

# Aquaponics: integrating fish feeding rates and ion waste production for strawberry hydroponics

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## Abstract

Aquaponics is the science of integrating intensive fish aquaculture with plant production in recirculating water systems. Although ion waste production by fish cannot satisfy all plant requirements, less is known about the relationship between total feed provided for fish and the production of milliequivalents (mEq) of different macronutrients for plants, especially for nutrient flow hydroponics used for strawberry production in Spain. That knowledge is essential to consider the amount of macronutrients available in aquaculture systems so that farmers can estimate how much nutrient needs to be supplemented in the waste water from fish, to produce viable plant growth. In the present experiment, tilapia (*Oreochromis niloticus* L.) were grown in a small-scale recirculating system at two different densities while growth and feed consumption were noted every week for five weeks. At the same time points, water samples were taken to measure pH, EC<sub>25</sub>, HCO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>, NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> build up. The total increase in mEq of each ion per kg of feed provided to the fish was highest for NO<sub>3</sub><sup>-</sup>, followed, in decreasing order, by Ca<sup>2+</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, K<sup>+</sup>, Mg<sup>2+</sup> and SO<sub>4</sub><sup>2-</sup>. The total amount of feed required per mEq ranged from 1.61-13.1 kg for the four most abundant ions (NO<sub>3</sub><sup>-</sup>, Ca<sup>2+</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and K<sup>+</sup>) at a density of 2 kg fish m<sup>-3</sup>, suggesting that it would be rather easy to maintain small populations of fish to reduce the cost of hydroponic solution supplementation for strawberries.

**Additional key words:** aquaculture; nutrients; recirculating system; tilapia; water quality.

## Resumen

### Aquaponica: integrando la tasa de alimentación de peces y la producción de iones residuales para la hidroponía de fresas

La acuaponía es la ciencia de integrar la acuicultura intensiva con la producción de plantas en sistemas con agua en recirculación. A pesar de que se ha encontrado que la producción de iones residuales de los peces no llega a satisfacer todos los requisitos de las plantas, se sabe menos sobre la relación entre el pienso total dispensado para los peces y la producción en milliequivalentes (mEq) de los diferentes macronutrientes para plantas, especialmente para hidroponía recirculante usada para la producción de fresas en España. Es esencial tener este conocimiento para calcular la cantidad de macronutrientes disponibles en sistemas de acuicultura para que los agricultores puedan estimar la cantidad de nutrientes que hay añadir al agua residual de los peces, para un crecimiento viable de plantas. En este experimento, se alimentó a tilapia (*Oreochromis niloticus* L.) en un sistema de recirculación a pequeña escala a dos densidades diferentes, controlando el crecimiento de los peces y el pienso consumido una vez cada semana durante cinco semanas. A la vez, se tomó muestras de agua para medir el pH, CE<sub>25</sub>, HCO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>, NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> y Mg<sup>2+</sup> que se acumulaba en los tanques. La ganancia total en mEq de cada ion por kg de pienso proporcionado a los peces fue mayor para NO<sub>3</sub><sup>-</sup>, seguido, en orden descendiente, por Ca<sup>2+</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, K<sup>+</sup>, Mg<sup>2+</sup> y SO<sub>4</sub><sup>2-</sup>. La cantidad total de pienso requerido por mEq varió entre 1 y 13 kg, lo cual sugiere que sería relativamente sencillo mantener poblaciones pequeñas de peces para reducir el coste de la solución fertilizante para las fresas.

**Palabras clave adicionales:** acuicultura; calidad de agua; nutrientes; sistema recirculante; tilapia.

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## Introduction

Aquaponics is the integrated production of plants and fish in a water recirculating system via a biofilter with nitrifying bacteria (Tyson *et al.*, 2007). Non-toxic nutrients that accumulate in recirculating aquaculture systems can be used to fertilize plant growth (Adler *et al.*, 1996; Seawright *et al.*, 1998), but the final amounts of dissolved anions or cations depend on a number of factors, such as fish species, system design, feed type and water source (Rakocy *et al.*, 1992). Knowledge about the daily increase of dissolved metabolic by-products from fish is essential to consider the amount of macronutrients available in aquaculture systems that integrate with plant production.

Recirculating aquaculture systems (RAS) are often designed in terms of fish biomass, which determines the amount of feed provided in tank water, or the feeding rate ( $\text{g day}^{-1}$ ) (Timmons *et al.*, 2002). Increasing fish biomass also implies increasing the total amount of feed provided per tank, which tends to increase the concentration of dissolved anions and cations involved in the nitrogen and carbon cycles (Seawright *et al.*, 1998) as well as dissolved minerals, such as  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ , P and S (Martins *et al.*, 2009). Theoretically, doubling the fish biomass should double ion production, but few studies have considered how ion production varies over time in relation to different fish densities or feeding levels.

In Spain, strawberries are intensively produced using the nutrient flow technique, as opposed to the media-filled raised bed or the floating raft system (López-Medina *et al.*, 2004). In the latter two systems, attempts have been made to supplement the hydroponic nutritive fertilizer solution with waste water from fish, thereby reducing fertilizer costs, increasing water use and producing two cash crops (Chow *et al.*, 1992; Tagliavini *et al.*, 2005).

This study describes temporal changes in ion concentrations over a period of five weeks in small scale recirculating units where solids were not removed and water was not added (zero-discharge). Those values were then compared to current knowledge on hydroponic nutrient solutions developed for strawberries in Spain, in order to help farmers estimate how much nutrient needs to be supplemented in the waste water from fish to produce viable plant growth.

## Material and methods

### Installations

The work was carried out using the recirculating aquaculture system (RAS) at the field station of the Agricultural College of the Polytechnic University of Madrid (Madrid, Spain). Six green fibre glass tanks were used (120 L capacity; height 0.38 m, lower diameter 0.56 m, upper diameter 0.56 m), with one filter (Eheim Classic 2217, 6 L capacity, 20 W,  $1,000 \text{ L h}^{-1}$  flow rate) per every two tanks ( $n = 3$  filters). All tanks were oxygenated from an outlet 20 cm above the tank bottom from one pump (the PUMP-20, Rolf C. Hagen Inc.). The flow rate of the system was 160-180  $\text{L h}^{-1}$ . Each tank was also covered with a 1 cm thick plastic square lid ( $75 \times 75 \text{ cm}$ ) to avoid fish jumping out of the tank and decrease evaporative losses. The temperature was maintained at  $22^\circ\text{C}$  using water heaters.

### Animals

Seventy tilapia (*Oreochromis niloticus*) were used, with an average weight ( $\pm$  sd) of 26.5 g ( $\pm 13.4$ ) at the beginning of the experiment (day 0), that had originally been purchased as larvae (approximately 1 g live weight) from a breeding population of genetically male tilapia (GMT<sup>®</sup>) from *Valenciana de Acuicultura* (Puçol, Valencia, Spain). All experimental fish originated from two tanks from another larger recirculating unit and had been handled and fed in a similar manner (twice a day) for three weeks before the experiment. At the beginning of the experiment (day 0), all the fish from the original two tanks were separated by live weight into five groups. Two fish were chosen randomly from each group for the low feeding rate ( $n = 10$ ) and five from each group for the higher feeding rate ( $n = 25$ ).

Tilapia were fed a commercial extruded diet (pellet diameter 3.5 mm) purchased from Dibaq Diproteg, S.A. (Segovia, Spain) with 35% crude protein, 10% ash, 6% crude fat, 3% crude cellulose and 1.4% phosphorus. Fish were fed three times a day using a programmable feeder (Eheim 3581) at 9:00, 13:00 and 17:00. Feed was provided at approximately 5% live weight.

## Productivity

All experimental fish were weighed individually at the beginning and end of the trial (day 0 and day 48) and feeding rate (grams of feed per fish) was calculated weekly. All fish in one tank per treatment were also weighed weekly, on the same day and shortly after water samples were taken. Using the data on feeding rate and live weight, the average initial weight, average final weight and weight gain were calculated.

## Water quality

Five weeks before placing the fish in the tanks, a fishless nitrogen cycle was completed. Tanks were filled with city water 30 days before introducing the experimental fish, loaded filters turned on and residual chlorine gas removed by aeration for 24 h. Three days later, tank water was inoculated with 5 mL Alken Clear-FLO® 1100 1x containing *Nitrosomonas* and *Nitrobacter* bacteria (Alken Murrarby Corporation). After that, 2 mL of ammonia and organic material were added once a week to each tank while continuing to add Alken Clear-FLO® 1100 1x at decreasing doses per week (2.2 mL first week, 1.1 mL second week, 0.6 mL third week). Tanks were topped up once a week with city water (approx 5-10 L) due to water losses from evaporation and daily cleaning. The photoperiod was 12:12 (light from 8:00 to 20:00h, closed room, only artificial lighting). After the fish were introduced, sodium bicarbonate ( $\text{NaHCO}_3$ ) was added daily (25% by weight of the feed provided) to each tank (except controls) to maintain appropriate pH levels (see Loyless and Malone, 1997). Since the total feed provided to the tanks was approximately 5% of the biomass in the tanks, the  $\text{NaHCO}_3$  added was 1.25% of the weight of the feed ( $0.5 \times 0.25$ ) or approximately 1.25 g for 2 kg fish  $\text{m}^{-3}$  and 3.75 g for 5 kg fish  $\text{m}^{-3}$ , which was maintained throughout the trial. Solid fish waste was not removed during the trial, but accumulated in the solids filter.

Dissolved oxygen and temperature were measured three times a week (Orion oxygen meter, model 810, Orion Research Inc.). The same day, and every week for five weeks, one water sample was taken from each tank at 10-15 cm from the water surface, between 8:00 and 9:30 a.m. using a wide mouth sterile transparent plastic jar (250 mL volume), to measure electrical conductivity ( $\text{EC}_{25}$ ) and the concentration of bicarbonates ( $\text{HCO}_3^-$ ), chloride ( $\text{Cl}^-$ ), ammonium ( $\text{NH}_4^+$ ), nitrite

( $\text{NO}_2^-$ ), nitrate ( $\text{NO}_3^-$ ), phosphate ( $\text{H}_2\text{PO}_4^-$ ), sulphate ( $\text{SO}_4^{2-}$ ), sodium ( $\text{Na}^+$ ), potassium ( $\text{K}^+$ ), calcium ( $\text{Ca}^{2+}$ ) and magnesium ( $\text{Mg}^{2+}$ ).

Mono and divalent cations were measured by ionic chromatography (Compact IC 761; Metrohm) column 6.1010.220 at a working pressure of 10-12 MPa and working flow of 1 mL  $\text{min}^{-1}$ . For anions, the column used was 6.1006.510, working pressure 7-8 MPa and working flow 0.7 mL  $\text{min}^{-1}$ . All water samples were filtered (0.45  $\mu\text{m}$ ) before analysis, and analysed automatically (Compact Autosampler Metrohm, mod. 813) with sample volume of 10 mL and injection volume 20  $\mu\text{L}$ .

## Strawberry hydroponic solution and calculation of requirements

The hydroponic nutritive solutions developed for strawberry production contain chemical profiles that can vary depending on the primary water source used for irrigation. However, a series of studies in recent years (Chow *et al.*, 1992; López-Medina *et al.*, 2004; Tagliavini *et al.*, 2005) have provided recommendations that are summarised in Table 1. Those approximate values were also approved by NGS®, a strawberry growing company in Spain that uses nutrient flow hydroponics (personal communication). Using the data on water quality, the increase of each ion per day was calculated as:

$$\Delta C_{\text{ion}} = C_f - C_i / t$$

where  $\Delta C_{\text{ion}}$  is the increase in concentration of any ion in  $\text{mEq day}^{-1}$ ,  $C_f$  is the final concentration,  $C_i$  is the

**Table 1.** Average requirements ( $\pm$  standard deviation) of the nutritive solution recommended by the NGS® company for strawberry production using hydroponics (strawberry  $\text{mEq L}^{-1}$ ) and average increase in concentration of different water quality variables ( $\Delta C_{\text{ion}}$ ) dissolved in tank water after 35 days in a small-scale experimental recirculating system with tilapia (*Oreochromis niloticus*). Treatments: low, 2 kg fish  $\text{m}^{-3}$  and high, 5 kg fish  $\text{m}^{-3}$

	Strawberry $\text{mEq L}^{-1}$	$\Delta C_{\text{ion}}$ low	$\Delta C_{\text{ion}}$ high
$\text{EC}_{25}$	< 1.2	$0.24 \pm 0.03$	$0.56 \pm 0.03$
$\text{NO}_3^-$	12.0	$2.31 \pm 0.02$	$3.35 \pm 0.14$
$\text{Ca}^{2+}$	11.0	$0.26 \pm 0.097$	$0.84 \pm 0.05$
$\text{H}_2\text{PO}_4^-$	1.8	$0.14 \pm 0.005$	$0.13 \pm 0.013$
$\text{K}^+$	5.2	$0.14 \pm 0.01$	$0.283 \pm 0.002$
$\text{Mg}^{2+}$	3.0	$0.037 \pm 0.048$	$0.277 \pm 0.028$
$\text{SO}_4^{2-}$	2.5	$0.010 \pm 0.015$	$0.122 \pm 0.023$
$\text{NH}_4^+$	1.0	0	0

initial concentration and  $t$  is the days of the trial (in this case 35 days).

Following that the increase in concentration of each ion per kg of feed was calculated as:

$$\Delta C_{\text{ion-feed}} = \Delta C_{\text{ion}} / F_{\text{fish}}$$

where  $\Delta C_{\text{ion-feed}}$  is the increase in concentration of any ion in mEq per kg feed provided to the fish,  $\Delta C_{\text{ion}}$  is as above and  $F$  is the amount of feed (in kg) provided to the fish  $\text{day}^{-1}$ .

Finally, knowing the strawberry requirements (in mEq), the amount of feed required to provide those requirements was calculated as:

$$F_{\text{stb}} = C_{\text{stb}} / \Delta C_{\text{ion-feed}}$$

where  $F_{\text{stb}}$  is the amount of feed (kg) that needs to be provided to fish in order to produce the necessary mEq for each ion, and  $C_{\text{stb}}$  is the average requirement that strawberries have for each ion.

## Experimental design and statistical analysis

Each pair of tanks was assigned a specific fish biomass (control: no fish or feed, low: 10 fish or 2 kg fish  $\text{m}^{-3}$ , and high: 25 fish or 5 kg fish  $\text{m}^{-3}$ ). The data on increase (initial and final data) were analyzed in a completely randomized design with biomass as the main source of variation using an analysis of variance (ANOVA) with the statistical package Statistix 8.1 (Analytical Software Inc., 2005). Means were compared using the least square difference (LSD) all-pairwise comparison test. The data on water quality (six measurements on days 1, 7, 14, 21, 28 and 35 of the trial) were compared using a repeated measures design where subject factor was fish biomass (three levels including the control water) and the within-subject factor was the date the water was sampled.

## Results

### Productive output

The automatic feeders were adjusted to deposit approximately 5% of the total biomass of fish in the tank. The average amount of feed available per fish in each tank was similar throughout the experiment ( $0.47 \pm 0.24$  g feed fish $^{-1}$  day $^{-1}$  at a density of 2 kg fish  $\text{m}^{-3}$ , range 0.15-0.86 g feed fish $^{-1}$  day $^{-1}$ ; and  $0.58 \pm 0.19$  g feed fish $^{-1}$  day $^{-1}$  at 5 kg fish  $\text{m}^{-3}$ , range 0.24-0.87 g feed

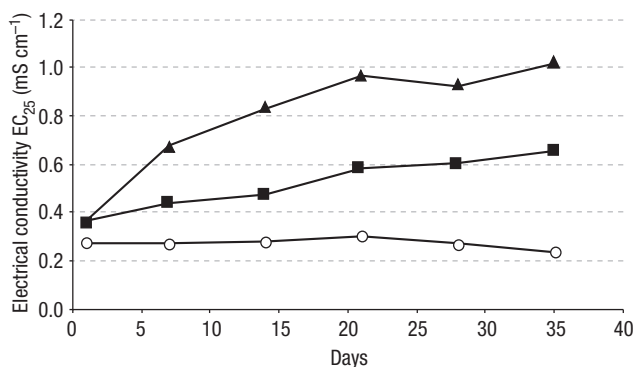
fish $^{-1}$  day $^{-1}$ ). One fish died at the high feeding rate on day 20 of the experiment (weight 30.4 g, cause unknown).

Regarding fish growth, there were no significant differences among the low and high biomass groups in terms of initial weight ( $26.0 \pm 1.9$  g at a density of 2 kg fish  $\text{m}^{-3}$ ,  $26.1 \pm 2.8$  g at a density of 5 kg fish  $\text{m}^{-3}$ ), final weights ( $44.4 \pm 2.9$  g at 2 kg fish  $\text{m}^{-3}$ ,  $53.1 \pm 3.3$  g at 5 kg fish  $\text{m}^{-3}$ ) or weight gain ( $18.4 \pm 4.8$  g at 2 kg fish  $\text{m}^{-3}$ ,  $26.9 \pm 6.1$  g at 5 kg fish  $\text{m}^{-3}$ ). The total amount of food provided per fish was also similar among treatments ( $23.60 \pm 2.72$  g at 2 kg fish  $\text{m}^{-3}$ ,  $29.35 \pm 1.52$  g at 5 kg fish  $\text{m}^{-3}$ ).

## Water quality

The average temperature of water during the trial was 22°C with no significant differences among treatments (control  $22.2 \pm 0.66^\circ\text{C}$ , 2 kg fish  $\text{m}^{-3}$   $21.9 \pm 0.87^\circ\text{C}$  and 5 kg fish  $\text{m}^{-3}$   $21.7 \pm 0.73^\circ\text{C}$ ). Dissolved oxygen levels were above 5 mg L $^{-1}$  in all tanks, within acceptable limits for tilapia. Before the fish were placed in the experimental tanks, a fishless cycling protocol produced a peak in NO $_3^-$  and then in NO $_2^-$ , suggesting the proper growth of *Nitrosomonas* and *Nitrobacter* autotrophic bacteria in the biofilter.

Average water quality data are summarised in Table 2, including statistical differences among treatments. The average pH was significantly higher in the high biomass treatment, and more acidic in the control and low biomass tanks. The electrical conductivity was low in the control tanks (no fish or feed), but tanks with fish at high biomass had approximately 150% more EC $_{25}$  than at low biomass, on average. Figure 1 describes the



**Figure 1.** Evolution of electrical conductivity EC $_{25}$  (mS cm $^{-1}$ ) throughout the trial where data are averages from two tanks per treatment (control: water with no fish or feed ○; low: 2 kg fish  $\text{m}^{-3}$  ■; high: 5 kg fish  $\text{m}^{-3}$  ▲).

**Table 2.** Average ( $\pm$  standard deviation) water quality variables in a small-scale experimental recirculating system with tilapia (*Oreochromis niloticus*). Treatments: control, no fish or feed; low, 2 kg fish m<sup>-3</sup>; and high, 5 kg fish m<sup>-3</sup>

Water quality variables <sup>1</sup>	Treatments <sup>2</sup>		
	Control	Low	High
pH	5.83 $\pm$ 0.38 <sup>a</sup>	6.12 $\pm$ 0.50 <sup>a</sup>	7.17 $\pm$ 0.38 <sup>b</sup>
EC <sub>25</sub>	0.28 $\pm$ 0.01 <sup>a</sup>	0.49 $\pm$ 0.10 <sup>b</sup>	0.75 $\pm$ 0.23 <sup>c</sup>
HCO <sub>3</sub> <sup>-</sup>	7.86 $\pm$ 2.90 <sup>a</sup>	19.0 $\pm$ 12.7 <sup>a</sup>	137.7 $\pm$ 70.0 <sup>b</sup>
Cl <sup>-</sup>	23.0 $\pm$ 0.92 <sup>a</sup>	23.8 $\pm$ 2.15 <sup>a</sup>	34.4 $\pm$ 7.36 <sup>b</sup>
Na <sup>+</sup>	32.9 $\pm$ 1.09 <sup>a</sup>	64.6 $\pm$ 20.03 <sup>b</sup>	96.9 $\pm$ 41.9 <sup>c</sup>
NH <sub>4</sub> <sup>+</sup>	0.47 $\pm$ 1.13 <sup>a</sup>	0.92 $\pm$ 0.81 <sup>a</sup>	8.05 $\pm$ 4.79 <sup>b</sup>
NO <sub>2</sub> <sup>-</sup>	0.00 $\pm$ 0.00 <sup>a</sup>	0.39 $\pm$ 0.76 <sup>a</sup>	6.76 $\pm$ 3.83 <sup>b</sup>
NO <sub>3</sub> <sup>-</sup>	60.9 $\pm$ 2.87 <sup>a</sup>	144.5 $\pm$ 66.4 <sup>b</sup>	197.4 $\pm$ 73.7 <sup>c</sup>
H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	4.81 $\pm$ 0.42 <sup>a</sup>	16.9 $\pm$ 4.6 <sup>b</sup>	23.5 $\pm$ 6.95 <sup>c</sup>
SO <sub>4</sub> <sup>2-</sup>	25.3 $\pm$ 0.51 <sup>a</sup>	29.2 $\pm$ 0.69 <sup>b</sup>	33.5 $\pm$ 4.79 <sup>c</sup>
K <sup>+</sup>	7.98 $\pm$ 4.35 <sup>a</sup>	9.52 $\pm$ 1.99 <sup>a</sup>	15.9 $\pm$ 5.37 <sup>b</sup>
Ca <sup>2+</sup>	13.0 $\pm$ 0.96 <sup>a</sup>	21.6 $\pm$ 3.49 <sup>b</sup>	36.3 $\pm$ 8.24 <sup>c</sup>
Mg <sup>2+</sup>	1.39 $\pm$ 0.77 <sup>a</sup>	3.89 $\pm$ 0.59 <sup>b</sup>	6.11 $\pm$ 1.92 <sup>c</sup>

<sup>1</sup> All concentrations in mg L<sup>-1</sup> except pH, and EC<sub>25</sub> = electrical conductivity, at 25°C, in mS cm<sup>-1</sup>.

<sup>2</sup> Averages with different superscript letters are significantly different among treatments ( $p < 0.05$ ).

evolution of EC<sub>25</sub> throughout the 28 day trial, showing a fairly consistent relationship between fish biomass (or feeding rate) and EC<sub>25</sub> accumulation.

The average amount of dissolved HCO<sub>3</sub><sup>-</sup> was higher in the high biomass group, while there was no significant difference among the low biomass group tank water and the control tank water. The HCO<sub>3</sub><sup>-</sup> concentration peaked on day 21 of the trial, and was always higher in the high biomass tanks (data not shown). Chloride ion (Cl<sup>-</sup>) concentration was similar among low biomass and control water but significantly higher in the high biomass tanks. Sodium was low in control water and significantly higher in low and high densities, and closely followed the trend in EC<sub>25</sub>.

Regarding nitrogen cycle ions, dissolved ammonium was similar among control and low biomass tank water but significantly higher at high biomass. At high biomass, NH<sub>4</sub><sup>+</sup> began to increase two weeks after the start of the experiment and reached a peak on day 21 (data not shown). Nitrite levels were significantly lower at 2 kg fish m<sup>-3</sup> where they were almost near zero, while they increased substantially at the higher biomass. As with NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup> peaked on day 21. Nitrate accumulation was significantly different in all three treatments (see Table 2), being lowest in control water, intermediate at low fish biomass and approximately 136% higher at high biomass.

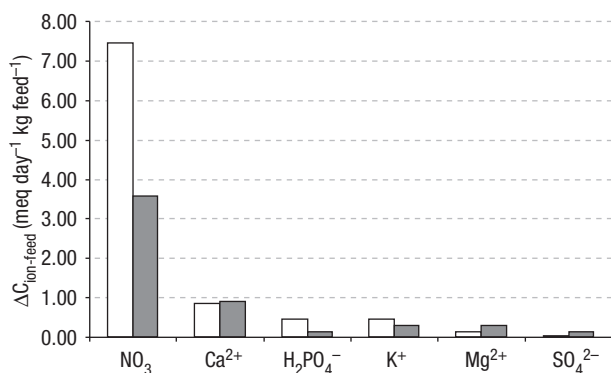
The average concentration of H<sub>2</sub>PO<sub>4</sub><sup>-</sup> was approximately four times higher on average at low biomass

compared to control water, and highest at high biomass. However, H<sub>2</sub>PO<sub>4</sub><sup>-</sup> concentration was only higher during the first two weeks, and then was more similar to low biomass concentrations. Sulphate ion (SO<sub>4</sub><sup>2-</sup>) was more similar among treatments, but followed the general trend of increasing biomass, and peaked on day 14 at high biomass.

Potassium was found in very low amounts, but was significantly higher at high biomass. Calcium was also low in control water and significantly different among low and high densities (approximately 160% higher at 5 kg fish m<sup>-3</sup>). Finally, magnesium had the lowest concentration among the dissolved minerals and was significantly different among treatments, being almost 160% higher at high biomass than at low biomass. Although values were similar in the first week, by day 14, Mg<sup>2+</sup> concentration at high biomass was double the concentration at low biomass.

### Strawberry hydroponic solution

The average requirements in mEq of the nutritive solution for strawberries is summarised in Table 1 as well as the total average increase ( $\Delta C_{ion}$ ) of each ion (in mEq) over the 35 day trial. The total amount of feed provided to each tank per day ( $F_{fish}$ ) was calculated from the productive data (8.87 g feed day<sup>-1</sup> at 2 kg fish m<sup>-3</sup> and 26.8 g feed day<sup>-1</sup> at 5 kg fish m<sup>-3</sup>) and used to



**Figure 2.** Total average increase in concentration ( $\Delta C_{\text{ion-feed}}$ ) of  $\text{NO}_3^-$ ,  $\text{Ca}^{2+}$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$  and  $\text{SO}_4^{2-}$  per kg of feed provided to fish ( $\text{mEq day}^{-1} \text{ kg feed}^{-1}$ ) for tank water with fish at 2 kg fish  $\text{m}^{-3}$  (white columns) and 5 kg fish  $\text{m}^{-3}$  (grey columns).

calculate  $\Delta C_{\text{ion-feed}}$  (Fig. 2). The amount of  $\text{Ca}^{2+}$  produced per kg of feed was only slightly higher at the higher biomass, while  $\text{H}_2\text{PO}_4^-$  and  $\text{K}^+$  increased at a higher rate at low density. Finally,  $\text{Mg}^{2+}$  and  $\text{SO}_4^{2-}$ , were both higher in the waste water from the higher biomass.

Finally, the  $F_{\text{strawberry}}$  (Table 3) reflects the  $\Delta C_{\text{ion-feed}}$ , showing that less feed is needed for similar increases in ion concentration for  $\text{NO}_3^-$ ,  $\text{H}_2\text{PO}_4^-$  and  $\text{K}^+$ , while more feed is needed for  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{SO}_4^{2-}$ .

## Discussion

In this study fish biomass, and therefore feeding regime, were manipulated to observe relative changes in mineral ion concentrations compared with the average requirements for strawberry hydroponic culture. As noted in previous studies (Nair *et al.*, 1985; Seawright *et al.*, 1998), the ions analysed did not accumulate at equal rates using a standard fish diet, and even differed between the two treatments with different initial fish

**Table 3.** Average amount (kg) of feed that should be provided to fish to fulfil the requirements (mEq) of strawberries ( $F_{\text{stb}}$ ), at low biomass (2 kg fish  $\text{m}^{-3}$ ) and high biomass (5 kg fish  $\text{m}^{-3}$ ) based on using juvenile tilapia (approximately 30 g) during a period of seven weeks.

Ion	$F_{\text{stb low}}$	$F_{\text{stb high}}$
$\text{NO}_3^-$	1.61	3.36
$\text{Ca}^{2+}$	13.1	12.3
$\text{H}_2\text{PO}_4^-$	4.0	13.0
$\text{K}^+$	11.5	17.3
$\text{Mg}^{2+}$	25.1	10.2
$\text{SO}_4^{2-}$	80.2	19.2

biomass, with a higher increase in some ions (mEq) at the lower fish biomass per kg of feed provided.

Our results coincide with Nair *et al.* (1985), Rakocy and Hargreaves (1993) and Seawright *et al.* (1998) who found that  $\text{NO}_3^-$  accumulated the most in waste water from the fish. Since  $\text{NaHCO}_3$  was used to maintain alkalinity,  $\text{Na}^+$  accumulation was also quite high, as in Seawright *et al.* (1998), who used the same base. The increase in phosphate was very similar in the two treatments (13 mg  $\text{L}^{-1}$ ) but different for potassium and calcium, which were twice and three times higher, respectively, in the high biomass treatment. As in the literature, sulfate and magnesium accumulation was very low, less than 6 and 4 mg  $\text{L}^{-1}$  respectively.

Tank water pH, which was similar among treatments at the beginning of the trial, was higher in tanks with more fish biomass, which may have had an effect on speciation of ions. Although water pH was not adjusted to a specific set point, it was maintained within appropriate levels for tilapia production and the more acidic levels commonly used in strawberry hydroponics, by adding sodium bicarbonate as a percentage of feed (25%). Surprisingly, the pH of water in control tanks was the lowest. However, as bacteria cells uptake ammonia, protons are lost to the water, acidifying it. In addition, after nitrifying bacteria were developed in the control filter (with sodium bicarbonate addition), no more bacteria, ammonia, or sodium bicarbonate was added to the control tanks after the trial began and fish were added to the treatment tanks. Thus, in the long term, the nitrifying bacteria in the control filter would begin to die/decompose, while the lack of sodium bicarbonate helped to decrease pH further. This same reasoning may also explain slightly higher levels of some ions (*i.e.*,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{K}^+$ ) in control tanks than expected.

Electrical conductivity ( $\text{EC}_{25}$ ) was a good general indicator of the increase in anions and cations in the treatment tanks, as found in other studies (Rafiee and Saad, 2005), but it does not indicate more subtle change in specific ions. Nonetheless, among the water quality indicators, the ratio of the average  $\text{EC}_{25}$  among treatments (see Table 2; 0.49  $\text{EC}_{25}$  at 2 kg fish  $\text{m}^{-3}$  / 0.75  $\text{EC}_{25}$  at 5 kg fish  $\text{m}^{-3}$  = 0.65) was more similar to the actual biomass values per tank (2 kg fish  $\text{m}^{-3}$  / 5 kg fish  $\text{m}^{-3}$  = 0.40), than the other ions. As seen in Table 1 the range of  $\text{EC}_{25}$  produced in the tanks was well below the maximum levels allowed for strawberry production. It should also be recalled that high water temperatures (required for tilapia) favour the degradation of

organic pollutants such as faeces or uneaten food, thus, to decrease  $EC_{25}$ , water temperature may be lowered. The quality and make-up of the feed used will also affect ion concentration and  $EC_{25}$ , but, as all fish were given the same feed, the effect of feed composition was not considered in this trial. Nevertheless, future trials could consider the effect of feed composition or of different commercial feeds on ion accumulation in aquaponics, for which there is little data at the moment.

The concentration of  $NO_2^-$  and  $HCO_3^-$  was higher at the higher biomass (23 and 13 times higher than at 2 kg fish  $m^{-3}$ , respectively). A greater concentration of dissolved  $NO_2^-$  indicates that autotrophic ammonia-oxidizing bacteria (AOB) are eliminating ammonia from the tank at a faster rate than chemolithoautotrophic  $NO_2^-$  oxidizing bacteria (NOB) can convert  $NO_2^-$  to  $NO_3^-$  (Ebeling *et al.*, 2006). However, after day 26,  $NO_2^-$  levels returned to near zero, indicating that the biofilter was working properly.

Bicarbonate concentration was consistently higher at the higher biomass and correlated with pH and feeding levels in both treatments. Unlike  $NO_3^-$ , which is produced by AOB and NOB and accumulates in RAS,  $HCO_3^-$  ions can be both produced (via fish respiration or addition of  $NaHCO_3$  buffer to the water) and consumed (by AOB and NOB). The  $NaHCO_3$  was added to all tanks at a rate of 25% of total feed, thus less total buffer was added to the lower biomass tanks. All  $HCO_3^-$  added or produced in the tanks at 2 kg fish  $m^{-3}$  appears to have been consumed, while it accumulated at 5 kg fish  $m^{-3}$ . However, the additional buffer in high biomass tanks does not alone account for the accumulation since  $Na^+$  increased four-fold in the first week and  $HCO_3^-$  twenty-fold. It seems reasonable to assume that  $HCO_3^-$  levels were also higher at higher biomass due to increased production of  $CO_2$  by the fish, which may have been more stressed. The increase in  $HCO_3^-$  helped to maintain pH above 7.0 throughout the experiment, and both values were clearly correlated with changes in feeding rate (grams of feed per fish) rather than total feed provided, underlying the importance of recording daily or weekly changes in feed provision as compared with total feed provided over the trial.

While the ions involved in the nitrogen and carbon cycles were more variable, the dissolved minerals were all present in lower concentrations. In our study, calcium concentration was within the range of 13-36 mg  $L^{-1}$ , while in a typical recirculating system cited in Martins *et al.* (2009), calcium concentrations were

higher (range 45-75 mg  $L^{-1}$ ). In Seawright *et al.* (1998), calcium concentrations were one of the first to decline in hydroponics with lettuce. Interestingly, those authors found a high concentration of calcium in fecal solids (70-170%), so they suggest that calcium has a low availability in the diet and that a significant fraction of the nutritional requirement was met through active uptake from the environment (fish absorbed  $Ca^{2+}$  from the tank water). As a result, most hydroponic integrated systems will need additional calcium to be added to the water (up to 200 mg  $L^{-1}$  in Seawright *et al.*, 1998), since it appears to be actively absorbed by both fish and plants.

Phosphate concentration was within the range of 17-24 mg  $L^{-1}$ , while in Martins *et al.* (2009) it was lower (range 0.61-17 mg  $L^{-1}$ ). Phosphates can precipitate as  $Ca_3(PO_4)_2$ , explaining near zero values in some cases (Seawright *et al.*, 1998), but they are one of the most important and expensive components of hydroponic solutions. In fish, phosphate retention is approximately 50% of that available in the diet, but it is also high in fecal solids 33-55% (Seawright *et al.*, 1998). The concentration of phosphates in fish feed is typically less than 1.5%, of which approximately 50% is retained in the carcass and the rest is lost in fecal solids or as urine (Flimlin *et al.*, 2003).

The dissolved potassium concentration was within the range of 7-16 mg  $L^{-1}$ , while in Martins *et al.* (2009) it was higher, reaching up to 112 mg  $L^{-1}$ . According to Seawright *et al.* (1998), the apparent retention of potassium in fish is 23-26%, and in solids 3-6%. Fish diets are quite low in  $K^+$  and whole body concentration of  $K^+$  in fish is approximately 1%. However, potassium concentrations decreased in Seawright *et al.* (1998), implying that  $K^+$  was absorbed by fish. With the diets used in the present study, it appears that there was enough dietary  $K^+$  in the diet to accumulate in the tank water. Seawright *et al.* (1998), report a strong correlation between fish biomass and change in the quantity of dissolved  $K^+$ . Our results support those findings since  $K^+$  was in the high biomass tanks was 167% the value in low biomass (Table 2). Sulfates were quite high, even in control water (25-33 mg  $L^{-1}$ ), compared to Martins *et al.* (2009) (8-39 mg  $L^{-1}$ ), which is most probably due to the water source.

Magnesium was within the range of 1-6 mg  $L^{-1}$ , while in Martins *et al.* (2009) it was 7.5-21 mg  $L^{-1}$ . Seawright *et al.* (1998) found that apparent fish retention of  $Mg^{2+}$  was 19-22% of  $Mg^{2+}$  in the diet, but presence in solids was fairly high (*i.e.*, 14-24%), so

they conclude that while the biological availability of  $Mg^{2+}$  in the diet is high, it is less than that of  $K^+$ . In natural waters,  $Mg^{2+}$  is often much higher, as in a recent study of aquaculture ponds with tilapia where levels were consistently above  $300 \text{ mg L}^{-1}$  (Mishra *et al.*, 2008). Thus, the levels found in this study are quite low, and should not have negative effects on tilapia health.

Regarding feed requirements, the increase in ion concentration was higher for tanks with less fish biomass for  $NO_3^-$ ,  $H_2PO_4^-$  and  $K^+$ , and very similar among the two treatments for  $Ca^{2+}$ , suggesting that less feed was needed to provide the mEq required by strawberries at the lower biomass. However, the lower concentration of those ions at the higher feeding rate could be a result of the accumulation of solid fish waste in the filters. Although not specifically measured during this trial, solid fish waste was presumably higher at the higher feeding rate, which may have captured ions from the water, making them unreadable in the water column analysis. This difference was especially acute regarding  $NO_3^-$  production, possibly reflecting the high sensitivity of nitrifying bacteria to waste build up in filters, thereby decreasing  $NO_3^-$  production. Greater waste build-up at the high biomass could also create anoxic pockets in the biofilters, where denitrification reactions may occur, thereby decreasing  $NO_3^-$  accumulation.

The total amount of feed required per mEq (Table 3) at the low biomass ( $2 \text{ kg fish m}^{-3}$ ) ranged from 1.61-13.1 kg for the four most abundant ions ( $NO_3^-$ ,  $Ca^{2+}$ ,  $H_2PO_4^-$  and  $K^+$ ), suggesting that it is feasible to integrate fish culture (at low densities) to reduce the cost of hydroponic solution supplementation for strawberries. On the other hand,  $Mg^{2+}$  and  $SO_4^{2-}$  would have to be supplemented for proper plant growth, since their production levels are several times less than the four main ions. Finally, care should be taken when choosing the proper feeding level or fish density since high levels (approaching  $5 \text{ kg fish m}^{-3}$ ) may have detrimental effects on the availability of some ions for aquaponics.

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