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Does fish stocking rate affect the photosynthesis of *Lactuca sativa* grown in an aquaponic system?

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Abstract: The depletion of natural resources such as freshwater and cropland makes it necessary to find a new solution for sustainable food production. Aquaponic systems seem to be a great alternative to traditional agriculture, however, there are still many unknowns that need to be explored. It is already known how fish stocking affects water quality in aquaponic systems, but not how it affects the plants' growth, and especially on chlorophyll fluorescence. In this study, we examined how the density of 0, 2, 4, 8, and 16 stocking fish in five aquaria affects lettuce growth. The first tank was only a hydroponic system with plants but without fish (control). In the remaining four aquaria – 2, 4, 8 and 12 specimens of common carp fry with an average weight of 20 grams (average 8.5–33.2 g) were placed in the aquaponic growing system. Physicochemical analysis of water was conducted to determine the levels of pH, electrical conductivity (*EC*), $N\text{-}NO_3$, $N\text{-}NO_2$, $N\text{-}NH_4$, $P\text{-}PO_4$, O_2 and physiological parameters of plants (nitrogen balance index – *NBI*, chlorophyll content index – *CCI*, quantum yield – *QY*, flavonoid content – Flv) were analysed. The results showed that fish stocking density has different effects on plant physiological parameters, but in most cases, was insignificant. It seems that the greater number of fishes and higher density indirectly causes growth inhibition (lower photosynthetic efficiency) due to the increase of $N\text{-}NO_3$ and a decrease of O_2 in the water.

Keywords: aquaponics, chlorophyll fluorescence, common carp, lettuce, plant production

INTRODUCTION

Aquaponics is the simultaneous (integrated) rearing of fish and growing plants (Buzby and Lin, 2014; Goddek *et al.*, 2015; Yavuzcan Yildiz *et al.*, 2017). The system is based on the principle that waste produced by feeding fish is assimilated by naturally occurring bacteria in the aquatic environment, which mineralise these substances and increase the uptake of key elements by plants, nourishing them and promoting their healthy growth. At the same time, the microorganisms purify the water circulating in the fish-plant system in the same way (Thorarinsdottir *et al.*, 2015; Lennard and Goddek, 2019).

Currently, aquaculture is one of the fastest growing sectors of the global economy, and the benefits of aquaponic systems have been published and implemented in crop production several times (Griessler Bulc *et al.*, 2012; Hamilton *et al.*, 2013; Petrea *et al.*, 2016; Sapkota, Sapkota and Liu, 2019; Majid *et al.*, 2021; Kolek and Irnazarow, 2022). One of the major advantages is the elimination of additional water purification facilities after production before the water is released into the environment.

Cultivation in aquaponic systems does not require a large area of land compared to field cultivation and is an excellent alternative for countries with nutrient-poor soils and in countries where pollution precludes the traditional cultivation system (Love *et al.*, 2014; Junge *et al.*, 2017; Mchunu, Lagerwall and Senzanje, 2018; Obirikorang *et al.*, 2021).

The cultivation of plants requires the adaptation of suitable growth conditions. Crop productivity is limited by various factors such as salinity, water scarcity, and heat stress (Hewedy *et al.*, 2022). Photosynthesis is the crucial process by which producers – green plants, some bacteria, and algae – convert light energy into chemical energy. This process involves a chain of reactions, each of which provides very specific information about a plant's physiological state.

The aim of this work was to investigate the relationship between the fish density in the aquarium of carp (*Cyprinus carpio* L.) and the efficiency of the photosynthetic apparatus of crisp lettuce – a variety of lettuce *Lactuca sativa* L. Moreover, the study was conducted with a view to future commercial cultivation of plants in aquaponic systems.

MATERIALS AND METHODS

The experiment was conducted from February 1 to March 25, 2022, in the laboratory of the Institute of Technology and Life Sciences – Branch of the National Research Institute in Szczecin (Szczecin) (Pol.: Instytut Technologiczno-Przyrodniczy – Państwowy Instytut Badawczy Oddział w Szczecinie) in two cropping systems: hydroponics and aquaponics.

Five aquariums equipped with filters and aerators, and filled with water (60 dm^3 capacity) were used. The plants were grown in hydroponic systems on floating styrofoam sheets (2 cm thick) and fixed in 7.5 cm diameter holes where mounting baskets were placed.

The plant material used in the experiment was crisp lettuce (*Lactuca sativa* L.). Eight lettuce seedlings were placed in each of the aquaria, for a total of 40 plants throughout the experiment. Light-expanded clay aggregate (LECA) served as a drainage material while stabilising the plants in the system. The plants were illuminated by means of two 4 m LED strips. Light intensity was about 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the lighting period was 14 hours per day. Plants were fertilised with Terra Aquatica Original FloraGro liquid fertiliser (108 $cm^3 \cdot 60\ dm^{-3}$ of water).

The first tank was only a hydroponic system with plants but without fish (control). In the remaining four aquaria – 2, 4, 8 and 12 specimens of common carp (*Cyprinus carpio* L.) fry with an average weight of 20 grams (average 8.5–33.2 g) were placed in the aquaponic growing system. The fish were fed daily with high-quality, medium-energy carp feed (size 3.0 mm) with protein (40%), fat (21%), fibre (1.8%) and ash (5.5%).

Every seven days water samples were taken from each system and the hydrochemical analyses were done, and water was replaced in the amount of 20% of the total tank volume.

PHYSIOLOGICAL PARAMETERS OF PLANTS

Nitrogen balance index (*NBI*) is defined as the ratio of chlorophyll to epidermal flavonoids (Chl:Flv), chlorophyll (Chl) and flavonoids (Flv) contents in the lettuce leaves was determined using the DUALEX FORCE A device (Force-A Company, Orsay, France). Before starting the measurements of chlorophyll fluorescence (*QY* parameter which is equal to *Fv*:*Fm* parameter – the maximum potential quantum efficiency of Photosystem II, where *Fv* is the variable and *Fm* is the maximal fluorescence) of plants using FluorPen FP100 (Photon Instruments Company, Drasov, Czech Republic), the plants were completely adapted in the dark for at least 30 minutes. Measurements were made on a single lettuce leaf in four replications for each experimental variant.

PHYSICOCHEMICAL ANALYSES OF WATER

The pH of the water in the collected water samples, electrical conductivity (*EC*), and oxygenation parameters were measured using a multi-parameter gauge HQD30 produced by Hach Company (Düsseldorf, Germany). The content of $N\text{-}NO_3$, $N\text{-}NO_2$ and $N\text{-}NH_4$ was determined in the collected water samples, for this purpose the Slandi250 photometer (Michałowice, Poland) was used, while the $P\text{-}PO_4$ content was determined by the AQUALYTIC PC-compact device (Dortmund, Germany). The temperature and air humidity were measured by the Testo 605-H1 hytherograph (Lenzkirch, Germany). All measurements were performed in four replications.

FISH PARAMETERS

The carp fry were obtained from the pond facility of the Polish Angling Association (Pol.: PZW – Polski Związek Wędkarski) in Goleniów (Poland). Prior to the arrival of the fish, each specimen was measured and weighed (total length (*TL*) and body length (*SL*) in cm, weight in g) by an ichthyologist from the PZW using a caliper with an accuracy of 1 mm. The unit weight of the fish was determined on a balance with an accuracy of 0.1 g and then assigned to the appropriate experimental variant (aquarium) before transport.

After the fish were brought to the laboratory, individual animals were released into the aquarium after a 20-minute acclimation period. All aquaria had the same water temperature, lighting, and oxygenation during the whole time of the experiment. All fish were fed every two days with the high-quality, medium-energy fattening feed for carp SteCo SuPreme-15. At the end of the experiment, the fish were handed over to the PZW ichthyologists and transported to the fish ponds, where again specialised personnel weighed and measured the individual fish, which were then released into the breeding ponds.

The condition of the fish was assessed on the basis of the condition factors: Fulton – *K*, (Czerniejewski *et al.*, 2019). This parameter was estimated according to the general formula:

$$K = \frac{W_1 \cdot 100000}{L^3} \tag{1}$$

where: W_1 = individual fish weight in g, L = body length (*SL*) of the fish in mm.

The statistical analysis of the data was performed on the basis of multifactor analysis of variance (ANOVA) using the Statistica 13.3 software (Statsoft Inc., Tulsa, OK, USA).

RESULTS

The nitrogen balance index (*NBI*) remained consistent across all treatments during the initial three measurement dates and exhibited no variations over time (refer to Fig. 1). Moving to the subsequent date (15.03.2022), the mean values displayed an increase compared to the preceding dates, yet no noteworthy variations were observed among the treatments. On the final date (22.03.2022), the *NBI* value within the hydroponic cultivation reached 8.95 rel. u. Meanwhile, values measured within the aquaponic cultivation with 2 and 4 fish demonstrated significant elevation in contrast to the control group. Concurrently, the *NBI* values observed in the aquaponic culture involving 8 and 16 fish exhibited notable increases when compared to the control group.

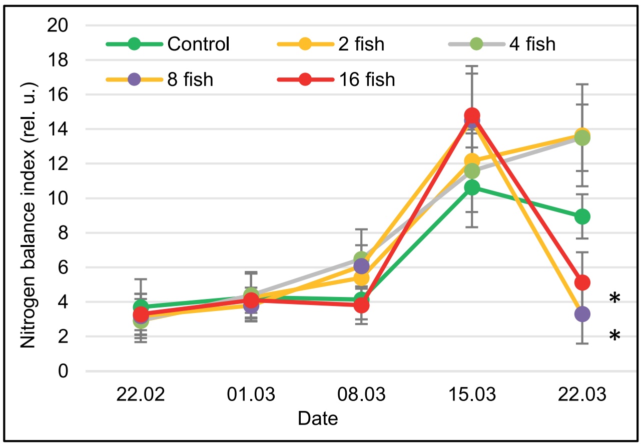


Fig. 1. Nitrogen balance index of *Lactuca sativa* L. in hydroponic (control) and aquaponic (2, 4, 8 and 16 fish) cultivation; means ±*SD*, values marked by an asterisk differ significantly from the control ones (n = 32, p = 0.05); source: own study

The chlorophyll content index (*CCI*) displayed significant changes solely on the last period day (22.03.2022). Throughout this timeframe, the *CCI* value within the hydroponic cultivation reached 4.84 rel. u. as depicted in Figure 2. Notably, values recorded within the aquaponic culture involving 2 and 4 fish exhibited a substantial increase compared to the control condition. Conversely, the *CCI* values within the aquaponic culture comprising of 8 and 16 fish did not exhibit any noteworthy deviations from those observed in the control group.

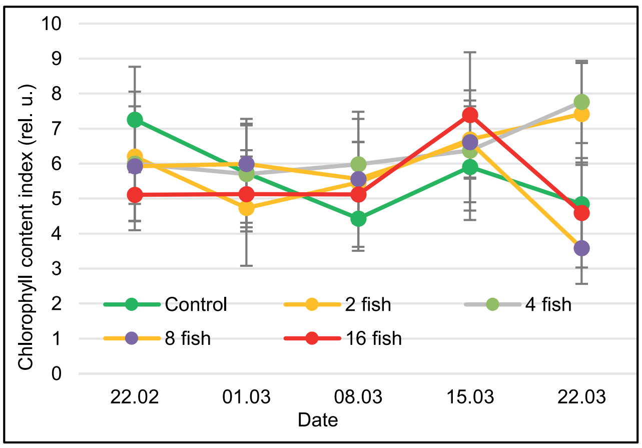


Fig. 2. Chlorophyll content index of *Lactuca sativa* L. in hydroponic (control) and aquaponic (2, 4, 8 and 16 fish) cultivation; means ±*SD*, (n = 32, p = 0.05); source: own study

The analysis of flavonoid content (Flv) revealed a consistent trend of diminishing levels across all treatments as time progressed, with the exception of the final date. Nonetheless, there were no statistically significant variations observed among the treatments on each individual date, as illustrated in Figure 3.

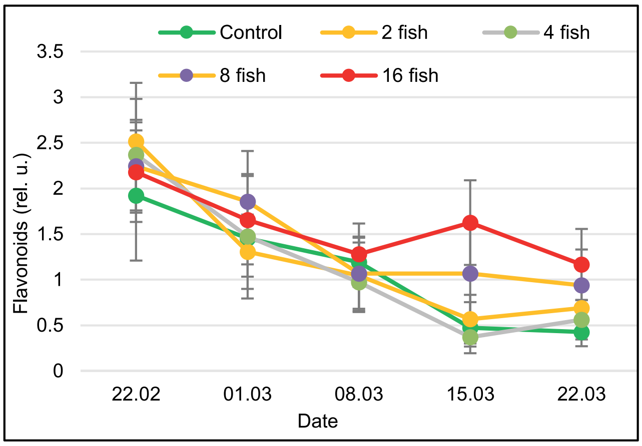


Fig. 3. Flavonoids content of *Lactuca sativa* L. in hydroponic (control) and aquaponic (2, 4, 8 and 16 fish) cultivation; means ±*SD*, (n = 32, p = 0.05); source: own study

The quantum yield (*QY*) of photosynthetic efficiency assessed in the control plants exhibited a gradual decline starting from 07.02.2022. A parallel trend was evident in plants cultivated aquaponically with 8 and 16 fish. However, no statistically significant disparities emerged between these treatments over the course of the experimental timeline. Notably, it's important to highlight that on the concluding day (22.03.2022), the parameter values for plants originating from aquaponic systems involving 2 and 4 fish were significantly greater in comparison to those observed in the control plant group (see Fig. 4).

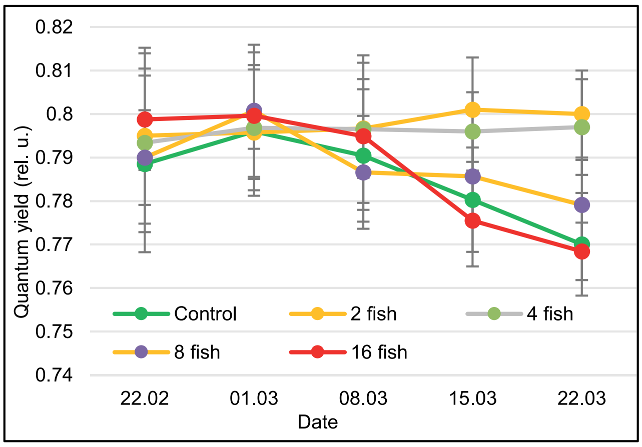
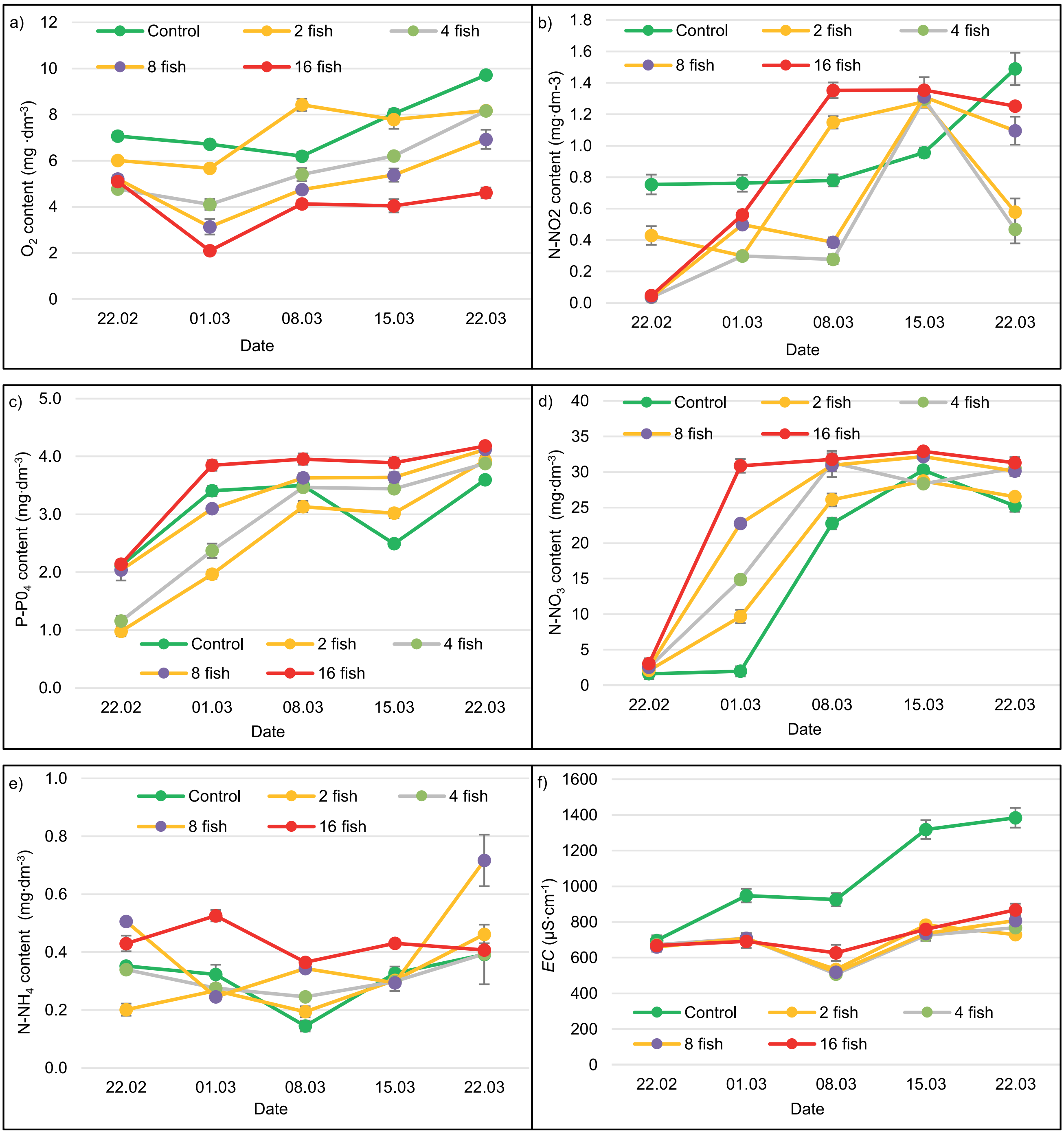


Fig. 4. Quantum yield of *Lactuca sativa* L. in hydroponic (control) and aquaponic (2, 4, 8 and 16 fish) cultivation; means ±*SD*, (n = 32, p = 0.05); source: own study

The quantity of fish within the aquarium distinctly influenced the physicochemical attributes of the water (see Fig. 5). In aquaria housing 8 and 16 fish, both oxygen content (O_2) and electrical conductivity (*EC*) experienced a significant decrease from 01.03.2022 until the experiment's culmination. Conversely, $P\text{-}PO_4$ and $N\text{-}NO_3$ levels exhibited a tendency to elevate over the course of time across all treatments; however, notable variations between the control group and aquaria containing 8 and 16 fish were evident. The trajectory of $N\text{-}NO_2$ values demonstrated an upward trend until 15.03.2022, following which these values continued to rise in the hydroponic setup but declined in the aquaponic system. The fish quantity had a discernible impact on the water's pH, as observed by lower values in the hydroponic culture when compared to the aquaponic culture on 15.03.2022 and 22.03.2022. Notably, no significant distinctions were identified between the $N\text{-}NH_4$ parameter values.

The results of the correlation analysis indicated several significant associations. The quantum yield (*QY*) parameter for *L. sativa* demonstrated a positive correlation with both the water's pH and the fish population within the aquarium, as detailed in Table 1. Conversely, there was a negative correlation observed between *QY* and *EC* as well as the $N\text{-}NO_2$ content in the water. On the other hand, the nitrogen balance index (*NBI*) of the plants displayed positive correlations with the $N\text{-}NO_3$, $N\text{-}NO_2$, and $P\text{-}PO_4$ content in the water. Please refer to Table 1 for a comprehensive overview of these correlations.

The *NBI* exhibited notable sensitivity to the timing of measurement, indicating a significant impact on its values. Regarding the stocking density factor, establishing a distinct



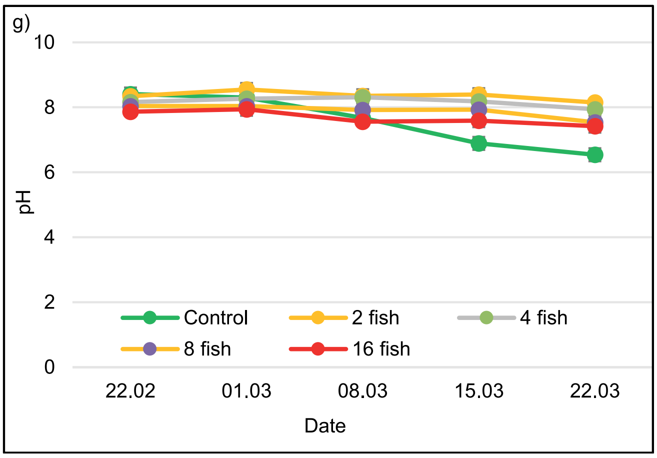


Fig. 5. Physicochemical properties of water in hydroponic (control) and aquaponic (2, 4, 8 and 16 fish) cultivation: a) O_2 content, b) N-NO_2 content, c) P-PO_4 content, d) N-NO_3 content, e) N-NH_4 content, f) electrical conductivity (*EC*), g) pH; $n = 4$, $p = 0.05$; source: own study

Table 1. Correlation coefficient between physiological parameters of *Lactuca sativa* L. (nitrogen balance index – *NBI*, chlorophyll content index – *CCI*, flavonoids – Flv and quantum yield – *QY*) and physicochemical properties of water (O_2, N-NO_2, N-NO_3, N-NH_4, *EC* and pH)

Parameter	*NBI*	*CCI*	Flv	*QY*
Control				
pH	−0.894*	0.533	0.969*	0.896*
EC	0.926*	−0.495	−0.982*	−0.796
N-NO_3	0.787	−0.610	−0.896*	−0.680
N-NO_2	0.674	−0.407	−0.757	−0.929*
N-NH_4	0.420	0.497	−0.171	−0.498
P-PO_4	−0.025	−0.886*	−0.361	−0.092
O_2	0.770	−0.135	−0.722	−0.944*
2 fish				
pH	−0.598	−0.870	0.186	−0.463
EC	0.584	0.475	−0.279	0.592
N-NO_3	0.790	0.440	−0.919*	0.830
N-NO_2	0.409	0.224	−0.568	0.575
N-NH_4	0.850	0.700	−0.590	0.709
P-PO_4	0.825	0.491	−0.904*	0.778
O_2	0.662	0.525	−0.700	0.671
4 fish				
pH	−0.664	−0.931*	0.266	−0.052
EC	0.455	0.527	−0.173	0.067
N-NO_3	0.782	0.493	−0.936*	0.778
N-NO_2	0.684	0.222	−0.768	0.307
N-NH_4	0.467	0.815	−0.024	−0.160
P-PO_4	0.849	0.593	−0.957*	0.817
O_2	0.917*	0.968*	−0.683	0.360

cont. Tab. 1

Parameter	*NBI*	*CCI*	Flv	*QY*
8 fish				
pH	0.133	0.896*	0.692	0.790
EC	0.047	−0.377	−0.111	−0.211
$N\text{-}NO_3$	0.491	−0.208	−0.920*	−0.360
$N\text{-}NO_2$	0.639	−0.194	−0.759	−0.501
$N\text{-}NH_4$	−0.470	−0.859	−0.172	−0.703
$P\text{-}PO_4$	0.307	−0.493	−0.952*	−0.540
O_2	0.055	−0.656	−0.512	−0.953*
16 fish				
pH	−0.304	−0.060	0.781	0.818
EC	0.333	0.015	−0.435	−0.899*
$N\text{-}NO_3$	0.387	0.234	−0.836	−0.459
$N\text{-}NO_2$	0.496	0.320	−0.856	−0.665
$N\text{-}NH_4$	−0.016	0.043	0.381	0.325
$P\text{-}PO_4$	0.298	0.102	−0.908*	−0.532
O_2	0.011	−0.048	0.130	−0.331

Explanations: values marked by asterisk are significant.
Source: own study.

relationship between this factor and the average *NBI* value proved challenging, as outlined in Table 2. Nevertheless, a statistically significant elevation in *NBI* values was observed concerning variants with 2 and 4 fish. Notably, statistical analysis revealed that the fish count (density) held no significant influence on chlorophyll levels. Conversely, the measurement date exhibited a significant effect on chlorophyll values; however, a clear relationship between the two couldn't be definitively established. With regard to flavonoid content, it's important to highlight that both studied factors displayed statistical significance, as highlighted in Table 2.

The decrease in the mean flavonoid value was evident across the provided data. Additionally, a general trend of increased flavonoid values at higher fish density was observed, with

Table 2. Changes of nitrogen balance index (*NBI*), chlorophyll content index (*CCI*), flavonoids (Flv) and quantum yield (*QY*) with results of ANOVA in the experiment

Variant	Value in period					
	I	II	III	IV	V	average
NBI						
Control	3.703^{abc}	4.272^{abcd}	4.141^{abcd}	10.631^{f}	10.419^{f}	$\mathbf{6.633}^{A}$
2 fish	2.925^{a}	4.311^{abcd}	5.400^{cde}	12.164^{fgh}	13.643^{hi}	$\mathbf{7.414}^{B}$
4 fish	2.928^{a}	4.387^{abcd}	6.484^{e}	11.587^{fg}	13.500^{ghi}	$\mathbf{7.631}^{B}$
8 fish	3.184^{ab}	3.791^{abc}	6.078^{de}	14.503^{i}	3.306^{ab}	$\mathbf{6.172}^{A}$
16 fish	3.297^{ab}	4.100^{abc}	3.812^{abc}	14.803^{i}	5.125^{bcde}	$\mathbf{6.227}^{A}$
Average	$\mathbf{3.207}^{I}$	$\mathbf{4.172}^{II}$	$\mathbf{5.183}^{III}$	$\mathbf{8.968}^{IV}$	$\mathbf{12.752}^{V}$	–
CCI						
Control	7.259^{ef}	5.728^{bcdef}	4.431^{ab}	5.903^{bcdef}	5.144^{abcde}	$\mathbf{5.693}^{A}$
2 fish	6.203^{bcdef}	4.731^{abc}	5.459^{abcdef}	5.686^{abcdef}	7.018^{def}	$\mathbf{5.791}^{A}$

cont Tab. 2

Variant	Value in period					
	I	II	III	IV	V	average
4 fish	6.000^{bcdef}	5.700^{abcdef}	5.978^{bcdef}	5.375^{abcdef}	6.761^{cdef}	**5.942^{A}**
8 fish	5.925^{bcdef}	5.991^{bcdef}	5.556^{abcdef}	6.616^{cdef}	3.584^{a}	**5.534^{A}**
16 fish	5.109^{abcd}	5.131^{abcd}	5.116^{abcd}	7.391^{f}	4.959^{abcd}	**5.541^{A}**
Average	**6.099 $^{II, III}$**	**5.456 $^{I, II}$**	**5.308 ^{I}**	**6.207 ^{III}**	**5.308 ^{I}**	–
Flv						
Control	1.922^{hij}	1.451^{efgh}	1.191^{cdefg}	0.474^{ab}	0.427^{ab}	**1.093^{A}**
2 fish	2.517^{j}	1.306^{defgh}	1.044^{abcdef}	0.567^{abc}	0.688^{abcde}	**1.256^{AB}**
4 fish	2.370^{j}	1.476^{efgh}	0.970^{abcdef}	0.370^{a}	0.561^{abc}	**1.164^{A}**
8 fish	2.242^{ij}	1.856^{ghij}	1.065^{bcdef}	1.067^{bcdef}	0.937^{abcdef}	**1.434^{BC}**
16 fish	2.178^{ij}	1.652^{fghi}	1.281^{defgh}	1.625^{fghi}	1.166^{cdef}	**1.580^{C}**
Average	**2.246 ^{IV}**	**1.548 ^{III}**	**1.110 ^{II}**	**0.827 ^{I}**	**0.763 ^{I}**	–
QY						
Control	0.788^{a}	0.799^{ab}	0.797^{ab}	0.794^{ab}	0.784^{a}	**0.792^{AB}**
2 fish	0.790^{ab}	0.801^{ab}	0.786^{a}	0.786^{a}	0.781^{a}	**0.789^{A}**
4 fish	0.793^{ab}	0.797^{ab}	0.796^{ab}	0.814^{b}	0.795^{ab}	**0.799^{B}**
8 fish	0.795^{ab}	0.796^{ab}	0.797^{ab}	0.800^{ab}	0.790^{ab}	**0.795^{AB}**
16 fish	0.799^{ab}	0.799^{ab}	0.795^{ab}	0.789^{a}	0.781^{a}	**0.792^{AB}**
Average	**0.793 $^{I. II}$**	**0.798 ^{II}**	**0.794 $^{I. II}$**	**0.797 ^{II}**	**0.786 ^{I}**	–

Explanations: the mean values designated with the same letter in the table line do not show a statistically significant difference (ANOVA), I–V = the period from 22.02 to 22.03 (22.02, 01.03, 08.03, 15.03, 22.03 respectively).
Source: own study.

noteworthy significance found in the 8 and 16 fish variants compared to the control. The statistical analysis revealed that, in the context of chlorophyll, the fish number (density) didn't exert a statistically significant influence. Conversely, for chlorophyll values, the measurement date emerged as a significant factor; however, definitively establishing the relationship between the two proved challenging. Turning to *QY*, it's important to mention that the studied factors demonstrated statistical significance, though no specific connections between the parameter studied and the factors were discernible.

Table 3 provides an overview of the average outcomes stemming from measurements of total fish length and biomass within the aquaponic system.

The results obtained from the fish measurements highlighted the most substantial length increments in the case of 8 fish, while the highest biomass was recorded with a reduced fish count (4 and 2 fish). Notably, the Fulton coefficient exceeded 1.90 after experiments, indicating that the fish were in good condition.

Table 3. Average values of total length (*TL*) and weight (*W*) of fish before (B) and after (A) the experiment

Specification	Variant							
	2 fish		4 fish		8 fish		16 fish	
	TL ±*SD* (cm)	*W* ±*SD* (g)	*TL* ±*SD* (cm)	*W* ±*SD* (g)	*TL* ±*SD* (cm)	*W* ±*SD* (g)	*TL* ±*SD* (cm)	*W* ±*SD* (g)
B	12.15 ±0.05	32.28 ±0.91	12.33 ±1.15	28.72 ±7.97	10.46 ±0.25	21.80 ±2.00	9.74 ±0.75	15.75 ±1.24
A	12.90 ±0.05	42.64 ±1.34	12.74 ±0.83	39.05 ±6.70	11.94 ±1.51	29.94 ±7.54	10.64 ±0.88	24.31 ±2.37
Difference (A–B)	0.75 ±2.85	10.36 ±7.61	0.42 ±1.77	10.32 ±2.21	1.48 ±1.78	8.13 ±1.38	0.91 ±1.43	8.56 ±0.99
K_B ±*SD*	1.79 ±0.03		1.86 ±0.20		1.89 ±0.49		1.98 ±1.01	
K_A ±*SD*	1.99 ±0.00		1.90 ±0.06		1.91 ±0.15		1.99 ±0.06	

Explanations: K_B = Fulton coefficient before the experiment, K_A = Fulton coefficient after the experiment, *SD* = standard deviation.
Source: own study.

DISCUSSION

In the context of emerging climate change, the imperative to reduce water usage for agricultural irrigation, and the pursuit of enhancing the quality of plant-based foods, the cultivation of plants in hydroponic and aquaponic systems has gained significant popularity over recent years. Research has underscored that growing crops within aquaponic systems not only offers economic advantages (Rizal *et al.*, 2018) but also demonstrates environmental friendliness with regard to crop and fish production, nutrient circulation, energy and water consumption (Delaide *et al.*, 2017).

The exploration of aquaponic systems encompasses various fish species. Among these, cyprinids, such as *Carassius auratus auratus*, have emerged as common choices for farming. Their presence notably stimulates shoot growth within aquaponic setups and augments plant nutrient content (Li *et al.*, 2020). Our research further corroborated that the common carp (*Cyprinus caprio*) is a suitable candidate for aquaponic cultivation. Notably, the value of common carp in our study aligns with that of other members of this species reared in ponds. However, an excess of fish within the culture did not prove advantageous for plant growth and led to a suboptimal condition for lettuce. Findings from Maucieri *et al.* (2019) demonstrated that lower stocking density improved carp and leafy vegetable production by enhancing water quality within the tested aquaponic system. Other studies (Knaus and Palm, 2017) revealed that distinct fish species utilised within identical aquaponic systems could influence oxygen levels and impact the growth of plant species. This highlights the potential to enhance plant yield through the inclusion of multiple fish species (polyponics) within conjugate aquaponic setups for improved breeding outcomes.

In comparison to hydroponic or recirculating systems for fish culture, aquaponic systems are considered more straightforward to operate. They necessitate less monitoring and generally offer a greater safety margin to ensure favourable water quality (Rakocy *et al.*, 2006 revision). In aquaponic systems, maximising nutrient uptake is essential to ensure robust plant biomass production without compromising optimal conditions for fish welfare in terms of water quality (Yavuzcan Yildiz *et al.*, 2017). Water quality, a pivotal determinant of fish health and well-being, constitutes a crucial consideration in all aquaponic systems. Buzby and Lin (2014), in a study evaluating new methods for assessing nutrient removal and achieving satisfactory water quality, revealed that lettuce solely removed total ammonium nitrogen among the tested plants, proving ineffective at nitrate removal. Furthermore, older lettuce plants exhibited improved efficiency in removing dissolved inorganic nitrogen, while younger plants excelled in phosphate removal.

Plant growth is inherently influenced by environmental factors (Hewedy *et al.*, 2022). Considerable efforts are being directed towards devising methods to accurately assess the impact of environmental parameters on plant development. Among the most informative techniques are those that investigate the process of photosynthesis. Particularly, non-destructive measurements of chlorophyll fluorescence, chlorophyll content index, nitrogen balance index, and flavonoids have witnessed rapid advancement (Dąbrowski *et al.*, 2015; Kalaji *et al.*, 2017; Dąbrowski *et al.*, 2021). During photosynthesis, chloroplasts' antenna pigments within leaves absorb solar radiation, releasing electrons that initiate the photochemical process (Richardson, Duigan and Berlyn, 2002). Chlorophyll molecules, predominantly chlorophyll *a* and *b*, are vital for converting light energy into chemical bonds. The quantity of solar energy absorbed by a leaf hinges on the leaf's pigment concentration. Diminished concentrations can detrimentally affect photosynthesis, resulting in decreased primary production (Curran, Dungan and Gholz, 1990). Quantifying chlorophyll content furnishes vital insights into the interplay between plants and their environment (Coste *et al.*, 2010). While our results did not establish a correlation between chlorophyll content and NO_3, the mean values of this parameter recorded in plants from aquacultures featuring 2 and 4 fish were notably higher than those in control plants. Furthermore, the majority of leaf nitrogen is bound within chlorophyll, making it feasible to indirectly evaluate the effect on photosynthesis by gauging the nitrogen balance index (*NBI*) (Moran, Abdulla and Smith, 2000). This index, representing the ratio between chlorophyll and polyphenols, inversely hinges on nitrogen nutrient status (Cartelat *et al.*, 2005). From our findings, it can be deduced that plants cultivated in aquacultures with 2 and 4 fish exhibit a higher nitrogen status, while those in aquacultures featuring 8 and 16 fish manifest lower nitrogen status.

Flavonoid content in leaves emerges as another intriguing parameter capable of serving as an indicator of plant status. These compounds arise from secondary metabolism in response to stressors (Aherne and O'Brien, 2002); however, they also hold significance for human health. Oh, Carey and Rajashekar (2009) suggested that mild stress can be harnessed to elevate phytochemical levels in health-promoting foods. Our observations did not reveal any discrepancies in this parameter's levels between hydroponically and aquaponically grown plants.

Quantum yield quantifies the proportion of light absorbed by chlorophyll linked with PSII and employed in photochemistry. It offers insights into the rate of linear electron transport and serves as an indicator of overall photosynthesis. A strong linear correlation exists between this parameter and carbon fixation efficiency (Maxwell and Johnson, 2000). Photosynthesis is acknowledged to be influenced by nutrition, and it diminishes with leaf longevity (Kalaji *et al.*, 2017). Our experiment led to the conclusion that a diet sourced from aquacultures housing 2 and 4 fish enhances photosynthetic efficiency.

CONCLUSIONS

Numerous research endeavours underscore the advantages of aquaponics as a promising alternative for crop cultivation. Our investigation has revealed a direct correlation between the number of fish in the aquaponic culture and the photosynthetic performance of lettuce plants. Optimal cultivation outcomes (best photosynthetic efficiency of plants) were observed with 2 or 4 fish in 60 dm^3 tanks. The exceptional photosynthetic performance, as indicated by elevated values of quantum yield (*QY*), nitrogen balance index (*NBI*), and chlorophyll content index (*CCI*) parameters under these conditions, underscores the plants' adept utilisation of the provided nutrients. To gain a more comprehensive understanding of the interdependencies within the aquaponic system's three key components – fish, water, and plants – further studies should be undertaken. These could involve the exploration of diverse fish and plant species, along with the examination of various lighting conditions.

All data generated or analysed throughout this study are thoroughly detailed in this published article.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

INSTITUTIONAL REVIEW BOARD STATEMENT

All methods for animal and plant handling were performed in accordance with the relevant guidelines and regulations. Ethical review and approval were waived for this study due to that all measurements that have been done on fishes were non-destructive and non-invasive (only growth parameters were estimated such as weight and length). The reporting in the manuscript follows the recommendations in the ARRIVE guidelines.

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