. Suárez-Cáceres, G.P.; Lobillo-Eguíbar, J.; Fernández-Cabanás, V.M.; Quevedo-Ruiz, F.J.; Pérez-Urrestarazu, L. Polyculture Production of Vegetables and Red Hybrid Tilapia for Self-Consumption by Means of Micro-Scale Aquaponic Systems. *Aquac. Eng.* **2021**, *95*, 102181. [CrossRef]
3. Goddek, S.; Delaide, B.; Mankasingh, U.; Ragnarsdottir, K.V.; Jijakli, H.; Thorarinsdottir, R. Challenges of Sustainable and Commercial Aquaponics. *Sustainability* **2015**, *7*, 4199–4224. [CrossRef]
4. Tidwell, J.H. *Aquaculture Production Systems*; John Wiley & Sons: New York, NY, USA, 2012; ISBN 978-1-118-25009-9.
5. Calone, R.; Pennisi, G.; Morgenstern, R.; Sanyé-Mengual, E.; Lorleberg, W.; Dapprich, P.; Winkler, P.; Orsini, F.; Gianquinto, G. Improving Water Management in European Catfish Recirculating Aquaculture Systems through Catfish-Lettuce Aquaponics. *Sci. Total Environ.* **2019**, *687*, 759–767. [CrossRef] [PubMed]
6. Yanes, A.R.; Martinez, P.; Ahmad, R. Towards Automated Aquaponics: A Review on Monitoring, IoT, and Smart Systems. *J. Clean. Prod.* **2020**, *263*, 121571. [CrossRef]
7. Suárez-Cáceres, G.P.; Pérez-Urrestarazu, L.; Avilés, M.; Borrero, C.; Lobillo Eguíbar, J.R.; Fernández-Cabanás, V.M. Susceptibility to Water-Borne Plant Diseases of Hydroponic vs. Aquaponics Systems. *Aquaculture* **2021**, *544*, 737093. [CrossRef]

8. Villarroel, M.; Junge, R.; Komives, T.; König, B.; Plaza, I.; Bittsánszky, A.; Joly, A. Survey of Aquaponics in Europe. *Water* **2016**, *8*, 468. [CrossRef]
9. Thomas, R.M.; Verma, A.K.; Prakash, C.; Krishna, H.; Prakash, S.; Kumar, A. Utilization of Inland Saline Underground Water for Bio-Integration of Nile Tilapia (*Oreochromis niloticus*) and Spinach (*Spinacia oleracea*). *Agric. Water Manag.* **2019**, *222*, 154–160. [CrossRef]
10. Chia, M.A.; Abdulwahab, R.; Ameh, I.; Balogun, J.K.; Auta, J. Farmed Tilapia as an Exposure Route to Microcystins in Zaria-Nigeria: A Seasonal Investigation. *Environ. Pollut.* **2021**, *271*, 116366. [CrossRef] [PubMed]
11. Guzmán-Luna, P.; Gerbens-Leenes, P.W.; Vaca-Jiménez, S.D. The Water, Energy, and Land Footprint of Tilapia Aquaculture in Mexico, a Comparison of the Footprints of Fish and Meat. *Res. Con. Recycl.* **2021**, *165*, 105224. [CrossRef]
12. Baßmann, B.; Brenner, M.; Palm, H.W. Stress and Welfare of African Catfish (*Clarias gariepinus* Burchell, 1822) in a Coupled Aquaponic System. *Water* **2017**, *9*, 504. [CrossRef]
13. Noble, C.; Gismervik, K.; Iversen, M.H.; Kolarevic, J.; Nilsson, J.; Stien, L.H.; Turnbull, J.F. *Welfare Indicators for Farmed Atlantic Salmon: Tools for Assessing Fish Welfare*; Nofima Marine: Trømso, Norway, 2018; ISBN 978-82-8296-556-9.
14. Hemathilake, D.M.K.S.; Gunathilake, D.M.C.C. Chapter 32—High-Productive Agricultural Technologies to Fulfill Future Food Demands: Hydroponics, Aquaponics, and Precision/Smart Agriculture. In *Future Foods*; Bhat, R., Ed.; Academic Press: Cambridge, MA, USA, 2022; pp. 555–567. ISBN 978-0-323-91001-9.
15. Schon, M. Tailoring Nutrient Solutions to Meet the Demands of Your Plants. In Proceedings of the 13th Annual Conference on Hydroponics, Hydroponic Society of America, Orlando, FL, USA, 9–12 April 1992.
16. Shao, M.; Liu, W.; Zha, L.; Zhou, C.; Zhang, Y.; Li, B. Differential Effects of High Light Duration on Growth, Nutritional Quality, and Oxidative Stress of Hydroponic Lettuce under Red and Blue LED Irradiation. *Sci. Hortic.* **2020**, *268*, 109366. [CrossRef]
17. Yavuzcan Yildiz, H.; Robaina, L.; Pirhonen, J.; Mente, E.; Domínguez, D.; Parisi, G. Fish Welfare in Aquaponic Systems: Its Relation to Water Quality with an Emphasis on Feed and Faeces—A Review. *Water* **2017**, *9*, 13. [CrossRef]
18. Goddek, S.; Joyce, A.; Wuertz, S.; Körner, O.; Bläser, I.; Reuter, M.; Keesman, K.J. Decoupled aquaponics systems. *Aquaponics Food Prod. Syst.* **2019**, *83*, 201–229.
19. de la Presidencia, M. Real Decreto 1201/2005, de 10 de Octubre, Sobre Protección de Los Animales Utilizados Para Experimentación y Otros Fines Científicos. 2005, Volume BOE-A-2005-17344, pp. 34367–34391. Available online: https://www.boe.es/buscar/doc.php?id=BOE-A-2005-17344#:~{}:text=Esta%20directiva%20pretende%20garantizar%20que,o%20la%20lesi%C3%B3n%2C%20prolongados%20innecesariamente (accessed on 7 February 2022).
20. Gullicks, H.A.; Cleasby, J.L. Nitrification Performance of a Pilot-Scale Trickling Filter. *Res. J. Water Pollut. Control Fed.* **1990**, *62*, 40–49.
21. López-Luna, J.; Ibáñez, M.A.; Villarroel, M. Using Multivariate Analysis of Water Quality in RAS with Nile Tilapia (*Oreochromis niloticus*) to Model the Evolution of Macronutrients. *Aquac. Eng.* **2013**, *54*, 22–28. [CrossRef]
22. Kittilsen, S.; Ellis, T.; Schjolden, J.; Braastad, B.O.; Øverli, Ø. Determining Stress-Responsiveness in Family Groups of Atlantic Salmon (*Salmo salar*) Using Non-Invasive Measures. *Aquaculture* **2009**, *298*, 146–152. [CrossRef]
23. Trinder, P. Determination of Glucose in Blood Using Glucose Oxidase with an Alternative Oxygen Acceptor. *Ann. Clin. Biochem.* **1969**, *6*, 24–27. [CrossRef]
24. Bucolo, G.; David, H. Quantitative Determination of Serum Triglycerides by the Use of Enzymes. *Clin. Chem.* **1973**, *19*, 476–482. [CrossRef] [PubMed]
25. Feller, I.C. Effects of Nutrient Enrichment on Growth and Herbivory of Dwarf Red Mangrove (*Rhizophora mangle*). *Ecol. Monogr.* **1995**, *65*, 477–505. [CrossRef]
26. Patrignani, A.; Ochsner, T.E. Canopeo: A Powerful New Tool for Measuring Fractional Green Canopy Cover. *Agron. J.* **2015**, *107*, 2312–2320. [CrossRef]
27. AOAC. Official Methods of Analysis, 17th Ed.—Google Académico. 2000. Available online: https://scholar.google.es/scholar?hl=es&as_sdt=0%2C5&q=AOAC.+2000.+Official+Methods+of+Analysis%2C+17th+ed.+Association+of+Official+Analytical+Chemists%2C+Washington+DC%2C+USA.&btnG= (accessed on 7 February 2022).
28. Gitelson, A.A.; Merzlyak, M.N. Signature Analysis of Leaf Reflectance Spectra: Algorithm Development for Remote Sensing of Chlorophyll. *J. Plant Physiol.* **1996**, *148*, 494–500. [CrossRef]
29. CARTER, G.A. Ratios of Leaf Reflectances in Narrow Wavebands as Indicators of Plant Stress. *Int. J. Remote Sens.* **1994**, *15*, 697–703. [CrossRef]
30. Suárez-Cáceres, G.P.; Fernández-Cabanás, V.M.; Lobillo-Eguíbar, J.; Pérez-Urrestarazu, L. Consumers' Knowledge, Attitudes and Willingness to Pay for Aquaponic Products in Spain and Latin America. *Int. J. Gastron. Food Sci.* **2021**, *24*, 100350. [CrossRef]
31. Broom, D.M. Assessing the Welfare of Transgenic Animals. In *Welfare Aspects of Transgenic Animals*; Van Zutphen, L.F.M., Van Der Meer, M., Eds.; Springer: Berlin/Heidelberg, Germany, 1997; pp. 58–67.
32. Conceição, L.E.C.; Aragão, C.; Dias, J.; Costas, B.; Terova, G.; Martins, C.; Tort, L. Dietary Nitrogen and Fish Welfare. *Fish. Physiol. Biochem.* **2012**, *38*, 119–141. [CrossRef] [PubMed]
33. Sánchez-Muros, M.J.; Villacreces, S.; Miranda-de la Lama, G.; de Haro, C.; García-Barroso, F. Effects of Chemical and Handling Exposure on Fatty Acids, Oxidative Stress and Morphological Welfare Indicators in Gilt-Head Sea Bream (*Sparus aurata*). *Fish. Physiol. Biochem.* **2013**, *39*, 581–591. [CrossRef] [PubMed]

34. Berg, T.; Erikson, U.; Nordtvedt, T.S. Rigor Mortis Assessment of Atlantic Salmon (*Salmo salar*) and Effects of Stress. *J. Food Sci.* **1997**, *62*, 439–446. [CrossRef]
35. Sigholt, T.; Erikson, U.; Rustad, T.; Johansen, S.; Nordtvedt, T.S.; Seland, A. Handling Stress and Storage Temperature Affect Meat Quality of Farmed-Raised Atlantic Salmon (*Salmo salar*). *J. Food Sci.* **1997**, *62*, 898–905. [CrossRef]
36. Uehara, S.A.; Walter, E.H.M.; Cunha, D.M.; Calixto, F.A.A.; Furtado, A.A.L.; Takata, R.; de Mesquita, E.F.M. Effects of Salinity Gradients on the Skin and Water Microbial Community and Physiological Responses of Tilapia (*Oreochromis niloticus*) in the Pre-Slaughter. *Aquac. Res.* **2021**, *52*, 4638–4647. [CrossRef]
37. Mohamed, N.A.; Saad, M.F.; Shukry, M.; El-Keredy, A.M.S.; Nasif, O.; Van Doan, H.; Dawood, M.A.O. Physiological and Ion Changes of Nile Tilapia (*Oreochromis niloticus*) under the Effect of Salinity Stress. *Aquac. Res.* **2021**, *19*, 100567. [CrossRef]
38. Kammerer, B.D.; Cech, J.J.; Kültz, D. Rapid Changes in Plasma Cortisol, Osmolality, and Respiration in Response to Salinity Stress in Tilapia (*Oreochromis mossambicus*). *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2010**, *157*, 260–265. [CrossRef]
39. Graber, A.; Junge, R. Aquaponic Systems: Nutrient Recycling from Fish Wastewater by Vegetable Production. *Desalination* **2009**, *246*, 147–156. [CrossRef]
40. Pacumbaba, R.O.; Beyl, C.A. Changes in Hyperspectral Reflectance Signatures of Lettuce Leaves in Response to Macronutrient Deficiencies. *Adv. Space Res.* **2011**, *48*, 32–42. [CrossRef]
41. Krom, M.D.; Ellner, S.; van Rijn, J.; Neori, A. Nitrogen and Phosphorus Cycling and Transformations in a Prototype "non-Polluting" Integrated Mariculture System, Eilat, Israel. *Mar. Ecol. Prog. Ser.* **1995**, *118*, 25–36. [CrossRef]
42. Monsees, H.; Suhl, J.; Paul, M.; Kloas, W.; Dannehl, D.; Würtz, S. Lettuce (Lactuca Sativa, Variety Salanova) Production in Decoupled Aquaponic Systems: Same Yield and Similar Quality as in Conventional Hydroponic Systems but Drastically Reduced Greenhouse Gas Emissions by Saving Inorganic Fertilizer. *PLoS ONE* **2019**, *14*, e0218368. [CrossRef] [PubMed]
43. Kloas, W.; Groß, R.; Baganz, D.; Graupner, J.; Monsees, H.; Schmidt, U.; Staaks, G.; Suhl, J.; Tschirner, M.; Wittstock, B.; et al. A New Concept for Aquaponic Systems to Improve Sustainability, Increase Productivity, and Reduce Environmental Impacts. *Aquac. Environ. Interact.* **2015**, *7*, 179–192. [CrossRef]
44. Delaide, B.; Goddek, S.; Gott, J.; Soyeurt, H.; Jijakli, M.H. Lettuce (Lactuca Sativa L. Var. Sucrine) Growth Performance in Complemented Aquaponic Solution Outperforms Hydroponics. *Water* **2016**, *8*, 467. [CrossRef]
45. Suhl, J.; Dannehl, D.; Kloas, W.; Baganz, D.; Jobs, S.; Scheibe, G.; Schmidt, U. Advanced Aquaponics: Evaluation of Intensive Tomato Production in Aquaponics vs. Conventional Hydroponics. *Agric. Water Manag.* **2016**, *178*, 335–344. [CrossRef]