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Stocking density optimisation of pangasius (*Pangasianodon hypophthalmus*) fingerlings with basil (*Ocimum basilicum* L.) in nutrient film technique (NFT) based aquaponics

Neerudu Harika, Ajit Kumar Verma*, Kishore Kumar Krishnani, Chandrakant Mallikarjun Hittinahalli, Tincy Varghese, Vidya Shree Bharti, Angom Lenin Singh and Aatira Farooq

ICAR-Central Institute of Fisheries Education, Off Yari Road, Versova, Andheri (W), Mumbai - 400 061, Maharashtra, India

Abstract

An experiment for 90 days was performed to optimise the stocking density of pangasius (*Pangasianodon hypophthalmus*) with basil (*Ocimum basilicum* L.) in a nutrient film technique (NFT) based aquaponics. The experiment had four treatments with different stocking densities of pangasius as T1 (2.5 kg m^{-3}), T2 (2.75 kg m^{-3}), T3 (3.0 kg m^{-3}) and T4 (3.25 kg m^{-3}) with a constant plant density of 24 plants m^{-2} and control *viz.,* C (2.5 kg m^{-3} without plants). Significant ($p<0.05$) difference in final body weight of fish was found among the treatments and control. The highest fish biomass was recorded in T3 (10.29±4.41 kg m^{-3}) followed by T4, T2, T1 and C. The highest plant yield was found in T4 (510.90±6.25 g); however, no significant difference was found between T4 and T3. No significant ($p>0.05$) difference in macro and micronutrient content of basil leaves was recorded in T4 and T3. The physiological parameters (hematological, serum biochemical and anti-oxidant stress enzymes) were found to be within the acceptable range. Considering the water quality parameters, fish growth, total biomass of fish, fish physiological responses, basil yield and nutrient content, the stocking density of 3.00 kg m^{-3} (pangasius) with 24 plants m^{-2} of basil could be recommended for basil-pangasius aquaponics.

*Correspondence e-mail:
akverma@cife.edu.in

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Introduction

The food demand has risen in tandem with global population growth; however, land and water resources are limited (Verma *et al.,* 2013; 2014). Furthermore, rapid urbanisation has led to a steady decline in the availability of fertile cultivable lands for conventional aquaculture and agriculture (Verma *et al.,* 2010; Saha *et al.,* 2016; Nuwansi *et al.,* 2020). To overcome these problems, aquaponics has been acknowledged as an eco-innovative, environmental-friendly, and resource-efficient close-loop technology for production of fish and plant under one roof (Thomas *et al.,* 2019; Meena *et al.,* 2022; Verma *et al.,* 2023). Aquaponics is combined culture of fish and plant (Seawright *et al.,* 1998; Angkha *et al.,* 2020) where wastewater from aquaculture is recycled and reused for plant growth in hydroponic system instead of getting discharged into nearby water bodies (Shete *et al.,* 2015; Thomas *et al.,* 2021).

The selection of fish and plant species is essential in aquaponics for higher returns. Pangasius (*Pangasianodon hypophthalmus*), a freshwater catfish commonly known as basa, sutchi catfish and iridescent shark; belongs to the Pangasidae family and contributes to 3% of the global production of finfish (FAO, 2018). In the traditional aquaculture systems, the culture of pangasius in higher stocking densities resulted in deterioration of water quality, biodiversity of that particular system and finally, led to the development of new diseases by spreading pathogens (Nageswari *et al.,* 2022). Hence, culturing pangasius in an aquaponic system is the best way to counter the above mentioned

issues by utilising aquaculture wastewater. Basil is a commercially important herb with medicinal properties (Ahmed and Masoud, 2014). Previous studies have suggested that basil is a high-value crop and its production is higher in aquaponic systems than in traditional agricultural and hydroponic systems (Rakocy *et al.,* 2004; Saha *et al.,* 2016; Patil *et al.,* 2019).

The most important aspect of an aquaponic system is determination of the optimal fish stocking density for increased productivity and profitability. The optimal stocking density determines numerous characteristics, such as growth, variation in size, level of comfort and survivability (Hussain *et al.,* 2014; Nuwansi *et al.,* 2021). The aquaponic system must be aptly sized to maintain a poise between nutrient production from fish culture and nutrient uptake by plants. Plant growth is hampered when nutrients from fish waste are in short supply; hence, it is preferable to maintain appropriate stocking densities that generate adequate nutrients for plant growth. Additionally, stocking density should not affect fish growth performance due to overcrowding (Buzby and Lin, 2014; Yildiz *et al.,* 2017). Studies conducted on stocking density optimisation strategies in aquaponics have focused mainly on tilapia with spinach (Hastuti and Subandiyono, 2020), koi carp with gotukola (Nuwansi *et al.,* 2021) and rohu with lemon grass (Mamatha *et al.,* 2020). Therefore, there is a need to undertake studies on utilisation of aquaculture wastewater nutrients for the optimum growth of pangasius and basil in aquaponic systems.

The present study aims to integrate pangasius with basil in a nutrient film technique (NFT) based aquaponic system to optimise the fish and plant component ratio so that optimum use of aquaculture wastewater nutrients could be achieved for better growth of pangasius and basil.

Materials and methods

Experiment set-up

The experiment was carried out for 90 days (November 2021 to February 2022) in the aquaponics unit of the Aquaculture Division, ICAR-Central Institute of Fisheries Education, Mumbai, India. The experimental set-up comprised 15 uniform and separate tanks of fish and NFT based hydroponic units (Fig. 1). The fish (pangasius) were stocked in rectangular fiber reinforced plastic (FRP) tanks (0.80×0.56×0.39 m) maintained with a water volume of 100 l throughout the experiment.

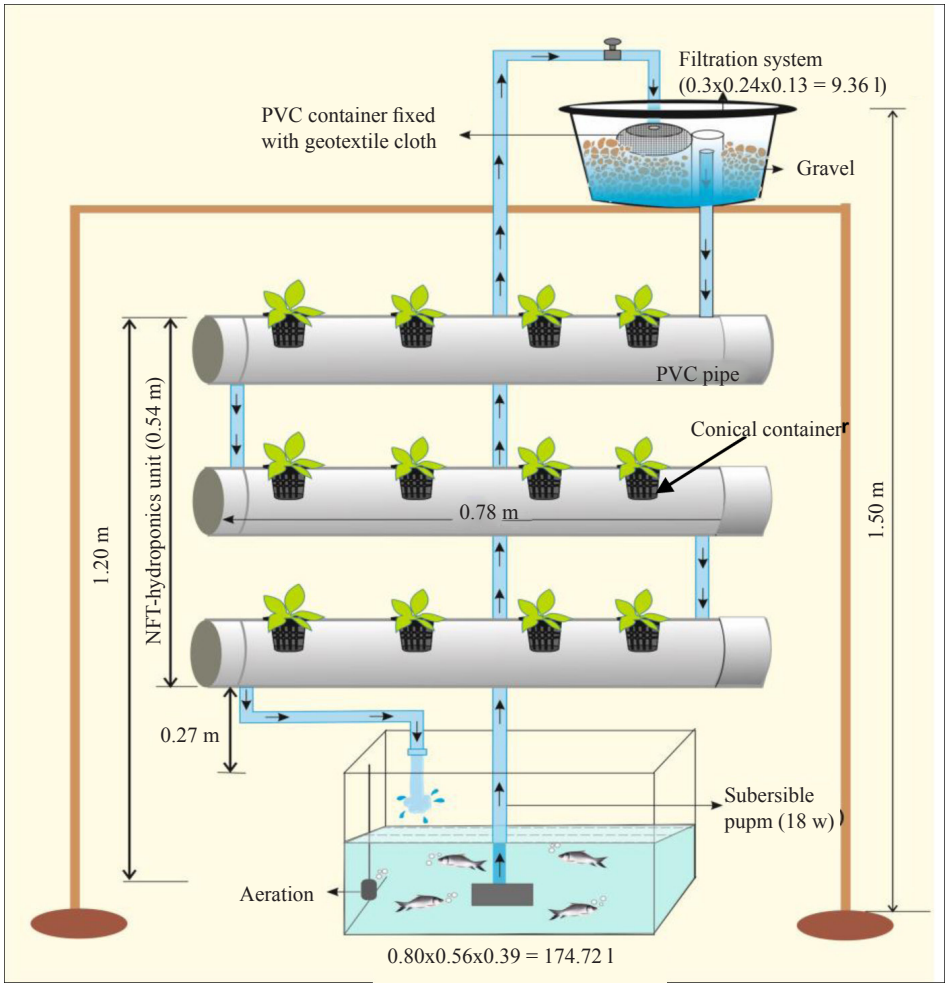

Fig.1. Schematic diagram of aquaponic system

For NFT based hydroponic unit, a polyvinyl chloride (PVC) pipe (4 inch dia) was installed with plastic conical net pots (3 inch deep) containing construction gravel (0.5-1.0 cm) which was used as a plant growing medium. The NFT hydroponic unit was strategically located above the fish tank, while the filtration system was constructed using a rectangular plastic container (measuring 0.30 x 0.24 x 0.13 m) filled with construction gravel ranging from 0.5 to 1 cm in size. A small plastic round bowl covered with geotextile cloth (0.3 x 0.3 = 0.9 m^2) was then placed inside the rectangular plastic container. The filtration unit was installed above the hydroponic unit. Each aquaponic system was maintained with a cumulative water volume of 121.51 l. A submersible pump (18 W, ELOVE, Hmax = 1.85/2.80 M) was installed in the fish culture tank for pumping water from the fish culture tank to the filtration system. From the filtration tank, the filtered water was routed *via* the NFT component before returning to the culture tank under gravity. Thus, a complete water recirculation system was established in the experimental set-up. An automatic timer (Hager, EH711 Nakoda Marketing, Chennai, India) was used to regulate the pumping frequency. A constant water flow rate of 7 l min^{-1} was maintained, for which the water was pumped for 3 min in every 10 min (*i.e.,* 18 min h^{-1}). Replenishing of water was done to compensate the evapotranspiration losses.

Plant and fish

Basil (*Ocimum basilicum* L.) seeds were procured from Go Green Nursery Pvt. Ltd. and sowed in a mixture of cocopeat (Kraft seeds Agro Ltd.) and vermicompost (Black gold, Kraft seeds Agro Ltd.). Following a growth period of 21 days, plantlets were transplanted into the aquaponic system. The pangasius fingerlings procured from Indepesca Aquaculture Pvt. Ltd. Maharastra, India, were acclimatised for 30 days. The fish were fed with Growel-Growfin floating feed containing 30% crude protein, 5% crude fat, 5.5% crude fiber, 11.5% moisture, macro-micro nutrients like, 5.67% nitrogen, 0.983% phosphate and 0.870% potash.

Experimental design and procedure

In this experiment, a completely randomised design was used with three replicates for each treatment. The experimental design consisted of four treatments and a control (C). Each treatment was assigned with different fish stocking densities *viz.,* T1, T2, T3 and T4 with 2.50, 2.75, 3.00 and 3.25 kg $m^{-3,}$ respectively and compared with control (aquaponics without plants having density 2.50 kg m^{-3}) (John *et al.,* 2022). Replenishing of water was done in all treatments and control to compensate the evapotranspiration losses in recirculating aquaponic system. The average length and weight of the pangasius was 9.46±0.03 cm and 6.39±0.09 g, while stocking and the average height of the basil plantlets during transplantation was 6.70±0.24 cm. At the experimental site; during the day time, natural light was available for 11 to 12 h throughout the experimental period. Following the installation of the system, the tanks were filled with water and a dry run (without fish) was performed for 15 days. Subsequently, fish were stocked in the culture tanks and a 30 day wet run was performed to acclimate the fish and colonisation of beneficial nitrifying bacteria to set up the nitrogen cycle. Basil plantlets (21 days old) were transferred into the troughs of the NFT hydroponic system. In each treatment, 12 basil plants (*i.e.* 24 plants m^2) were planted in the hydroponic system (Selek *et al.,* 2017). In all treatments and control, the fingerlings were fed at a fixed feeding rate of 3% of body weight divided into two doses twice a day at 09:30 hrs and 16:30 hrs. The parameters related to water quality, fish and plants were analysed fortnightly. Fish physiological parameters and plant macro-micro nutrient analysis were performed at the end of the experimental period.

Water quality

Water quality analysis was performed by collecting water from fish tanks. The water temperature was analysed with the help of a mercury thermometer and pH was measured with a Labindia digital pico+ pH meter. The dissolved oxygen (DO), alkalinity, total hardness, total ammoniacal nitrogen (TAN), nitrite-nitrogen (NO2-N), nitrate-nitrogen (NO_3-N) and phosphate were estimated following (APHA, 2017). The potassium content in the water was analysed by flame photometer (Microcontroller Flame Photometer, Labard 122 Instruchem Pvt. Ltd., LIM-204).

Growth of fish

In order to reduce the stress experienced by the fish during sampling, they were not provided any food for 24 h prior to the procedure. Further, they were anesthetised using clove oil. An electronic weighing balance (Sitrus digital balance, SF-400) was used to take the fish weight and length was recorded using a graduated ruler. Other important growth parameters like weight gain percentage (WGP), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), feed efficiency ratio (FER) and survival rate (%) were studied at the end of the experimental period by using equations given by Nuwansi *et al.* (2020) and Haridas *et al.* (2017).

Growth and nutrients of the plant

The height, length and width of the leaf of basil were recorded with a ruler (Ikis stainless steel ruler). A sample of 6 plants was chosen randomly from each replicate to determine plant growth. During the harvest (30, 60 and 90th day), an electronic weighing balance was used to weigh the yield of fresh basil leaves. Recording of mean stem fresh weight (SFW), mean root fresh weight (RFW), stem dry weight (SDW) and mean root dry weight (RDW) was done at the final harvest on the 90th day. Micro-macro nutrient analysis of basil leaves was performed at the end of the experimental period by following standard methods prescribed by Singh *et al.* (1999). Nitrogen content was estimated by using Kjeldahl method. Phosphorus and sulphur was estimated by spectrophotometric method. Potassium content was estimated by flame photometer. Calcium, magnesium and micronutrients like copper, iron and zinc were estimated using atomic absorption spectrophotometer (AAS). Method of John *et al.* (2022) was followed for drying and storing plant samples. Dry and wet weights of roots and stems were recorded at the end of the experimental period. The total chlorophyll content was estimated following Kamble *et al.* (2015).

Fish health

Hematological parameters and serum studies were conducted by collecting the blood samples as per Latha *et al.* (2020) and Nageswari *et al.* (2022), respectively. In contrast, the hematological parameters were analysed in a certified laboratory (Shruti Clinical Laboratory, Mumbai, India). Serum glucose (Coral Clinical Systems) and serum cortisol (Cayman Kit) were determined following the methods prescribed by the commercial diagnostics manufacturers. The procedure prescribed

by Misra and Fridovich (1972) was used for analysing catalase and superoxide dismutase (SOD).

Ethics statement

The animals used in this experiment were cared for in line with the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests (Animal Welfare Division), Government of India.

Statistical analysis

Data analysis was performed using a statistical analysis tool (*i.e.,* IBM SPSS statistics 22.0 version). All statistical analyses were performed on replicate tanks (N=3). One-way analysis of variance (ANOVA) was used to examine the results of water quality, plant nutrient analysis, chlorophyll estimation of plant leaves and the fish health parameters. Duncan's Multiple Range Test was used to assess the significance of differences between treatment means. Fish and plant growth data were analysed using repeated-measures ANOVA with Bonferroni's test. The data were presented as mean (±SE). Differences were deemed statistically significant at $p<0.05$ for all statistical methods.

Results and discussion

Water quality

The quality of water is the most critical environmental consideration to optimise production of the aquaponic system as it affects the growth of fish, feed efficiency, fish physiology and plant nutrition (Yildiz *et al.,* 2017). All the water quality parameters were found to be in the optimal range throughout the experimental period. No significant ($p>0.05$) difference was recorded between the treatment and control groups in the water temperature and pH during the entire experimental period (Table 1).Temperature of water is one of the key parameters which influences the optimal growth of fish, yield of the plant and performance of nitrifying bacteria in aquaponics (Shete *et al.,* 2016).

In the present experiment, the water temperature was observed to be in the range of 24.05 to 26.86^{0}C. Pangasius has a temperature tolerance range of 22 to 32^{0}C and an ideal temperature range of 28 to 32^{0}C resulted in enhanced growth rate and metabolic activity (Islam *et al.,* 2019). A temperature of 25.1±0.72^{0}C was found to be favourable for basil plant growth (Saha *et al.,* 2016). Somerville *et al.* (2014) reported a temperature range of 17 to 34^{0}C as ideal for optimal performance of nitrifying bacteria. The temperature recorded in the present experiment was found to be within the acceptable range for better fish, plant and growth of nitrifying bacteria. The pH of the water varied in the range of 7.26 to 7.34. The best stability between nitrification and nutrient availability can be observed at pH close to 7 (Wurts and Durborow, 1992; Rakocy *et al.,* 2006). Similar observation for pH was found in the present study. The DO during experimental period was above 5 mg l^{-1} which is considered to be optimum for fish growth (Masser *et al.,* 1999). Similar observations were also reported by Peter *et al.* (2019). Total hardness ranged from164.4 to 170.82 mg l^{-1} and similar observations were made by Peter *et al.* (2019). Comparatively higher hardness values were recorded in the controls compared to the treatments. The reduction in hardness levels may be due to the absorption of calcium and magnesium by plant roots resulting in lesser hardness in treatments with plants. Total alkalinity concentrations showed a similar trend as that of total hardness concentrations. Total alkalinity levels in the control and treatments were found to be in the acceptable limit of 5-500 mg l^{-1} throughout the experimental period (Lawson, 1995).

Nitrogenous compounds *viz,* total ammoniacal nitrogen (TAN), nitrite-nitrogen and nitrate-nitrogen, play a critical role in an aquaponic system. Ammonia is a nitrogenous waste material excreted by fish. The ammonia is further oxidised into nitrite and nitrate-nitrogen with the help of nitrifying bacteria. In the present experiment, the TAN values were high in T4 (0.22 ±0.03 mg l^{-1}) because of the higher fish stocking density and the lowest was in T1 (0.10±0.01 mg l^{-1}) with lower fish stocking density. The presence of plants in aquaponics plays an important role in the removal of TAN. Similar observations were recorded by Hussain *et al.* (2014). Recommended ambient concentration of TAN in a recirculating aquaculture system (RAS) should be less than 1.0 mg l^{-1} (van Rijn and Rivera, 1990; Graber and Junge, 2009). Similar observations of an increased ammonia content with an increase in stocking density were made by Peter *et al.* (2019) and Nuwansi *et al.* (2019). Nitrite-nitrogen (NO_2-N) is an intermediate product in the nitrification process. Similar to ammonia, higher nitrite-nitrogen levels are also toxic to fish. Significantly ($p<0.05$) higher nitrite-nitrogen (NO_2) concentration was observed in T4 (0.056±0.01 mg l^{-1}) and the lowest value was found in T1 (0.041±0.00 mg l^{-1}). Nitrite-nitrogen concentration in all the treatments was within the desired level of less than 0.2 mg l^{-1} as suggested by Graber and Junge (2009). Selek *et al.* (2017) also observed a lower level of nitrite-nitrogen (0.05 to 0.21 mg l^{-1}) in aquaponics. The nitrite-nitrogen levels showed a declining trend as the experiment proceeded, and this is due to the accumulation of nitrifying bacteria in the biofilter. In the

Table 1. Water quality parameters observed during the experimental period

Parameter	C (2.50 kg m^{-3})	T1 (2.50 kg m^{-3})	T2 (2.75 kg m^{-3})	T3 (3.00 kg m^{-3})	T4 (3.25 kg m^{-3})
Temperature (°C)	24.08±0.03	24.08±0.03	24.07±0.02	24.08±0.03	24.07±0.03
pH	7.28±0.09	7.26±0.11	7.30±0.01	7.33±0.01	7.34±0.01
DO (mg l^{-1})	6.97^{b}±0.02	6.76^{b}±0.06	6.40^{a}±0.17	6.25^{a}±0.10	6.13^{a}±0.14
Hardness (mg l^{-1})	170.82±0.87	169.78±3.77	166.86±1.84	164.45±1.54	164.40±1.24
Alkalinity (mg l^{-1})	171.74±1.22	171.39±1.54	169.66±0.34	168.64±1.79	168.34±0.58
Total ammoniacal nitrogen (mg l^{-1})	0.13^{a}±0.02	0.10^{a}±0.01	0.15^{ab}±0.01	0.20^{bc}±0.03	0.22^{c} ±0.03
Nitrite-N (mg l^{-1})	0.046^{a}±0.00	0.041^{a}±0.00	0.047^{a}±0.00	0.049^{a}±0.00	0.056^{b}±0.01
Nitrate-N (mg l^{-1})	4.28^{c}±0.04	2.23^{a}±0.01	2.27^{a}±0.01	3.26^{b}±0.01	3.30^{b}±0.02
Phosphate (mg l^{-1})	0.91^{b}±0.01	0.75^{a}±0.02	1.10^{c}±0.01	1.13^{cd}±0.01	1.16^{d}±0.01
Potassium (mg l^{-1})	13.81^{c}±0.84	11.13^{b}±0.28	10.67^{ab}±0.46	9.40^{a}±0.9.40	8.63^{a}±0.59

Mean values (Mean±S.E.) with the same superscript in each row did not show significant difference ($p>0.05$).

nitrification process, nitrate-nitrogen is the end product and compared to ammonia and nitrite-nitrogen, nitrate-nitrogen is less toxic to fish and easily available for the plants (Somerville *et al.,* 2014). In aquaponics, nitrate-nitrogen levels lower than 150 mg l^{-1} are acceptable (Graber and Junge, 2009). Moreover, nitrate-nitrogen above 50 mg l^{-1} in aquaponics will lead to development of algal blooms (Poxton, 2003). In the present study, the lowest concentration of nitrate-nitrogen was found in T1 (2.23±0.01 mg l^{-1}), while the highest was recorded in C (4.28±0.04 mg l^{-1}). The higher nitrate-nitrogen in C is due to the absence of plants. The treatments with plants have lesser nitrate-nitrogen compared to C, which may be due to the utilisation of nitrate by plants. Mamatha *et al.* (2020) recorded the nitrate content in rohu and lemon grass biointegrated aquaponic system in the range of 2.74 to 5.30 mg l^{-1} and suggested that the treatments without plants have higher nitrate content compared to the treatments with plants. In the current study, the nitrate-nitrogen increased in the treatments with increasing stocking density. Similar observations of increasing nitrate content with increasing stocking density was reported by Rana *et al.* (2011), Mamatha *et al.* (2020) and Nuwansi *et al.* (2021).

Phosphorus is an important nutrient for plant growth, which is utilised by plants in the form of ionic orthophosphate (Eck *et al.,* 2019). Most plants need a phosphate concentration of 1.9 to 2.8 mg l^{-1} for adequate growth in solution culture (Asher and Loneragan, 1967; Kasozi *et al.,* 2021). The concentration of orthophosphate in the present experiment was found lower than the desired concentrations. Significantly ($p<0.05$) higher phosphate levels were observed in T4 (1.16±0.01 mg l^{-1}) and the lowest in T1 (0.75±0.02 mg l^{-1}). Cerozi and Fitzsimmons (2016a) reported that the orthophosphate concentration in the untreated aquaponics never exceeded 2 mg l^{-1}. When the pH of aqueous solutions rises over 7.0, most of the dissolved phosphorus combines with calcium to generate calcium phosphate, which makes phosphate unavailable to plants (Cerozi and Fitzsimmons, 2016b). Lower levels of orthophosphate in the present study were due to higher pH. Potassium is an important nutrient required for plant growth and it is involved in osmatic regulation, growth, enzyme activation and development of plants (Caliskan and Caliskan, 2019). Significantly ($p<0.05$) higher potassium content was found in control while the lowest was found in in T4. The higher potassium in C was due to the absence of plants in the system; whereas, in other treatments, the plants utilised the potassium resulting in lower potassium concentrations.

Growth of fish

In an aquaponic system, profits are dependent on the growth performances of the fish. The growth and survival performances of pangasius are presented in Table 2 . There was no significant ($p>0.05$) difference of mean body weights between the control and treatments (T1, T2 and T3). Comparatively higher final body weight was recorded in T1 (22.58±0.30 g) followed by T2, C and T3. Significantly ($p<0.05$) lower body weight was observed in T4 (20.14±0.21 g).

The lower growth performance of fish in higher stocking densities was due to an increase in competition for space and food among the fish due to overcrowding (Pankhurst and Kraak, 1997). Nuwansi *et al.* (2021) investigated the growth performance of koi carp at stocking densities of 1.4 kg m^{-3}, 2.1 kg m^{-3} and 2.8 kg m^{-3} and recorded the final body weight of 12.53±0.20, 9.92±0.20 and 8.40±0.06 g, respectively, which suggests the reduction in body weight with an increase in the stocking density, which is also reflected in T4 of the present study and similar observations were recorded by many researchers (Shelton *et al.,* 1981; Jha and Barat, 2005; Haridas *et al.,* 2017; Wang *et al.,* 2017). Mean body length of fish showed a similar trend as that of body weight. WGP and SGR were reduced with an increase in the stocking density; similar observations were also made by Shete *et al.* (2013). In the current study, SGR was recorded above 1%. Farooq *et al.* (2023) reported SGR more than 1% in pangasius reared in aquaponics. Significantly ($p<0.05$) higher total biomass was recorded in T3 with 10.29±4.41 kg m^{-3} followed by T4, T2, T1 and C. Higher fish biomass in T3 reflects the healthy stocking density and reduction of biomass in T4 indicates that the further increase in stocking density will hinder the fish growth performance, which is also reflected in SGR and WGP. No significant difference was observed in FCR with an increase in stocking density from control to T3 and there was a significant increase in FCR with further increase in the stocking density (T4) compared, with control. Effective utilisation of food in the treatments with lower stocking densities compared to higher stocking density might be the reason for the lower FCR in the lower stocking density treatments. A similar observation of an increased FCR with increased stocking density was observed in rohu by Mamatha *et al.* (2020), Nuwansi *et al.* (2021) and Hussain *et al.* (2014) in Koi carp. In the present experiment, the values of FCR ranged between 2.28 and 2.52. The higher FCR in the present study is due to lower temperature (24.05 to 24.08^{0}C) during the experimental period. Islam *et al.* (2019) investigated the effect of temperature on the

Table 2. Fish growth parameters in different treatments

Parameter	C (2.50 kg m^{-3})	T1 (2.50 kg m^{-3})	T2 (2.75 kg m^{-3})	T3 (3.00 kg m^{-3})	T4 (3.25 kg m^{-3})
Weight (g)					
Initial	6.42±0.02	6.40±0.02	6.45±0.02	6.43±0.02	6.41±0.02
Final	22.58^{b}±0.30	23.59^{bc}±0.18	22.99^{b}±0.06	22.39^{b}±0.10	20.14^{a}±0.21
Final biomass (kg m^{-3})	8.95^{a}±0.16	9.19^{a}±0.07	9.88^{b}±0.02	10.29^{c}±0.04	10.06^{b}±0.10
Length (cm)					
Initial	9.44±0.06	9.48±0.02	9.47±0.03	9.44±0.04	9.48±0.02
Final	16.27^{b}±0.14	17.28^{d}±0.06	16.82^{c}±0.09	16.10^{b}±0.04	15.07^{a}±0.05
Weight gain percentage (WGP)	251.95^{b}±4.83	268.34^{c}±1.62	256.44^{b}±1.19	247.99^{b}±2.10	214.33^{a}±4.19
Specific growth rate (SGR) (% day^{-1})	1.13^{b}±0.01	1.18^{c}±0.00	1.14^{b}±0.00	1.12^{b}±0.00	1.03^{a}±0.01
Feed conversion ratio (FCR)	2.30^{a}±0.04	2.28^{a}±0.02	2.28^{a}±0.02	2.28^{a}±0.01	2.52^{b}±0.05
Feed efficiency ratio (FER)	0.44^{b}±0.01	0.44^{b}±0.01	0.44^{b}±0.00	0.44^{b}±0.00	0.40^{a}±0.01
Protein efficiency ratio (PER)	1.45^{b}±0.03	1.46^{b}±0.02	1.46^{b}±0.01	1.46^{b}±0.01	1.32^{a}±0.03
Survival percentage (%)	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

Mean values (Mean±S.E.) with the same superscript in each row did not show significant difference ($p>0.05$).

growth performance of Thai pangas and reported higher FCR (2.10) at 24^{0}C and stated that during lower water temperatures, more feed is required to gain unit body weight. John *et al.* (2022) reported higher FCR (2.35 to 2.52) in pangasius grown in aquaponics due to lower water temperature (25.29 to 25.95^{0}C). The FCR of 1.82 to 5.60 in pangasius juveniles in RAS was reported by Meilisza *et al.* (2010). PER values were recorded in the range of 1.32 to 1.45 in the current investigation. A significant decrease in PER with an increase in stocking density was observed in T4 compared to the control and other treatments and it was also reflected in SGR and WGP. Similar observations were made by Nageswari *et al.* (2022) in pangasius reared in a biofloc system. Feed efficiency ratio (FER) was found to reverse the trend of FCR and recorded in the range of 0.40 to 0.44. No mortality of pangasius was observed throughout the experimental period and other researchers also observed similar findings (Hussain *et al.,* 2014).

Growth and nutrient content of basil

The growth of plants is equally important, like fish growth, for attaining higher profits in the aquaponic system, which can generate double income. The parameters related to plant as well as macro and micronutrients of basil leaves are presented in Table 3 and 4 . At the end of the experiment, the highest leaf height and width were observed in T4. The highest SFW and RFW observed were in T4. In the present study, the total yield of 3 harvests (combined) was calculated. The highest total yield was observed in T4 with 510.90±6.25 g followed by T3, T2 and T1; whereas, no significant (p>0.05) difference was found between T4 and T3. The current findings imply that higher fish stocking densities increased plant yield, which may be due to an increase in the availability of nutrients to plants from fish. It may be attributed to the higher amount of nutrients from fish excrement available to plants in treatments with higher stocking densities (Wang *et al.,* 2017; Mamatha *et al.,* 2020; Nuwansi *et al.,* 2021). The highest plant height was observed in T4, followed by T3, T2 and T1 in the present study. Similar findings were made by Saha et al. (2016) in aquaponics with a combination of basil and crayfish.

The highest total chlorophyll content was found in T4, while the lowest was found in T1. Result suggests that the higher fish stocking densities may benefit plant growth by providing more nutrients (Wang *et al.,* 2017; Nuwansi *et al.,* 2019; Mamatha *et al.,* 2020) .

Leaf nutrient analysis is one of the effective methods to analyse the nutritional quality of basil leaves. Nitrogen (N) levels were higher in T4 followed by T3, T2 and lower nitrogen was recorded in T1. A similar trend was observed with phosphorus (P), potassium (K) and calcium (Ca). Other micronutrients like magnesium, sulphur, copper, iron and zinc also found the same trend with the highest level recorded in T4 while the lowest was in T1. Overall, the highest nutrients were observed in T4 compared to other treatments, which may be due to the higher feeding rate in T4 owing to the higher stocking density of fish. The feed may also incorporate nutrients into the culture system; thus slightly higher micro and macronutrients were recorded in basil leaves from T4 treatment. Similar observations in the micro and macronutrients were made by Saha *et al.* (2016) in basil grown in aquaponics. However, the findings of the present experiment contradict Saha *et al.* (2016), which stated that

Table 3. Growth parameters of basil and chlorophyll content in different treatments at the end of the experiment

Parameter	T1 (2.5 kg m^{-3})	T2 (2.75 kg m^{-3})	T3 (3.0 kg m^{-3})	T4 (3.25 kg m^{-3})
Yield (g)				
1st harvest (30th day)	62.54^{a}±1.37	74.51^{b}±1.16	87.30^{c}±1.34	88.81^{c}±1.43
2nd harvest(60th day)	106.07^{a}±3.88	130.12^{b}±2.32	151.54^{c}±1.83	153.47^{c}±3.44
3rd harvest (90th day)	197.69^{a}±1.50	236.68^{b}±1.63	264.69^{c}±0.50	268.62^{c}±1.38
Total yield	366.3^{a}±6.75	446.42^{b}±5.11	503.53^{c}±3.67	510.90^{c}±6.25
Plant height (cm)				
Initial	6.69±0.24	6.61±0.12	6.70±0.18	6.82±0.15
30th day	34.52^{a}±2.51	36.09^{a}±1.05	42.88^{a}±0.55	44.51^{ab}±0.46
60th day	52.91^{a}±1.93	58.78^{a}±1.85	63.83^{ab}±1.36	65.05^{ab}±0.83
90th day	67.04^{a}±0.90	74.00^{b}±0.94	89.40^{c}±0.82	89.60^{c}±0.45
Leaf length (cm)				
Initial	1.40±0.03	1.39 ± 0.03	1.37±0.03	1.46±0.01
30th day	3.59^{a}±0.09	3.26^{a} ± 0.12	5.14^{b}±0.08	5.39^{b}±0.27
60th day	6.06^{a}±0.09	6.81^{b} ± 0.09	7.86^{c}±0.12	8.71^{d}±0.14
90th day	6.08^{a}±0.12	7.08^{b} ± 0.09	8.98^{c}±0.06	9.01^{c}±0.05
Leaf width (cm)				
Initial	0.91±0.05	0.89±0.06	0.87±0.06	0.91±0.05
30th day	2.21^{a}±0.60	2.45^{a}±0.10	2.89^{ab}±0.08	3.05^{ab}±0.11
60th day	3.68^{a}±0.09	4.03^{a}±0.09	4.87^{b}±0.13	4.79^{b}±0.13
90th day	3.85^{a}±0.05	4.26^{b}±0.14	5.12^{c}±0.04	5.19^{c}±0.04
Mean stem fresh weight (g)	87.26^{a}±1.53	113.14^{b}±1.54	182.54^{c}±0.62	183.64^{c}±1.06
Mean stem dry weight (g)	8.74^{a}±0.32	11.92^{b}±0.78	18.35^{c}±0.22	18.65^{c}±0.15
Mean root fresh weight (g)	89.98^{a}±2.03	106.07^{b}±3.06	134.01^{c}±1.96	137.56^{c}±1.32
Mean root dry weight (g)	5.92^{a}±0.16	8.18^{b}±0.14	11.52^{c}±0.35	11.73^{c}±0.25
Chlorophyll (a + b) (mg g^{-1})	0.72±0.03	0.72±0.02	0.82±0.07	0.84±0.02

Mean values (Mean±S.E.) with the same superscript in each row did not show significant difference (p>0.05).

Table 4. Macro and micro-nutrient content in basil leaves

Element	T1 (2.50 kg m^{-3})	T2 (2.75 kg m^{-3})	T3 (3.00 kg m^{-3})	T4 (3.25 kg m^{-3})
Nitrogen (%)	3.74^{a}±0.03	3.78^{a}±0.05	3.98^{ab}±0.06	4.17^{b}±0.04
Phosphorus (%)	0.38^{a}±0.00	0.38^{ab}±0.00	0.39^{b}±0.00	0.39^{b}±0.00
Potassium (%)	0.79±0.02	0.80±0.03	0.82±0.03	0.83±0.02
Calcium (%)	2.71 ±0.02	2.73 ±0.02	2.78±0.03	2.82±0.04
Magnesium (%)	0.64^{a}±0.01	0.64^{a}±0.00	0.66^{ab}±0.01	0.67^{c}±0.00
Sulfur (%)	0.15^{a}±0.00	0.16^{b}±0.00	0.16^{b}±0.00	0.17^{c}±0.00
Copper (mg kg^{-1})	10.86±0.01	10.86±0.01	10.88±0.01	10.91±0.02
Iron (mg kg^{-1})	97.24±0.21	97.56±0.36	98.40±0.42	98.85±0.20
Zinc (mg kg^{-1})	72.45^{a}±1.45	72.91^{a}±0.79	76.40^{ab}±1.88	77.62^{b}±1.01

Mean values (Mean±S.E.) with the same superscript in each row did not show significant difference (p>0.05).

additional nutrients supplied by crayfish waste had no impact on the nutrient levels of the leaves. In the present experiment, there was a slight increase in the nutrient contents with an increase in the stocking density.

Fish health monitoring

Haematological observations

Haematological parameters are crucial factors for assessing physiological health of a fish. When fish are stocked in high stocking densities, fish will undergo stress and this reflects in the hematological parameters. Thus, evaluation of hemoglobin, haematocrit (%), RBC and WBC have been known to be effective for disease diagnosis and monitoring of the physiological condition of fish (Caruso *et al.,* 2005; Carbonara *et al.,* 2010) . No significant (p>0.05) difference was found in haematological parameters in T1, T2, T3 compared to control whereas significantly (p<0.05) lower levels were recorded in T4 (Table 5).

In the present study, all the haematological parameters were in the range as mentioned by Latha *et al.* (2020) in pangasius. The findings of the present experiment showed a significant increase in the concentration of blood cells with an increase in the stocking density, which suggests a slight increase in stress on organisms with an increase in stocking density. Similar observations of increase in haemoglobin, haematocrit, and RBC count with an increase in stocking density were recorded by Ni *et al.* (2014) in juvenile Amur sturgeon and Upadhyay *et al.* (2022) in *Puntius sarana.* Yarahmadi *et al.* (2015) reported a reduction in WBC count with an increase in the stocking density of rainbow trout stocked at different stocking densities. Stocking density stress might be the reason for the reduction in the WBC count and the reduction in WBC count will negatively affect the fish by making fish vulnerable to diseases.

Anti-oxidant stress enzyme and serum biochemical parameters

Overcrowding will stress the organism, which can be immediately reflected in increased serum glucose levels. Analysing the serum glucose levels will clearly show stress levels in the fish (Yarahmadi *et al.,* 2015). The parameters related to anti-oxidant stress enzyme and serum biochemical are presented in Table 6 . A significantly higher serum glucose levels was recorded in T4 as compared to control. This rise in glucose level is likely due to the chronic stress response triggered by an increase in the stocking density, which causes the fish to engage in gluconeogenesis and glycogenolysis to produce the extra energy needed to cope with crowding stress. Galagarza *et al.* (2017) reported blood glucose levels in pangasius in the range of 82.8 to 136.8 mg dl^{-1}.

Table 5. Haematological parameters of *P. hypophthalmus*

Parameter	C (2.50 kg m^{-3})	T1 (2.50 kg m^{-3})	T2 (2.75 kg m^{-3})	T3 (3.00 kg m^{-3})	T4 (3.25 kg m^{-3})
Haemoglobin (g dl^{-1})	6.73^{a}±0.12	6.63^{a}±0.03	6.77^{a}±0.03	6.87^{ab}±0.15	6.95^{b}±0.12
Haematocrit (%)	21.73^{a}±0.87	21.23^{a}±0.38	21.80^{ab}±0.56	21.90^{ab}±0.35	23.54^{b}±0.25
Red blood cell (10^{6} cells mm^{-3})	1.76^{a}±0.10	1.87^{ab}±0.03	1.89^{ab}±0.04	1.88^{ab}±0.09	1.99^{b}±0.01
White blood cell (10^{3} cells mm^{-3})	20.92^{b}±0.15	21.03^{b}±0.17	20.92^{b}±0.15	20.58^{b}±0.31	19.74^{a}±0.03

Mean values (Mean±S.E.) with the same superscript in each row did not show any significant difference (p>0.05).

Table 6. Serum biochemical and anti-oxidant stress enzyme parameters *P. hypophthalmus*

Parameter	C (2.50 kg m^{-3})	T1 (2.50 kg m^{-3})	T2 (2.75 kg m^{-3})	T3 (3.00 kg m^{-3})	T4 (3.25 kg m^{-3})
Glucose (mg dl^{-1})	69.24^{ab}±0.41	68.95^{a}±2.09	71.35^{ab}±0.63	72.46^{ab}±0.85	73.27^{b}±0.55
Cortisol (ng ml^{-1})	33.72^{a}±4.40	31.03^{a}±1.65	38.16^{ab}±1.65	43.22^{b}±1.59	50.95^{c}±0.78
SOD (U mg $protein^{-1}$)					
Liver	31.84^{ab}±0.07	30.98^{a}±0.38	31.26^{a}±0.75	33.01^{bc}±0.34	34.55^{c}±0.61
Gill	41.30^{a}±1.99	39.87^{a}±1.50	43.05^{ab}±1.55	47.47^{bc}±1.63	49.93^{c}±2.40
Catalase (U mg $protein^{-1}$)					
Liver	1.50±0.05	1.53±0.04	1.54±0.04	1.59±0.03	1.61±0.05
Gill	1.40±0.03	1.38±0.02	1.41±0.03	1.46±0.04	1.48±0.04

Mean values (Mean±S.E.) with the same superscript in each row did not show significant difference (p>0.05).

The present study results were lesser than the above study. Serum cortisol is also known as the primary stress parameter, breaking the glycogen from the liver and producing glucose (Morgan and Iwama, 1981).

The increase in serum cortisol is linked with the increase in serum glucose levels. Cortisol levels in the present study ranged from 31.05 to 50.95 ng ml^{-1}. A significant increase in the cortisol levels was recorded with an increase in stocking density. Similar findings were made by Yin *et al.* (1995) in *C. carpio*, with an increase in stocking density leading to increased the stress parameters and Montero *et al.* (1999) also reported an elevated level of cortisol due to overcrowding.

The antioxidant stress enzymes include SOD and CAT. These enzymes play a crucial role in protecting cells from the harmful effects of free radicals by converting them into less reactive forms. SOD (superoxide dismutase) converts superoxide radicals into hydrogen peroxide, while CAT (catalase) converts hydrogen peroxide into water and oxygen. The higher SOD and CAT values represent higher stress on the organism. In the current study, the SOD values for liver were in the range of 30.98 to 34.55 U mg $protein^{-1}$; whereas; for gill tissues, it ranged from 39.87 to 49.93 U mg $protein^{-1}$. A significant increase in the SOD levels was observed with increasing stocking density in treatments and highest liver and gill SOD was recorded in T4. Nuwansi *et al.* (2021) reported increasing SOD levels with increasing stocking density in the aquaponic system. Catalase activity of liver and gill was found in the range of 1.50 to 1.61 U mg $protein^{-1}$ and 1.38 to 1.48 U mg $protein^{-1}$, respectively. No significant difference in the CAT values of liver and gill tissues was recorded when compared to control.

To be the most efficient, the aquaponic system must be appropriately sized with an optimal poise of nutrients from fish culture wastewater and nutrients utilised by the plant component. In the present study, both the plants and fish in all the treatments showed better growth, and the water quality and health parameters were found to be within the optimum ranges. The highest plant yield was recorded in T4 (3.25 kg m^{-3}) and there was no significant difference with T3 (3.00 kg m^{-3}). However, the highest fish biomass was achieved in T3 (3.00 kg m^{-3}). In this context, by considering the total biomass of fish, plant yield, quality of water, growth of fish, fish physiological responses and basil nutrient content, the stocking density of 3.00 kg m^{-3} of pangasius with 24 plants m^{-2} of basil is recommended as the optimum stocking density for the best growth of the basil and pangasius using aquaculture wastewater nutrients in a nutrient film technique-based aquaponics.

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References

Ahmed, A. A. and Masoud, R. A. 2014. Cardioprotective potential of basil oil and vitamin E against oxidative stress in experimental myocardial infarction induced by epinephrine in rats. *AAMJ,* 12(4): 204: 237.

Angkha, B., Verma, A. K., Kumar, S. H., Prakash, C. and Thomas, R. M. 2020. Mobilisation of mica by *Bacillus* sp. and its effect on nile tilapia (*Oreochromis niloticus*) cum holy basil (*Ocimum tenuiflorum*)-based aquaponic system. *Aquac. Int.,* 28(5): 2045-2058. https://doi.org/10.1007/s10499-020-00575-4.

APHA 2017. *Standard methods for examination of water and wastewater,* 23st edn. American Public Health Association (APHA), Washington D. C., USA.

Asher, C. J. and Loneragan, J. F. 1967. Response of plants to phosphate concentration in solution culture: I. Growth and phosphorus content. *Soil Sci.,* 103(4): 225-233. https://doi.org/10.1097/00010694-196704000-00001.

Buzby, K. M. and Lin, L. S. 2014. Scaling aquaponic systems: Balancing plant uptake with fish output. *Aquac. Eng.,* 63: 39-44. https://doi.org/10.1016/j.aquaeng.2014.09.002.

Caliskan, B. and Caliskan, A. C. 2019. Potassium nutrition in plants and its interactions with other nutrients in hydroponic culture. In: *Improvement of quality in fruits and vegetables through hydroponic nutrient management.* IntechOpen, London, UK, pp. 9-22. https://doi.org/10.5772/intechopen.71951.

Carbonara, P., Corsi, I., Focardi, S., Lembo, G., Rochira, S., Scolamacchia, M., Teresa Spedicato, M. and Scott McKinley, R. 2010. The effects of stress induced by cortisol administration on the repeatability of swimming performance tests in the European sea bass (*Dicentrarchus labrax* L.). *Mar. Freshw. Behav. Physiol.,* 43(4): 283-296. https://doi.org/10.1080/10236244.2010.504046.

Caruso, G., Genovese, L., Maricchiolo, G. and Modica, A. 2005. Haematological, biochemical and immunological parameters as stress indicators in *Dicentrarchus labrax* and *Sparus aurata* farmed in off-shore cages. *Aquacult. Int.,* 13(1): 67-73. https://doi.org/10.1007/s10499-004-9031-5.

Cerozi, B. da S. and Fitzsimmons, K. 2016a. Use of *Bacillus* spp. to enhance phosphorus availability and serve as a plant growth promoter in aquaponics systems. *Sci. Hortic.,* 211: 277-282. https://doi.org/10.1016/j.scienta.2016.09.005.

Cerozi, B. da S. and Fitzsimmons, K. 2016b. The effect of pH on phosphorus availability and speciation in an aquaponics nutrient solution. *Bioresour. Technol.,* 219: 778-781. https://doi.org/10.1016/j.biortech.2016.08.079.

Eck, M., Korner, O. and Jijakli, M. H. 2019. Nutrient cycling in aquaponics systems, in: Goddek, S., Joyce, A., Kotzen, B., Burnell, G. M. (Eds.), *Aquaponics food production systems.* Springer, Cham, pp. 231-246.

FAO 2018. *The state of world fisheries and aquaculture 2018 (SOFIA):* Meeting the sustainable development goals. Food and Agriculture Organisation of the United Nations, Rome, Italy.

Farooq, A., Verma, A. K., Hittinahalli, C. M., Harika, N. and Pai, M. 2023. Iron supplementation in aquaculture wastewater and its effect on the growth of spinach and pangasius in nutrient film technique based aquaponics. *Agric. Water Manag.,* 277: 108126. https://doi.org/10.1016/j.agwat.2022.108126.

Galagarza, O. A., Kuhn, D. D., Smith, S. A. and Hrubec, T. C. 2017. Hematologic and plasma chemistry RIs for cultured Striped catfish (*Pangasius hypophthalmus*) in recirculating aquaculture systems. *Vet. Clin. Pathol.,* 46(3): 457-465. https://doi.org/10.1111/vcp.12513.

Graber, A. and Junge, R. 2009. Aquaponic systems: Nutrient recycling from fish wastewater by vegetable production. *Desalination,* 246(1): 147-156. https://doi.org/10.1016/j.desal.2008.03.048.

Haridas, H., Verma, A.K., Rathore, G., Prakash, C., Sawant, P. B. and Babitha Rani, A. M. 2017. Enhanced growth and immuno-physiological response of Genetically Improved Farmed Tilapia in indoor biofloc units at different stocking densities. *Aquacult. Res.,* 48(8): 4346-4355. https://doi.org/10.1111/are.13256.

Hastuti, S. and Subandiyono, S. 2020. Aminotransferase, hematological indices and growth of tilapia (*Oreochomis niloticus*) reared in various stocking densities in aquaponic systems. *Aquac. Aquar. Conserv. Legis.,* 13(2): 813-824. http://www.bioflux.com.ro/docs/2020.813-824.pdf.

Hussain, T., Verma, A. K., Tiwari, V. K., Prakash, C., Rathore, G., Shete, A. P. and Nuwansi, K. K. T. 2014. Optimizing koi carp, *Cyprinus carpio* var. *koi* (Linnaeus, 1758), stocking density and nutrient recycling with spinach in an aquaponic system. *J. World Aquac. Soc.,* 45(6): 652-661.

Islam, S. M. M., Rahman, Md. A., Nahar, S., Uddin, Md. H., Haque, Md. M. and Shahjahan, Md. 2019. Acute toxicity of an organophosphate insecticide sumithion to striped catfish *Pangasianodon hypophthalmus*. *Toxicol. Rep.,* 6: 957-962. https://doi.org/10.1016/j.toxrep.2019.09.004.

Jha, P. and Barat, S. 2005. The effect of stocking density on growth, survival rate, and number of marketable fish produced of koi carps, *Cyprinus carpio* vr. *koi* in concrete tanks. *J. Appl. Aquac.,* 17(3): 89102. https://doi.org/10.1300/J028v17n03_07.

John, V. C., Verma, A. K., Krishnani, K. K., Chandrakant, M. H., Bharti, V. S. and Varghese, T. 2022. Optimization of potassium (K+) supplementation for growth enhancement of *Spinacia oleracea* L. and *Pangasianodon hypophthalmus* (Sauvage, 1878) in an aquaponic system. *Agric. Water Manag.,* 261: 107339. https://doi.org/10.1016/j.agwat.2021.107339.

Kamble, P. N., Giri, S. P., Mane, R. S. and Tiwana, A. 2015. Estimation of chlorophyll content in young and adult leaves of some selected plants. *Univers J. Env. Res. Technol.,* 5: 306-310.

Kasozi, N., Kaiser, H. and Wilhelmi, B. 2021. Effect of *Bacillus* spp. on lettuce growth and root associated bacterial community in a small-scale aquaponics system. *Agronomy,* 11(5): 947. https://doi.org/10.3390/agronomy11050947.

Latha, C., Krishnakumar, V. and Munuswamy, N. 2020. Growth, survival and haemato-biochemical profiles of the freshwater catfish, *Pangasius sutchi* (Fowler, 1937) fingerlings fed on *Tinospora cordifolia* leaf extract supplemented diet. *Egypt. J. Aquat. Biol. Fish.,* 24(1): 249-262. https://doi.org/10.21608/ejabf.2020.70288.

Lawson, T. B. 1995. Water quality and environmental requirements. In: Lawson, T.B. (Ed.), *Fundamentals of aquacultural engineering.* Springer US, Boston, USA, pp. 12-39. https://doi.org/10.1007/978-1-4615-7047-9.2.

Mamatha, D., Verma, A. K., Tiwari, V. K., Chandrakant, M. H., Nayak, S. K. and Javed, H. 2020. Biointegration of rohu (*Labeo rohita*) fry and lemon grass (*Cymbopogon citratus*) in a recirculating aquaponic system. *J. Indian Soc. Coast. Agric. Res.,* 38(2): 132-139.

Masser, M. P., Rakocy, J. and Losordo, T. M. 1999. *Recirculating aquaculture tank production systems-management of recirculating systems.* SRAC Publication, No. 452, USDA.

Meena, L. L., Verma, A. K., Bharti, V. S., Nayak, S. K., Chandrakant, M. H., Haridas, H., Reang, D., Javed, H. and John, V. C. 2022. Effect of foliar application of potassium with aquaculture wastewater on the growth of okra (*Abelmoschus esculentus*) and *Pangasianodon hypophthalmus* in recirculating aquaponic system. *Sci. Hortic.,* 302: 111161. https://doi.org/10.1016/j.scienta.2022.111161.

Meilisza, N., Moreau, Y., Baras, E. and Hirnawati, R. 2010. Influence of feeding time on feed utilization by Siamese catfish, *Pangasius hypophthalmus* Juvenile. *Indones. Aquac. J., 5*(1): 83-89. https://doi.org/10.15578/iaj.5.1.2010.83-89.

Misra, H. P. and Fridovich, I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.,* 247(10): 3170-3175. https://doi.org/10.1016/S0021-9258(19)45228-9.

Montero, D., Izquierdo, M. S., Tort, L., Robaina, L. and Vergara, J. M. 1999. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. *Fish Physiol. Biochem.,* 20(1): 53-60. https://doi.org/10.1023/A:1007719928905.

Morgan, J. D. and Iwama, G. K. 1981. Measurements of stressed states in the field. *Fish Stress Health Aquac.,* 62: 247.

Nageswari, P., Verma, A. K., Gupta, S., Jeyakumari, A. and Chandrakant, M. H. 2022. Optimisation of stocking density and its impact on growth and physiological responses of *Pangasianodon hypophthalmus* (Sauvage, 1878) fingerlings reared in finger millet based biofloc system. *Aquaculture,* 551: 737909. https://doi.org/10.1016/j.aquaculture.2022.737909.

Ni, M., Wen, H., Li, J., Chi, M., Bu, Y., Ren, Y., Zhang, M., Song, Z. and Ding, H., 2014. The physiological performance and immune responses of juvenile Amur sturgeon (*Acipenser schrenckii*) to stocking density and hypoxia stress. *Fish Shellfish Immunol.,* 36(2): 325-335. https://doi.org/10.1016/j.fsi.2013.12.002.

Nuwansi, K. K. T., Verma, A. K., Chandrakant, M. H., Prabhath, G. P. W. A. and Peter, R. M. 2021. Optimisation of stocking density of koi carp (*Cyprinus carpio* var. *koi*) with gotukola (*Centella asiatic*a) in an aquaponic system using phytoremediated aquaculture wastewater. *Aquaculture,* 532: 735993. https://doi.org/10.1016/j.aquaculture.2020.735993.

Nuwansi, K. K. T., Verma, A. K., Rathore, G., Chandrakant, M. H., Prabhath, G. P. W. A. and Peter, R. M. 2020. Effect of hydraulic loading rate on the growth of koi carp (*Cyprinus carpio* var. *koi*.) and Gotukola (*Centella asiatica* (L.)) using phytoremediated aquaculture wastewater in aquaponics. *Aquac. Int.,* 28(2): 639-652. https://doi.org/10.1007/s10499-019-00485-0.

Nuwansi, K. K. T., Verma, A. K., Rathore, G., Prakash, C., Chandrakant, M. H. and Prabhath, G. P. W. A. 2019. Utilization of phytoremediated aquaculture wastewater for production of koi carp (*Cyprinus carpio* var. *koi*) and gotukola (*Centella asiatica*) in an aquaponics. *Aquaculture,* 507: 361-369. https://doi.org/10.1016/j.aquaculture.2019.04.053.

Pankhurst, N. W. and Kraak, G. van der. 1997. Effects of stress on reproduction and growth of fish. *Fish Stress Health Aquac.,* 73-93.

Patil, P. A., Dube, K., Verma, A. K., Chadha, N. K., Sundaray, J. K. and Jayasankar, P., 2019. Growth performance of goldfish, *Carassius auratus* and basil, *Ocimum basilicum* in media bed aquaponics. *Indian J. Fish.,* 66(1): 112-118. https://doi.org/10.21077/ijf.2019.66.1.78353-15.

Peter, R. M., Verma, A. K., Neelam, S., Tiwari, V. K. and Thomas, R. M. 2019. Optimizing pearlspot, *Etroplus suratensis* (Bloch, 1790) stocking density with tomato (*Solanum lycopersicum*) in Recirculating Aquaponic System. *J. Exp. Zool. India,* 22(2): 1109-1113.

Poxton, M. 2003. Water quality, In: *Aquaculture: Farming aquaculture animals and plants*. Blackwell Publishing, Oxford. UK, pp. 47-73.

Rakocy, J. E., Bailey, D. S., Shultz, R. C. and Thoman, E. S. 2004. Update on tilapia and vegetable production in the UVI aquaponic system. In: *New dimensions on farmed tilapia: Proceedings of the Sixth International Symposium on tilapia in aquaculture,* Manila, Philippines. pp. 676-690.

Rakocy, J. E., Masser, M. P. and Losordo, T. M. 2006. Recirculating aquaculture tank production systems: Aquaponics-integrating fish and plant culture. *SRAC Publ. South. Reg. Aquac. center,* 454: 1-16.

Rana, S., Bag, S. K., Golder, D., Roy, S. M., Pradhan, C. and Jana, B. B. 2011. Reclamation of municipal domestic wastewater by aquaponics of tomato plants. *Ecol. Eng.,* 37(6): 981-988. https://doi.org/10.1016/j.ecoleng.2011.01.009.

Saha, S., Monroe, A. and Day, M. R. 2016. Growth, yield, plant quality and nutrition of basil (*Ocimum basilicum* L.) under soilless agricultural systems. *Ann. Agric. Sci.,* 61(2): 181-186. https://doi.org/10.1016/j.aoas.2016.10.001.

Seawright, D. E., Stickney, R. R. and Walker, R. B. 1998. Nutrient dynamics in integrated aquaculture-hydroponics systems. *Aquaculture,* 160(3-4): 215-237. https://doi.org/10.1016/S0044-8486(97)00168-3.

Selek, M., Endo, M., Yigit, M. and Takeuchi, T. 2017. The integration of fish and plant production: Nile tilapia (*Oreochromis niloticus*) and basil (*Ocimum basilicum*) culture in recirculating and aquaponic systems. *J. Aquac. Eng. Fish. Res.,* 3(1): 28-43.

Shelton, W. L., Smitherman, R. O. and Jensen, G. L. 1981. Density related growth of grass carp, *Ctenopharyngodon idella* (Val.) in managed small impoundments in Alabama. *J. Fish Biol.,* 18(1): 45–51. https://doi.org/10.1111/j.1095-8649.1981.tb03758.x

Shete, A. P., Verma, A. K., Chadha, N. K., Prakash, C. and Chandrakant, M. H. 2015. A comparative study on fish to plant component ratio in recirculating aquaponic system with common carp and mint. *J. Environ. Bio-Sci.,* 29(2): 323-329.

Shete, A. P., Verma, A. K., Chadha, N. K., Prakash, C., Peter, R. M., Ahmad, I. and Nuwansi, K. K. T. 2016. Optimization of hydraulic loading rate in aquaponic system with Common carp (*Cyprinus carpio*) and Mint (*Mentha arvensis*). *Aquac. Eng.,* 72-73: 53-57. https://doi.org/10.1016/j.aquaeng.2016.04.004.

Shete, A. P., Verma, A. K., Kohli, M. P. S., Dash, A. and Tandel, R. 2013. Optimum stocking density for growth of goldfish, *Carassius auratus* (Linnaeus, 1758), in an aquaponic system. *Isr. J. Aquac. Bamidgeh,* 65: 1-6.

Singh, D., Chhonkar, P. K. and Pandey, R. N. 1999. *Soil, plant and water analysis - A method manual.* Indian Agriculture Research Institute, New Delhi, India.

Somerville, C., Cohen, M., Pantanella, E., Stankus, A. and Lovatelli, A. 2014. Small-scale aquaponic food production. Integrated fish and plant farming. *FAO Fish. Aquac. Tech., Pap.,* 589. Food and Agriculture Organisation of the United Nations, Rome, Italy.

Thomas, R. M., Verma, A. K., Krishna, H., Prakash, S., Kumar, A. and Peter, R. M. 2021. Effect of salinity on growth of Nile tilapia (*Oreochromis niloticus*) and spinach (*Spinacia oleracea*) in aquaponic system using inland saline groundwater. *Aquac. Res.,* 52(12): 6288-6298. https://doi.org/10.1111/are.15492.

Thomas, R. M., Verma, A. K., Prakash, C., Krishna, H., Prakash, S. and Kumar, A. 2019. Utilization of Inland saline underground water for bio-integration of Nile tilapia (*Oreochromis niloticus*) and spinach (*Spinacia oleracea*). *Agric. Water Manag.,* 222: 154-160. https://doi.org/10.1016/j.agwat.2019.06.001.

Upadhyay, A., Swain, H. S., Das, B. K., Ramteke, M. H., Kumar, V., Krishna, G., Mohanty, B. P., Chadha, N. K. and Das, A. K. 2022. Stocking density matters in open water cage culture: Influence on growth, digestive enzymes, haemato-immuno and stress responses of *Puntius sarana* (Ham, 1822). *Aquaculture,* 547: 737445. https://doi.org/10.1016/j.aquaculture.2021.737445.

van Rijn, J. and Rivera, G. 1990. Aerobic and anaerobic biofiltration in an aquaculture unit-Nitrite accumulation as a result of nitrification and denitrification. *Aquac. Eng.,* 9(4): 217-234. https://doi.org/10.1016/0144-8609(90)90017-T.

Verma, A. K., Chandrakant, M. H., John, V. C., Peter, R. M. and John, I. E., 2023. Aquaponics as an integrated agri-aquaculture system (IAAS): Emerging trends and future prospects. *Technol. Forecast. Soc. Change,* 194: 122709. https://doi.org/10.1016/j.techfore.2023.122709.

Verma, A. K., Gupta, S. K. and Isaac, R. K. 2010. Long-term use of saline drainage waters for irrigation in subsurface drained lands: simulation modelling with swap. *J. Agric. Eng.,* 47(3): 15-23.

Verma, A. K., Gupta, S. K. and Isaac, R. K. 2013. Long-term cyclic irrigation in subsurface drained lands: simulation studies with SWAP. *J. Agric. Sci.,* 5(1): 84-94. https://doi.org/10.5539/jas.v5n1p84.

Verma, A. K., Gupta, S. K. and Isaac, R. K. 2014. Calibration and validation of SWAP to simulate conjunctive use of fresh and saline irrigation waters in semi-arid regions. *Environ. Model. Assess.* 19(1): 45–55. https://doi.org/10.1007/s10666-013-9379-x.

Wang, C., Chang, C., Dahms, H. U. and Lai, H. 2017. Effects of stocking density of tilapia on the performance of a membrane filtration-recirculating aquaponic system. *Desalination Water Treat.,* 96: 22-32.

Wurts, W. A. and Durborow, R. M. 1992. *Interactions of pH, carbon dioxide, alkalinity and hardness in fish ponds. Publication No. 464,* Southern Regional Aquaculture Center, Stoneville, Mississippi, USA.

Yarahmadi, P., Miandare, H. K., Hoseinifar, S. H., Gheysvandi, N. and Akbarzadeh, A. 2015. The effects of stocking density on hemato-immunological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). *Aquac. Int.,* 23(1): 55-63. https://doi.org/10.1007/s10499-014-9797-z.

Yildiz, H. Y., Robaina, L., Pirhonen, J., Mente, E., Domínguez, D. and Parisi, G. 2017. Fish welfare in aquaponic systems: its relation to water quality with an emphasis on feed and faeces-A review. *Water,* 9(1): 13. https://doi.org/10.3390/w9010013.

Yin, Z., Lam, T. J. and Sin, Y. M. 1995. The effects of crowding stress on the non-specific immuneresponse in fancy carp (*Cyprinus carpio* L.). *Fish Shellfish Immunol.,* 5(7): 519-529. https://doi.org/10.1016/S1050-4648(95)80052-2.