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Effects of Fish Stocking Density on Water Quality, Growth Performance of Tilapia and Yield of Butterhead Lettuce Grown in Decoupled Recirculation Aquaponic Systems

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ABSTRACT

This study was conducted over a period of 52 days to determine the effects of fish stocking density on the water quality, growth performance of tilapia and yield of butterhead lettuce cultivated in decoupled recirculation aquaponic systems (DRAPS). In this study, three respective tilapia stocking densities (treatments) of 8 $kg \cdot m^{-3}$, 10 $kg \cdot m^{-3}$, and 12 $kg \cdot m^{-3}$ were used to evaluate the butterhead lettuce in the DRAPS, which consist of two independent loops. All treatments were done in triplicates. The results showed with increased stocking density, the electrical conductivity, total dissolved substances and salinity increased and dissolved oxygen decline. The results showed that the highest stocking density produced the highest nutrients accumulation of ammonia-nitrogen (NH_3-N), ammonium (NH_4), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) and potassium (K) except for phosphorus (P). Nevertheless, based on the conversion of fish feed to NO_3-N and P per kilogram of feeds, the lowest stocking density provided the highest concentration of NO_3-N and P. It was documented that DRAPS relied solely on the fish waste produced an insufficient concentration of N, P, K and iron. The average survival rate of tilapia in all treatments was above 94% and was not a significant difference among the treatments.

Keywords: Decoupled aquaponics systems, stocking density, tilapia, butterhead lettuce (*Lactuca sativa*).

INTRODUCTION

Aquaponics system (APS) is a modern agricultural technique that provides a more sustainable food production system through a synergistic combination of recirculation aquaculture system (RAS) (Love et al. 2015) and hydroponic (HP) (Resh 2012) where the biological processes in the system produce nitrogen (N) in the form of nitrate (NO_3) as the main end product (Graber and Junge 2009; Rakocy et al. 2006; Rakocy 2012). However, integration tilapia and lettuce being as one of the most significant integrations in APS and the success of this symbiotic plays a crucial role in improving the sustainability of the agriculture production system (Ajitama et al. 2018; Jordan et al. 2018a; 2018b; Sreejariya et al. 2016).

The concentrations of macronutrients such as N, phosphorus (P), and potassium (K) produced through the breakdown of fish waste in APS are inadequate compared to the levels of nutrients in HP systems (Rakocy et al. 2004). Several studies reported that the levels of nutrients in APS were inadequate as the ratio of the fish and plants was imbalanced, resulting in NO_{3-} depletion (Buzby and Lin. 2014). Studies also showed that an imbalance ratio of fish and plants might lead to the accumulation of NO_{3-} (Liang and Chien 2013). Therefore, stocking density should be optimal to maintain suitable water quality for fish and plant growth as well as the proper functioning of the system.

There were a few studies reported the integration of tilapia and lettuce in the DRAPS (Delaide et al. 2016). In 2015, a German professor, Kloas, conducted the first study on the integration of tomato and tilapia in DRAPS using the nutrient film technique (NFT) HP unit. Then, a few other studies were conducted to evaluate the efficiency of DRAPS by integrating tilapia and tomatoes (Karimanzira et al. 2016), or African catfish and tomato (Suhl et al. 2018a), tilapia and lettuce (Monsees et al. 2019), tilapia and cucumber (Blanchard et al. 2020). However, Suhl et al. (2016) reported that the fish to plant ratio and the production of the nutrients were not optimized to integrate different species of both fish and plants in DRAPS. To date, there were limited studies on DRAPS (Monsees et al. 2019; Suhl et al. 2018b) and no study on the effects of fish stocking density on the lettuce growth and production. Nevertheless, according to previous studies, different plants and fishes will have a different optimal ratio and are highly dependent on factors such as fish stocking density, APS type, plant species, planting density, type of HP or soilless culture production system, water flow rate, and external factors such as light, air, water temperature, and pH. However, the data generated in this study can be used to estimate the optimal fish ratio to obtain an adequate range of N level for high growth of lettuce and to further support the importance of DRAPS as the new sustainable agriculture production system. The data is also important for the generation of the baseline data on the applicability of DRAPS in tropical regions as a small-scale production in order to improve food security, reduce poverty, and maximize the availability of fish and plants to the economy of the country.

There were two objectives in this study: (I) to determine the ideal stocking density of tilapia (*Oreochromis niloticus*) and its effects on the water quality, growth performance of fish, and yield of lettuce (*Lactuca sativa*) in the DRAPS with non-controlled conditions and without the addition of inorganic fertilizers and (II) to determine the composition of nutrients, which produced through the biological processes (fish metabolism) under the non-controlled condition, in the solution.

MATERIALS AND METHODS

The setting of DRAPS

This study was conducted in a shelter at Ladang 15, Faculty of Agriculture, UPM Serdang, Selangor, Malaysia (longitude 101° 44’ N and latitude 2° 58’S, 68 m above sea level). The total area involved was 85 m^2. This study was carried out under the tropical temperature range between 26°C and 37°C. The DRAPS used in this study was adapted from the system developed by Kloas et al. (2015), which is composed of two loops, with slight modification done to the system, as illustrated in Figure 1.

Water circulation in DRAPS

The setting of the first loop was adapted from Rakocy (2007). The water circulation in the first loop started from a single 350 L fish tank and was then connected to a 45 L mechanical filtering tank to remove solid particles and minimize floating debris as much as possible so that the aggregate formation would not impact the yield of the fish and plants in the system. After the mechanical filtering stage, the filtered water flowed to a 45 L biofilter tank, which contained bio balls and bio rings filter materials for the biological processes (nitrification) to take place. From the biofilter tank, the water was then connected to a 300 L sump tank for the collection of nitrified water and to complete the first loop. The total volume for the first loop was 740 L with a flow rate of 9.2 $m^3 \cdot day^{-1}$ or 6.4 $L \cdot min^{-1}$ (Endut et al. 2010), which enabled the water retention time of 50 minutes in the fish tank. The second loop of the DRAPS used in this study was based on the setup design by Kloas et al. (2015). It received the nutrient solution from the first loop and was then connecting the nutrient solution to an additional reservoir with a one-way-valve, which made the water in the DRAPS recirculated independently in one direction from the first loop to the HP unit in the second loop. The additional 300 L tank or reservoir was connected to an NFT HP. The flow rate of the circulation in the second

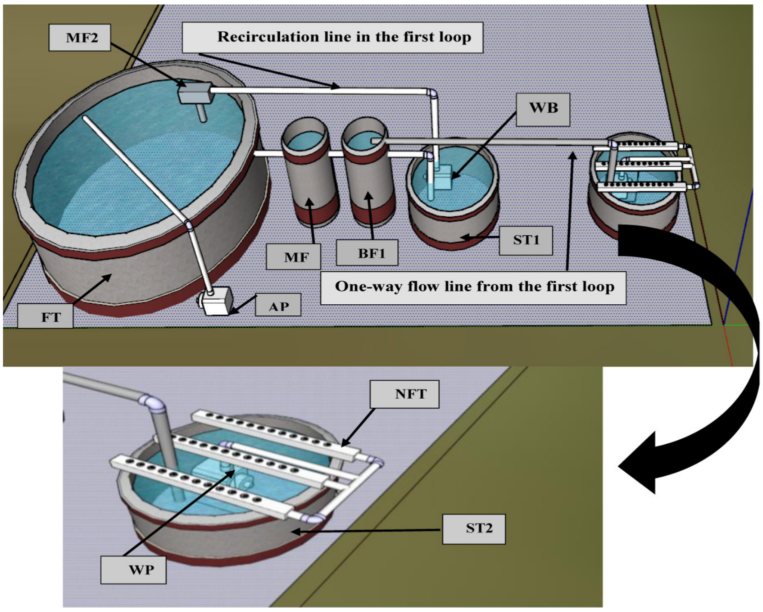

Figure 1. A schematic illustration of NFT of original DRAPS was used in Universiti Putra Malaysia. (AP) Air pump, (FT) fish tank, (MF) mechanical filter, (BF) biological filter, (ST1) sump tank 1, (WP) water pump, (MF2) mechanical filter 2. The second loop is composed; (NFT) hydroponics unit (NFT trials), (ST2) sump tank 2, (WP) water pump

loop was between one to two liters per minute (Resh 2013). The total volume of water in the first and second loops of the DRAPS was maintained at 1040 L. There was no water discharge during the experimental period except for the water lost through evaporation, transpiration, and sludge removal at less than 5% under tropical conditions.

Plant and fish materials

The butterhead lettuce (*Lactuca sativa*) seeds were obtained from a company known as (Green World Genetics). The lettuce seeds were sown and grown in a seed tray for 14 days before the transplantation to match the size of seedlings at the time of planting. The seeds were watered daily until it starts to germinate. After two weeks, the germinated lettuces were then transferred to the HP cups. The seedlings from the third to fourth true-leaf stages (14 days old) were shifted to the NFT HP with a planting density of 32 plants m^{-2} and with a spacing of 15×15 cm^2. No external factors such as the usage of fertilizers were introduced to the DRAPS, both air and water temperature were also not controlled in the study. The red tilapia (*Oreochromis niloticus*) used in this study were obtained from the aquaculture farm in UPM in Puchong, where the tilapia were grown in a conventional aquaculture system. All the red tilapia with an initial average weight of 125 ± 20 g were stocked simultaneously in all nine of the DRAPS. The daily feeding rate for each stocking density of tilapia in the DRAPS was 2% of the bodyweight of tilapia. The feed type was a commercial floating pellet with a size of 3.2 mm from Dindings Company. The proximate nutrient composition of the fish feed was 32% protein, 5% fat, 10% ash, 5% fiber, and 10% moisture content. The fish were fed twice a day manually for 30 minutes at 9.00 and 5.00 pm.

Experimental design set up and treatments

The period for completing this study was 52 days, starting from April 6, 2019, until May 16, 2019. However, including the time taken for the establishment of the study site and system set up for this study, the total period for the completion of this study was 120 days. The completely Randomized Complete Block Design (RCBD) with three replicates was used in this study. The stocking densities were determined based on the minimum density required to provide sufficient N for the growth of butterhead lettuce in a small-scale APS. A total of nine independent DRAPS, which consisted of three fish stocking densities, were set up, with three replicates for each fish stocking density. The stocking densities (treatments) of 12 $kg·m^{-3}$, 10 $kg·m^{-3}$, and 8 $kg·m^{-3}$ were used in this study and assigned as T1, T2, and T3. The stock densities per 350 L fish tank were 4.2 $kg·tank^{-1}$, 3.5 $kg·tank^{-1}$, and 2.8 $kg·tank^{-1}$, respectively. Each stocking density had three replicates and each replicate was connected to an HP unit. This gave a total of nine HP units and each HP unit have three troughs of NFT. The experiment was conducted under a 12-h light (07.00–17.00 h)/12-h dark (17.00–07.00 h) natural light cycle

Measurement of water quality paraments

Various parameters were measured and recorded on a daily and weekly basis as an indication of the water quality. The dissolved oxygen (DO; $mg·L^{-1}$), oxygen saturation (%), temperature (T; °C), pH, electric conductivity (EC; mS cm^{-1}), total dissolved solids (TDS; g L^{-1}), and salinity (S; ppt) of the water in the fish tanks and HP units were measured daily using a YSI 556 Multiparameter meter (YSI Inc. USA) before feeding the fish at 9 am and 4 pm. The aquarium thermostat heaters did not use for controlling the T of the fish tanks and HP units. The pH and EC in both the fish tanks and HP units were also not controlled throughout the study. Therefore, the concentrations of ammonia-nitrogen (NH_3-N) (Silva et al. 2017), ammonium (NH_4) (Geisenhoff et al. 2016) nitrate-nitrogen (NO_2-N) (Emerson et al. 1975) in each of the fish tanks were frequently measured (in terms of mg L^{-1}). In addition, parameters such as nitrate-nitrogen (NO_3-N), orthophosphate (PO_4-P), potassium (K), iron (Fe), calcium (Ca), and alkalinity ($CaCO_3$ mg L^{-1}) were analyzed at the beginning and the end of the study using a multiparameter spectrophotometer (HI 83200, HANNA instruments, Woonsocket, RI, USA).

Growth and yield measurements of lettuce

Fresh yields of the lettuce were measured at the harvest stage. The lettuce samples were divided into leaf, stem and root and weighted by using a ME analytical Weighing Balance (Mettler Toledo Inc.). The number of leaves was determined by counting the total number of leaves per plant at the harvest stage. Total leaf area was measured using a leaf area meter (Li-Cor LI-3100C) at the harvest stage. The leaves were cut off from the stem and entered to leaf area meter and the reading showed on the screen recorded in square centimeters cm^2.

Growth and yield characteristics of tilapia

The health condition and mortality rate of the tilapia were monitored twice a day, which were in the morning and evening. The data of this study were categorized into two categories. The first category was the initial measurements that were recorded at the beginning of the study, and this included the initial stocking densities ($kg·m^{-3}$). The second category of data was recorded at the end of this study, which included the final stocking density ($kg·m^{-3}$), weight gain (WG; %), fish increment (%), feed conversion ratio (FCR), and survival rate (SR; %). The tilapia from all nine of the treatment tanks were weighted in order to compare the growth rate and yield of the tilapia. Furthermore, the growth performances of the tilapia and the feed utilization were calculated as described by Sveier et al. (2000) and Jimoh et al. (2019) using the following formulas.

The WG was estimated using the following formula.

$$\text{WG} = final\ fish\ weight\ (g) - initial\ fish\ weight\ (g) \quad (1)$$

The FCR was calculated using the following formula.

$$\text{FCR} = \frac{weight\ of\ feed\ given\ (g)}{fish\ weight\ gain\ (g)} \quad (2)$$

The SR was calculated with the following formula.

$$\text{SR (\%)} = 100 \times \frac{(final\ number\ of\ fish\ survived}{total\ number\ of\ fish\ stocked)} \quad (3)$$

Statistical analysis

The RCBD with three replicates was used in this study. The data were analyzed using analysis of variance (ANOVA) in the Statistical Analysis System (SAS), version 9.4 (SAS Institute Inc., Cary, NC, USA). The means were compared using the least significant difference (LSD) test with a significance level of 0.05.

RESULTS

Physical water parameters of fish tank

The physical water parameters such as water T, pH, DO, EC, TDS and salinity in all the treatment tanks are presented in Table 1. There was no significant difference in the T and pH among all the treatment tanks ($p>0.05$). However, there was a significant difference ($p<0.05$) in the DO among the treatments, with the lowest mean of DO observed in T1, which had the highest stocking density (Table 1). The EC was also significantly different among the treatments ($p<0.05$), with mean values of 0.367 $ms\cdot cm^{-1}$, 0.362 $ms\cdot cm^{-1}$, and 0.326 $ms\cdot cm^{-1}$ for T1, T2, and T3, respectively. Moreover, the TDS was significantly higher in T1 and T2, as compared to that of T3 (Table 1). Finally, the average salinity of the water was also significantly higher in T1 and T2 as compared to that of T3, which were 0.166 ppt, 163 ppt, and 0.146 ppt, respectively. It has been documented that with increased stocking density, the EC, TDS and salinity increased and DO decrease.

Values reported are mean for three replications. Mean values±(SD) with a different letter in the same row are significantly different ($p<0.05$).

Chemical water parameters of fish tanks

Chemical water parameters were monitored and recorded in Table 2. The mean ± standard deviation (SD) values for the N compounds such as (NH_3-N), (NH_4^+), (NO_2-N), and NO_3-N, PO_4-P, K, Ca, and Fe that were measured throughout the study are presented in Table 2. The differences in the concentrations of NH_3-N, NH_4^+, and NO_2-N were insignificant ($p>0.05$) among the treatments, but the concentrations increased in higher stocking density. However, the difference in NO_3-N concentration was significant ($p<0.05$) between all three treatments. T1 had the highest concentration of NO_3-N, whereas T3 had the lowest concentration of NO_3-N (Table 2). The percentage of nutrient reduction (NH_3-N, NH_4^+ and NO_2-N) in the stocking density treatments were relatively decreased when increasing stocking density. The concentrations of PO_4-P, Ca, and Fe were not significantly different among all three treatments, except for K^+ ($p<0.05$). In addition, The Ca and the alkalinity values were not significantly different among all the treatments ($p>0.05$) (Table 2). Finally, it has been observed that the concentration NH_3-N, NH_4^+, NO_2-N and NO_3-N

Table 1: Physical water parameters of tilapia tanks in response to different stocking densities in DRAPS conditions during the study period

Physical water parameters	Treatments		
	T1	T2	T3
T (°C)	28.39± 1.38a	28.10± 1.48a	28.36± 1.45a
pH	6.89± 0.20a	6.92± 0.17a	6.90± 0.20a
DO (mg L^{-1})	4.44 ± 0.79b	4.78± 0.71a	4.76± 0.72a
EC (ms cm^{-1})	0.368± 0.073a	0.362± 0.066a	0.326± 0.049b
TDS (g L^{-1})	0.229± 0.046a	0.227± 0.050a	0.203± 0.035b
Salinity(ppt)	0.166± 0.035a	0.163± 0.031a	0.146± 0.026b

Table 2: Chemical water parameters of tilapia tanks in response to different stocking densities in DRAPS conditions during the study period

Chemical water parameters	Treatments		
	T1	T2	T3
NH_3-N (mg L^{-1})	2.09± 2.02a	1.32± 1.02ab	0.99± 0.73b
NH_4 (mg L^{-1})	2.58±2.47a	1.58±1.17ab	1.24±0.87b
NO_2-N (mg L^{-1})	1.80± 3.80a	1.53± 3.22a	1.40± 2.92a
NO_3-N (mg L^{-1})	36.7± 3.70 a	30.77 ± 2.2b	26.13± 0.25b
PO_4-P (mg L^{-1})	15.53± 3.07a	15.83± 2.25a	19.13± 7.14a
K (mg L^{-1})	22.06± 3.43a	22.66± 1.52a	13.81± 2.16b
Ca (mg L^{-1})	107±16a	109± 21.28a	98.67± 45.08a
Fe (mg L^{-1})	0.10± 0.047a	0.08± 0.03a	0.10± 0.020a
Alkalinity ($CaCO_3$ mg L^{-1})	59±. 36.5a	37.33± 1.52a	63.67± 16.50a

and K increased with increased stocking density, while P decreased.

Values reported are mean for three replications. Mean values±(SD) with a different letter in the same row are significantly different($p<0.05$).

Physical water parameters of hydroponic units

Table 3 reports the descriptive statistics of water quality parameters for HP units in different treatments. In this study, the T in the morning and evening, pH, DO, TDS and salinity varied within a small range and were insignificantly different ($P>0.05$) among the treatments.

Values reported are mean for three replications. Mean values±(SD) with a different letter in the same row are significantly different($p<0.05$).

Lettuce growth performance

Table 4 shows the potential of using the nutrient solution from the fish tank to support the growth and production of lettuce. The results showed significant differences ($p<0.05$) in the shoot fresh weight and root fresh weight except for the number of leaves and leaf area. The shoot fresh weight at T1 and T2 were significantly decreased by 23.3% than T2. In contrast, the root fresh weight at T1 and T2 were significantly reduced by 77.2% and 72.7% than T3.

Values reported are mean for three replications. Mean values±(SD) with a different letter in the same row are significantly different($p<0.05$).

Fish growth performance

The growth performances of tilapia are shown in Table 5. The results of the statistical analysis using one-way ANOVA showed that the differences in the final weight, weight gain and feed conversion ratio of tilapia among the stocking density treatments were significant ($p<0.05$). The mean of the final biomass of tilapia in T1 was higher than that in T2 and T3, respectively (Table 5). The highest weight gain was found in T1, whereas the lowest was found in T3. The results also showed that lower FCR were obtained from T3 and T2, respectively. There is no significant difference ($P>0.05$) in the SR of tilapia was observed among all the treatments.

Values reported are mean for three replications. Mean values±(SD) with a different letter in the same row are significantly different($p<0.05$).

The amount of nitrate per 1,000 g of feeds is shown in Figure 2. The results showed that a higher NO_3-N concentration per 1,000 g of feeds, which was 9.12 $mg \cdot L^{-1}$, was obtained from T3, followed by 8.45 $mg \cdot L^{-1}$ and 8.40 $mg \cdot L^{-1}$ from T2 and T1, respectively. Also, the results showed that a higher P concentration was 2.13 $mg \cdot L^{-1}$, was obtained from T3, followed by 1.41 $mg \cdot L^{-1}$ and 1.15 $mg \cdot L^{-1}$ from T2 and T1. The overall view of Figure 6, that low stocking density yields higher NO_3-N and P.

Table 3: Physical water quality parameters in the hydroponic tank in response to different stocking densities in DRAPS conditions

Parameters	Treatments		
	T1	T2	T3
T (°C) morning	28.06± 0.98a	28.01± 0.86a	28.10± 0.92a
T (°C) evening	31.69± 0.97a	31.80±1.03a	31.76±0.87a
pH	7.28± 0.42a	7.28± 0.42a	7.29± 0.40a
EC (ms cm^{-1})	0.337± 0.05a	0.345± 0.08a	0.331± 0.06a
TDS (g L^{-1})	0.209± 0.03a	0.214± 0.05a	0.201± 0.04a
Salinity(ppt)	0.15± 0.02a	0.15± 0.04a	0.14± 0.03a

Table 4: Growth and yield of lettuce in response to different tilapia stocking densities in DRAPS conditions

Growth and yield parameters	Treatments		
	T1	T2	T3
Shoot fresh weight (g $plant^{-1}$)	10.73± 1.63b	9.46± 0.466b	14.00± 1.00a
Root fresh weight (g $plant^{-1}$)	0.40± 0.10 b	0.48± 0.15b	1.76± 0.26 a
Number of leaves (number $plant^{-1}$)	22± 2.00a	18.34± 1.15a	21.67± 3.21a
Leaf area (cm^2 $plant^{-1}$)	180.72±33.50a	162.42±42.99a	212.69±67.05a

Table 5: Growth and yield of tilapia reared for 52 days at different stocking densities under the DRAPS condition

Fish yield Parameters	Treatments		
	T1	T2	T3
Initial biomass (kg)	4.2	3.5	2.8
Final biomass (kg)	7.633±51.61a	6.565±54.08b	5.357±81.66c
WG (kg)	3.433± 51.60 a	3.065± 54.08b	2.557± 81.63 c
FCR	1.27± 0.02 a	1.18±0.02 b	1.14±0.03 b
SR (%)	94.81±1.50 a	95.48±1.51 a	97.05±2.61a

DISCUSSION

Water quality parameters of the fish tank are crucial in APS as they will directly affect the growth, weight gain of the fish (Harmon 2009). In this study, the physical water quality parameters of the tanks, which were T, pH, DO, EC, TDS, and salinity, in all treatments fell within the recommended limits. In this study, the T was within the recommended range for tilapia between 27°C and 30°C (El-Sayed 2006; Delong et al., 2009). Besides T, pH is also one of the most critical factors for the survival of the components in APS, which includes fish, microbes, and plants. Ross (2000) and Delong et al. (2009) and reported that the optimum pH for tilapia growth was between 6.0 and 9.0. With reference to that, the pH of the rearing tanks in this study fell within the range of 6.0 to 7.0, which was similar to the findings of that in Rakocy et al. (2006). Optimizing and maintaining the pH within the range of 6.0 to 7.0 can keep the ammonia in the form of NH_4^+, thus lowering the toxicity level caused by the NH_3. In APS, it is crucial to optimize the T and pH as both parameters correlate strongly to NH_3 concentration. The pH of the water is not only a requirement for fish growth but also to ensure the availability of nutrients and allow optimum nutrient absorption by plants for effective plant growth and development in soilless culture systems. However, the pH levels in this study were higher than the optimum level for plant growth and development, as recommended by Resh (2012), which is between 5.5 and 6.5. The mean values of the water T in this study for both time intervals were higher than the recommended range for lettuce plant, that is, 20°C to 26°C (Resh 2012). Other than T and pH, DO is also one of the most critical environmental factors linked to the proper physiological functions of tilapia and is a limiting factor for the lifespan of fish (Zhao et al., 2018; Li et al., 2018). In this study, it was observed that higher stocking density had a lower DO level. This observation is congruent with the findings of Mahfouz et al. (2015) and Zaki et al. (2020). The mean values of the DO obtained from this study were within the recommended range by Eding et al. (2009), which is between 4.0 mg L^{-1} and 6.0 mg L^{-1}.

The chemical composition of the nutrient solution in the fish tanks was composed of dissolved ions and organic substances produced from the biological processes of nitrifying bacteria. In this study, it was observed that the concentrations of NH_3-N, NH_4, NO_2-N, NO_3-N, and K increased in higher stocking density except for P. According to Durborow et al. (1997), Hargreaves and Tucker (2004), Zaki et al. (2020), and Capkin et al. (2010), the accumulation of feces and N in the form of NH_3 and NO_2 will negatively affect the water quality. The TAN concentration for T2 and T3 was within the recommended value by Timmons et al. (2002), which is below 3.0 mg·L^{-1} whereas that of T1 exceeded the recommended value. During the first week of the

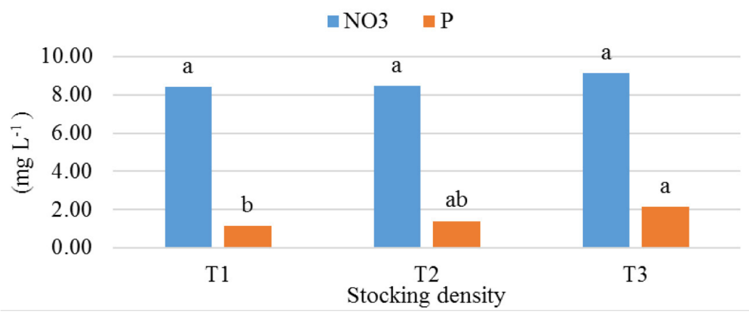

Figure 2. The concentration of nitrate and phosphorus per 1000g feeding under three stocking density

study period, the NO_2-N concentration increased with the stocking density, and it was higher than that of the recommended value by Timmons et al. (2002), which is below 1.0 mg·L^{-1}. Additionally, DeLong et al. (2009) found out that the optimal NO_2-N lower than 5 mg·L^{-1}. In DRPAS, NO_3-N is also one of the most critical end products from the nitrification process. The NO_3-N level in this study did not meet the optimum level of NO_3-N for the growth of lettuce in HP recommended by Resh (2012), which is 165.0 mg·L^{-1}, but fell within the range of 26.3 mg·L^{-1} to 42.0 mg·L^{-1}, which was the recommended range for APS by Rakocy et al. (2006). Also, the concentrations of P and K in this study did not fell within the recommended range for optimum lettuce growth by Resh (2012). However, studies by Adler et al. (1996), Seawright et al. (1998), and Graber and Junge (2009) reported that APS, which relies solely on fish waste as the source of nutrients for plants, had a lower concentration of P and K. Hence, the inorganic P and K supplies in different types of APS become the main factor for optimum growth and development, resulting in the improvement of quality and quantity of plants. Furthermore, it was observed that the Fe production from the nitrification process was minimal. This phenomenon was supported by Adler et al. (1996), Rakocy et al. (2006), Graber and Junge (2009), Roosta and Hamidpour (2011), and Nozzi et al. (2018), who reported that Fe was one of the most limited micronutrients that were produced from the fish waste in APS. As such, throughout the entire period of this study, the effects of Fe deficiency, such as suppressed growth and leaf chlorosis, were observed. This condition was further explained by Briat (2007), which reported that Fe deficiency affected both of the plant's physiology and morphology by suppressing the growth of leaves, causing leaf chlorosis, and loss of turgidity. Kosegarten and Koyro (2001) also reported that Fe deficiency inhibited the formation of new leaves. In this study, the plant roots did not have any significant role in the removal of nutrients such as NH_3-N, NH_4^+, and NO_2-N, which were toxic to the fish, from the first loop of the DRAPS because the plants were located in the second loop. Instead, the mechanical and biofilter tanks installed in the first loop played a major role in removing the toxic nutrients. Moreover, in contrast to SRAPS, the plant roots provided a surface area for the nitrifying bacteria to oxidize the toxic ammonia to nitrates. Fully developed roots have larger surface areas that can provide more space for the inhabitation of nitrifying bacteria. This catalyzes the nitrification process, which significantly lowered the concentration of NH_3-N, NH_4^+, and NO_2-N by converting them to soluble nitrates (Estim et al., 2018). Furthermore, Trang and Brix (2012) stated that (*Canna glauca L.*) can be used as a biofilter for removing NH_4-N from the nitrification-denitrification process. Besides that, Moya et al. (2016) also suggested some herbs such as basil, peppermint, and spearmint as the biofilter in SRAPS. The results from these previous studies indicated that nitrifying bacteria were associated with the root surface area of the plants, and this maintained the water quality in SRAPS (Knaus and Palm 2017). While in DRAPS, the only advantage of having a larger root surface area is to increase the nutrients absorption rate of the lettuce.

In this study, three different stocking densities of tilapia were tested in order to observe its effects on lettuce growth in small-scale production. The main difference among the stocking densities was the concentrations of nutrients produced, which in turn, affected the growth and development of the lettuce. According to Pérez-Urrestarazu et al. (2019), the productivity of APS is highly dependent on the type of lettuce and environmental conditions. In this study, the air and water T, EC, and pH were not controlled and there were no external inorganic nutrients supplied to the HP units. In terms of lettuce yield in all the treatments, the observed shoot fresh weight, number of leaves, root fresh weight and leaf area were lower than the values reported in the studies conducted by Licamele (2009), Schmautz et al. (2017), Nozzi et al. (2018), and Madar et al. (2019). Being nitrate has a crucial role in determining the effectiveness of a DRAPS and determining the optimal growth of leafy plants such as lettuce. The high planting density of 32 plant·m^{-2} together with the low nitrate level in all the treatments, has contributed to the poor growth performances of the lettuce. Rakocy et al. (2006) recommended the range from 26.3 mg·L^{-1} to 42.0 mg·L^{-1} as the optimum nitrate level for the healthy growth of leafy plants in APS. Regarding that, the nitrate levels in this study were between 26.13 mg·L^{-1} and 36.7 mg·L^{-1}, which fell within the recommended range. Despite that, the lettuce still ceased to grow. This can be explained by using the results of other water quality parameters such as T and pH, which were not in the optimal range. The elevated T and pH may have contributed to the stunted growth of the lettuce by decreasing the absorption of the nutrients. During the period of vegetative growth in this study, the Fe deficiency symptoms (chlorosis and suppressed growth) began to be visible on the new lettuce leaves in all the NFT units. According to Jones et al. (2005), the nutrient requirements for leafy plants such as lettuce increases with time during vegetative growth. However, the concentrations of the nutrients (N, P, K, Mg, Ca, and Fe) that were produced in the first loop were extremely low and in turn, stunted the growth of lettuce. Regarding this, Rakocy et al. (2007) also stated that low yield in APS might be associated with low K, P, Fe, and Mn concentrations in the nutrient solution. Nozzi et al. (2018) also reported that P deficiency in APS led to low nitrogen uptake by lettuce. One of the main limitations when comparing the yields of lettuce in APS is that the background data, such as the lettuce type, seedling age, planting density, and growth period, are not available. Besides, optimization of physical water quality like T and pH to ensure nutrients availability in APS is a complex process.

The stocking density of tilapia is one of the most significant factors that have direct impacts on the weight gain, behavior and yield in APS. The tilapia were gaining more weight in higher stocking density. While, the FCR values ranged between 1.14 and 1.27, which were similar to the productive recirculating aquaculture performance with an FCR value of 1.25 reported by El-Sayed (2006) and Timmons and Ebeling (2013). Besides, the FCR values in this study were lower and more preferable than those reported by Rakocy et al. (2006), which were between 1.70 to 1.80. The SR of tilapia was higher in lower stocking density, which was within the normal range for tilapia as reported by El-Sayed (2006).

CONCLUSION

The results showed that the stocking density of fish had impacted the tilapia growth, water quality and production of lettuce. The lowest stocking density of gave the highest yield of lettuce. The uncontrolled temperature and pH, along with the low nitrogen, phosphorus, potassium and iron concentrations, were the major reasons for the low growth and yield of lettuce. This study approves the importance of supplementing inorganic nutrients and optimizing the pH in decoupled recirculation aquaponic systems. This study validated that decoupled recirculation aquaponic systems that relied solely on fish waste have a low concentration of elements such as nitrate, phosphorus, potassium and iron to supply nutrients for plants in contrast to hydroponics. The total fish biomass yield and weight gain, feed conversion ratio increased in higher stocking density. Finally, considering the specific decoupled recirculation aquaponic systems design used in this study, the lettuce roots did not play any role in removing ammonia, ammonium, and nitrite, but only for nutrients uptake.

REFERENCES

1. Adler, P., Takeda, F., Glenn, D., & Summerfelt, S. 1996. Utilizing byproducts to enhance aquaculture sustainability. World Aquaculture, 27(2), 24–26.
2. Ajitama, P., Effendi, H., & Hariyadi, S.2018. Usage of fisheries rearing waste for butterhead lettuce (Lactuca sativa L. var. capitata) cultivation in recirculation. Nature Environment and Pollution Technology, 17(1), 145–151.
3. Blanchard, C., Wells, D. E., Pickens, J. M., & Blersch, D. M. 2020. Effect of pH on Cucumber Growth and Nutrient Availability in a Decoupled Aquaponic System with Minimal Solids Removal. Horticulturae, 6(1), 10.
4. Briat, J. F., Curie, C., & Gaymard, F.2007. Iron utilization and metabolism in plants. Current opinion in plant biology, 10(3), 276–282. https://doi.org/10.1016/j.pbi.2007.04.003
5. Brown, S., McIvor, K., & Snyder, E. H. (Eds.) .2016. Sowing seeds in the city: Ecosystem and municipal services. Dordrecht: Springer
6. Buzby, K. M., & Lin, L. S.2014. Scaling aquaponic systems: Balancing plant uptake with fish output. Aquacultural Engineering, 63, 39–44. https://doi.org/10.1016/j.aquaeng.2014.09.002
7. Capkin, E., Kayis, S., Boran, H., & Altinok, I. 2010. Acute toxicity of some agriculture fertilizers to rainbow trout. Turkish Journal of Fisheries and Aquatic Sciences, 10(1), 19–25. https://doi.org/10.4194/trjfas.2010.0103
8. Delaide, B., Goddek, S., Gott, J., Soyeurt, H., & Jijakli, M. H.2016. Lettuce (Lactuca sativa L. var. Sucrine) growth performance in complemented aquaponic solution outperforms hydroponics. Water, 8(10), 467. https://doi.org/10.3390/w8100467
9. DeLong, P.D., Losordo, M.T. and Rakocy, J.E.2009. Tank Culture of Tilapia. SRAC Publication, No. 282, Texas, USA, 7 pp

10. Eding, E. H., Kamstra, A., Verreth, J. A. J., Huisman, E. A., & Klapwijk, A.2006. Design and operation of nitrifying trickling filters in recirculating aquaculture: a review. Aquacultural engineering, 34(3), 234–260. https://doi.org/10.1016/j.aquaeng.2005.09.007
11. Ebeling, J. M., Timmons, M. B., & Bisogni, J. J.2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia–nitrogen in aquaculture systems. Aquaculture, 257(1–4), 346–358. https://doi.org/10.1016/j.aquaculture.2006.03.019
12. El-Sayed, A.-F.M. Tilapia Culture; CABI Publishing: Oxfordshire, UK, 2006.
13. Emerson, K., Russo, R.C., Lund, R.E. and Thurston, R.V.1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. Journal of the Fisheries Board of Canada, 32(12), pp.2379–2383.
14. Endut, A., Jusoh, A., Ali, N., Nik, W. W., & Hassan, A.2010. A study on the optimal hydraulic loading rate and plant ratios in recirculation aquaponic system. Bioresource technology, 101(5), 1511–1517. https://doi.org/10.1016/j.biortech.2009.09.040
15. Espinosa Moya, E. A., Angel Sahagún, C. A., Mendoza Carrillo, J. M., Albertos Alpuche, P. J., Álvarez-González, C. A., & Martínez-Yáñez, R.2016. Herbaceous plants as part of biological filter for aquaponics system. Aquaculture Research, 47(6), 1716–1726. https://doi.org/10.1111/are.12626
16. Estim, A., Saufie, S., & Mustafa, S.2019. Water quality remediation using aquaponics sub-systems as biological and mechanical filters in aquaculture. Journal of Water Process Engineering, 30, 100566. https://doi.org/10.1016/j.jwpe.2018.02.001
17. Geisenhoff, L. O., Jordan, R. A., Santos, R. C., Oliveira, F. C. D., & Gomes, E. P. 2016. Effect of different substrates in aquaponic lettuce production associated with intensive tilapia farming with water recirculation systems. Engenharia Agrícola, 36(2), 291–299. https://doi.org/10.1590/1809–4430-Eng.Agric.v36n2p291–299/2016.
18. Graber, A., & Junge, R. 2009. Aquaponic Systems: Nutrient recycling from fish wastewater by vegetable production. Desalination, 246(1–3), 147–156. https://doi.org/10.1016/j.desal.2008.03.048
19. Hargreaves, J.A. and Tucker, C.S.2004. Managing ammonia in fish ponds (Vol. 4603). Stoneville, MS: Southern Regional Aquaculture Center.
20. Harmon, T. S. 2009. Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. Reviews in Aquaculture, 1(1), 58–66. https://doi.org/10.1111/j.1753–5131.2008.01003.x
21. Jimoh, W.A., Kamarudin, M.S., Sulaiman, M.A. and Dauda, A.B.2019. Assessment of prebiotic potentials in selected leaf meals of high dietary fibre on growth performance, body composition, nutrient utilization and amylase activities of a tropical commercial carp fingerlings. Aquaculture Research, 50(11), 3401–3411. https://doi.org/10.1111/are.14298
22. Jones, J. B., 2005. Hydroponics: a practical guide for the soilless grower. CRC Press
23. Jordan, R.A., Geisenhoff, L.O., Oliveira, F.C.D., Santos, R.C. and Martins, E.A. 2018a. Yield of lettuce grown in aquaponic system using different substrates. Revista Brasileira de Engenharia Agrícola e Ambiental, 22(1), 27–31. https://doi.org/10.1590/1807–1929/agriambi.v22n1p27–31
24. Jordan, R. A., Ribeiro, E. F., Oliveira, F. C. D., Geisenhoff, L. O., & Martins, E. A.2018. Yield of lettuce grown in hydroponic and aquaponic systems using different substrates. Revista Brasileira de Engenharia Agrícola e Ambiental, 22(8), 525–529. https://doi.org/10.1590/1807–1929/agriambi.v22n8p525–529
25. Karimanzira, D., Keesman, K. J., Kloas, W., Baganz, D., & Rauschenbach, T. 2016. Dynamic modeling of the INAPRO aquaponic system. Aquacultural engineering, 75, 29–45. https://doi.org/10.1016/j.aquaeng.2016.10.004
26. Kloas, W., Groß, R., Baganz, D., Graupner, J., Monsees, H., Schmidt, U., Staaks, G., Suhl, J., Tschirner, M., Wittstock, B. and Wuertz, S.2015. A new concept for aquaponic systems to improve sustainability, increase productivity, and reduce environmental impacts. Aquaculture Environment Interactions 7(2), 179–192. https://doi.org/10.3354/aei00146
27. Knaus, U., & Palm, H. W. 2017. Effects of the fish species choice on vegetables in aquaponics under spring-summer conditions in northern Germany (Mecklenburg Western Pomerania). Aquaculture, 473,62–73. https://doi.org/10.1016/j.aquaculture.2017.01.020
28. Kosegarten, H., & Koyro, H. W. 2001. Apoplastic accumulation of iron in the epidermis of maize (Zea mays) roots grown in calcareous soil. Physiologia plantarum, 113(4), 515–522. https://doi.org/10.1034/j.1399–3054.2001.1130410.x
29. Li, M., Wang, X., Qi, C., Li, E., Du, Z., Qin, J. G., & Chen, L. 2018. Metabolic response of Nile tilapia (Oreochromis niloticus) to acute and chronic hypoxia stress. Aquaculture, 495, 187–195. https://doi.org/10.1016/j.aquaculture.2018.05.031
30. Liang, J. Y., & Chien, Y. H. 2013. Effects of feeding frequency and photoperiod on water quality and crop production in a tilapia–water spinach raft aquaponics system. International Biodeterioration & Biodegradation, 85, 693–700. https://doi.org/10.1016/j.ibiod.2013.03.029
31. Licamele J. 2009. Biomass production and nutrient dynamics in an aquaponics system. PhD thesis.

Department of Agriculture and biosystems engineering, University of Arizona

32. Love, D.C., Fry, J.P., Li, X., Hill, E.S., Genello, L., Semmens, K., Thompson, R.E.2015. Commercial aquaponics production and profitability: Findings from an international survey. Aquaculture. 435. 67–74. https://doi.org/10.1016/j.aquaculture.2014.09.023
33. Madar, Á.K., Rubóczki, T. and Hájos, M.T. 2019. Lettuce production in aquaponic and hydroponic systems. Acta Universitatis Sapientiae, Agriculture and Environment, 11(1),51–59. https://doi.org/10.2478/ausae-2019–0005
34. Mahfouz, M. E., Hegazi, M. M., El-Magd, M. A., & Kasem, E. A.2015. Metabolic and molecular responses in Nile tilapia, Oreochromis niloticus during short and prolonged hypoxia. Marine and Freshwater Behaviour and Physiology, 48(5), 319–340. https://doi.org/10.1080/10236244.2015.1055915
35. Monsees, H., Kloas, W. and Wuertz, S. 2017. Decoupled systems on trial: Eliminating bottlenecks to improve aquaponic processes. PloS one, 12(9), p.e0183056. https://doi.org/10.1371/journal.pone.0183056
36. Monsees, H., Suhl, J., Paul, M., Kloas, W., Dannehl, D. and Würtz, S.2019. Lettuce (Lactuca sativa, variety Salanova) production in decoupled aquaponic systems: Same yield and similar quality as in conventional hydroponic systems but drastically reduced greenhouse gas emissions by saving inorganic fertilizer. PLoS ONE 14(6): e0218368. https://doi.org/10.1371/journal.pone.0218368
37. Nozzi, V., Graber, A., Schmautz, Z., Mathis, A., & Junge, R. 2018. Nutrient management in aquaponics: comparison of three approaches for cultivating lettuce, mint and mushroom herb. Agronomy, 8(3), 27. https://doi.org/10.3390/agronomy8030027
38. Pérez-Urrestarazu, L., Lobillo-Eguíbar, J., Fernández-Cañero, R., & Fernández-Cabanás, V. M.2019. Suitability and optimization of FAO's small-scale aquaponics systems for joint production of lettuce (Lactuca sativa) and fish (Carassius auratus). Aquacultural Engineering, 85, 129–137. https://doi.org/10.1016/j.aquaeng.2019.04.001
39. Durborow, R. M., Crosby, D. M., & Brunson, M. W. 1997. Nitrite in fish ponds. Southern Regional Aquaculture Center. SRAC Publication No. 462. http://www.aces.edu/dept/fisheries/aquaculture/pdf/462fs.pdf
40. Rakocy, J.E, Shultz, R.C., Bailey, D.S., Thoman, E.S. 2004. Aquaponic production of tilapia and basil: comparing a batch and staggered cropping system. Acta Horticulturae. (ISHS) 648:63–69. https://doi.org/10.17660/ActaHortic.2004.648.8
41. Rakocy, J.E., Masser, M.P., Losordo, T.M.2006. Recirculating aquaculture tank production systems: aquaponics–integrating fish and plant culture. SRAC publication, 454, 1–16.
42. Rakocy, J.2007. Ten Guidelines for Aquaponic Systems. Aquaponics Journal, 46, 14–17. Retrieved from http://santarosa.ifas.ufl.edu/wp-content/uploads/2013/06/Aquaponics-Journal-10-Guidelines.pdf.
43. Rakocy, J.E. 2012. Aquaponics: integrating fish and plant culture. Aquaculture production systems, 1, 344–386. https://doi.org/10.1002/9781118250105.ch14
44. Resh, H.M. 2012. Hydroponic Food Production: A Definitive Guidebook for the Advanced Home Gardener and the Commercial Hydroponic Grower. Boca Raton, Florida: CRC Press. Retrieved from http://howardresh.com/dr-howard-resh-hydroponic-services/hydroponic-lettuce-production-i/
45. Resh, H.M. 2013. Hydroponic food production: a definitive guidebook for the advanced home gardener and the commercial hydroponic grower. CRC Press.
46. Roosta, H. R., & Hamidpour, M. 2011. Effects of foliar application of some macro- and micro-nutrients on tomato plants in aquaponic and hydroponic systems. Scientia Horticulturae, 129(3), 396–402. https://doi.org/10.1016/j.scienta.2011.04.006
47. Ross, L.G .2000. Environmental physiology and energetics. In Tilapias: Biology and Exploitation; McAndrew, B.J., Ed.; Springer Netherlands: Dordrecht, The Netherlands,89–128
48. Schmautz, Z., Graber, A., Jaenicke, S., Goesmann, A., Junge, R., & Smits, T. H. 2017. Microbial diversity in different compartments of an aquaponics system. Archives of microbiology, 199(4), 613–620. https://doi.org/10.1007/s00203–016–1334–1
49. Seawright, D.E., Stickney, R. R., & 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
50. Silva, L., Escalante, E., Valdés-Lozano, D., Hernández, M. and Gasca-Leyva, E. 2017. Evaluation of a semi-intensive aquaponics system, with and without bacterial biofilter in a tropical location. Sustainability, 9(4), 592. https://doi.org/10.3390/su9040592
51. Sreejariya, P., Raynaud, T., Dabbadie, L. and Yakupitiyage, A.2016. Effect of water recirculation duration and shading on lettuce (Lactuca sativa) growth and leaf nitrate content in a commercial aquaponic system. Turkish Journal of Fisheries and Aquatic Sciences, 16 (2): 311–319. http://dx.doi.org/10.4194/1303–2712-v16_2_11
52. Suhl, J., Dannehl, D., Kloas, W., Baganz, D., Jobs, S., Scheibe, G. and Schmidt, U.2016. Advanced aquaponics: Evaluation of intensive tomato production in aquaponics vs. conventional hydroponics.

Agricultural Water Management, 178,.335–344. https://doi.org/10.1016/j.agwat.2016.10.013

53. Suhl, J., Dannehl, D., Baganz, D., Schmidt, U. and Kloas, W.2018a. An innovative suction filter device reduces nitrogen loss in double recirculating aquaponic systems. Aquacultural engineering, 82,.63–72. https://doi.org/10.1016/j.aquaeng.2018.06.008.
54. Suhl, J., Dannehl, D., Zechmeister, L., Baganz, D., Kloas, W., Lehmann, B., Scheibe, G. and Schmidt, U .2018b. Prospects and challenges of double recirculating aquaponic systems (DRAPS) for intensive plant production. Acta horticulturae. 1227, 449–456. https://doi.org/10.17660/ActaHortic.2018.1227.56.
55. Sveier, H., Raae, A.J. and Lied, E. 2000. Growth and protein turnover in Atlantic salmon (Salmo salar L.); the effect of dietary protein level and protein particle size. Aquaculture, 185(1–2), pp.101–120. https://doi.org/10.1016/S0044–8486(99)00344–0
56. Timmons, M.B., and Ebeling, J, M.2013. Recirculating Aquaculture (3rd ed.). Reading, United Kingdom: Ithaca Publishing Company. Retrieved from https://www.amazon.in/Recirculating-Aquaculture-3rd-Michael-Timmons/dp/0971264651
57. Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt,S.T. & Vinci, B.J.2002 Recirculating Aquaculture Systems (2nd Ed.). Cayuga Aqua Ventures, Ithaca, NY, Northeastern Regional Aquaculture Center Publication 01–002
58. Trang, N. T, D., and Brix, H. 2014. Use of planted biofilters in integrated recirculating aquaculture-hydroponics systems in the Mekong Delta, Vietnam. Aquac Res, 45: 460–469. https://doi:10.1111/j.1365–2109.2012.03247.
59. Zaki, M.A., Alabssawy, A.N., Nour, A.E.A.M., El Basuini, M.F., Dawood, M.A., Alkahtani, S. and Abdel-Daim, M.M. 2020. The impact of stocking density and dietary carbon sources on the growth, oxidative status and stress markers of Nile tilapia (Oreochromis niloticus) reared under biofloc conditions. Aquaculture Reports, 16, p.100282. https://doi.org/10.1016/j.aqrep.2020.100282
60. Zhao, Z., Dong, S. and Xu, Q. 2018. Respiratory response of grass carp Ctenopharyngodon idellus to dissolved oxygen changes at three acclimation temperatures. Fish physiology and biochemistry, 44(1), pp.63–71. https://doi.org/10.1007/s10695–017–0413–9