Aquaculture Research

Aquaculture Research, 2014, 1–11 doi: 10.1111/are.12626

Herbaceous plants as part of biological filter for aquaponics system

Elvia A Espinosa Moya[1], César A Angel Sahagún[2], J Mario Mendoza Carrillo[2], Pedro J Albertos Alpuche[2], C Alfonso Álvarez-González[3] & Rosario Martínez-Yáñez[2]

[1]Maestría en Biociencias, División de Ciencias de la Vida, Campus Irapuato-Salamanca, Universidad de Guanajuato, Ex Hacienda El Copal, Irapuato, Guanajuato, México

[2]División de Ciencias de la Vida, Universidad de Guanajuato, Campus Irapuato-Salamanca, Ex Hacienda El Copal, Irapuato, Guanajuato, México

[3]Laboratorio de Acuicultura Tropical, Universidad Juárez Autónoma de Tabasco, Villahermosa, Tabasco, México

Correspondence: R Martínez-Yáñez, Departamento de Agronomía, División de Ciencias de la Vida (DICIVA), Campus Irapuato-Salamanca (CIS), Universidad de Guanajuato (UGTO), ExHacienda El Copal, km 7 carretera Irapuato-Silao, CP. 36824, Irapuato, Guanajuato, México. E-mail: ar.martinez@ugto.mx

Abstract

Aquaponics is a recirculating aquaculture system (RAS), where plants and aquatic animals are grown using the same water. In these systems, plants act as part of biological filters. The cultivation of *O. basilicum, Menta x piperita* and *M. spicata* is commonly integrated to the production of *O. niloticus* in aquaponics. The aim of this study was to evaluate the ability of these herbs as part of biological filters for tilapia intensive production in aquaponics. Various physicochemical parameters were evaluated as water quality indicators. N and P content in the different elements of the system were also measured. Results showed that for tilapia growing the three herbaceous evaluated could be used as part of the biological filters in aquaponics, because they remove significant concentration in nitrogen compounds and phosphates; however, there were no differences among species. There was a positive relationship between the time and the levels of NH_4 and therefore NO_3^- in the water. The pH, temperature and dissolved oxygen were kept at appropriate ranges for tilapia. The electrical conductivity and total dissolved solids were in suitable levels for growing herbaceous, which adapted to flooded substrates, with water constantly moving and high concentration of dissolved oxygen. A key parameter to consider is the oxygen concentration in water when herbaceous is used in aquaponics, due to the high input of this element for these species need, especially basil. Tilapia largely incorporated N and P entering the system.

Keywords: herbaceous, tilapia, biological filters, nitrogen, phosphorus, aquaponics

Introduction

Aquaponics is an intensive recirculating aquaculture system (RAS) where growing plants are integrated to the production of aquatic animals (Ramírez, Sabogal, Jiménez & Hurtado-Giraldo 2008) and, could be used as an alternative for the aquaculture waste management system (Adler, Harper, Wade, Takeda & Summerfelt 2000). Aquaponics offers an economical and cost effective option for the treatment of aquaculture discharges by mitigating pollution and increasing water use efficiency, thereby reducing environmental impact (Zhang, Li, Wu, Liu, Yao, Tao & Liu 2011). The basic principle of aquaponics system is to recycle the nutrient rich wastewater generated from the aquaculture system which nutrients are taken by plants preventing their accumulation, as they act as a natural filter, extending the use of the water returning to the aquatic organisms (Rakocy, Masser & Losordo 2006). This results in an integrated sustainable production system. The aquaculture wastewater contains high levels of nitrogen and phosphate metabolites (Dosdat, Ruyet, Covès, Dutto, Gasset, Le Roux & Lemarié 2003). The nitrogenous metabolites include unionized ammonia

(NH_3), ammonium (NH_4^+), nitrite (NO_2) and nitrate (NO_3^-) of which unionized ammonia and nitrite are detrimental to fish. NH_3 is excreted by fish, through diffusion in the gills, urine or faeces, plus other nitrogenous wastes accumulate from organic waste or leaching foods (Lazzari & Baldisserotto 2008; Timmons & Ebeling 2010). Nitrite is the intermediate product of nitrification and can be accumulated in water due to incomplete nitrification. If these elements are not removed from the RAS properly, the health and welfare of the animals under culture is at risk (Mateus 2009). Therefore, plants as biofilters can be considered one of the main components in RAS in reducing these wastes (Hall 1999).

One of the most common fish species in aquaponics is Nile Tilapia (*Oreochromis niloticus*), because it has good meat flavour, faster growth, and strong and broad market acceptance (Rosas 2002; Nelson 2004). The variety of plants that can be grown as part of biofilters in aquaponics is vast. The most recommended species are herbaceous plants, such as basil (*Ocimum basilicum*), peppermint (*Mentha x piperita*) and spearmint (*Mentha spicata*), because of their fast growth, adaptability and various utility such as culinary, medicinal and aromatic (Kintzios & Makri 2007; Rains 2007; Ramírez *et al.* 2008; ASA 2009). There is limited information on the capability of herbaceous plants as part of the biological filter in aquaponics. Rakocy, Shultz, Bailey and Thoman (2004) and Ronzón-Ortega, Hernández-Vergara and Pérez-Rostro (2012) reported basil production integrated to growing tilapia and prawn respectively. Wahap, Estim, Seok-Kiang, Senoo and Mustafa (2010), integrated mint and tilapia in aquaponics system two more research findings are reported where the use and production of oregano and spearmint were evaluated (Ramírez-Sánchez, Pérez-Trujillo, Jiménez, Hurtado-Giraldo & Gómez-Ramírez 2011; Campos-Pulido, Alonso-López, Ávalos-de la Cruz, Asiain-Hoyos & Reta-Mendiola 2013). However, all these reported research have focused only on the plant production parameters. The biofiltration role and water purifying capacity of plants in aquaponics systems neglecting the biofiltration role that plants have to purify water in recirculating aquaculture systems, is an area that has recently begun to be explored (Trang & Brix 2012). Therefore, the main objective of this study is to evaluate the ability of basil, peppermint and spearmint as part of biological filters to purify water in intensive tilapia production in aquaponics systems and their N and P absorption and integration capability.

Materials and methods

This study was conducted at the Aquaponics Experimental Unit from the Agriculture Department, Life Sciences Division (DICIVA), Campus Irapuato-Salamanca, University of Guanajuato (20°44′34.42″N 101°19′50.7″W; 1745 m.s.n.m.), and lasted for 30 days.

Systems

The experimental aquaponics system was designed according to Regalado (2013). All of them were located inside a greenhouse (144 m^2) walls made of anti-aphid mesh and roof made of light diffusing plastic covered with sun shade mesh (50%). Three similar and independent aquaponics systems, each consisting of a pond (V_{Ef} = 1.5 m^3) and three hydroponic beds (HB) with 0.270 m^3 total volume and 0.9 m^2 total crops area. In each HB 0.076 m^3, volume of river stone were used as substrate (1″ particle-size, previously washed and disinfected, NaClO 5%) and a volume of 0.104 m^3 of water, to give a total of 0.180 m^3 V_{Ef} (Fig. 1). Each HB had a physical filter (V_{Ef} = 10 L, clarifier with plastic mesh 0.4 mm hole). PVC pipe was used for water pipelines, the internal motion was carried out with a submersible pump (BOYU, ACQ-6000) and to each pond air was injected (53.5 L min^{-1}, compressor BOYU ACQ-009) by silicone hose (Ø 4 mm) connected to four stone diffusers. Adjustments to the flow rate for hydraulic retention time (HRT) on each HB was 50 min (Regalado 2013).

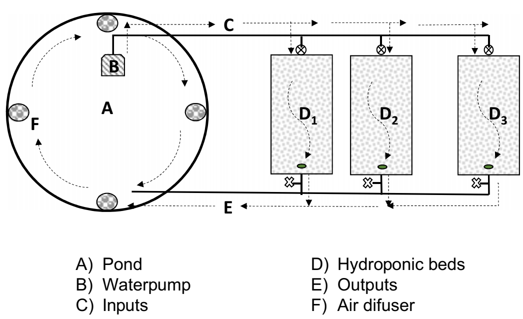

Figure 1 Items in the aquaponic system used. Not to scale.

Water

To test the herbaceous as part of biological filter, physicochemical parameters of un-ionized ammonia (NH_3-N), ammonium (NH_4), nitrite (NO_2), nitrate (NO_3^-), phosphate (PO_4), pH, temperature, electrical conductivity, total dissolved solids and dissolved oxygen were evaluated as water quality indicators. All systems were filled with water from a single source, and recirculation and oxygenation launched on the same day; 24 h later, 30 g balanced fish food (53% crude protein and 0.35 mm particle) were directly supplied to the water in each pond. Biological filters were inoculated after 24 h taking into consideration the entire system water volume (V_{Ef}), with aerobic bacteria lyophilized (AZOO – NitriPro, *Nitrosomona* and *Nitrobacter*) at 3 g of bacteria per 250 L of water. 48 h later, feed was supplied again to the ponds (20 g each), and after 24 h a second bacterial inoculation was performed at 1 g per 250 L of water. After 48 h of second inoculation, fish was stocked into the ponds, and bacterial inoculation was done for a third time to the filters (1 g per 250 L of water). In the next day, herbaceous plants were transplanted to HB (Day 0). Every other day starting from 15th to 30th day of the experimental period, nutrient removal rates by herbaceous plants was determined for NH_3-N, NH_4 and PO_4 in HB inputs (water coming from fish pond) and outputs (water leaving HB) as: concentration HB_{input} – concentration HB_{output} (mg L^{-1}). For this, we conducted a pre-sample (9:00 am zero hour and then every 30 min until 13:00 h) to determine the time when maximum concentrations of NH_3-N, NH_4 and PO_4 reached in the pond water after the first fish feeding. This peak time of the day was used as for water sampling to determine the difference in the concentration of these metabolites between inputs and outputs. The measurement was made on 200 mL water samples directly taken from individual faucet water supply (input) and each HB output 50 min later (observing HRT). Water loss due to sample taken and evapotranspiration was compensated. The nutrient concentrations (mg L^{-1}) were measured using an aquaculture spectrophotometer (Hanna HI 83203-01), and reagents for NH_3-N and NH_4 (HI 93700-03), NO_2 (HI 93707-0), NO_3^- (HI 93728-01) and PO_4 (HI 93713-0), following the protocols specified by the manufacturer. The other water quality variables pH, electrical conductivity (EC, mS cm^{-3}), total dissolved solids (TDS, ppm) were measured on daily basis (10:00 h) in ponds, and HB inputs and outputs were determined the with a Hanna equipment (model 98129), and dissolved oxygen (DO, mg L^{-1}) and temperature (°C), with an OAKTON equipment (model DO-300). Oxygen consumption was also determined according to the herb as concentration at HB_{input} – concentration HB_{output} (mg L^{-1}). To obtain all these parameters HRT was taken into account.

Fish

In each pond, 80 specimens of Nile Tilapia (*Oreochromis niloticus* L. var. Stirling; 137.6 ± 20.4 g wet weight and 20.3 ± 1.0 cm in length) were stocked. A commercial floating pellet (dry matter: 93.17% moisture: 6.83%, crude protein: 24.60%, ether extract: 5.19%, ash: 6.56%, crude fibre: 6.77% and nitrogen free extract: 50.05%, El Pedregal) was supplied to the pond at a rate of 3% body weight and divided into three doses throughout the day (9:00, 13:00 and 17:00 hours.). At the beginning and end of the experiment, fish were measured from the tip of the snout to the end of the caudal fin with an acrylic measuring board and a measuring tape, and weighed on a Labtronic Scientific digital scale (model 21-2544-09). The survival (taking into account the initial number and the final number of fish) and biomass gain (g of harvested tilapia – g stocked tilapia) were calculated in each pond. Feed intake was calculated as the sum of the food supplied per pond during the experimental period. Specific growth rate was determined as: SGR (% animal day^{-1}) = $[(LnPh2 - LnPh1/t)] * 100$, where Ph2 Ph1 are final and initial fish wet weight, Ln is the natural logarithm and t is the number of days in the experimental period, and daily gain as: DGW (g $animal^{-1}$ day^{-1}) = $(Ph2 - Ph1)/t$, where Ph2 Ph1 are fish final and initial wet weight and t is the number of days in the experimental period (Martínez, Gallardo, Pech, Navarro, Sánchez, Caamal-Monsreal & Rosas 2014). Corporal condition ratio (Fulton) was determined as: $k = (W/L^3) * 100$, where W is the individual wet weight (g) and L is total length (cm). The feed conversion ratio was determined as: FCR = feed, kg per net tilapia production, kg (Boyd, Tucker, McNevin, Bostick & Clay 2007).

Herbaceous

Herbaceous plants used (90 days old, cultivated in a greenhouse) were basil (*Ocimum basilicum*, 21.6 ± 6.8 g), peppermint (*Mentha x piperita*, 25.8 ± 10.3 g) and spearmint (*Mentha spicata*, 26.1 ± 7.3 g). Randomly selected 10 plants were placed per HB (placed direct in the gravel), the three species separately representing each system. At the beginning and end of the experiment, the plants were weighted (including root). To avoid micronutrient deficiencies every week a foliar micronutrients solution for organic agriculture (Mg: 325 ppm, B: 70 ppm, Cu: 12 ppm, Fe: 150 ppm, Mo: 1 ppm y Zn: 90 ppm, Greenforce) was sprayed to all plants accordingly to the manufacturer instructions (0.15 mL by m^2 per week). Production was calculated by the plant biomass (g m^2) on a wet (WM) and dry matter (DM) per plant.

N and P balance in the system

Determining concentrations of N and P in the system were used as the feed (nutrient input) consumption per tank (mean ± SD) and the incorporation in fish, removal by the plants, the recovery of sludge and presence of these elements in water (nutrient output). For this, DM values were used, N and P contained in the tilapia reported by Tian, Li, Dong, Yan, Qi, Liu and Lu (2001). For the sludge produced calculation was determined the dry matter ratio as DMR = FCR * (% DM in feed/% DM in tilapia). This data were necessary to determine the waste production rate as: WPR = (DMR − 1) * (% DM in tilapia/100) (Boyd *et al.* 2007). Throughout the experimental period were taken samples of water and sludge; the latter were taken from the clarifiers. Water was frozen (−40°C) and sludge was dried (60°C, to constant weight) for later analysis. With these sample pools (one for water and one for sludge) of each system were sent to a specialized laboratory to analyse the concentration of N (Kjeldahl method; Chang 2010) and P (digestion with nitric-perchloric acid; AOAC 1997). At the end of the experiment, three plants were randomly selected from each HB which were cut into small pieces (including root) and set out to dry for the % DM, and then determine the concentration of N and P (methods described above).

Statistical analysis

The homogeneity of variances and normality of data were checked and transformed where necessary. The removal capacity of MH_3-N, NH_4 and PO_4 between filters with herbaceous and concentration of NO_2 and NO_3^- in HB were analysed using a factorial ANOVA (A: species, B: sampling day). The relation between the experimental period (sampling days, independent variable) and the concentration (mg L^{-1}, dependent variable) of NH_3-N, NH_4 and PO_4 inputs and NO_2 and NO_3^- in HB medium was analysed by linear regression. DM, plant production and herbaceous dissolved oxygen consumption were analysed by a one-way ANOVA. Obtained pH values were converted into the hydrogen ion concentration (M) (Brown, LeMay, Bursten & Burdge 2004). By the nature of the differences between the data inputs and outputs pH, EC, TDS, temperature and DO were identified by means of a Kruskal–Wallis test. Comparison of means among three different herbaceous plants species was performed using Tukey's test. Data were reported as mean ± SE and statistically significant differences when $P < 0.05$ and indicated with different letters.

Results

Herbaceous plants as part of biological filters

The peak concentrations (mg L^{-1}) of NH_3-N, NH_4 and PO_4 in the ponds were recorded three hours after the first feeding (12:00 hour). The three filters with herbs removed nitrogen and phosphorus compounds from the water. However, no significant differences between plant species were recorded. Differences in NH_3-N and NH_4 were observed during the experimental period ($P = 0.0015$ and 0.0039 respectively), on the 21 and 24 days the greater removal of NH_3-N and NH_4 was shown, and on day 30 lower removal (Fig. 2a and b). No statistically significant differences in sampling day were observed in the removal of phosphates (Fig. 2c).

Nutrients concentrations in water

The concentrations of NO_2 and NO_3^- in the HB (Fig. 3a and b), showed significant differences among the sampling dates ($P = 0.0000$ and 0.0017 respectively), but not among the three plant species. On Day 15, the concentrations were the lowest and increased by Day 30 of the experiment. Regression analyses show that the NH_3-N, NH_4 y PO_4 concentration in the inputs (Figure 2a-c), and the NO_2 and NO_3^- concentration in the HB

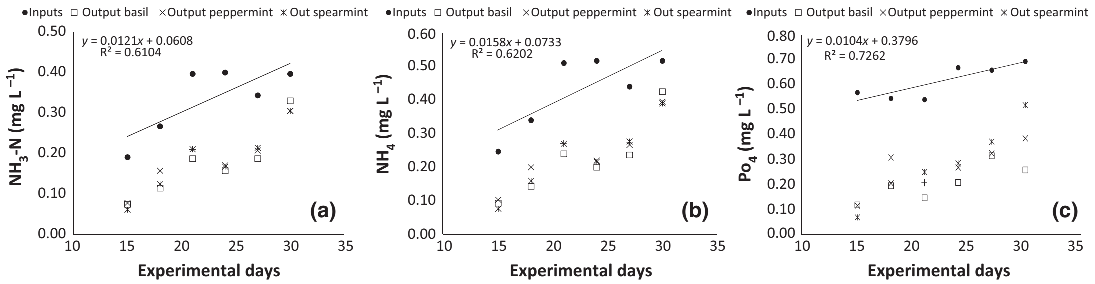

Figure 2 Metabolites concentration in water (mg L^{-1}) inputs and outputs of biological filters according to the herb species used, for tilapia culture in aquaponics systems. (a) Un-ionized ammonia (NH_3-N), (b) Ammonium (NH_4) and (c) Phosphates (PO_4).

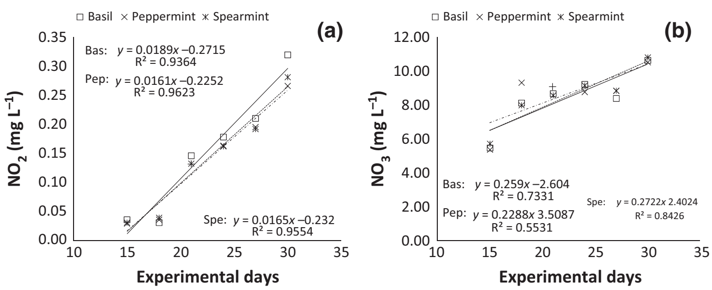

Figure 3 Metabolites concentration in water (mg L^{-1}) of hydroponic beds (HB) according to the herbaceous species used as part of biological filters for tilapia culture in aquaponics systems. (a) Nitrite (NO_2) and (b) Nitrate (NO_3).

(Figure 3a and 3b) are positively related to the experimentation time. For the NH_3-N, NH_4 and PO_4 concentration in inputs, the correlation coefficients are 0.7732 ($P = 0.0713$), 0.7892 ($P = 0.0620$) and 0.8537 ($P = 0.0305$), respectively, indicating a moderately strong relationship between these variables and time. The NO_2 and NO_3^- concentrations in HB were consistently increased throughout the experiment.

Water physicochemical parameters

The pH values in the ponds showed a fluctuation between 7.4 and 7.9. The electrical conductivity showed a range from 0.676 to 0.876 mS cm^3, remaining stable throughout the experiment. Total suspended solids, also remained stable, between 478 and 712 ppm. The temperature fluctuated between 21.1 and 26.1°C (Fig. 4a–d). Dissolved oxygen in the water kept a range between 4.6 and 6.0 mg L^{-1} (Fig. 5). In Fig. 4a, can be observed pH values in ponds, inputs and outputs from HB throughout the experimental period. Significant differences ($P = 0.0000$) between the inputs (7.6) and outputs (7.5), but not between herbaceous species were observed. The same behaviour was presented for temperature (Fig. 4b, P = 0.0000). No differences in EC and TDS variables (Figs 4c and 3d) were presented. The dissolved oxygen concentration presented significant differences between inputs (5.79 mg L^{-1}) and outputs (2.26 mg L^{-1}), as well as between herbaceous (Fig. 5, P = 0.0000), been basil the species with more oxygen consumption (3.71 ± 0.15 mg L^{-1} in 50 min). Peppermint and spearmint are the herbs that require less oxygen (3.29 ± 0.15 and 3.20 ± 0.14 mg L^{-1} in 50 min respectively).

Fish and plants production

The final individual weight and size of fish (mean ± SD) were 168.78 ± 29.8 g per animal and 21.2 ± 1.1 cm per animal respectively. The fish survival was 100% in the three systems. Feed intake during the experimental period was 3436.93 ± 45.56 g per pond (mean ± SD). The average EGR and DGW were 0.67 ± 0.001% animal day^{-1} and 1.04 ± 0.004 g $animal^{-1}$ day^{-1} respectively. The initial and final k values were 1.64 ± 0.01 and 1.76 ± 0.006, respectively, and the FCR was 1.37 ± 0.05 (mean ± SD). Table 1 shows that the peppermint had the highest value in DM (%), and basil and spearmint lowest values ($P = 0.0036$). The biomass yield (g m^2), in terms of DM varied

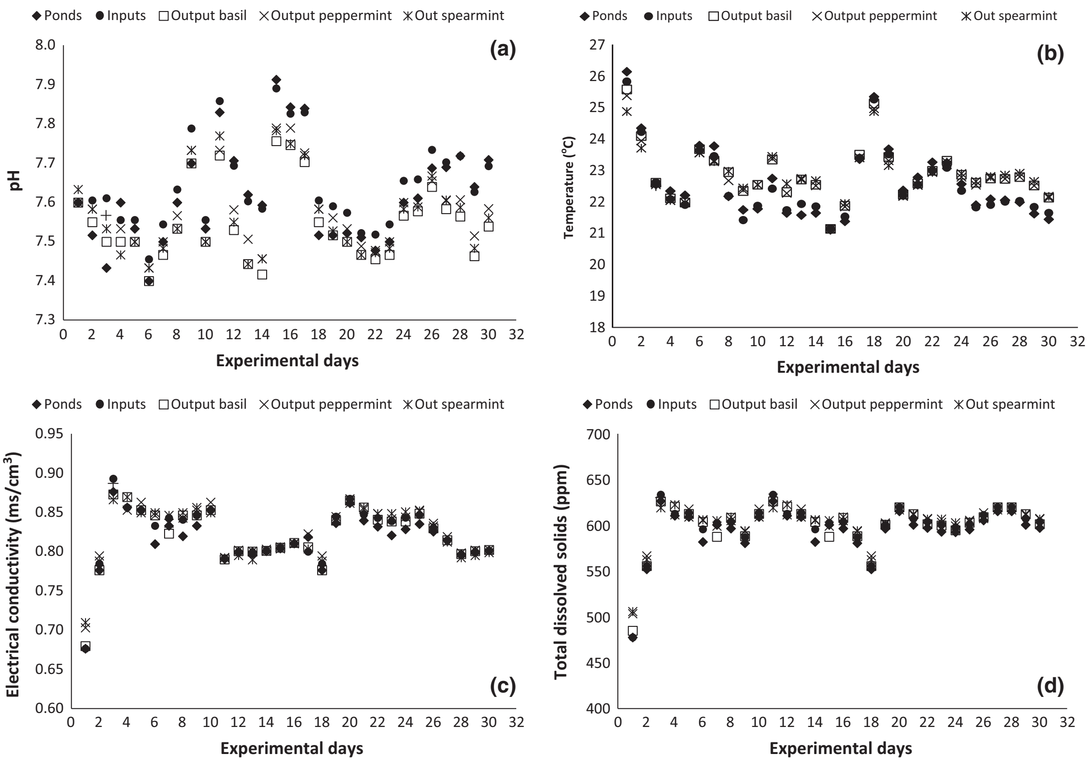

Figure 4 Values throughout the experimental period (a) pH, (b) Temperature (°C), (c) Electrical Conductivity (mS cm^{-3}) and (d) Total Dissolved Solids (ppm) in ponds, inputs (n = 9) and outputs (n = 3) from hydroponic beds according to the herb used as part of biological filter for tilapia culture in aquaponics systems.

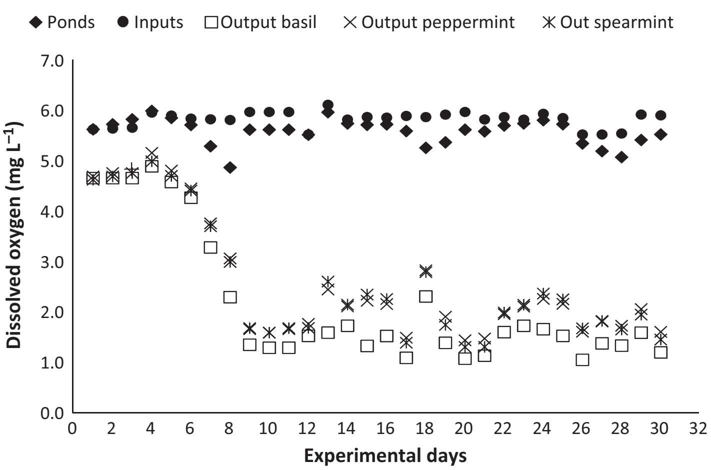

Figure 5 Dissolved oxygen values (mg L^{-1}) in ponds, inputs (n = 9) and outputs (n = 3) from hydroponic beds according to the herb used as part of biological filter for tilapia culture in aquaponics systems, along experimental period.

significantly among the plant species (P = 0.0116), whereas WM did not vary significant (Table 1).

N and P balance in the system

N and P registered values in the herbaceous can be seen on Table 2. The DMR and WPR were 5.13 ± 0.18 and 1.03 ± 0.05 respectively (mean ± SD). The N and P content balance in the various components of the system is presented in Table 3. Both nutrients entered the systems through feed supply, and subsequently trapped into various biological components including fish flesh, bacteria and herbaceous plants, and the

Table 1 Biomass production values for herbaceous used as part of biological filters, N y P compounds removal in aquaponics systems

Variable	Basil (*O. basilicum*)	Peppermint (*M. x piperita*)	Spearmint (*M. spicata*)
Dry matter (%)	13.6 ± 0.46 b	16.02 ± 0.61 a	12.42 ± 0.15 b
Production (g m^2 WM)	531.85 ± 49.56	567.92 ± 31.58	647 ± 28.43
Production (g m^2 DM)	72.33 ± 6.74 b	101.25 ± 2.68 a	80.43 ± 3.53 b
Removal NH_4 (%)	49.70	44.45	48.10
Removal PO_4 (%)	62.59	56.54	54.88

Means ± SE. DM, dry matter; WM, wet matter; NH_4, ammonia; PO_4, phosphates; N, nitrogen; P, phosphorus.

Table 2 Total nitrogen and potassium comparison (dry matter, DM) of herbaceous grown in aquaponics systems (g/100 g DM)

Variable	Basil	Peppermint	Spearmint
Nitrogen	2.64 ± 0.54	1.75 ± 0.47	2.81 ± 0.45
Phosphorus	0.30 ± 0.05	0.25 ± 0.07	0.32 ± 0.07

Means ± SE.

Table 3 N and P balance content in the aquaponics systems biological components (Mean ± SD, *n* = 3)

Variable	N	P
Feed (input, DM g day^{-1})	4.93 ± 0.065	1.260 ± 0.017
Fish (incorporated, DM g day^{-1})	2.00 ± 0.067	0.879 ± 0.030
Plants (removal) DM g day^{-1}:		
Basil (*Ocimum basilicum*)	0.064 ± 0.023	0.007 ± 0.002
Peppermint (*Mentha x piperita*)	0.059 ± 0.028	0.009 ± 0.005
Spearmint (*Mentha spicata*)	0.075 ± 0.021	0.009 ± 0.003
Slodges (recovered, DM g day^{-1})	0.022 ± 0.003	0.007 ± 0.001
Water (g day^{-1})	0.081 ± 0.055	0.020 ± 0.000
Not accounted	2.62	0.33

DM, dry matter.

remainder distributed to the sludge and water. The nitrogen and phosphorus incorporated into the fish corresponding to 40.6% and 69.8% respectively. As for the removal of nutrients from the herbs, basil removed the 1.29% and 0.58%, peppermint 1.20% and 0.69%, and spearmint 1.53% and 0.69% N and P respectively. In the sludge was recovered 0.44% and 0.54% of N and P entering the system respectively. In the water was 1.65% and 1.59% of N and P respectively.

Discussion

This study provides for the first time the performance of *Ocimum basilicum*, *Mentha x piperita* and *Mentha spicata* as part of a biological filter in aquaponic systems particularly, their uptake capability of N and P. As any other recirculating aquaculture system (RAS), one of the key parameters to keep in such systems is the nitrogen compound concentration. When aquatic organisms are chronically exposed to elevated concentrations of these compounds, different pathologies and finally death occur (Navarro & Gutiérrez 1993; Ip, Chew & Randall 2001). According to our results, nitrogenous wastes levels were observed at lower ranges than those reported by Trang and Brix (2012), using plants as biofilters in integrated recirculating aquaculture-hydroponics systems and optimal for the freshwater fish culture (Navarro & Gutiérrez 1993). The results showed that there is adequate removal of these molecules by filters with herbaceous, which is reflected in the prominent difference in NH_3-N and NH_4 concentration between inputs and outputs from the biological filters, in addition, the biomass production from each herbaceous species in the aquaponics system. According to the results, PO_4 levels from water ponds (inputs) are higher than the optimum range for growing tilapia (0.15–0.2 mg L^{-1}, Saavedra-Martínez 2006) but, in sufficient concentration to be assimilated by plants (Amirkolaie 2011), in this regard, we noted that this compound also showed significant differences between the inputs and outputs of HB because herbaceous remove and uptake it. It is possible that the PO_4 levels observed in the pond water being the result of the phosphorus leaching from uneaten feed since there has been reported that near 50% of the phosphorus in the fish feed can be lose in a period of three hours (Sales, Britz & Viljoen 2003), together with the animals excretion (Timmons & Ebeling 2010).

It is important to mention the direct relationship between sampling days and the nitrogen compounds and phosphates concentration in the water. This positive relationship indicates accumulation of these metabolites in the system, which should be consistently and efficiently removed to avoid health problems in fish. Aquacultural effluents containing a high concentration of N and P, are a major problem in production as they contribute to eutrophication (Smith 2003), although the RAS considerably reduce the effluent to remove solids and perform biofiltration while the water is reused, a significant amount of N and P is discharged (Timmons & Ebeling 2010). One of the major aquaponics advantages is the high amount of plant biomass that can be obtained. In a single fish farming cycle, it is possible to harvest several plant cycles mainly attributable to the constant nutrients supply in the water (Rakocy *et al.* 2004). Commercial tilapia culture goes from 6 to 9 months (depending on the water temperature, Diario Oficial de la Federación (DOF) 2011), based on our results, using a small scale aquaponics system (similar to the one used in this study) it would be feasible in one fish cycle to obtain a 30–35 k of fish and between 9.6–14.3, 10.2–15.3 and 11.6–17.4 (k, wet matter) of basil, peppermint and spearmint respectively.

In aquaponics, plants play a predominant role in maintaining water quality by removing from the system dissolved nutrients which they use for their growth, allowing reusing this important resource, at the same time reducing significantly the wastewater discharges and minimizing the water turnover rate (Timmons & Ebeling 2010) which means a minor environmental impact. The use of plants with nitrates and phosphates absorption capability in biological filters in RAS may be a viable option to reduce these elements concentrations in the discharged water from the aquaculture production. N and P contribute to eutrophication (Smith 2003) and should be controlled as much as possible. In Mexico, the NOM-001-ECOL-1996 establishes the by-law limits and allowable pollutant discharges of waste water in national waters, in particular, indicates the basic pollutants concentration in mg L^{-1} such as total nitrogen and total phosphorus. These effluents can be used for agricultural irrigation and urban use; however, it is important to note that for the aquatic life protection, the maximum permissible limit of N and P in effluents discharged to rivers and coastal waters is 15 and 5 mg L^{-1} respectively (Diario Oficial de la Federación (DOF) 1996). In the present study, were registered lower concentrations of N and P from those in aforementioned norm, therefore the system's water can be used for irrigation or be discharged without any environmental risk.

Nitrate and phosphorus accumulate in the water in recirculation systems during intensive fish culture (Rodehutscord & Pfeffer 1995; Barak & Van Rijn 2000). The observed values in the N and P addition in the various elements show the balance that this type of production systems can present. According to our results, the N and P total proportion entering which is incorporated into the fish is similar than that reported by Trang and Brix (2012) generally only the 35–40% of the consumed feed by the tilapias is assimilated and transformed into flesh meanwhile the remainder (60–65%) is excreted to the water (Chapell, Brown & Purcell 2008). Nitrate usually does not reach levels lethal to fish in RAS (Timmons & Ebeling 2010), in the case of phosphorus, which is not assimilated by the fish accumulates in the water (Rodehutscord & Pfeffer 1995; Barak & Van Rijn 2000). The observed N and P concentration in plants, reinforces the results of water quality in terms of the similar behaviour of these species as part of the biological filter, and indicates that the nitrification process and the constant degradation of organic matter with consequent production nitrates and phosphates also occur significantly in the hydroponic beds used specifically in the substrate where the roots are. It is noteworthy that both peppermint like spearmint, assimilate nutrients better when growing in aquaponics, this very likely due to the constant water and nutrients supply. The three evaluated herbaceous absorption capability in this study is lower than reported for *I. aquatica* and greater than *L. sativa* (Trang & Brix 2012); however, noteworthy that the first is an aquatic plant and the second has less DM than recorded in the studied herbaceous.

The NO_2 and NO_3^- levels observed in the HB over the experimental period, shows that the nitrification is carried out appropriately. In aquaponics, removing nitrogen compounds takes place through bacteria and plants action. Ammonium tends to be oxidized to nitrate in a two-step process ($NH_4 \rightarrow NO_2 \rightarrow NO_3^-$) by aerobic chemoautotrophic bacteria (*Nitrosomonas* and *Nitrobacter*, primarily) (Madigan, Martink, Stahl & Clark 2012), even if levels of dissolved oxygen decline to a value as low as 1.0 mg L^{-1} (Stumm & Morgan 1996; Wetzel 2001). Due to this process in aquaponics systems NO_3^- concentrations are greater than NH_4 and

NO_2, this relationship is also observed in freshwater ecosystems (Gleick 1993; Wetzel 2001; Rabalais 2002). In RAS, denitrifying bacteria transforms nitrate or nitrites to nitrogen gas (Timmons & Ebeling 2010); however, in aquaponics systems plants absorb a big amount of nitrates. Plants assimilate most of the absorbed nitrate in organic nitrogen compounds; the first stage of this process is the reduction in nitrate to nitrite in the cytoplasm. Because this is a potentially toxic and highly reactive ion immediately after generated, the nitrite is transported from the cytoplasm into chloroplasts in the leaves and in the roots plastids. In these organelles, nitrite reductase enzyme reduces nitrite to ammonium. At the same time, plant cells prevent ammonium toxicity incorporating it quickly in amino acids, by forming glutamine. Once nitrogen assimilated in glutamine this is incorporated into other amino acids by transamination reactions. Subsequently, the amino acids are used in protein synthesis (Sinha 2004) which results in growth to obtain biomass, thus showing that the nutrients are absorbed and utilized by plants.

In wetlands, essential nutrients such as nitrate, ammonium and phosphate are taken easily by the plants for growth and even accumulate them in tissues; it has been observed that the herbaceous uptake rate is higher than that in woody plants (Llagas & Guadalupe 2006). In traditional culture (open ground) were obtained fresh matter yields between 10 and 15 kg m^2 of basil (Muñoz 1987; Gill & Randhawa 1996), higher than the value reported in this work. Regarding peppermint, apparently this herbaceous growth is better in aquaponics tan in traditional culture systems (Campos-Pulido *et al.* 2013). However, basil production in such systems is considered high compared to that reported by Carrasco, Ramírez and Vogel (2007) using Nutrient Film Technique (NTF) hydroponic system. Bareño (2006) reports for open ground cultivation a production of 2000 g m^2 per year (WM) for spearmint, lower than the results observed in our data, and according to the literature, the present study seems to be the first report of production for this species in aquaponics.

Physicochemical water parameters, especially pH, temperature and DO fluctuations play an important and decisive role in the fish health in aquaponics systems, as they can affect the oxidation of NH_4 and NO_2, besides affecting some fish and bacteria metabolic processes (Chen, Ling & Blancheton 2006; Timmons & Ebeling 2010). Regarding pH, temperature and dissolved oxygen observed in the pond during this study, these parameters were at appropriate levels for tilapia culture (Fragoso-Cervón & Auró de Ocampo 2006; Saavedra-Martínez 2006; Timmons & Ebeling 2010; Diario Oficial de la Federación (DOF) 2011).

DO levels in the water have a direct impact on the fish welfare, nitrogenous compounds oxidation processes bearing out by nitrifying bacteria (Timmons & Ebeling 2010), and also in plants, considering that in aquaponics, usually the plants roots are kept flooded. Plants like animals require oxygen to carry out various metabolic processes. When plants are located in flooded soils, there can reach quickly a lack of oxygen in the roots, affecting the cultivation growth and performance (Kozlowski 1984; van Patten 2002). Note also that it is important that the engineering design and ensure the most oxygen in the water returning to the fish, especially when basil is used as part of the biological filter because of its greater demand for this element.

Conclusions

The results of this research showed that for tilapia culture, the three evaluated herbaceous can be used as part of the biological filters in aquaponics systems, because it removes significant concentration in nitrogen compounds and phosphates. There is a positive relationship between time and NH_4 concentration and therefore NO_3^- in the system. The pH, DO and temperature parameters were maintained in appropriate ranges for tilapia. The EC and TDS were present in suitable levels for growing herbaceous, which are adapted to flooded substrates, with constantly moving water and high oxygen concentration. A key parameter to consider is the oxygen concentration in water when herbaceous is used in aquaponics systems, due to the high input of this element these species need, specifically basil.

Acknowledgment

This experiment was part of Espinosa Moya Master's Thesis in Biosciences, held in facilities Life Sciences Division, Universidad de Guanajuato, Campus Irapuato-Salamanca, with funding from the Research Support and Graduate Office. To Dr Juan José Peña Cabriales, CINVESTAV Irapuato, Guanajuato, for the N and P determination analyses. We also thank the support received from JAPAMI to provide potable water for the ponds.

References

Adler P.R., Harper J.K., Wade E.M., Takeda F. & Summerfelt S.T. (2000) Economic analysis of an aquaponic system for the integrated production of rainbow trout and plants. *International Journal of Recirculating Aquaculture* **1**, 10–13.

Amirkolaie A.K. (2011) Reduction in the environmental impact of waste discharged by fish farms through feed and feeding. *Reviews in Aquaculture* **3**, 19–26. Department of Fisheries, Faculty of Animal Sciences and Fisheries, Sari Agricultural and Natural Resources University, Sari, Iran.

AOAC (1997) *Officials Methods of Analysis of AOAC International*. Fertilizers 16th edn. 3rd revision. pp.5. AOAC International, Gaithersburg, MD, USA. Vol 1, chapter 2, method 957.02.

ASA (2009) *Alliance for Sustainable Aquaculture and Food and Water Watch*. Land-Based Recirculating Aquaculture Systems: a more sustainable approach to aquaculture. pp. 9. Available at http://www.recirculatingfarms.org/downloads/RAS.pdf

Barak Y. & Van Rijn J. (2000) Biological phosphate removal in a prototype recirculating aquaculture treatment system. *Aquaculture Engineering* **22**, 121–136.

Bareño P. (2006). Hierbas aromáticas culinarias para exportación en fresco, manejo agronómico, producción y costos. In: *Últimas Tendencias en Hierbas Aromáticas Culinarias Para Exportación en Fresco. Curso de Extensión* (ed by Universidad Nacional de Colombia), pp. 65–72. Produmedios, Bogotá.

Boyd C.E., Tucker C., McNevin A., Bostick K. & Clay J. (2007) Indicators of resource use efficiency and environmental performance in fish and crustacean aquaculture. *Reviews in Fisheries Science* **15**, 327–360.

Brown T., LeMay E., Bursten B. & Burdge J. (2004) Apéndice A Operaciones matemáticas (pH). In: *Química: La Ciencia Central* 9th edn (ed by Pearson-Prentice Hall), pp. 1038. Pearson Educación de México, SA de CV, México.

Campos-Pulido R., Alonso-López A., Ávalos-de la Cruz D.A., Asiain-Hoyos A. & Reta-Mendiola J.L. (2013). Caracterización fisicoquímica de un efluente salobre de tilapia en acuaponía. *Revista Mexicana de Ciencias Agricolas* Pub. Esp. Núm. **5**, 939–950.

Carrasco G., Ramírez P. & Vogel H. (2007) Efecto de la conductividad eléctrica de la solución nutritiva sobre el rendimiento y contenido de aceite esencial en albahaca cultivada en NFT. *IDESIA* **25**, 59–62.

Chang S.K.C. (2010) Chapter 9. Protein analysis. In: *Food Analysis*, (4th edn) (ed. by S.S. Nielsen), pp. 135–138. Springer, USA.

Chapell J.A., Brown T.W. & Purcell T. (2008) A demostration of tilapia and tomato culture utilizing an energy efficient integrated system approach. 8th International Symposium on Tilapia in Aquaculture 2008. pp. 23–32.

Chen S., Ling J. & Blancheton J.P. (2006) Nitrification kinetics of biofilm as affected by water quality factors. *Aquaculture Engineering* **34**, 179–197.

Diario Oficial de la Federación (DOF) (1996) Norma Oficial Mexicana 001-ECOL-1996. Límites máximos permisibles para contaminantes básicos en descargas de aguas residuales en aguas y bienes nacionales Mexican regulations, DOF 1996. Available at http://www.ordenjuridico.gob.mx/Documentos/Federal/wo68981.pdf

Diario Oficial de la Federación (DOF) (2011) *Carta Nacional Acuícola*. México. pp. 39-40, 57-59. Mexican regulations, DOF 2011. Available at http://www.inapesca.gob.mx/portal/documentos/publicaciones/2011/SAGARPA_CNA.pdf

Dosdat A., Ruyet J.P., Covès D., Dutto G., Gasset E., Le Roux A. & Lemarié G. (2003) Effect of chronic exposure to ammonia on growth, food utilization and metabolism of the European sea bass (*Dicentrarchus labrax*). *Aquatic Living Resources, Paris* **16**, 509–520.

Fragoso-Cervón M. & Auró de Ocampo A. (2006) Capítulo 9. Zootecnia Acuícola. In: *Introducción a la Zootecnia* (ed. by O.M.E. Trujillo), pp. 358–359. Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de México (UNAM), México, DF.

Gill B. & Randhawa G. (1996) Effect of different transplanting dates and harvesting stages on the quality of French basil oil. *Journal of Herbs, Spices and Medicinal Plants* **4**, 35–42.

Gleick P.H. (1993) *Water in Crisis: A Guide to the World's Fresh Water Resources*. Oxford University Press, New York.

Hall A.G. (1999). A comparative analysis of three biofilter types treating wastewater produced in recirculating aquaculture systems. Thesis for Master of Science in Fisheries and wildlife sciences. pp. 1-3. Faculty of Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Ip Y.K., Chew S.F. & Randall D.J. (2001) Ammonia toxicity, tolerance, and excretion. In: *Fish Physiology*, vol. **20** (ed by P.A. Wright & P.M. Anderson), pp. 109–148. Academic Press, New York.

Kintzios S. & Makri O. (2007) *Ocimum sp.* (Basil): botany, cultivation, pharmaceutical properties, and biotechnology. *Journal of Herbs, Spices & Medicinal Plants* **13**, 123–150.

Kozlowski T.T. (1984). Extent, causes and impacts of floodingIn: *Flooding and Plant Growth*. (ed by TT Kozlowski), pp. 1–7. Academic Press, New York.

Lazzari R. & Baldisserotto B. (2008). Nitrogen and phosphorus waste in fish farming. *Boletim do Instituto de Pesca, São Paulo*, **34**, 591–600.

Llagas W. & Guadalupe E. (2006) Diseño de humedales artificiales para el tratamiento de aguas residuales en la UNMSM. *Revista del Instituto de Investigaciones FIGMMG* **5**, 85–96.

Madigan M., Martink J., Stahl D. & Clark D. (2012) *Biology of Microorganisms* (13th edn). Pearson Education, California, USA.

Martínez R., Gallardo P., Pech K., Navarro J., Sánchez A., Caamal-Monsreal C. & Rosas C. (2014) Growth, survival and physiological condition of *Octopus maya* when fed a successful formulated diet. *Aquaculture* **426–427**, 310–317.

Mateus J. (2009). *Acuaponía: Hidroponía y Acuacultura, Sistema Integrado de Producción de Alimentos*. Centro de Investigación Tibaitatá-CORPOICA, Colombia. Red Hidroponía, Boletín No. 44. Lima, Perú. pp. 7-10.

Muñoz F. (1987) *Plantas Medicinales y Aromáticas*. pp. 85-87. Ediciones Mundi-Prensa, Madrid, España.

Navarro I. & Gutiérrez J. (1993) Respiración y excreción. In: *Acuicultura Marina: Fundamentos Biológicos y Tecnología de la Producción* (ed by O.F. Castelló), pp. 309. Universidad de Barcelona, España.

Nelson R.L. (2004) Tilapia. Fast growing, hardy and tasty. *Aquaponics Journal* **35**, 16–17.

van Patten G. (2002) Soilless gardening. Hydroponics for the rest of us. In: *The Best of The Growing Edge* (ed by D. Parker), pp. 2. New Moon Publishing, Inc, USA.

Rabalais N.N. (2002) Nitrogen in aquatic ecosystems. *Ambio* **31**, 102–112.

Rains J. (2007) Plants that grow well in aquaponics. *Backyard Aquaponics Magazine* **1**, 20.

Rakocy J., Shultz R.C., Bailey D.S. & Thoman E.S. (2004). Aquaponic production of tilapia and basil: comparing a batch and staggered cropping system. ISHS Acta Horticulturae 648: South Pacific Soilless Culture Conference – SPSCC.

Rakocy J.E., Masser M.P. & Losordo T.M. (2006). *Recirculating Aquaculture Tank Production Systems: Aquaponics-Integrating Fish and Plant Culture*. SRAC Publication No. 454. Southern Regional Aquaculture Center. Texas A & M University, Texas, USA.

Ramírez D., Sabogal D., Jiménez P. & Hurtado-Giraldo H. (2008) La acuaponía: una alternativa orientada al desarrollo sostenible. *Revista Facultad de Ciencias Básicas* **4**, 32–51.

Ramírez-Sánchez L.M., Pérez-Trujillo M.M., Jiménez P., Hurtado-Giraldo H. & Gómez-Ramírez E. (2011) Evaluación preliminar de sistemas acuapónicos e hidropónicos en cama flotante para el cultivo de orégano (*Origanum vulgare*: Lamiaceae). *Revista Facultad de Ciencias Básicas* **7**, 242–259.

Regalado A.J.R. (2013) Diseño y evaluación de un Sistema acuapónico para la producción de animales acuáticos y plantas para consumo humano. Tesis, Maestría en Ciencias del Agua. Universidad de Guanajuato, México.

Rodehutscord M. & Pfeffer E. (1995) Effects of supplemental microbial phytase on phosphorus digestibility and utilization in rainbow trout (*Oncorhynchus mykiss*). *Water Science Technology* **31**, 143–147.

Ronzón-Ortega M., Hernández-Vergara M.P. & Pérez-Rostro C.I. (2012) Producción hidropónica y acuapónica de albahaca (*Ocimum basilicum*) y langostino malayo (*Macrobrachium rosenbergii*). *Tropical and Subtropical Agroecosystems* **15**, S63–S71.

Rosas A. (2002) *Granja Integral Dimensional*. Rojas Eberhard Editores, Bogotá, Colombia.

Saavedra-Martínez M.A. (2006) *Manejo del Cultivo de Tilapia*. Facultad de Ciencia, Tecnología y Ambiente, Universidad Centroamericana, Managua, Nicaragua. [En línea, consulta: 2014]. http://pdf.usaid.gov/pdf_docs/PNADK649.pdf

Sales J., Britz P.J. & Viljoen J. (2003) Dietary phosphorus leaching and apparent phosphorus digestibility from different inorganic phosphorus sources for South African abalone (*Haliotis midae* L.). *Aquaculture Nutrition* **9**, 169–174.

Sinha R.K. (2004). *Modern Plant Physiology*, pp. 290–309. Alpha Science International, India.

Smith V.H. (2003) Eutrophication of frehwater and coastal marine ecosystems. A global problem. *Environmental Science and Pollution Research* **105**, 126–139.

Stumm W. & Morgan J.J. (1996) *Aquatic Chemistry* (3rd edn). John Wiley and Sons, New York.

Tian X.L., Li D., Dong S., Yan X., Qi Z., Liu G. & Lu J. (2001) An experimental study on closed-polyculture of penaeid shrimp with tilapia and constricted tagelus. *Aquaculture* **202**, 57–71.

Timmons M.B. & Ebeling J.M. (2010). *Recirculating Aquaculture* (2nd edn). Editorial Cayuga Aqua Ventures, USA. pp. 53-55, 251, 294-295.

Trang N.T.D. & Brix H. (2012) Use of planted biofilters in integrated recirculating aquaculture-hydroponic systems in the Mekong Delta. Vietnam. *Aquaculture Research* **45**, 1–10.

Wahap N., Estim A., Seok-Kiang A.Y., Senoo S. & Mustafa S. (2010) Producing organic fish and mint in an aquaponic system. A model of green technology in action. *Aquaponics Journal* **58**, 28–33.

Wetzel R.G. (2001) *Limnology* (3rd edn). Academic Press, New York, USA.

Zhang S.-Y., Li G., Wu H.-B., Liu X.-G., Yao Y.-H., Tao L. & Liu H. (2011) An integrated recirculating aquaculture system (RAS) for land-based fish farming: the effects on water quality and fish production. *Aquacultural Engineering* **45**, 93–102.