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Effects of stocking density on the growth and flesh quality of rainbow trout (*Oncorhynchus mykiss*) reared in a low-tech aquaponic system

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ABSTRACT

In the present study, we evaluated the effects of two stocking densities (low - ALD, 3.81 kg m^{-3} vs. high - AHD, 7.26 kg m^{-3}) on the growth, health, and flesh quality of rainbow trout (*Oncorhynchus mykiss*) and the yield and microbiological quality of lettuce (*Lactuca sativa*) produced in a low-tech aquaponic system compared to hydroponic cultivation (HYP). Nine experimental units (three replications per treatment) were utilised. A total of 123 rainbow trout (initial body weight: 142 ± 35 g) were randomly distributed in six 500-L tanks (3 per stocking density) and monitored during a 117 day trial period. The final weight (331 g on average), specific growth rate (0.73% d^{-1}), feed conversion ratio (1.58), and mortality (3%) of the fish did not differ between stocking densities. The morphometric indices, slaughter results, and flesh quality were not affected. Similarly, the quantity of lettuce produced during two consecutive cycles was similar among treatments (2.4 kg m^{-2} on average). At harvest, microbial contamination (total viable count, *E. coli*, *Enterobacteriaceae*, *Pseudomonas*, mould, and yeasts) was similar in the fish skin and lettuce produced in aquaponic systems with different stocking densities, as well as in the lettuce produced in aquaponic and hydroponic systems. In conclusion, rainbow trout and lettuce productions were successful in the tested aquaponic system, whereas stocking density did not affect fish growth or flesh quality.

1. Introduction

Aquaponics is an emerging sustainable food production system, which combines fish farming (aquaculture) and soilless crop cultivation (hydroponics) in integrated multi-trophic systems where animals, plants, and microorganisms are in symbiosis (Rakocy et al., 2006; Goddek et al., 2015; König et al., 2018). These systems provide short and eco-friendly food supply chains with increased resource-use efficiency, high economic and environmental sustainability, and food resilience (Van Woensel et al., 2015). Low-tech aquaponic systems based on a simple design, easy management, and low capital costs are suitable for implementation in different geographic regions and production areas (Palm et al., 2018; Palm et al., 2019). Moreover, aquaponics could also play a key role in future urban farming developments and socio-economic progress in smart cities (dos Santos, 2016).

A vital requirement for one-loop aquaponic systems is the need for maintaining the optimal levels for fish and plants concerning the water temperature, pH, and chemical composition (Monsees et al., 2017; Gichana et al., 2018). In this regard, the stocking density of fish is a key factor for balancing aquaponic ecosystems, since it directly affects water quality in terms of nutrients, gases, and waste by-products, thus influencing plant growth as well as fish health and growth (Somerville et al., 2014; Yildiz et al., 2017; Palm et al., 2019).

Several 'easy-to-produce' fish species, such as Blue tilapia (*Oreochromis aureus*), Nile tilapia (*Oreochromis niloticus*), Common carp (*Cyprinus carpio*), tench (*Tinca tinca*), and African catfish (*Clarias gariepinus*), have been successfully reared in aquaponic farming (Somerville et al., 2014; Goddek et al., 2015). Most of this research has focused on warm-water fish species, whereas, to our knowledge, no data are available on cold-water species, such as rainbow trout (*Oncorhynchus mykiss*). Indeed, the choice of fish species depends upon its economic values, the market demand, and the geographic localisation of the production system (Forchino et al., 2017).

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On the other hand, several crops have been grown in aquaponics, including herbs, fruiting species, and leafy vegetables (Thorarinsdottir, 2015). Lettuce (*Lactuca sativa*) is one of the main plant species used in aquaponic systems (Love et al., 2015), showing growth rates similar to those in hydroponics (Lennard and Ward, 2019; Monsees et al., 2019). Moreover, all studies have focused on fish performance/mortality and vegetal biomass production, whereas, to our knowledge, no information is available on the rheological and microbiological quality of the fish produced in aquaponic systems. Finally, few studies have tested the microbiological quality and safety of the vegetables in aquaponics (Elumalai et al., 2017; Schmautz et al., 2017; Mori and Smith, 2019).

Thus, our study aimed at evaluating the effects of two stocking densities in fish tanks on the growth performance and flesh quality traits of rainbow trout (*Oncorhynchus mykiss*) as well as the microbiological load of the end-products (both fish and vegetables) in a low-tech aquaponic system.

2. Materials and methods

2.1. Experimental conditions of the aquaponic and hydroponic systems

The research was conducted at the experimental farm of the University of Padova, North-East Italy (45°20′N, 11°57′E, 6 m a.s.l.), inside a plastic greenhouse. The experiment consisted of nine identical independent units (Fig. 1), i.e., three hydroponic units (HYP) without fish, three aquaponics units with a low stocking density (ALD), and three aquaponics units with a high stocking density (AHD). The hydroponic section worked as a biofilter. The system size was designed basing on the recommendations of Somerville et al. (2014) for small-scale aquaponics units, i.e. 10 kg maximum fish biomass in a 500-L fish tank coupled with a biofilter having a minimum volume equal to 10–30% of the total fish tank volume. Before the start of the present trial, the system had been previously used for another cycle with European carp, which guaranteed about the regular functioning of the biofilter (Maucieri et al., 2019). Municipal water was used without any pre-treatment to fill the systems at the start of the European carp cycle. Then, to restore losses due to fish splashing, evaporation and transpiration during the trial, municipal water was used after stocking for at least 48 h. No energy to regulate water temperature, no probe for the continuous evaluation of water quality or remote management, and no device for water sanitation were used. No correction for alkalinity was performed.

Each independent unit consisted of: 1) a main tank (volume 500 L, height 0.80 m, diameter 0.90 m), in which the fish were kept in the aquaponic units or where the nutritive solution was present in the hydroponic units; 2) two tanks for vegetables (volume 275 L each, height 0.35 m, diameter 1.00 m) for a total crop area of 1.6 m^2, filled with 225 L of light expanded clay aggregates (specific area 250 m^2 m^{-3}, packing density 300 kg m^{-3}, total porosity 0.55 m^3 m^{-3}; LECA Laterlite, Solignano, Italy), which received water from the main tank and acted both as a biofilter and hydroponic substrate for vegetable growth; and 3) a storage tank (volume 50 L, height 0.45 m) for collecting water from the vegetable tanks, before pumping it back into the main tank (Fig. 1). The three parts of the system had water at different heights so that the water flow was guaranteed by the overflow (from the main tank to the vegetable tanks, and then to the storage tank). A single pump (Newa Jet 1700, NEWA TecnoIndustria Srl, Loreggia, Italy) returned the water from the storage tank to the main tank. The flow rate was 300 L h^{-1}, which corresponded to a complete recirculation of water every 2 h. The nine main tanks were aerated by a porous stone (4.0 cm × 4.0 cm × 15.0 cm, 14 L min^{-1}; Sweetwater® AS15S, Pentair, Cary, NC, USA) connected to an aerator (Scubla D100, Scubla Srl, Remanzacco, Italy) and covered with a net to prevent fish from jumping.

Fig. 1. Rendering of the aquaponic unit. A, main tank for fish (500 L); B, tanks for vegetables/biofilters (275 L); C, storage tank for water collection (50 L) and pumping to A tank.

2.2. In vivo trial and recordings

The experiment was run during the winter season (November–February), under a natural photoperiod, and lasted 117 days. In all 9 units before the beginning of the trial, 132 g $unit^{-1}$ of KH_2PO_4, 197 g $unit^{-1}$ of K_2SO_4, 273 g $unit^{-1}$ of $MgSO_4{\cdot}7H_2O$, 10 g $unit^{-1}$ of Fe-EDTA, and 5 g $unit^{-1}$ of micronutrients were added. On day one, fish were placed in the 3 ALD and 3 AHD units. Meanwhile, 333 g $unit^{-1}$ of $Ca(NO_3)_2$ and 480 g $unit^{-1}$ of (NH_4NO_3) were added to the 3 HYP units. The nutrient solution was calculated by the free software HydroBuddy based on optimal conditions for lettuce in hydroponics.

Six of the nine main tanks were stocked with a total of 123 rainbow trout, with an initial live weight of 142 ± 35 g, obtained from a commercial farm (Troticoltura Santa Cristina, Treviso, Italy). The three ALD units received 14 fish per tank (average initial stocking density of 3.81 kg m^{-3}), while the three AHD units received 27 fish per tank (average initial stocking density of 7.26 kg m^{-3}). The low and the high stocking density were chosen based on recommendations for small-scale aquaponics systems, i.e. 10–20 kg m^{-3} (Somerville et al., 2014). The high stocking density was chosen also in view of the maximum final biomass of the fish (estimated as 3.0–3.5 times the initial weight), consistent with recommendations for organic aquaculture (i.e., 25 kg m^{-3} for rainbow trout reared in freshwater; Commission Regulation 889/2008). Consistently with practice in not intensive farms, the fish were manually fed once a day, until apparent satiation and in two rounds separated by 20–30 min, with a commercial diet of extruded pellets (Skretting, Verona, Italy; composition: 40% crude protein, 11.5% crude fat, 4% crude fibre, 8% ash, 0.2% sodium, 1.5% calcium, and 0.8% phosphorus, as-fed basis). The quantity of feed administered daily was calculated for each aquaponic unit on the basis of the biomass present at the moment of each weighing, i.e., 1.5% of the biomass from the beginning of the experiment to 20 days, 1.0% from 23 to 53 days, and 1.5% from 56 days to the end of the experiment.

During the trial, two crop cycles of lettuce (*Lactuca sativa* L.) were cultivated in succession during 77 days for the first cycle and 44 days for the second one. At the beginning of each cycle, 20 plants per experimental unit (10 plants per tank, plant density of 13 plants m^{-2}) were transplanted during the third true leaf stage. The first cycle began 3 days before fish addition in the systems, the second cycle was harvested the day after fish harvesting. Plants were obtained from an external supplier. Neither pesticides nor antibiotics were used in the water or feed during the entire experiment.

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2.3. Water quality

Throughout the trial, water lost from each unit was daily recorded and manually refilled. Two times per week, the outflow water of the fish tanks was monitored for the temperature, dissolved oxygen (DO), pH, redox potential (ORP), and electric conductivity (EC), using a portable multi-parameter apparatus (HQ40d Portable Multi-Parameter Meter, Hach Lange GmbH, Germany). The chlorophyll content was measured with a fluorescence detector (HHLD Fluorescence-Chlorophyll, Turner Designs, USA). The anion (NO_2^-, NO_3^-, and PO_4^{3-}) and cation (NH_4^+) contents in the water were determined by ion chromatography (Maucieri et al., 2019).

2.4. In vivo recordings of fish

Fish health was monitored daily. Fish were individually weighed by a scale (precision 1 g; Wunder Sa.Bi. srl, Trezzo sull'Adda, Italy) at the beginning of the trial (day 0), and then on a monthly basis at 22, 55, 84, and 117 days (end of the rearing period). For this, fish were removed from their tank, placed in a separate one, and anaesthetised with 10 mg L^{-1} of clove oil containing 87% eugenol. Fish were not fed for 48 h before and 24 h after weighing. The specific growth rate (SGR) and feed conversion ratio (FCR) were calculated as follows:

$$\text{SGR } (\%\ d^{-1}) = \left[\left(Log_e\ Final\ weight - Log_e\ Initial\ weight\right)/No.of\ days\right] \times 100 \quad (1)$$

$$FCR = weight\ of\ dry\ feed\ distributed/weight\ gain\ of\ fish \quad (2)$$

2.5. Recordings at fish slaughtering and vegetable harvest

At the end of the rearing period, all fish were slaughtered. Before slaughtering, fish were fasted for 24 h. Fish were harvested with a handling net, anaesthetised with 10 mg L^{-1} of clove oil in a separate tank, and then manually restrained using a plastic knob so that a percussion could be applied to the head of fish. All fish were weighed and 36 fish (6 per tank; 18 per experimental treatment), representative of their experimental groups regarding average body weight and variability, were selected, individually tagged, and stored in polystyrene boxes with ice in a cold room (0 to 2 °C). Eighteen fish (3 per tank; 9 per experimental treatment) were analysed one day after slaughter, whereas the other eighteen fish (3 per tank; 9 per experimental treatment) were analysed seven days after slaughter. The *rigor mortis* index (RI) was determined using the following formula (Bito et al., 1983):

$$RI\ (\%) = \left[\left(L_0 - L_t\right)/L_0\right] \times 100 \quad (3)$$

where, L_0 (cm) is the vertical distance between the base of the caudal fin and the table surface, measured immediately after death, and L_t (cm) is the vertical distance between the base of the caudal fin and the table surface at 1 and 7 days after slaughter.

For each storage time, the total length, standard length, head length, and maximum height were measured (Luxinger et al., 2018), and the following morphological indices (Di Marco et al., 2017) were calculated:

$$Condition\ factor = body\ weight/total\ length^3 \quad (4)$$

$$Cranial\ index = head\ length/total\ length \quad (5)$$

$$Relative\ profile = maximum\ height/total\ length \quad (6)$$

Thereafter, the L*, a*, and b* colour indices of the skin were measured at three points on the dorsal side with a Minolta CM–508C spectrophotometer (Minolta Corp., Ramsey, NJ, USA). A texture profile analysis (TPA) was performed at a central position on the lateral side under the first dorsal fin, using a TA.XT.plus Texture Analyser (Stable Micro Systems, Godalming, UK) with a 20-mm diameter cylindrical probe, with 5 mm compressions at a constant speed of 2 mm/s for two consecutive cycles, separated by a 5-s interval. The muscle pH was measured at three points on the dorsal side with a pH meter (Basic 20; Crison Instruments Sa, Carpi, Italy) equipped with a specific electrode (cat. # 5232; Crison Instruments Sa).

Then, the fish were dissected and the carcasses were weighed. The carcass and fillet yields were calculated using the following formulae:

$$Carcass\ yield\ (\%) = (carcass\ weight/slaughter\ weight) \times 100 \quad (7)$$

$$Fillet\ yield\ (\%) = (fillet\ weight/slaughter\ weight) \times 100 \quad (8)$$

The skin was separated from the fillets, and then the flesh colour indices were measured at three points on the dorsal side of the fillets, taken from the right side of the fish (the right fillets) according to the procedure describe above. The left fillets were used to measure the total volatile base-nitrogen (TVB-N) content (EEC, 1995).

At harvesting (77 days: first crop cycle; 44 days: second crop cycle), all lettuce plants were divided into aboveground and belowground parts, and the fresh weight of the leaves was immediately recorded to determine the marketable yield following the same procedure reported by Maucieri et al. (2019).

2.6. Microbiological analysis of vegetables and fish

At the time of fish slaughter, leaves from all plants (i.e. those of the second cycle) were collected and pooled (one pool per tank, 9 pools). The pooled samples were rinsed with 400 mL of Buffer Peptone Water (BPW) and rinsates were used for the detection and enumeration of different microbial targets.

Then, the same 36 fish selected for rheological analyses were used for sampling at the skin level immediately after slaughter (18 specimens; 3 fish × 3 tanks × 2 groups) and 7 days later, after storage at 2 °C (18 specimens; 3 fish × 3 tanks × 2 groups). For sampling, a standard area of the skin (25 cm^2) was swabbed with sterile cotton swabs, which were placed in 10 mL of Maximum Recovery Diluent (8 g NaCl/L, 1 g of bacteriological peptone/L) and then serially diluted.

Targets bacteria included specific spoilage organisms (SSO) associated with fish spoilage during storage (i.e. psychotrophyc bacteria such as Pseudomonas and putative H2S producers), besides bacteria associated with faecal contamination (i.e. *Escherichia coli*). Total mesophilic count (as total viable count), mould, yeast, and *Enterobacteriaceae* were also evaluated being generic food quality indicators.

In both vegetables and all fish sampled at slaughter and after 7 d, the total viable count (TVC) was evaluated on Plate Count Agar (Biokar Diagnostics, Beauvais, France), incubated at 30 °C for 72 h (ISO 4833-1:2013). The contamination provided by *Enterobacteriaceae* was assessed using Violet Red Bile Glucose Agar (Biokar Diagnostics), incubated at 37 °C for 24 h (ISO 21528-2:2017). *Escherichia coli* counts were performed on a Tryptone Bile X-Glucuronide (TBX, Oxoid Ltd., Basingstoke, Hampshire, UK) medium, incubated at 44 °C for 18–24 h (ISO 7251:2005). The count of H_2S-producing bacteria (putative *Shewanella* spp.) was carried out on iron agar (Lyngby, Laboratorios Conda, Torrejón de Ardoz, Spain), incubated at 25 °C for 48 h (Andreani and Fasolato, 2016). The *Pseudomonas* spp. count was evaluated on *Pseudomonas* Agar Base supplemented with cetrimide, fucidine, and cephaloridine (Oxoid), incubated at 25 °C for 48 h (Andreani and Fa-

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solato, 2016). In trout samples, yeast and mould counts were performed on Oxytetracycline Glucose Yeast Extract Agar (OGYE, Oxoid), incubated at 25 °C for 3–5 days (ISO 21527-1:2008). The results were reported as $\log_{10}$ CFU/cm^2 or mL of rinsate.

2.7. Ethics statement

The study was approved by the Ethical Committee for Animal Experimentation (Organismo per la Protezione del Benessere Animale, OPBA) of the University of Padova (project no. 6/2017; prot. n. 15,132). All animals were handled according to the principles stated by the EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes. Research staff involved in animal handling were animal specialists (PhD or MS in Animal Science) and veterinary practitioners.

2.8. Statistical analysis

The data concerning the water quality, vegetable yields and microbiological quality were analysed by a one-way ANOVA with the experimental group (ALD, AHD, and HYP) as the main effect. The growth performance and slaughter data of the fish were analysed by a one-way ANOVA with the stocking density (ALD and AHD) as the main effect. The *rigor mortis*, physicochemical traits, and microbiological quality data of the fish were analysed by a two-way ANOVA with the stocking density, storage time (1/7 days), and their interaction as the main effects. The PROC GLM of SAS (Statistical Analysis System, 2013) was used for all analyses. Bonferroni's test was used to compare means. Differences among means with $p < .05$ were assumed to be statistically significant.

3. Results

3.1. Water characteristics

Throughout the trial, the daily water losses averaged 2.45 L d^{-1}, i.e., 0.41% of the total water contained in each unit, without significant differences among groups (data not reported in tables). The EC, redox potential, and contents of ammonium, nitrite, and nitrate in the water varied according to the following pattern: ALD < AHD < HYP ($p < .001$). However, the phosphate content was higher in aquaponic units compared to hydroponic ones, and increased significantly with the stocking density of fish ($p < .001$; Table 1).

Table 1
Physicochemical traits of water over the experimental period (117 days).

	Experimental groups				
	ALD	AHD	HYP	*P*-value	RMSE
Tanks (n)	3	3	3		
Temperature (°C)	10.8	10.8	10.6	0.722	2.0
Dissolved oxygen (mg L^{-1})	9.58 ^{A}	8.69 ^{B}	11.31 ^{C}	<0.001	0.85
pH	7.43 ^{B}	7.24 ^{A}	7.25 ^{A}	0.005	0.45
Chlorophyll (μg L^{-1})	55.3 ^{B}	80.2 ^{C}	14.1 ^{A}	<0.001	38.2
Electrical conductivity (ds cm^{-1})	1.63 ^{A}	1.89 ^{B}	2.81 ^{C}	<0.001	0.20
Redox potential (mV)	75.2 ^{A}	83.1 ^{AB}	90.6 ^{B}	<0.001	24.9
NH_4^+ (mg L^{-1})	0.55 ^{Aa}	0.80 ^{Ab}	21.20 ^{B}	<0.001	13.41
NO_2^- (mg L^{-1})	0.07 ^{Aa}	0.18 ^{Ab}	7.74 ^{B}	<0.001	7.74
NO_3^- (mg L^{-1})	314 ^{A}	417 ^{B}	1405 ^{C}	<0.001	202
PO_4^{3-} (mg L^{-1})	86.3 ^{B}	106.5 ^{C}	42.9 ^{A}	<0.001	16.8

ALD: aquaponic units at low stocking density of fish; AHD: aquaponic units at high stocking density of fish; HYP: hydroponic units. RMSE: Root mean square error.
Means with different superscript letters are statistically different (a, b: $p < .05$; A, B: $p < .01$).

Trends in water characteristics during the trial are shown in Fig. 2. Water temperature changed with external environmental conditions (Fig. 2a); dissolved oxygen showed a similar pattern in the three groups, being always higher in HYP tanks (Fig. 2b); pH decreased in all groups from about 8.00 until about 7.00 (Fig. 2c). Ammonia added with the fertilizer in the HYP tanks at the beginning of the trial disappeared by the 60th d of trial (Fig. 2d), being converted in nitrite (Fig. 2e) and then in nitrate (Fig. 2f). Ammonia produced by fish was higher in AHD tanks compared to ALD tanks during the first weeks; it was similar in the two groups in following period, whereas it showed an increase after 100 d when fish biomass increased (Fig. 2d). Nitrite sharply increased in AHD tanks from 100 d onwards (Fig. 2e) whereas nitrate showed a constant increase in both groups until about 600 mg L^{-1} at the end of the trial (Fig. 2f).

3.2. Fish growth performance and biomass production

Throughout the trial, only four fish died (2 from ALD and 2 from AHD treatments) without showing any previous symptoms of disease. The stocking density did not affect the live weight, SGR, or FCR of the fish (Table 2). At the end of the trial, the live weight and SGR averaged 331 ± 78 g and 0.73 ± 0.06% d^{-1}, respectively, while the FCR reached 1.58 ± 0.16 (average of AHD and ALD fish). The stocking density at the end of the experiment reached 8.86 and 16.94 kg m^{-3} in ALD and AHD groups, respectively (Table 2).

3.3. Biometric traits and flesh quality of fish

The stocking density did not affect the morphologic traits/indices, slaughter results (Table 3), or flesh quality of the fish (Table 4). On average, the carcass yield was 89.0% of the slaughter weight. Meanwhile, the fillet weight and yield were 163 g and 49.1% of the slaughter weight, respectively.

Regarding the storage time, after 7 days the rigor index (−75.5 units), muscle pH (−0.095 units), hardness (−15.8 N), and chewiness (−5.09 units) significantly decreased ($.001 < p < .01$). Meanwhile, the lightness of the skin (+12.7 units) and fillet (+5.7 units) and the yellowness of the skin (+2.8 units) increased ($p \leq .001$). Whereas, the redness of the skin (−3.6 units) and the yellowness of the fillet (−1.8 units) decreased ($p < .001$).

3.4. Marketable yield of vegetables

The marketable lettuce yield was 2.82 ± 0.64 kg m^{-2} at the end of the first cycle and 1.89 ± 0.18 kg m^{-2} at the end of the second cycle (average of the three groups). On average for the two cycles, the lettuce production in the aquaponic system was similar to that of the hydroponic cultivation, even if a higher production was recorded in aquaponic units compared to hydroponic ones in the second cycle (on average 1.98 vs. 1.75 kg m^{-2}, $p < .01$; Fig. 3).

3.5. Microbiological quality of fish and vegetables

At the skin level, the TVC and *Enterobacteriaceae* counts were not affected by the stocking density or sampling time (Table 5). *Pseudomonas* increased (0.36 to 0.81 $\log_{10}$ CFU cm^{-2}; $p = .024$) and H_2S-producing bacteria tended (0.41 to 0.86 $\log_{10}$ CFU cm^{-2}; $p = .072$) to increase from ALD to AHD groups. *Escherichia coli,* moulds and yeasts were not found in the skin or lettuce and thus their counts are not given in tables. In addition, regarding the lettuce, the TVC, *Enterobacteriaceae*, and *Pseudomonas* counts did not differ between the aquaponic and hydroponic systems (Table 6).

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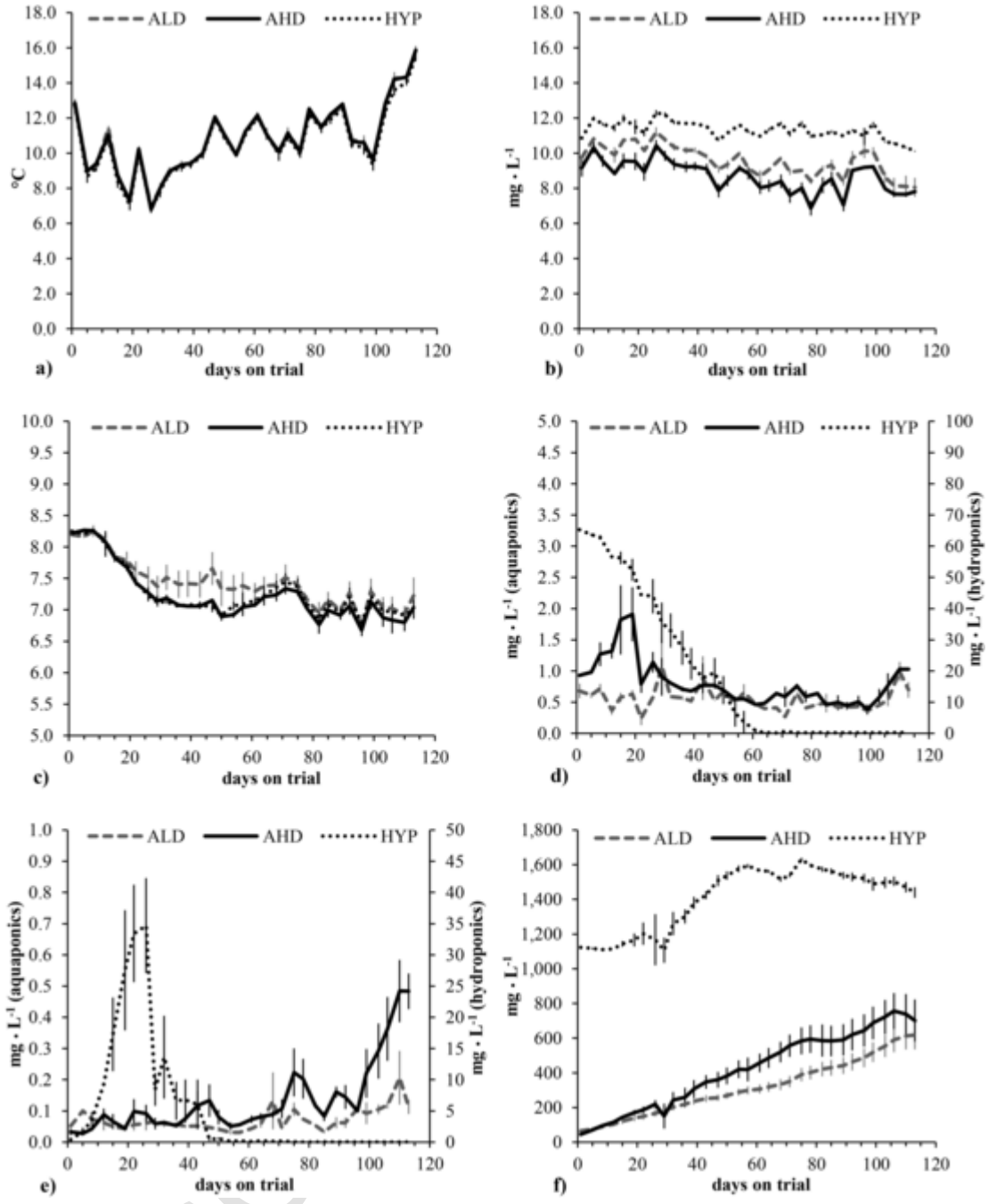

Fig. 2. Temperature (a), dissolved oxygen (b), pH (c), ammonium (d), nitrite (e) and nitrate (f) values (means ± SE) measured in water of the main tanks with fish during the trial. ALD: aquaponic system with low stocking density of fish; AHD: aquaponic system with high stocking density of fish; HYD: hydroponic system.

4. Discussion

4.1. Growth performance of fish

In the present study, the stocking density did not affect the growth performance of rainbow trout reared in low-tech aquaponic systems. The water conditions were maintained within acceptable values for trout growth (Azevedo et al., 1998; Bregnballe, 2015). In fact, the water temperature ranged from 7.0 to 15.9 °C (Fig. 2a), with an average of 10.7 °C. The DO values were consistently within suitable ranges for the health and welfare of rainbow trout (Ellis et al., 2002), nutrient adsorption capacity of plant roots, and productivity of nitrifying bacteria (Somerville et al., 2014). As expected, the lowest DO values were recorded in the AHD units (Fig. 2b) due to the higher fish and bacteria respiration (Hussain et al., 2014; Rayhan et al., 2018; Maucieri et al., 2019). The water pH (on average 7.3, range 8.1–6.7; Fig. 2c) was also consistent with the requirements for rainbow trout ($7.0 < pH < 8.0$; Bregnballe, 2015) and bacteria ($7.0 < pH < 9.0$; Antoniou et al., 1990; Goddek et al., 2015). Considering the water pH and temperature during the trial, the content of unionised ammonia was consistently below the critical threshold for rainbow trout (0.06–1.10 mg L^{-1}; Ellis et al., 2002) in both ALD and AHD treatments. Similarly, the nitrites remained within a safe range (Kroupova et al., 2008). On the other hand, the water nitrate content overpassed values recommended for cold species in aquaponic systems

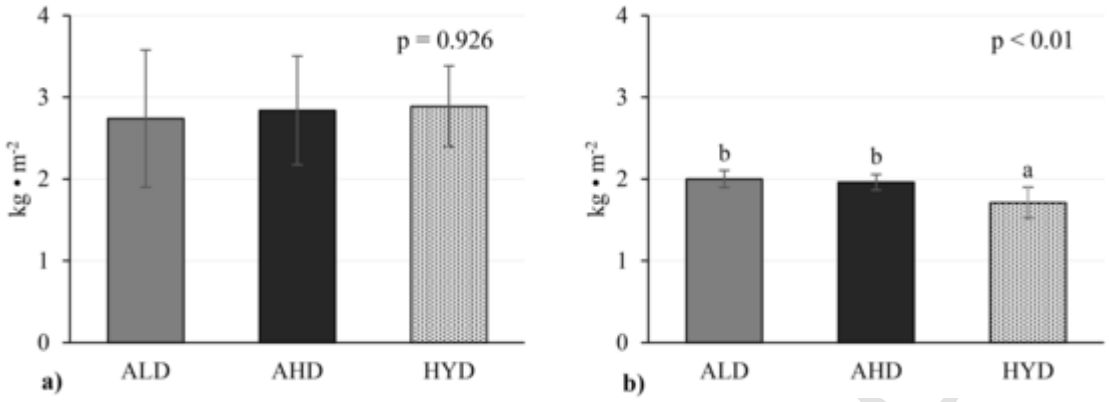

Fig. 3. Lettuce production in the first cycle (a) and in the second cycle (b). ALD, aquaponic system with low stocking density of fish; AHD: aquaponic system with high stocking density of fish; HYD: hydroponic system.

Table 2
Growth performance of rainbow trout.

	Stocking density		P-value	RMSE
	ALD	AHD		
Total fish per treatment (n)	40	78		
Tanks (n)	3	3		
Fish weight (g)				
0 days	143	140	0.657	6
22 days	170	166	0.726	12
55 days	217	215	0.886	19
84 days	264	256	0.660	20
117 days	333	329	0.871	26
Specific growth rate (% d^{-1})				
0–22 days	0.79	0.76	0.850	0.17
22–55 days	0.74	0.77	0.693	0.08
55–84 days	0.69	0.62	0.298	0.06
84–117 days	0.70	0.76	0.449	0.08
0–117 days	0.72	0.73	0.928	0.04
Feed conversion ratio				
0–22 days	1.52	1.58	0.868	0.38
22–55 days	1.28	1.14	0.317	0.15
55–84 days	1.84	1.87	0.842	0.20
84–117 days	1.73	1.44	0.255	0.27
0–117 days	1.65	1.51	0.323	0.15
Biomass (kg m^{-3})				
0 days	3.81	7.26	< 0.001	0.49
22 days	4.53	8.57	< 0.001	0.36
55 days	5.78	11.04	< 0.001	0.29
84 days	7.04	13.20	< 0.001	0.49
117 days	8.86	16.94	< 0.001	0.51
Biomass growth (kg m^{-3})				
0–22 days	0.72	1.31	0.031	0.22
22–55 days	1.25	2.47	0.002	0.21
55–84 days	1.26	2.16	0.010	0.24
84–117 days	1.82	3.74	< 0.001	0.21
0–117 days	5.05	9.68	< 0.001	0.08

ALD: aquaponic units at low stocking density of fish; AHD: aquaponic units at high stocking density of fish. RMSE: Root mean square error.

Table 3
Morphometric indices and slaughter results of rainbow trout.

	Stocking density			
	ALD	AHD	P-value	RMSE
Fish (n)	18	18		
Total length (mm)	289	290	0.858	15
Standard length (mm)	250	248	0.797	13
Head length (mm)	61	61	0.644	3
Maximum height (mm)	71	73	0.402	7
Condition factor	1.37	1.35	0.774	0.17
Relative profile	0.25	0.25	0.299	0.02
Cranial index	0.21	0.21	0.356	0.01
Carcass weight (g)	287	289	0.881	73
Carcass yield (%)	89.0	89.0	0.825	0.5
Fillet weight (g)	165	160	0.632	32
Fillet yield (%)	49.9	48.3	0.095	2.9

ALD: aquaponic units at low stocking density of fish; AHD: aquaponic units at high stocking density of fish. RMSE: Root mean square error.

(400 mg L^{-1}; Somerville et al., 2014) for about half of the trial in the AHD tanks and about one third in the ALD tanks (Fig. 2f).

The activity of nitrifying bacteria in ALD and AHD units was proven by the nitrate content, which was higher than previously observed in other one-loop aquaponic systems (Lennard and Leonard, 2006; Palm et al., 2014; Maucieri et al., 2019). The progressive accumulation of nitrates in the aquaponic systems could be related to the reduction in the nutrient uptake by plants at higher pH values (Wortman, 2015) or an unbalance between available nitrate and vegetable biomass. Nevertheless, changes in water ammonia and nitrite during the trial can be related to changes in water temperature and pH, which can affect the bacterial nitrification activity (Chen et al., 2006), besides fish biomass and fish feeding rate which affect nitrogen production. On the other hand, importantly, performance of a biofilter in RAS systems can be optimized by a rigorous designing that take into account all working factors, among which fish total ammonia nitrogen excretion, dietary nitrogen content, biofilter substrate, nitrification rate, biofilter efficiency under different operating conditions (temperature, salinity, and alkalinity), and mass nitrogen load rate (Drennan II et al., 2006).

The mortality rate was very low (3.3% on average), as previously observed for *Cyprinus carpio* farmed in the same aquaponic system at similar stocking densities (initial values: 2.5 and 4.6 kg m^{-3}; final values: 6.9 and 11.5 kg m^{-3}) (Maucieri et al., 2019). To our knowledge, only one study is available concerning trout reared in aquaponic systems focused on microbiological analysis of lettuce (Alcarraz et al., 2019), whereas commercial aquaponics systems are successfully working in Columbia, Chile and Canada. Other authors found that the mortality rate simultaneously increased when the initial stocking density increased, both in *Cyprinus carpio* var. Koi (from 2.1 to 2.8 kg m^{-3}) (Hussain et al., 2014) and *Oreochromis niloticus* (from 0.5 to 1.6 kg m^{-3}) (Rayhan et al., 2018) cultured in aquaponics. However,

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Table 4
Rigor index and flesh quality measured at different times (1 day and 7 days after slaughter) in rainbow trout stored in ice (0 to 2 °C).

Stocking density (D)	ALD		AHD		P-value			
Time (T)	1 d	7 d	1 d	7 d	D	T	D × T	RMSE
Fish (n)	9	9	9	9				
Whole fish								
Rigor index (%)	92.4	18.7	89.2	11.6	0.216	< 0.001	0.645	0.1
Muscle pH	6.55	6.45	6.54	6.45	0.956	0.014	0.885	0.11
Hardness (N)	25.0	6.9	20.2	6.7	0.279	< 0.001	0.329	6.8
Cohesiveness	0.82	0.77	0.83	0.71	0.606	0.101	0.457	0.15
Springiness (mm)	0.39	0.34	0.32	0.45	0.902	0.807	0.598	0.47
Chewiness (N mm)	7.74	1.88	5.59	1.28	0.105	< 0.001	0.357	2.47
TVB-N (mg 100 g^{-1})	16.3	16.5	16.9	16.7	0.293	0.931	0.540	1.1
Skin								
L*	41.0	48.6	37.1	54.8	0.544	< 0.001	0.136	12.8
a*	4.06	0.02	3.34	0.11	0.324	< 0.001	0.201	0.93
b*	6.94	11.00	7.40	9.03	0.321	0.001	0.117	2.27
Fillets								
L*	38.3	43.8	39.1	45.0	0.111	< 0.001	0.775	1.8
a*	−1.71	−1.32	−1.65	−1.06	0.558	0.088	0.724	0.84
b*	9.72	7.58	9.78	8.33	0.402	0.001	0.466	1.42

ALD: aquaponic units at low stocking density of fish; AHD: aquaponic units at high stocking density of fish. RMSE: Root mean square error.

Table 5
Total viable count, *Enterobacteriaceae*, *Pseudomonas* and H_2S-producing bacteria (Log_{10} CFU 20 cm^{-2}) measured at skin level at different times (at slaughter, 0 d, and 7 d after slaughter) in rainbow trout stored in ice (0 to 2 °C).

Stocking density (D)	ALD		AHD		P-value			
Time (T)	0 d	7 d	0 d	7 d	D	T	D × T	RMSE
Total viable count	2.06	2.41	2.59	2.44	0.401	0.982	0.119	0.64
Enterobacteriaceae	0.63	1.00	0.92	0.93	0.665	0.469	0.502	0.78
Pseudomonas	0.39	0.33	0.87	0.74	0.024	0.637	0.846	0.57
H_2S-producing bacteria	0.41	0.41	0.89	0.82	0.072	0.889	0.890	0.72

ALD: aquaponic units at low stocking density of fish; AHD: aquaponic units at high stocking density of fish. RMSE: Root mean square error.

Table 6
Total viable count, *Enterobacteriaceae* and *Pseudomonas* (Log_{10} CFU ml^{-1}) measured in lettuce at harvesting.

	Experimental group			P-value	RMSE
Parameters	ALD	AHD	HYD		
Lettuce					
Total viable count	5.41	5.89	5.64	0.536	0.50
Enterobacteriaceae	3.43	2.44	1.98	0.274	1.00
Pseudomonas	6.04	5.70	6.03	0.492	0.38

ALD: aquaponic units at low stocking density of fish; AHD: aquaponic units at high stocking density of fish; HYP: hydroponic units. RMSE: Root mean square error.

the response to different stocking densities could vary dramatically among fish species (Ashley, 2007), and is largely affected by the biological requirements of the fish and water quality.

Nevertheless, in the present trial, the SGR of trout were considerably lower and FCR higher than that observed in open or recirculating aquaculture systems (Naderi et al., 2017; Zahedi et al., 2019). Low water temperatures (10.7 °C on average) and moderate feeding levels likely accounted for these results. However, rainbow trout show feeding activity at water temperatures as low as 6 °C, while growth and feed conversion are optimized at 15–16 °C (Azevedo et al., 1998; Bregnballe, 2015).

In intensive aquaculture systems, a high stocking density can impair the feed efficiency and growth performance of trout (see Ellis et al., 2002 for a review). Recent studies have confirmed the negative impacts of a high stocking density on the growth performance of different fish species (Li et al., 2012; de las Heras et al., 2015; Ni et al., 2016), including trout (Suárez et al., 2014; Naderi et al., 2017; Zahedi et al., 2019). However, regarding rainbow trout, these effects are detectable only when the fish biomass surpasses 24 kg m^{-3} (677 g final weight) (Zahedi et al., 2019), which did not occur in the current experiment. On the other hand, North et al. (2006) did not observe significant differences in the final weight of female rainbow trout (180 g initial weight; approximately 400 g final weight) stocked at densities of 10, 40, and 80 kg m^{-3}. Indeed, multiple factors, such as fish age, fish size, and water quality, could modify the effects of stocking density on the growth performance and welfare of fish (Ashley, 2007; Poli, 2009). Furthermore, the competition for food and physiological stress due to crowding conditions may also reduce the growth performance of fish (Qi et al., 2016).

4.2. Rheological and microbiological shelf life of fish

As for the growth performance, the stocking density did not modify the slaughter results or morphometric indices of trout. In contrast, Suárez et al. (2014) found a decreased final weight, total length, fillet weight, and viscerosomatic index when the initial stocking density

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increased from 15 to 40 kg m^{-3} in one-year-old rainbow trout (100 g initial weight) cultured in tanks continuously supplied with 1 L s^{-1} of water at 14 °C.

In our trial, we did not detect significant differences in the flesh quality according to the stocking density. In standard land-based production systems, a high stocking density is a major chronic stressor in farmed fish (Hoyle et al., 2007), which may also affect the end-product quality. In fact, a high rainbow trout stocking density (40 kg m^{-3}) has been known to reduce the muscle pH and water holding capacity, and enhance the rigor strength and firmness (Suárez et al., 2014). Nevertheless, in the low-tech aquaponic system we tested, the highest stocking density used did not produce stressful conditions for the rainbow trout and, as a consequence, there were no negative effects on the flesh quality.

The changes in fish texture during storage could be related to autolytic and microbial processes that take place after death, which make the muscle softer and less elastic (Li et al., 2011; Cai et al., 2014). This is usually associated with an increase in flesh lightness during cold storage, as observed in our trial and extensively reported in fillets of rainbow trout (Jouki et al., 2014; Dehghani et al., 2018) and European sea bass (*Dicentrarchus labrax*; Chéret et al., 2005). Moreover, after one week of storage in ice, lighter skin has also been found in the Atlantic salmon (*Salmo salar*; Erikson and Misimi, 2008). However, other authors have found a decreased L* (Álvarez et al., 2008) with an increasing storage time. Therefore, in the present study, the reduction in the rigor index, and hardness and chewiness of the flesh was expected and consistent with previous results concerning trout fillets (Jouki et al., 2014; Concollato et al., 2016), whole gilthead seabream (*Sparus aurata*; Álvarez et al., 2008), and whole red seabream (*Pagrosomus major*; Cai et al., 2014). In fact, the L* index changes based on the muscle structure and amount of free water, which affects light scattering (Chéret et al., 2005). Moreover, significant a* and b* changes can occur in fish during storage because of the oxidation of heme pigments and lipids, respectively (Dehghani et al., 2018).

Under the conditions used in this trial, the flesh pH decreased from 24 h to 7 days of storage, which could be attributed to the anaerobic process of muscle glycogen breakdown with the consequent production and accumulation of lactic acid (Grigorakis et al., 2003; Daskalova, 2019). Nevertheless, several authors have reported that the muscle pH of fish remained stable (Grigorakis et al., 2003; Álvarez et al., 2008) or increased (Dehghani et al., 2018; Secci et al., 2018) after 6–8 days of cold storage, due to the formation of alkaline by-products of microbial origin (Mokrani et al., 2018). In the current study, the TVB-N concentration in the fish muscle did not change after one week of storage in ice, and remained below the acceptability threshold (25–27 mg TVB-N 100 g^{-1} of sample) (Giménez et al., 2002; Ninan et al., 2011).

The shelf life for rainbow trout stored on ice is approximately 8–11 days (Ninan et al., 2011; Sampels, 2014). In our study, the skin microbial contamination remained stable during the 7-day storage, which is consistent with the absence of change concerning TVB-N. The low levels of spoiler targets, such as *Pseudomonas* and *Shewanella,* explain the dynamics of chemical markers of freshness, such as TVB-N. Nevertheless, the surface washing of fish by melting ice could have also contributed to this result, as reported in other studies on rainbow trout and other species using slurry ice (Aubourg et al., 2009; Rodríguez et al., 2006). Moreover, in our trial, the initial and final mesophilic counts were lower than those previously measured in other species reared in aquaponics (Elumalai et al., 2017), whereas the initial skin contamination was consistent with previous recordings in the same species farmed under conventional aquaculture systems (Aubourg et al., 2009).

4.3. Lettuce production and microbiological contamination

The increasing concerns regarding water consumption in food production should be solved by a comprehensive approach that includes several strategies focused on the improvement of water-use efficiency, especially where the availability of water for agricultural purposes is a critical factor (Goddek et al., 2015). In the present trial, the daily water consumption was within ranges reported in most of the literature (0.05–5.0%; see Maucieri et al., 2018 for a review). As expected, results obtained during winter were 70% lower than those achieved in the same aquaponic system during summer (Maucieri et al., 2019).

Other authors have reported that aquaponic systems can equal or even overcome the hydroponic method in the production of lettuce, herbs, and fruiting vegetables (Suhl et al., 2016; Lennard and Ward, 2019). This is consistent with our results that showed the comparable (first crop cycle) or greater (second crop cycle) marketable yield of lettuce in aquaponics compared with the HYP units. The vegetable production in aquaponics is supported by the continuous supply of N compounds from the fish and the dense community of microbial flora in the system, which may assist plants in nutrient access and uptake (Lennard and Ward, 2019). Despite Pantanella et al. (2012) reporting that the yield of lettuce decreased in aquaponics with respect to hydroponics when the fish density dropped from 8 to 5 kg m^{-3}, we did not find any effect of the stocking density on lettuce production which means that nutrient supply was sufficient also in the case of the low stocking density. In addition, Maucieri et al. (2019) observed similar yields of lettuce in hydroponics and aquaponics at a low fish stocking density (*Cyprinus carp*, 2.5 kg m^{-3}). Overall, the marketable yield of lettuce achieved in the present study was lower than that reported by other authors (Lennard and Leonard, 2006; Maucieri et al., 2019). Differences in the variety of lettuce, environmental conditions, nutrient concentrations, water temperature, and solar irradiation justify the different results among studies.

Regarding the microbial quality of the lettuce, leafy vegetables are often largely contaminated. The results of the present trial are consistent with previous ones concerning whole vegetables, such as red chicory, on which *Pseudomonas* (6 Log_{10} CFU g^{-1}) and *Enterobacteriaceae* (4 Log_{10} CFU g^{-1}) were dominant microorganisms (Alfonzo et al., 2018). Under the conditions of the present study, the average mesophilic count (reported as TVC) at harvest in the lettuce rinsate (5.65 Log_{10} CFU mL^{-1}) was comparable to the values measured after 63 days (4.5 Log_{10} CFU g^{-1}) but higher than those after 118 days of cultivation (2.8–3.5 Log_{10} CFU g^{-1}) in a previous study cultivating lettuce in aquaponics (Elumalai et al., 2017). Moreover, we did not find differences in the counts of mesophilic bacteria and *Enterobacteriaceae* between lettuce leaves produced in aquaponics or in hydroponics as already found by Alcarraz et al. (2019) who did not find differences neither in psychrophilic bacteria. Finally, in lettuce, as with fish samples, the absence of faecal indicator bacteria, such as *E. coli*, suggests the aquaponic system was suitably hygienic (Elumalai et al., 2017). On the other hand, a recent study (Wang et al., 2020) highlighted the risk for foodborne pathogens (e.g. *E. coli* STEC) in aquaponic and hydroponic systems.

5. Conclusions

The economic margin of aquaponic systems deployed in cold environments could be increased by rearing species characterised by a higher market value than the warm-water species usually cultured, such as rainbow trout. Based on our results, rainbow trout can be successfully farmed in a low-tech aquaponic system until a final density of approximately 17 kg m^{-3} without negative effects on the growth and flesh quality. The marketable yield of lettuce, similar to that obtained in hydroponics, confirmed that aquaponics is a viable integrated pro-

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duction system for both fish and vegetables. The chemical, physical, and microbiological indicators proved that food-safe products can be obtained from a low-tech aquaponic system.

Uncited references

Drennan II et al., 2006
Palm et al., 2018
Rodríguez et al., 2006
Sampels, 2014
Suhl et al., 2016

Declaration of Competing Interest

None.

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