

Journal of Aquaculture Engineering and Fisheries Research

E-ISSN 2149-0236

ORIGINAL ARTICLE/ORIJİNAL ÇALIŞMA

FULL PAPER

TAM MAKALE

THE INTEGRATION OF FISH AND PLANT PRODUCTION: NILE TILAPIA (*Oreochromis niloticus*) AND BASIL (*Ocimum basilicum*) CULTURE IN RECIRCULATING AND AQUAPONIC SYSTEMS

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Received: 27.09.2016

Accepted: 29.11.2016

Published online: 29.12.2016

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E-mail: muratvigit@comu.edu.tr**Abstract:**

In the present study, tilapia and basil production was performed in an aquaponic system developed with the integration of fish and plant production in the same culture environment. The variation of elements in the water was monitored and their effects on fish growth performance and feed utilization together with the plant growth were recorded. Triplicate groups of fish tanks were used in two different culture systems (recirculating-RS and aquaponic system-AS). The RS consisted of a water filtration unit whereas the AS was set with the incorporation of basil (*Ocimum basilicum*) production with the roots in water instead of soil, absorbing the discharge water from fish tanks through their roots. Nile tilapia (*Oreochromis niloticus*) with an initial mean weight of 5.65 g were introduced into both RS and AS systems and fed a commercial diet (50% protein) for a period of 75 days. At the same time basil was set into the AS with roots in water, a photoperiod of 12:12 dark:light regime was applied for the monitoring of plant growth. Water physico-chemical parameters were recorded in both culture environments throughout the study period. Based on the

findings in both culture systems, at the end of the experiment, it was found that the variations of ammonium (NH₄), nitrite (NO₂) and nitrate (NO₃) recorded in the AS were lower than those measured in the RS culture unit.

Keywords: Aquaponic system, Recirculating system, Sustainable aquaculture, Soil-free agriculture

Introduction

The world population is in a rapid growth period with a total of about 7.4 billion people (Worldometer, 2016). According to the estimations of FAO, the world population over the next 34 years is expected to reach about 9.6 billion by 2050 (FAO, 2012). The daily birth of around 265000 a day (Worldometer, 2016) links to a challenge in food supply forcing the global food industry for increase their capacity with new investments. However, considering the limited resources in terms of soil and water suitable for food production is another important problematic issue. Eventhough, rich marine and inland water resources in Europe with its increasing aquaculture industry seems to be capable to meet the increasing demand for food with high quality protein from aquaculture (Yigit et al., 2016), freshwater resources are also vital for drink water. Hence it is an important approach to use water in a rational way with new technology or production systems that minimize the use of water for food production. Aquaponics is a new approach for fish farming with the integration of vegetable production. This new technique can utilize the outflow water from the fish farm in a hydroponic system with the production of vegetables, herbs or flowers. Aquaponics is a culture system with the integration of fish and plant production in a closed recirculating system. Practically, nutrient elements, excreted by the fish or supplied by the microbial breakdown of organic waste materials are directly absorbed by plants produced in the same system without the use of soil. In the fish farming environment, almost all required nutrients for plant growth can be provided by the post-prandial waste material excreted by the fish into the water environment. Since the aquaculture effluent flows through the hydroponic structure of the recirculating system, waste metabolites from fish are removed by nitrification and then absorbed by the plants, acting as natural biological filtration in the system that afterwards flows back for reuse in the fish culture ponds. In a traditional fish farm, the nutrients provided by fish feeding are normally discharged into the water environment that contributes to water pollution in a long term acumulaiton effect. Recirculating aquaculture systems (RAS) are minimizing this effluent effect with the reuse of water flowing through a biofiltration unit. However, with the integration of plant production, a separate biofilter is not necessary and the removal of nutrients by plants

may prolong the use of water with a minimized discharge to the environment. The other advantage of aquaponics to the hydroponic system is that it requires less monitoring for water quality in both fish and hydroponic plant production units. In general, a cost effective production is generated due to the elimination of nutrient cost for plants production, and the elimination of separate biofilter units, less water requirements, and the share of operation costs in the entire system. Basil is a fast growing herb with a high economic value, is widely being produced commercially and suitable for aquaponic systems (Rackocy and Hargreaves, 1993). Besides, basil is accepted as a medicinal herb with various health benefits, such as reduction of inflammation and swelling, anti-aging properties, effective antioxidant activities (Nordqvist, 2016). The present study describes the integration of fish and plant production, where Nile tilapia (*Oreochromis niloticus*) and basil (*Ocimum basilicum*) production was performed in recirculating and aquaponic systems. This research focused on the suitability of the new integrated production system for fish farmers, with the aim comparing fish growth performance and plant productivity in aquaponic and in traditional recirculating aquaculture systems.

Materials and Methods

The present study was conducted in the laboratories of Tokyo University of Marine Science and Technology, Faculty of Marine Science, Department of Marine Biosciences in Tokyo – Japan. Two different culture systems were designed and deployed for the experimentations. One was prepared as a traditional recirculation aquaculture system (RS) with water filtration, and the other was designed as an aquaponics system (AS) using plant roots as a biofiltration of the water effluent from fish culture tanks. Triplicate groups of fish tanks were stocked with Nile tilapia (*Oreochromis niloticus*) (initial mean weight: 5.65 g) in both culture environments (RS and AS). For the plant production in the AS, basil (*Ocimum basilicum*) absorbing the discharge from fish tanks through their roots was used. Commercial diet with a protein content of 50% was fed to the experimental fish for a period of 75 days.

System Design and Operation

The experimental setup used in the present study has been given in Figure 1, which consisted of (a)

recirculating aquaculture system (RS) set with fish tanks and filtration equipments, and (b) aquaponic system (AS) set with fish tanks and the plant production units serving as bio-filtration. A factorial design of 2 x 3, with 2 groups and triplicate tanks of 30 L volume (44 x 28 x 26 cm) (6 tanks in total) were used in the experiment. Group 1 (RS) consisted of triplicate groups of fish tanks made of glass aquariums with plastic bottom layers and was designed to have a filtration unit (Figure 1), whereas Group 2 (AS) consisted of triplicate groups of plastic containers of 26 L volume (59 x 37 x 12 cm) and used a plant production unit serving as the filtration system (Figure 2). Different then the RS, the outflow water from the fish culture tank in the AS system was linked to the plant culture tanks settled above the fish tanks for the bio-filtration of the waste water, which then was directed back to the fish tanks for re-use. A water pump (EHEIM; 100 V, 50/60 Hz, 5/6 Watt) was used for water circulation and the photoperiod was arranged using fluorescent light sources. Aeration was maintained using an aerator SLL-40 (40 L/min, 11,8 kPa, 100 V, 50/60 Hz, 30/35 W) and air stones set in to the experimental tanks. Water temperature was controlled using an aquarium heater (100 V, 50/60 Hz, 100~300 W) and set to 23 °C. In order to ensure an effective plant growth, constant and suitable environmental conditions such as room temperature and humidity were controlled with an air conditioner.

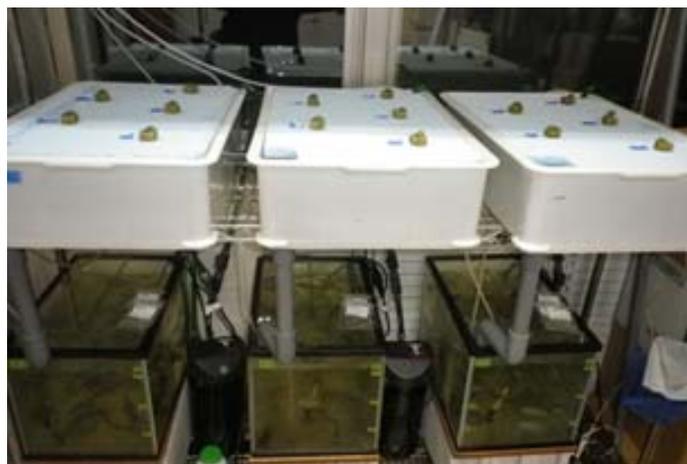


Figure 2. Aquaponic system setup used in the experiment

Basil seeds were placed into stone wool peaces with 3 seed planting in each hole (Figure 3) and left for germination of 10 days' period. After having reached an average weight of 20 g, plants were set into the culture environment, consisting of styrofoam layers (37 x 59 cm) set into plastic containers where the roots of the plants were met with water. The styrofoam layer were drilled with holes each within a distance of 20 cm, ensuring proper plant growth in the system. Five plants were inserted into each tank of the triplicate groups of plastic water containers through the styrofoam layers on the water surface (3 tanks x 5 plants with a total number of 15 basil roots in the AS system) (Figure 4).



Figure 1. Experimental setup and design of the recirculating system

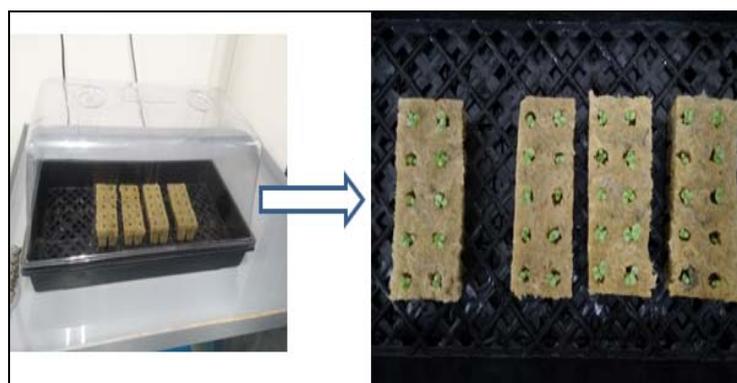


Figure 3. Basil (*O. basilicum*) germination

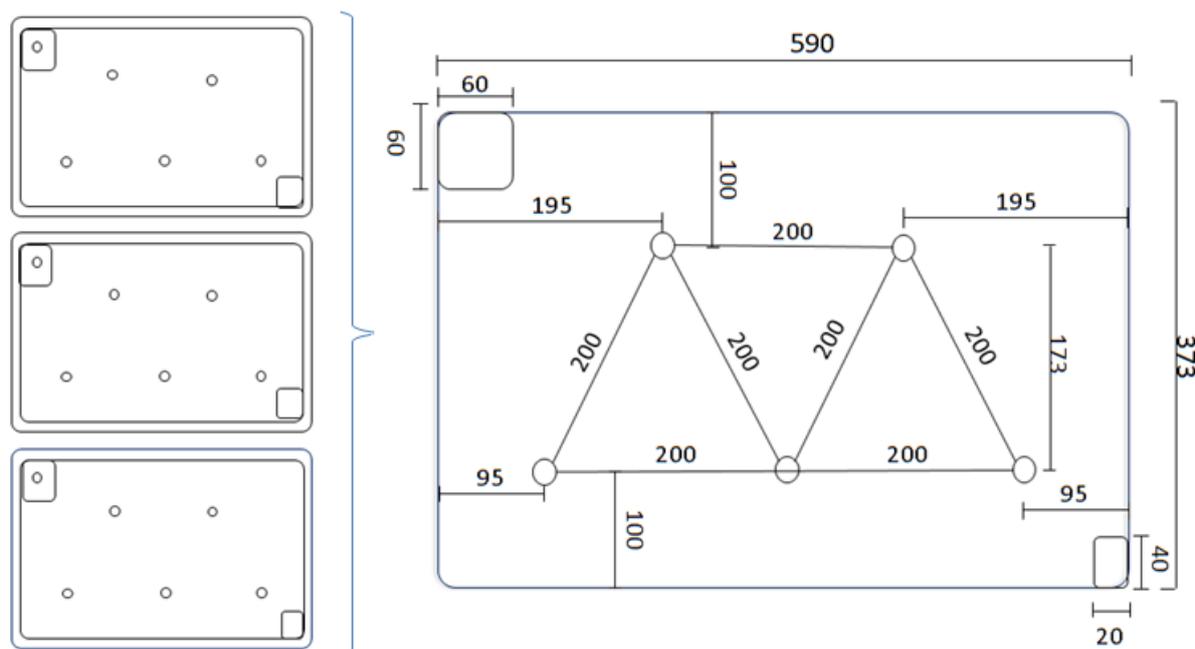


Figure 4. Planting design on styrofoam layers set on plastic water containers

After preparation of the plant production unit, Nile tilapia (*O. niloticus*) with initial mean weights of 5.0-6.0 g were stocked into the glass aquariums located below the plant growing tanks (Figure 2). Prior to fish stocking, all experimental fish were deprived from feed for 3 days. With the beginning of the feeding trial, all experimental fish were fed a commercial diet with 50% protein at 80 % of the biomass level. A photoperiod regime of 12:12 dark:light was controlled with an automatic timer and measured using a LI-COR (LI-1400) data logger. The humidity in the experimental area was measured by EXTECH Humidity and Temperature Recorder (RH-520).

Water Quality and Analyses

During the course of the 75-day experimental period, pH, oxygen and water temperature were measured daily using multi-probe water analyser. Furthermore, water samples (initial and final) were taken from both culture systems (RS and AS) for measuring ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), phosphate (PO_4), color and turbidity tests. Ammonia, nitrite, nitrate, and phosphate were measured by the phenol-hypochlorite method of Strickland and Parsons (1977).

Color and turbidity in the fish tanks and the plant production containers were measured using a UV-Spectrophotometer (SHIMADZU UV-1800, JAPAN). For the color tests, water samples from both fish tanks and plant containers were filtered

through Whatman GF/A filters prior to the spectrophotometer readings, whereas water samples for the turbidity were directly read in the spectrophotometer without any treatment. Water temperature and humidity were controlled and daily measurements were performed at four intervals (10:00, 13:00, 16:00, 18:00 hour) using a multi-probe water analyzer.

Light intensity was measured using a LI-COR (LI-1400) data logger, and the humidity in the experimental area was measured by EXTECH Humidity and Temperature Recorder (RH-520). Light intensity for the plants was measured at 5 different area of the AS system and at the daylight area.

Fish Growth Performance and Calculations

Growth performance of experimental fish and feed utilization have been calculated using the following equations as described by Yiğit and Yiğit (2003), Yigit et al. (2006, 2010), Bulut et al. (2014a,b), Kesbic et al. (2016a,b):

$$\text{RGR (relative growth rate, \%)} = (W_2 - W_1 / W_1) \times 100$$

$$\text{SGR (specific growth rate, \% growth per day)} = ((\ln W_2 - \ln W_1) / (t_2 - t_1)) \times 100$$

$$\text{FCR (feed conversion rate)} = \text{FC (g)} / \text{WG (g)}$$

where; W2: final weight, W1: initial weight, t2-t1: days in total, FC: feed consumption, WG: weight gain.

Results and Discussion

In the present study, survival was 100 % for all experimental treatments, showing that the culture system had no effect on fish survival. Growth performance of Nile tilapia was satisfactory in both culture systems (RS and AS). There were no significant differences ($p > 0.05$) between the experimental groups in terms of wet weight gain (WWG), relative growth rate (RGR), specific growth rate (SGR) or feed conversion rate (FCR) (Table 1). Growth rates and feed utilization data recorded for Nile tilapia in the present study were comparable to those of previous reports (Fasakin et al., 1999; Cremer et al., 2002; Rakocy et al., 2004; Rakocy et al., 2006; Ogunji et al., 2008; Yıldırım et al., 2009; Chowdhury, 2011; Antache et al., 2013; Madalla et al., 2013; Mensah et al., 2013; Ferdous et al., 2014; Githukia et al., 2015; Kaya and Bilgüven, 2015; Day et al., 2016).

Variations of ammonium (NH_4 ; 0.14-2.21 mg/L), nitrite (NO_2 ; 0.09-0.28 mg/L), nitrate (NO_3 ; 3-

175 mg/L) and phosphate (PO_4 ; 2.25-40.1 mg/L) recorded in the aquaponic system (AS) were lower than those recorded in the recirculating system (RS; 0.08-0.39 mg/L, 0.05-0.21 mg/L, 11-106 mg/L, 0.41-22.7 mg/L, respectively) throughout the study period. With the incorporation of plant production in the fish culture system, water quality was increased that might have led to an improved growth performance of fish in the AS system compared to the RS, however the differences were not significant ($p > 0.05$) (Figure 5-8).

Overall the concentrations of NH_4 , NO_2 , NO_3 and PO_4 in the water of both culture environments (RS and AS) were recorded within safe limits (0-2.5 mg/L, 0.05 mg/L, 100-200 mg/L, 1-20 mg/L, respectively) stated by Bregnballe (2015) for aquaculture operations, except for phosphate in the RS culture environment, which increased two-fold of the preferable level (40.1 versus 20.0 mg/L). In the AS culture environment, however phosphate concentration (1.41-22.7 mg/L) remained lower than that of the RS, and did not increase over the acceptable limits reported as 1-20 mg/L by Bregnballe (2015) (Table 2).

Table 1. Growth performance and feed utilization of Nile tilapia in the experimental conditions of recirculating- and aquaponic systems.

	Recirculating System	Aquaponic System
Initial weight (g)	21.99 ± 0.55 ^a	22.16 ± 0.52 ^a
Final weight (g)	36.47 ± 1.36 ^a	36.82 ± 0.94 ^a
WWD (g)	14.48 ± 1.25 ^a	14.65 ± 0.43 ^a
RGR (%)	62.46 ± 2.08 ^a	66.10 ± 0.55 ^a
SGR (%/day)	1.80 ± 0.13 ^a	1.81 ± 0.01 ^a
FCR	1.31 ± 0.09 ^a	1.30 ± 0.03 ^a

WWG (wet weight gain, g) = (Wfinal – Winitial)

RGR (relative growth rate, %) = (Wfinal – Winitial / Winitial) x 100

SGR (specific growth rate, % growth/day) = ((lnWfinal - lnWinitial) / (total time in days)) x 100

FCR (feed conversion rate) = feed consumption (g) / weight gain (g)

Table 2. Acceptable limits for different physico-chemical water quality parameters in a recirculating system and data recorded in the present study.

Parameter	Formula	Unit	Normal condition RS (Bregnballe, 2015)	Present study	
				RS	AS
pH	-	-	6.5-7.5	5.56-7.09	5.72-7.28
Temperature	-	°C	Species specific	24-25	25
Oxygen	O_2	%	70-100	80	86
Ammonium	NH_4	mg/L	0-2.5 (pH influenced)	0.14-2.21	0.08-0.39
Ammonia	NH_3	mg/L	< 0.01 (pH influenced)	N/A	N/A
Nitrite	NO_2	mg/L	0-0.5	0.09-0.28	0.05-0.21
Nitrate	NO_3	mg/L	100-200	3-175	11-106
Phosphate	PO_4	mg/L	1-20	2.25-40.1	0.41-22.7

RS: Recirculating system, AS: Aquaponic system

The levels of pH in both experimental setups (RS and AS) were between 5.56 and 7.28, with an average rate of 6.3 and 6.5, respectively. The pH levels in the AS tanks were higher than the RS tanks throughout the study period (Figure 9), but pH in both culture systems were within acceptable limits of 6.5-7.5 as described by Bregnballe (2015).

Dissolved oxygen level in both experimental set-up was recorded as 7.13 mg/L, initially, which than showed fluctuation throughout the study, and were recorded as 6.53 ± 0.29 mg/L (80 %) and 7.08 ± 0.14 mg/L (86 %) for the RS and AS culture environments, respectively and remained within acceptable limits (70-100 %) reported by Bregnballe (2015) for recirculating culture systems. The fluctuation of oxygen in the culture environments during the course of the study was possibly due to feeding and excretory end-

products from fish into the water environment. Similar to the pH values, dissolved oxygen and temperature levels were also higher in the AS tanks than those of the RS environment (Figure 10, 11).

In the present study, water color was more constant in the aquaponic system compared to the recirculating culture environment, where the change in water color increased to more than two-fold over the value measured in the aquaponic system (Figure 12). The change in water color might due to the accumulation of refractory organic compounds (e.g., tannic acid) as described by Rakocy et al. (2004). Water condition in the AS group was more clear compared to the RS group, which could be explained with a low level of suspended solids in the water environment of the AS culture environment.

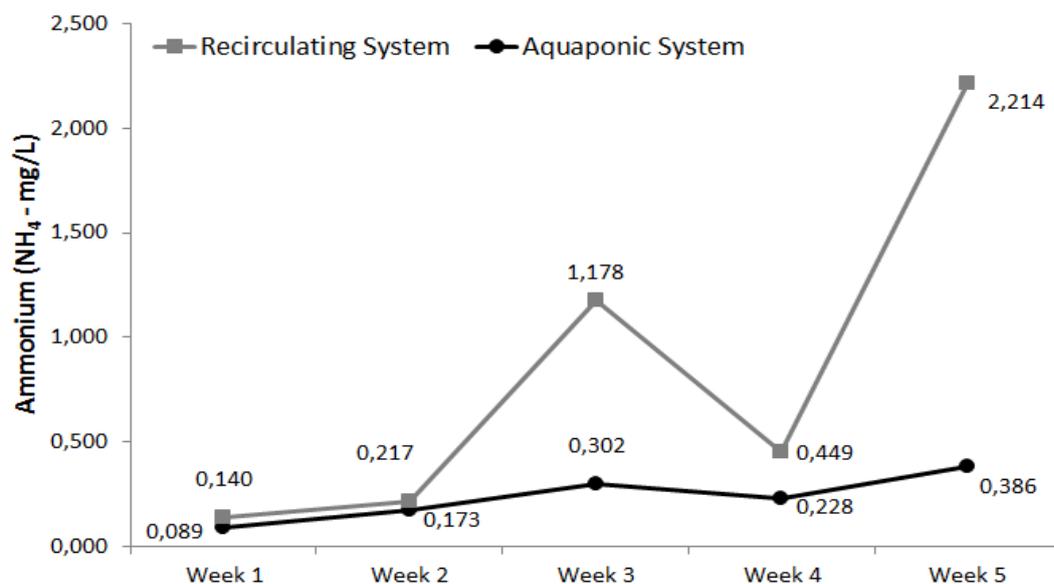


Figure 5. Weekly variations (06 January 2015 – 03 February 2016) of ammonium (NH_4) in the aquaponic system integrated with basil culture

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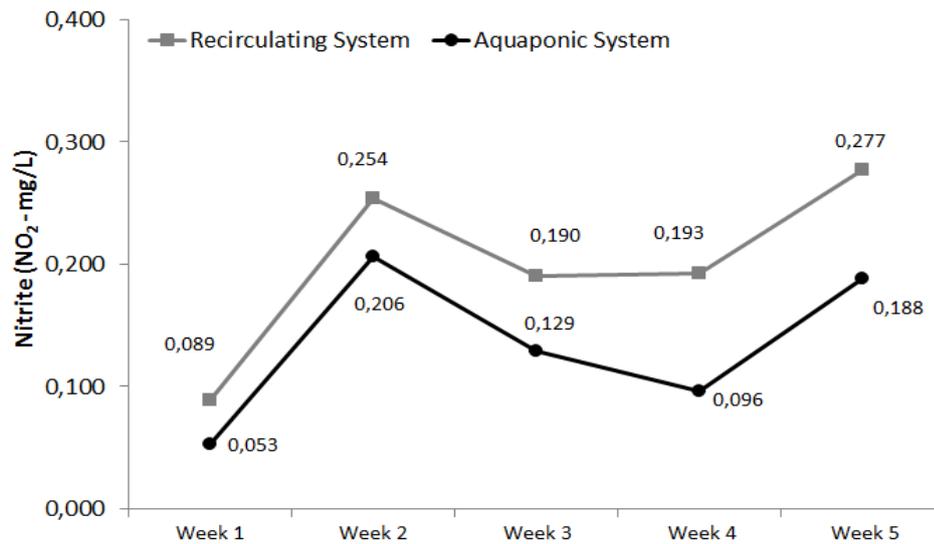


Figure 6. Weekly variations (January 2015 – February 2016) of nitrite (NO₂) in the aquaponic system integrated with basil culture

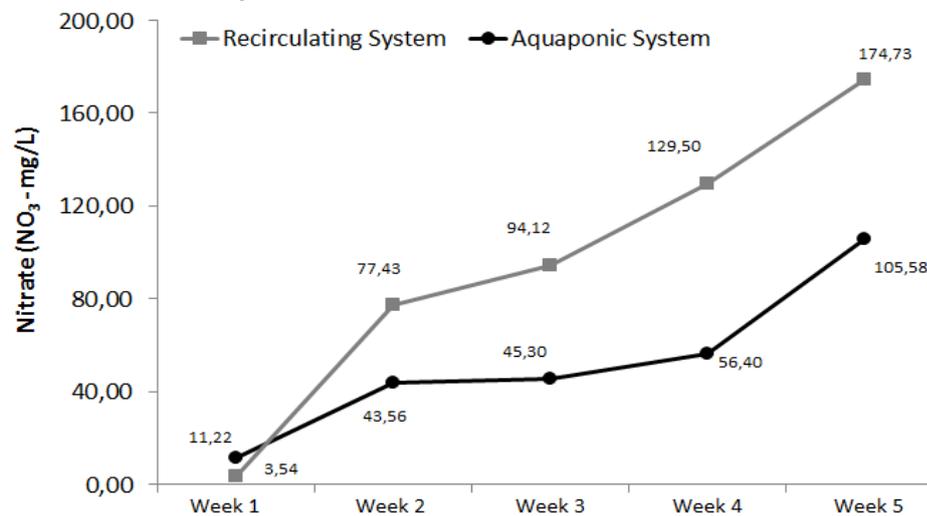


Figure 7. Weekly variations (January 2015 – February 2016) of nitrate (NO₃) in the aquaponic system integrated with basil culture

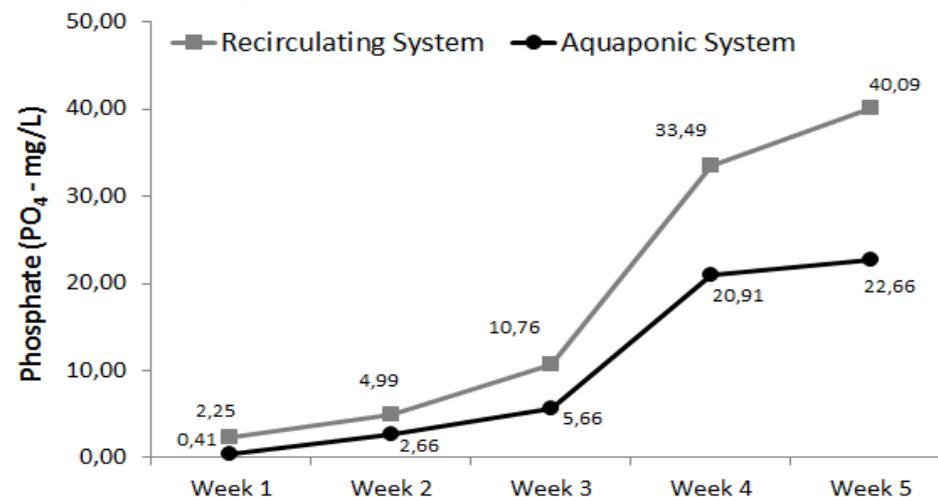


Figure 8. Weekly variations (January 2015 – February 2016) of phosphate (PO₄) in the aquaponic system integrated with basil culture

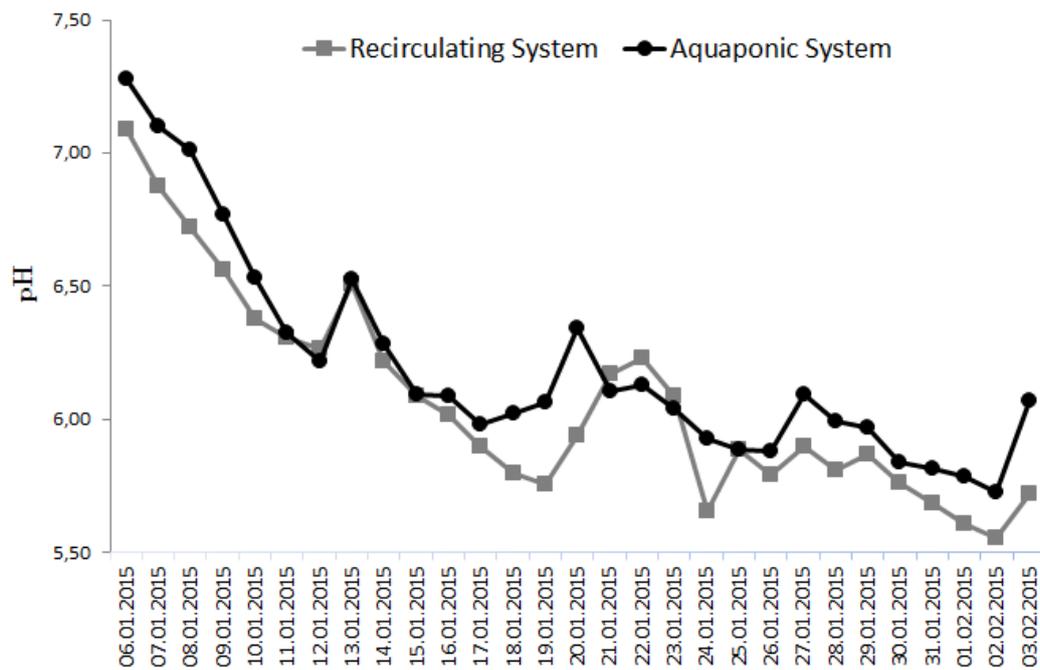


Figure 9. Daily measurement of pH levels in the experimental tanks

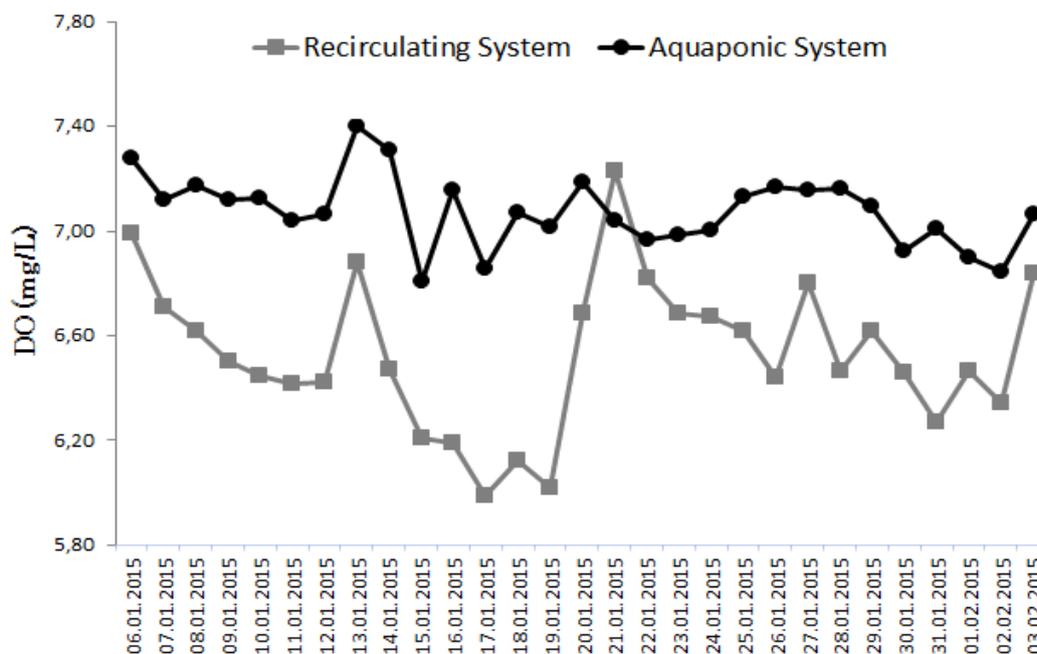


Figure 10. Dissolved oxygen (mg/L) levels in the experimental tanks

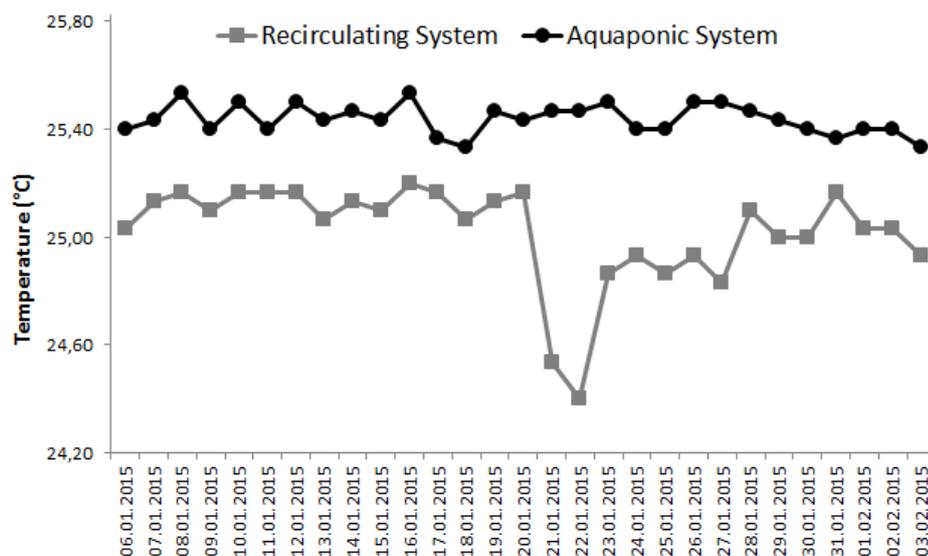
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Figure 11. Daily water temperature (°C) variations in the experimental tanks

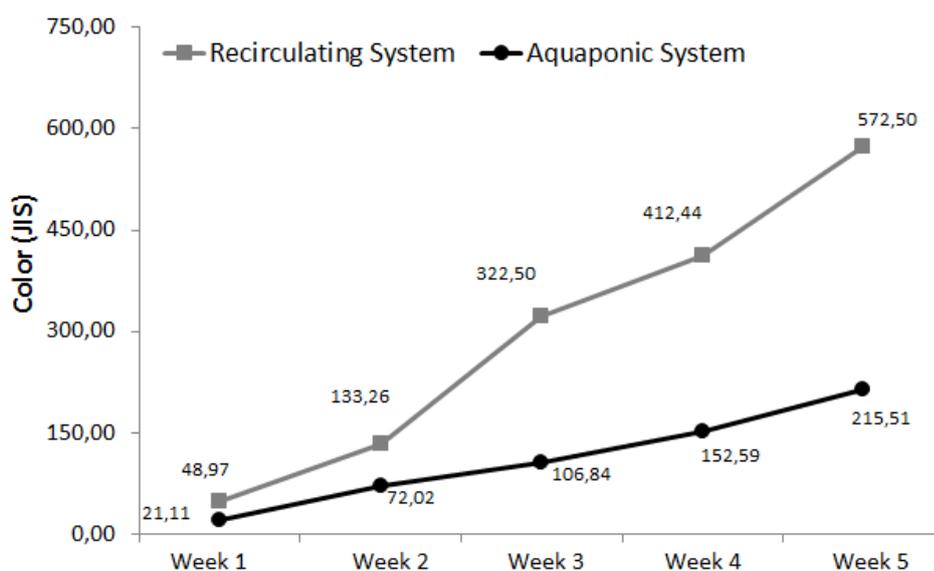


Figure 12. Weekly variations (January 2015 – February 2016) of water color in the aquaponic system integrated with basil culture

Turbidity, a measure of the strength of water clarity, may decrease the amount of light that can penetrate the water body, hence the rate of photosynthesis might be decreased. Turbidity in natural waters such as lakes and reservoirs can range between 1-20 mg/L (ANZECC, 2000), which can also be acceptable for carp or tilapia culture operations. In the present study, turbidity measurements in the aquaponic system were within the range of the values reported by ANZECC (2000) for lakes and reservoirs, while the turbidity in the

recirculating system increased to almost three-fold of the aquaponic culture environment (Figure 13). Higher turbidity in the RS group resulted in a lower dissolved oxygen concentration in the water, which might be attributed to a reduced photosynthetic activity due to a reduced light penetration. Similar results were reported by Rakocy et al. (2004), where dissolved oxygen levels in the rearing tanks decreased when water became turbid in an aquaponic system integrated with basil and tilapia culture.

Despite the room temperature of the experimental setup remained constant, outside ambient weather conditions affected temperature and humidity and variations in these parameters were observed at different time intervals during the course of this study. Negative correlation was observed between room temperature and humidity rate. The temperature values in the morning and noon hours were higher than those recorded in the afternoon and evening hours. Humidity however showed contrast results, compared to the temperature values, with lower humidity in the morning and noon hours, but higher rates of humidity in the afternoon and evening hours. It was also recorded that humidity rates in the experimental room environment lowered during rainy or cloudy days and vice versa (Figure 14 and 15).

Light intensity through penetration from outer environment (sun light) was visibly higher than those each plant was exposed to in the morning

and noon hours. However at afternoon and evening hours, light penetration from outer environment dropped and was equal to those measured in the plant growth area. Eventhough the light intensity in the experimental setup was controlled by fluorescent lights, sun light penetrated from outer environment fortified the light effect during the morning and noon hours, which were consequently higher than those measured in the afternoon and evening periods (Figure 16).

In the present experiment, basil with an initial mean weight of 20.54 ± 0.73 g reached a final marketable size of 131.02 ± 16.77 g with a weight gain of 110.48 ± 17.02 g after a 75 days growth period. Specific growth rate was recorded as 6.16 ± 0.47 %/day. Growth performance of basil in the aquaponic system during the course of the trial has been given in Table 3, and weekly harvest has been shown in Figure 17.

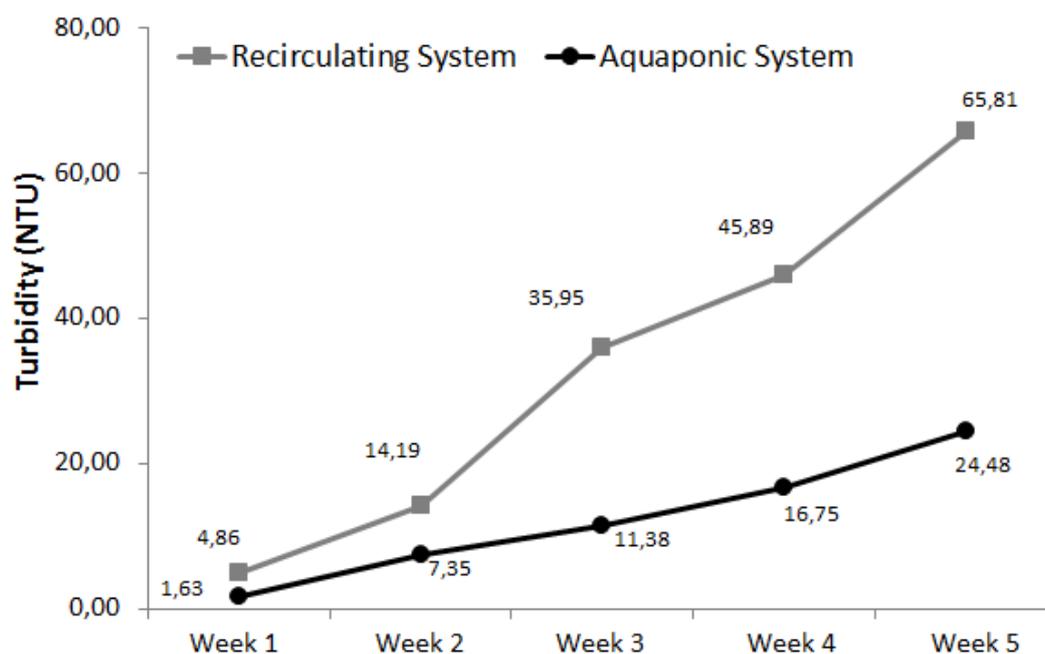


Figure 13. Weekly variations (January 2015 – February 2016) of water turbidity in the aquaponic system integrated with basil culture

Journal abbreviation: J Aquacult Eng Fish Res

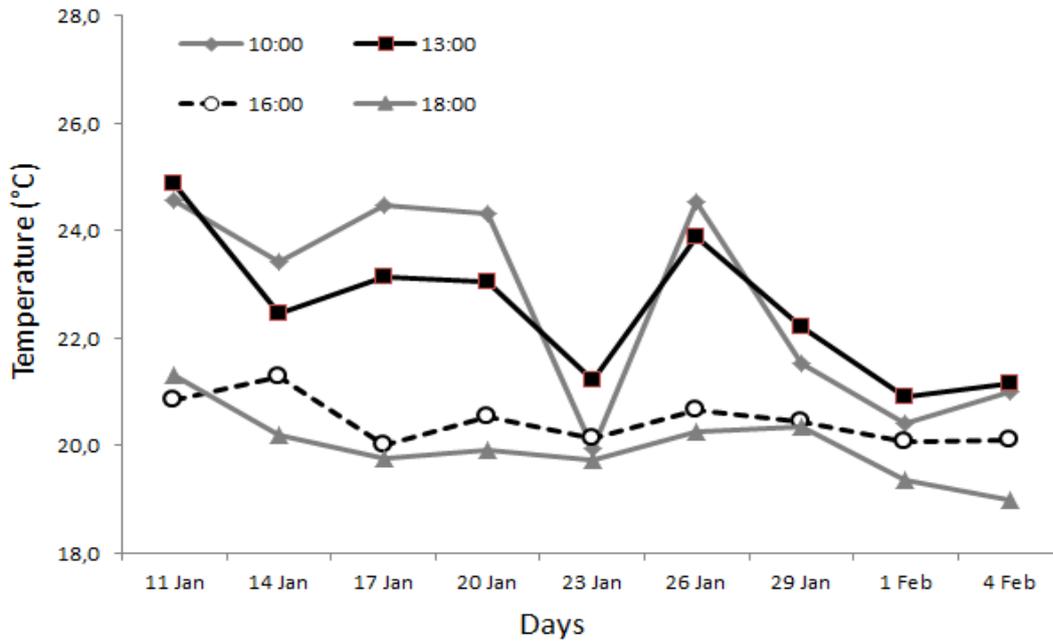


Figure 14. Tertian variations of room temperature in the experimental area at four time intervals during the trial

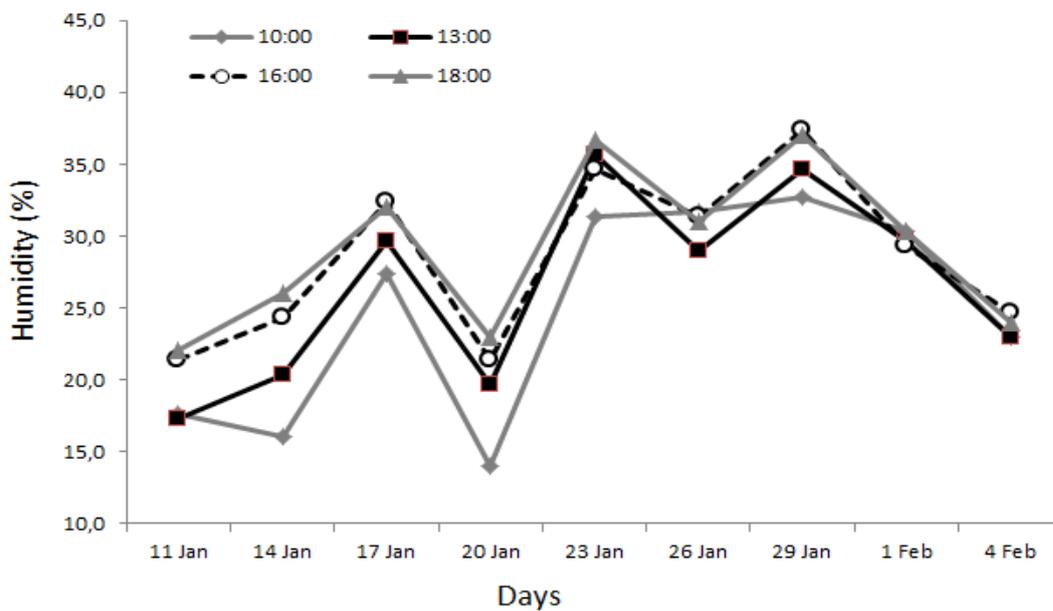


Figure 15. Tertian variations of room humidity in the experimental area at four time intervals during the trial

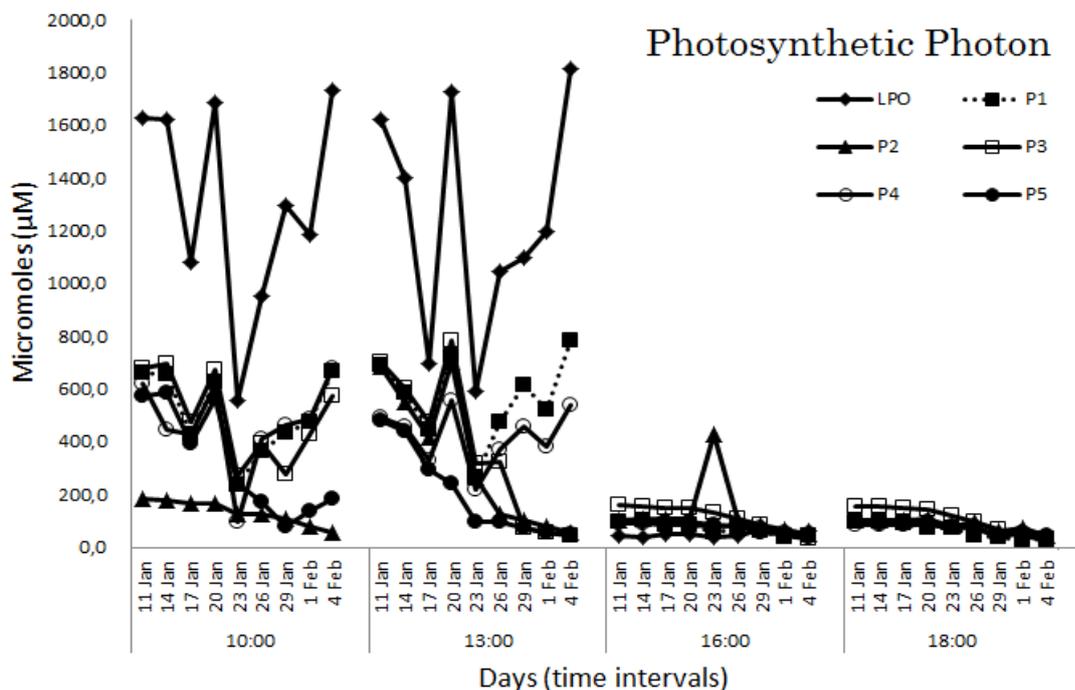


Figure 16. Tertiary variations of light intensities (Photosynthetic Photon) in the plant culture environment (LPO: light penetration from outside; P1-5: positioning of planted basil on syrofoam layers in the aquaponics system)

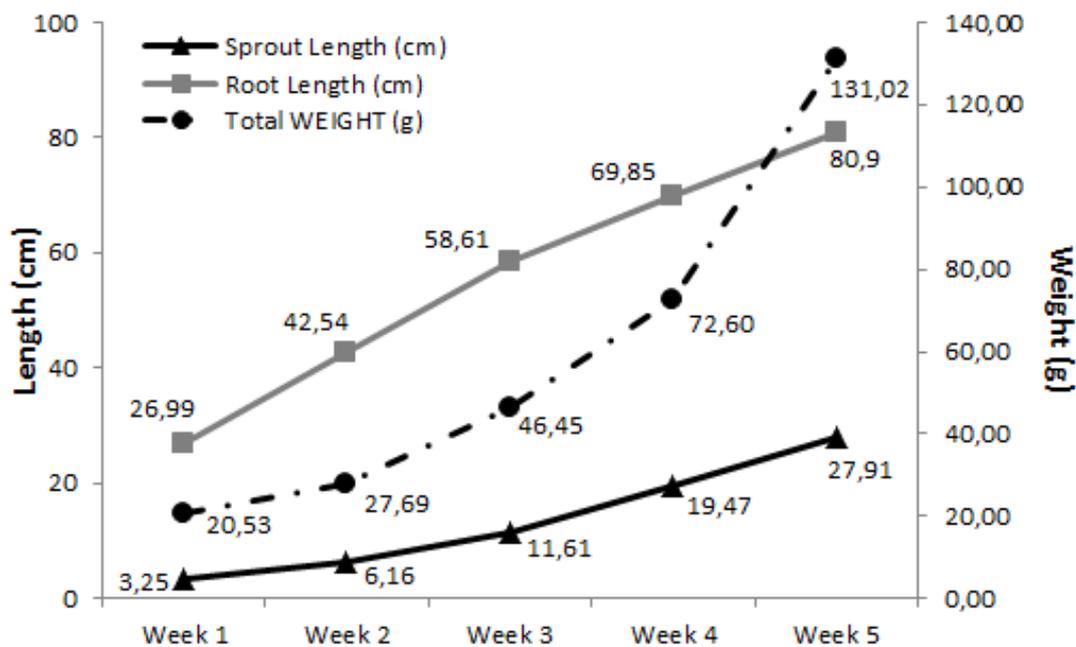


Figure 17. Increase of root and sprout length (cm) and harvest yield (g) of Basil (*O. basilicum*) (January 2015 – February 2016) in the aquaponic system integrated with tilapia culture

Basil harvest steadily increased during the trial and the end production per plant averaged 131 g with a yield of 5 kg/m³ (600 g/m²) (Table 3). Weight of basil doubled over the initial value 3 weeks after the start of the experiment, and at the final harvest basil showed a weight increase of more than 6 times over the initial value. Cumulative relative growth rate of basil increased from 34 % in the first week to 539 % at the end of 5-week experimental period (Table 3). Initially, basil showed a slow growth one week after the start of the trial and reached a mean weight gain of 28 g compared to the initial weight of 21 g. The first harvest gave a yield of 127 g/m² (28 g), while the yield almost doubled to 213 g/m², tripled to 330 g/m² and became 5 times higher over the first harvest at the second, third and last harvest, respectively.

Harvest results for basil in the present study are comparable with earlier reports. The harvest rate of 600 g/m² in the present study was lower than that reported by Rakocy et al. (2004) (1.8 kg/m²) for basil production (8 plants/m²) in an aquaponic system integrated with tilapia culture. Higher yield of basil was recorded at a rate of 6.25 kg/m² by Bradley and Marulanda (2001) in a hydroponic system. Our findings are in agreement with the yields of basil in field production (0.6 kg/m², mean weight of 104.4 g) that was reported by Rakocy et al. (2004). In the study of Bradley and Marulanda (2001), the number of basil planted into the production system was reported as 25 plants/m² which was more than the planting density of 23 plants/m² applied in the present study.

The discrepancies between the results of the present and earlier studies in terms of the harvest amount of basil in a square meter might be attributed to several factors, such as different diet composition used for fish feeding, protein level and digestibility of the diet, which may affect the diurnal pattern of ammonia excretion in fed fish, nutrient availability and amount of nutrients in the production system, culture conditions such as water quality, temperature fluctuations, length of growth period, or any combination of all these factors. However, from the results of the present study and those of earlier reports, it can be suggested that a higher planting density of basil might be applied in aquaponic culture systems.

The results in the present study shows the efficient use of water resources in an aquaponic system, in terms of the integration of plant production with tilapia culture that created a sustainable and eco-friendly food production system through the uptake of nutrients excreted postprandially into the water environment.

After the end of 5-weeks experimental period, there was no sign of nutrient deficiency as no chlorosis of the leaves was seen. Rakocy et al. (2004) reported nutrient deficiencies by the fourth harvest in a batch production system, due to a possible reduction of some nutrients as water passed through a long distance pipe between two sets of hydroponic tanks. Furthermore, the authors assumed that a batch production of basil might have exceeded the nutrient production capacity in their water system.

Table 3. Growth of Basil in the Aquaponic system integrated with Tilapia culture

	Aquaponic System
Initial weight (g)	20.53 ±0.73
Final weight (g)	131.02 ±16.76
Weight gain (g)	110.49 ±17.02
Specific growth rate (%/day)	6.16 ±0.46
Relative growth rate (%)	539.22 ±91.78
Final Harvest (kg/m ³)	5.002 ±0.64
Final Harvest (g/m ²)	600.18 ±0.08

SGR (specific growth rate, % growth/day) = ((lnW_{final} - lnW_{initial}) / (total time in days)) x 100

Conclusion

Results in the present study reveal lower variations of ammonium, nitrite and nitrate in the aquaponic system compared to the recirculating fish culture system. Different from traditional flow-through fish culture facilities, aquaponic systems can operate with lower amount of water. Hence, the reuse of freshwater in the aquaculture facility may support less water usage for food production, but more for drink water supply. The results in the present study shows the efficient use of water resources in an aquaponic system, in terms of the integration of plant production with tilapia culture that created a sustainable and eco-friendly food production system through the uptake of nutrients excreted postprandially into the water environment.

Acknowledgement

The first author received financial support from Tokyo University of Marine Science and Technology (TUMSAT), Tokyo-JAPAN as a research student for one year. TUMSAT-JAPAN is gratefully acknowledged for the financial support and for the use of experimental facilities and laboratories throughout the study, which has been performed in partial fulfillment of requirements for the degree of Master of Science of the first author.

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